1 CELL AND INTEGRATION OF METABOLISM

Cell and integration of metabolism

Q. The marker enzyme of microsomes (Endoplasmic Reticulum) is?

a. Galactosyl Transferase
b. Cathepsin
c. Lactate Dehydrogenase
d. Glucose 6 Phosphatase

- Microsome is nothing but Endoplasmic Reticulum and in Endoplasmic Reticulum part of Gluconeogenesis happens.
- Galactosyl Transferase is an important enzyme involved in glycoprotein Synthesis. The sub organelle involved in this is Golgi Complex.
- Cathepsin are Proteases. Present in Lysosomes and enzyme marker of Lysosomes.
- Lactate Dehydrogenase is the enzyme of Glycolysis which happens in Cytoplasm. It is a marker for Cytoplasm.

Marker Enzymes

00:02:15

S no.	Organelle	Marker Enzyme
1	Nucleus	DNA & RNA Polymerases
2	Endoplasmic Reticulum	Glucose 6 Phosphatase
3	Golgi Complex	Galactosyl Transferase
4	Mitochondria Outer Membrane (OM) Inner Membrane	Monoamine Oxidase ATP Synthase / Succinate Dehydrogenase
5	Lysosome	Cathepsin
6	Cytoplasm	Lactate Dehydrogenase
7	Peroxisome- Very long chain Fatty acid oxidation, concerned with Ether Lipid Synthesis (Plasmalogen), Related to BCFA Oxidation (Refsum's Disease).	Catalase

Q. Cytoplasmic Proteins are synthesized in?

a. Ribosomes
b. Nucleus
c. Smooth Endoplasmic Reticulum
d. Rough Endoplasmic Reticulum

- Rough Endoplasmic Reticulum is related to Protein Synthesis.
- Smooth Endoplasmic Reticulum is related to Steroid Synthesis.
- All protein synthesis is initiated by a Free Ribosome

Where are proteins Synthesized?

00:07:00

- Free Ribosomes
- These Free Ribosomes read the MRNA from 5' to 3'. They read every codon one by one. And depending upon the codon, ribosomes are capable of recruiting a complementary anticodon containing tRNA.
- And these tRNA, drag along with them an Amino acid and this is how nucleotide sequences of mRNA get translated as Amino acid sequence or Polypeptide chains.
- This process is known as the Translation process.
- When Ribosomes reach the mRNA from 5' to 3', the polypeptide chain will grow from Amino terminal (First amino acid) end to Carboxyl terminal end (last Amino acid).
- The synthesis is done for the Mitochondrial, Nuclear and cytoplasmic protein.
- For other types of protein Ribosomes are not sufficient.

Protein Synthesis And Sorting

00:10:20

- All free ribosomes start reading mRNA from 5' to 3'.
- Depending upon the codon, they recruit a complex with anticodon called tRNA.
- And this is how translation happens and the Polypeptide chain will start growing from the amino terminal end to the Carboxyl Terminal end.
- But if the protein getting synthesized is one of the three types- Lysosomal, membrane or Secretary Protein because you want specific targeting to target these proteins to respective organelles.
- For example- Not all proteins just like that can be targeted to Lysosomes. If you want any protein to target it, then it needs a label and that label is Mannose 6 Phosphate.
- Mannose is a Carbohydrate. Only ribosomes are not sufficient in these type syntheses.
- So, you need other organelles in the form of endoplasmic reticulum and Golgi Complex to help in attaching this signal / label.
- Similarly, not all proteins like that can reach the membrane.
- For a protein to reach the membrane, it needs a label which is Lipid side chain because the membrane is lipid bilayer and for a protein to become a membrane protein, it needs a Lipid side chain.

- In this also you need the endoplasmic reticulum and Golgi Complex to support as free Ribosomes individually are not sufficient.
- So, in all the three types- Lysosomal, Membrane and Secretary because you want specific sequences to target them to respective organelles and because that targeting sequence can only be attached by Endoplasmic Reticulum and Golgi Complex, you want rough Endoplasmic Reticulum to be formed.
- But the rough Endoplasmic Reticulum is directly not formed. It is initiated by a free Ribosomes.
- Again, they read mRNA, the polypeptide chain changes but the amino terminal end of these three types of proteins alone have a signal recognition peptide sequence.
- The function of the Signal sequence is to guide the Ribosomes to get attached to Endoplasmic Reticulum forming Rough Endoplasmic Reticulum within the same cell.
- There will definitely be a nucleus and the outer membrane of this nucleus will attach Endoplasmic Reticulum.
- But this is a smooth Endoplasmic Reticulum. Once the Signal recognition peptide sequence is synthesized, they will guide the ribosomes to attach to ER forming Rough ER.
- And when the Ribosomes come, they don't come freely, it comes with mRNA which it is translating so that the polypeptide chain now starts growing into the vesicles of Endoplasmic Reticulum.
- Once Rough Endoplasmic Reticulum is formed, the signal wouldn't be required and removed.
- That is how the pre-pro proteins to Proproteins (Same happens with Preproamino acid or others).
- Also, when the signal is removed, some modifications happen on the polypeptide chains because these modifications are happening along with translation.
- Still the polypeptide chain is going. And along with translation, some modifications are happening simultaneously.
- The modifications are called co-translational modifications which are happening because of rough Endoplasmic Reticulum.
- After co-translational modifications, all these proteins will be folded.
- The first protein folding happens within the Endoplasmic Reticulum. After folding they leave the Endoplasmic Reticulum and reach the Golgi Complex.
- The proteins pass through the Cyst, Medial and Trans Golgi.
- During the transit through Trans Golgi Complex, some modifications happen which are called post translational modifications.
- After post translational modifications, the proteins get packed and are sorted out.
- The sorting depends on the label present.
- If the protein has Mannose 6 phosphate residue, the protein is targeted to Lysosome.
- If the protein has got a Lipid side chain, it will target the Membrane protein.
- If the protein carries neither Mannose or lipid, by deform it gets secreted as a Secretory Protein.

MCQ's 00:21:20

Q. Secretory Proteins are Synthesized in:

a. Ribosomes
b. Smooth Endoplasmic Reticulum
c. Rough Endoplasmic Reticulum
d. First in Ribosomes, and then in Endoplasmic Reticulum

Q. A child present with coarse features, HSM and mental retardation. The Clinicians suspect a LSD and ask the IEM lab to perform a few Lysosomal enzyme activities including Hexosaminidase. All Lysosomal enzyme activities were high. What is probably the diagnosis?

a. Tay Sachs Disease
b. Mucopolysaccharidosis I
c. Mucopolysaccharidosis II
d. I Cell Disease

I Cell Disease 00:21:20

- It is Inclusion Cell Disease.
- It is a Lysosomal storage Disorder caused by the defect of an enzyme in Acetylglucosamine Phosphotransferase. Also known as the Phosphotransferase Effect.
- The function of Phosphotransferase is to form Mannose 6 Phosphate which is the label for a protein to be targeted to Lysosome.
- So, when the Phosphotransferase is defected, the label for Lysosomal enzyme is missing.
- So, all Lysosomes will be empty in this condition.
- When the Lysosomes are empty, as all cells are in Pinocytosis.
- A part of the cell forms an indentation. That indentation deepens, it gets pinched out of the cell membranous endocytic vesicle.
- That way, it is nonspecifically talking the part of interstitial fluid into the endocytic vesicle.
- And this endocytotic vesicle content has to be metabolized, which is done by primary Lysosomes.
- Primary Lysosomes fuse with endocytotic vesicles to form a secondary Lysosome.
- Within the secondary Lysosome, the Lysosomal enzymes are expected to digest the content.
- But in this child, there is a defect of Phosphotransferase because its Lysosomal enzymes are not targeted properly and are empty.

- And there are multiple inclusions in the connective tissue that cause coarse facial features.
- Multiple inclusions and parenchymal cells will present to us Hepatosplenomegaly.
- Accumulation neurons will cause mental retardation.
- Similarly, all Lysosomal activities will be hyped because when a protein carries neither Mannose nor lipid, it gets secreted as secretory protein.
- So, in this condition, the Lysosomal proteins have not reached lysosomes. Instead they are into circulation. Plasma Lysosomal activity will be hyped.
- Within the cell, Lysosomal enzyme activity will be low. So, tissue Lysosomal activity will be less in this condition.
- The triad that is used for diagnosing I cell disease will be Clinical Features resembling a Lysosomal storage Disorder with a paradox (plasma Lysosomal activities high, Tissue Lysosomal activities high).
- Caused by the defect of Phosphotransferase, a defective labeling of Lysosomal enzymes.

Q. A 39-year-old man came to the emergency department because of severe back pain. An ultrasound was taken and renal stones were found. Following lithotripsy, a sample of the stone was sent for biochemical analysis. Results revealed the presence of oxalate and glyoxylate. He was diagnosed with primary hyperoxaluria Type I. It is caused by?

a. Protein Folding defect
b. Silent Mutation
c. Protein Targeting Defect
d. Acceptable Mutation

Hyperoxaluria 00:29:30

Primary Hyperoxaluria

- It has two types: Type I & Type II. Both are related to Glycine metabolism.
- **Metabolism of Glycine:**
  - Glycine gets metabolized by reacting with keto acid which is Pyruvate (Most enzymes in the body react with Keto acid).
  - Glycine gives off its amino group to Pyruvate converting it into amino acid which is Alanine. This way Glycine amino acid becomes a keto acid which is Glyoxylate.
  - This reaction is happening in Peroxisome.
  - The enzyme is Glyoxylate Alanine aminotransferase which helps in the conversion of Glycine and Pyruvate to Glyoxylate and Alanine.
  - This reaction is reversible.
  - The Glyoxylate which is formed will again react and form Glycine.
  - If reverse reaction does not happen, the excess Glyoxylate will be reduced by Glyoxylate Reductase and will be converted to Glycolate.
  - If Glyoxylate cannot become Glycolate, it becomes Oxalic Acid.

Type I Primary Hyperoxaluria

- It is a protein Targeting Defect.
- The Glyoxylate Alanine Aminotransferase, instead of being there in Peroxisome, is miss placed in mitochondria.
- When in Mitochondria, the reversible reaction is not taking place. So, any Glycine consumed will be converted to Glyoxylate.
- So, the excess Glyoxylate will be converted to Oxalic Acid (Oxalate) and cause Type I Hyperoxaluria.

Type 2 Primary Hyperoxaluria

- It is caused by Glyoxylate Reductase.
- When it is defective, the Glyoxylate is Converted to Oxalate.

Secondary Hyperoxaluria

- It is most common because of Dietary effects (Oxalate rich food). Dietary effects can be when a person eats a lot of chocolates, beetroots, green leafy vegetables.

Enteric Hyperoxaluria

- Usually 5-10% of dietary oxalate will get absorbed. Remaining will form stones with calcium. The insoluble calcium oxalate is excreted in feces.
- When there is Fat malabsorption, fatty acids will get accumulated in the intestinal lumen. These fatty acids will form complexes with calcium and they get excreted with Faeces.
- The calcium is not available for forming calcium oxalates. So, there will be free oxalates accumulating in the lumen. And these will be absorbed paracellularly causing secondary Hyperoxaluria.

Introduction To Metabolism 00:35:50

- Metabolism is the process by which we assimilate the food we intake. For assimilating, there are two parts of Metabolism-
  - Catabolism
  - Anabolism

Catabolism

- It is the process by which we break down complex molecules into simpler substances.
- This is done by breaking covalent linkages in which the energy is liberated.
- Depending upon how you will trap the energy liberated in the form of ATP, Catabolism is of two types:

Substrate Level Phosphorylation

- In this you will see a set of substrates converted to a set of products. And simultaneously the ADP is converted to ATP.
- The four examples of Substrate level Phosphorylation are:
  - Phosphoglycerate Kinase (glycolysis step)
  - Pyruvate Kinase (glycolysis step)
  - Succinate Tyrosinase step of citric acid cycle
  - Creatine kinase of muscle

Oxidative Phosphorylation

- Even here the set of substrates is converted to a set of products.
- Energy is liberated too but it is not directly trapped as ATP.
- It is trapped as Reducing Equivalence like NADH / FADH 2.
- After this, the oxidized NADH / FADH 2, they leave electrons and they transport the electrons in the electron transport chain which in a complex way liberates energy and that energy is used for Phosphorylation of ADP to ATP.
- The energy is trapped in the form of ATP in Catabolism because energy will be liberated as heat if we don't use it. Another reason is that the energy should be liberated in a usable form, which is the energy currency of the body (ATP).
- Oxidative Phosphorylation is more common in human metabolism because human beings are aerobic organisms.

Integration Of Metabolism 00:42:10

- Most of the fuels in the body would be carbohydrates.
- First, we get glucose and most of it will get into Glycolysis.
- When glucose gets into Glycolysis, one molecule of glucose will split into 2 pyruvates. This way you expect 7 ATPs.
- This step is known as Aerobic Glycolysis.
- When the same process happens in the absence of oxygen, the 2 pyruvates will be converted to 2 Lactate.
- And when the final product is Lactate, only 2 ATPs are expected.
- In the presence of Oxygen, after the formation of Pyruvate the process continues because pyruvate is an acid and if you allow this acid to accumulate, it will cause metabolic acidosis.
- All fuels get finally metabolized through the citric acid cycle.
- To enter the Citric Acid cycle, the 2 pyruvate is converted to 2 Acetyl CoA and the enzyme used is Pyruvate Dehydrogenase.
- This Pyruvate Dehydrogenase removes hydrogen from Pyruvate, gives it to its coenzyme NAD and converts it into NADH, which is equal to 2.5 ATP.
- For 2 pyruvates, you expect 5 ATPs.
- Now the 2 Acetyl CoA goes into the Citric Acid Cycle and finally comes as CO2 which can be exhaled out.
- This is called Complete Oxidation of Glucose.
- Every Citric Acid Cycle gives 10 ATP.
- So, 2 Acetyl CoA give 20 ATPs.
- So, when glucose is completely oxidized, it gives 32 ATPs.
- When the energy is high, then also Glucose enters into a cell.
- And forms 2 pyruvate, then 2 Acetyl CoA.
- But during this when you have adequate pyruvate and Acetyl CoA, there will be feedback inhibition of Glycolysis.
- At one point, Glucose 6 Phosphate, instead of getting into Glycolysis, gets into Glycogen Synthesis which is stored.
- Some Glucose 6 Phosphate will start entering into HMP Shunt.
- HMP Shunt Significance:
  - It acts as a source of NADPH
  - It acts as a source of Ribose 5 Phosphate. Ribose 5 Phosphate is like a foundation based on which you built all nucleotides.

Difference Between Nadph Vs Nadh 00:50:30

S. No	NADH	NADPH
1	Can enter into ETC and give rise to 2.5 ATPs. It is going to be a part of Oxidative Phosphorylation.	• Necessary for reductive biosynthesis of all lipids. • It is also necessary for regenerating Glutathione which is an important antioxidant mechanism of RBCs. • Necessary as a coenzyme for Ribonucleotide Reductase • Necessary as a coenzyme for Cytochrome P450 Enzymes
2	**Sources**- all fuel Oxidative pathways. • Glycolysis • PDH • Citric Acid Cycle • Amino acid oxidation	**Sources-** • The major source of it HMP Shunt • Minor sources include Cyt ICDH • Malic Enzyme

- RBCs act as a major source of Oxidative stress in the body because they carry oxygen. And when they carry there is a high proficiency that they donate an electron converting oxygen to Superoxide radical. This radical becomes hydrogen peroxide that becomes peroxyl radical that causes lipid peroxidation.

- Suppose Hydrogen Peroxide is generated in RBCs, immediately Glutathione will come for rescue. Two molecules of Glutathione will come. Both these molecules will donate one hydrogen each. So, hydrogen Peroxide changes to 2 molecules of water. In this process Glutathione has been oxidized. The enzyme that catalyzes this reaction is Glutathione Peroxidase.

- Now, you have to regenerate Glutathione. For this the enzyme necessary is Glutathione Reductase. For this reduction a hydrogen source is needed which is NADPH.

Defect of Hmp Shunt

- In HMP Shunt, there is an enzyme Glucose 6 Phosphate Dehydrogenase.
  - Glucose 6 Phosphatase and Glucose 6 Phosphate Dehydrogenase are different.
  - Glucose 6 Phosphatase is an enzyme of Gluconeogenesis which increases blood glucose.
  - Glucose 6 Phosphate Dehydrogenase is an enzyme of HMP Shunt which is a source of NADPH.
- So, when HMP Shunt (G6PD) is defective, there is no NADPH. And without NADPH you won't be able to regenerate Glutathione.
- When it is not regenerated, RBCs miss an effective antioxidant mechanism. So, RBCs undergo Lysis following exposure to Oxidative Stress.
  - The history is very classical.
  - There is a child presenting with Hemolytic Anemia after intake of Fava Beans or Primaquine (Prophylactic drug for malaria). Any of these, means Defect in HMP Shunt, G6PD.

Fate of Acetyl Coa

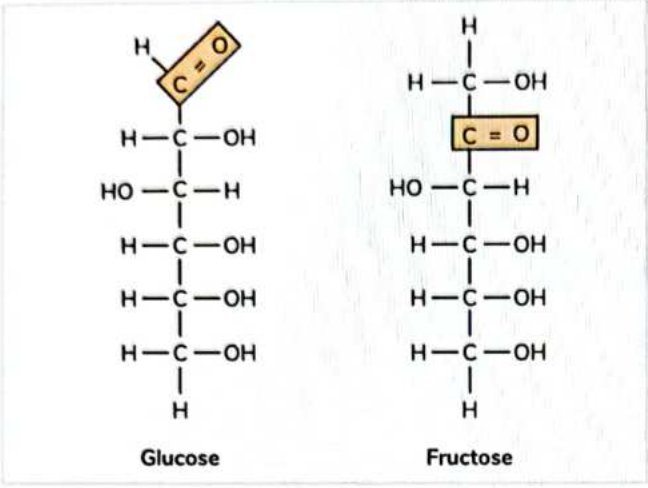
- When there was low energy, all Acetyl CoA got into the Citric Acid Cycle.
- Acetyl CoA is a building block of Fatty acid and Cholesterol.
  - Excess fatty acid will be stored as Triacylglycerol.
  - Excess cholesterol will be stored as cholesterol esters.
- It is a Carbohydrate rich diet which is lipogenic. It is because of carbohydrate Glucose which becomes pyruvate. It is pyruvate which becomes Acetyl CoA which is the building block of Fatty acid and Cholesterol.
- If you want to avoid Hypertriglyceridemia or Hypercholesterolemia, carbohydrate intake has to be restricted otherwise it would result in Lipogenesis.

Preferred Fuel 01:03:30

- Anaerobic Glycolysis gives rise to 2 ATP. So, the only pathway which can generate ATP in the absence of oxygen being anaerobic Glycolysis, the only fuel which can be used in anaerobic condition is glucose.
- Fatty and amino acids can only be used in aerobic conditions.
- The choice of fuel will depend on Aerobic or anaerobic.
- If the cells are anaerobic, they have to use only glucose. These are:
  - RBCs, Retinal cells and Corneal cells
  - White muscle fibers (stores glucose as glycogen) without myoglobin
  - The renal medulla.
- If the cells are aerobic, they can use either glucose or fatty acids. But the cells will choose fatty acid because glucose on complete oxidation gives only 32 ATPs whereas Fatty acid can give 106 ATPs. Stearic acid will give 120 ATP.
- So, all Aerobic cells choose Fatty acids as preferred fuel. Cardiac muscle fibers, red muscle fibers use fatty acids.
- But there is one aerobic cell which uses glucose which is Neurons. The neurons are covered by the blood brain barrier. In this condition any fatty acid present in circulation will be attached to albumin and not be able to cross BBB. That is why neurons have evolved to use glucose as their preferred Fuel.
- RBCs use glucose anaerobically while Neurons use aerobically. In RBCs, 1 glucose will be 2 Lactate and will give 2 ATP. The same glucose in Neurons will break to give carbon dioxide and each will give 32 ATPs.

2 CLASSIFICATION OF CARBOHYDRATES SIMPLE CARBOHYDRATES

What are Carbohydrates?



Basic similarity: Both have multiple hydroxyl groups

Basic difference

- Glucose has got an Aldehyde group.
- Fructose has got a ketone group.

Definition

- That is why we define all carbohydrates as polyhydroxy Aldoses or Ketoses.
- Having general molecular formula: $C_nH_{2n}O_n$

Classification of Carbohydrates

Carbohydrates

Simple carbohydrates	Complex carbohydrates
• They have carbohydrates units only	• Carbohydrate attaches to protein or lipid and of 3 types • Glycoproteins • proteoglycans • Glycolipids o Point to remember: Glycoproteins & proteoglycans are not at all the same things.

Q. How to classify the simple carbohydrate where only carbohydrate units are present?

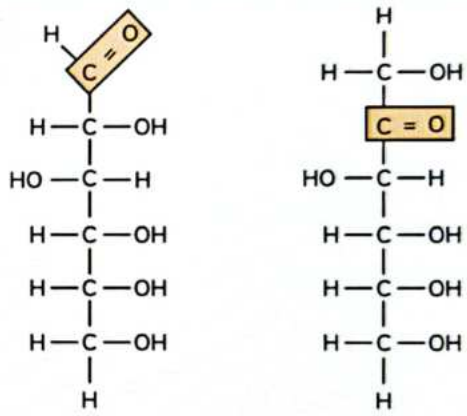
Ans. The classification of simple carbohydrates is based on the number of carbohydrate units– logically -into three types

- Monosaccharides: Single carbohydrate unit in them
- Oligosaccharides: 2 to 10 units of carbohydrates. E.g., disaccharides
- Polysaccharides: These have more than 11 units of polysaccharides

Monosaccharide

- Two ways to classify the monosaccharides.
  - Number of carbon atoms
  - Functional group

Based on Number of carbon atoms	Based on functional group
• Trioses: 3 carbon atoms. • Tetroses: 4 carbon atoms. • Pentoses: 5 carbon atoms. • Hexoses: 6 carbon atoms	• Aldoses: Aldehyde group • Ketoses: Ketone group



Q. In glucose, how many functional groups do you find?

Ans.

- Only one functional group is present in glucose and that is monosaccharide. The functional group that presents in glucose is aldehyde. Therefore, glucose is an Aldose.
- Glucose is a hexose → glucose has 6 carbon atoms.
- **Quick tips**
  - Based on the no. of carbon atoms - glucose is a hexose
  - Based on the functional group - glucose is an aldehyde

Q. In fructose, how many functional groups do you find?

Ans.

- In Fructose, only one functional group is present that is monosaccharide because it got only one sugar unit.
- The functional group that presents in fructose is Ketone group- makes fructose ketosis.

- **Quick tips**
  - Based on the no. of carbon atoms - 6 carbon atoms - fructose is a hexose
  - Based on the functional group - one sugar unit makes it a ketose - ketone group.
- Glucose and fructose share same molecular formula: $C_6H_{12}O_6$

Two facts

- Based on a misnomer - Triose is a monosaccharide - 3 carbon atoms
  - Misnomer is maltotriose - not a triose - it's a trisaccharide with 3 glucose residues → 3 * 6 = 18 atoms
- If you see "Mal" in a compound's names, it means made up of multiple glucose residues.
  - Maltose: 2 glucose residues
  - Isomaltose: 2 glucose residues
  - Maltotriose: 3 glucose residues
- How do you name a ketose from a corresponding aldose name?
  - Ribose is aldose and ribulose is a ketose
  - Erythrose is an aldose erythrulose is a ketose
  - Cidoheptose is an aldose, and cidoheptulose is a ketose
  - The name is given by adding UL to the corresponding aldose name.

Disacccharides

- One of the types of oligosaccharides having - Two sugar units

Two must know facts are

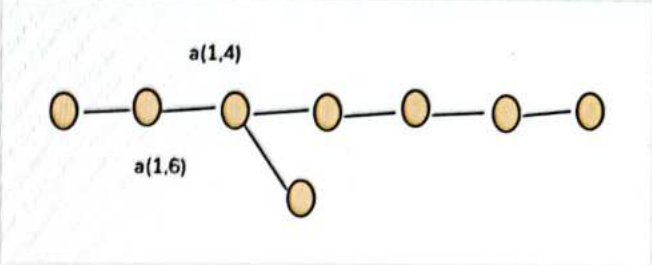
- What is the individual sugar present?
- What is the linkage that is present?

Disaccharide	Sugars present	Linkage
Maltose (Basic thumb rule)	2 glucose residues– glu+glu	**α(1,4)**
Isomaltose (Six atoms)	2 glucose residues– glu+glu	**α(1,6)**
Trehalose	2 glucose residues– glu+glu	**α(1,1)**
Sucrose (non-reducing- although made up of reducing agents) Also called table sugar	Glucose and fructose – glu+fru	**α(1,2)**
Lactose (Present in milk)	Galactose + glucose	**β(1,4)**

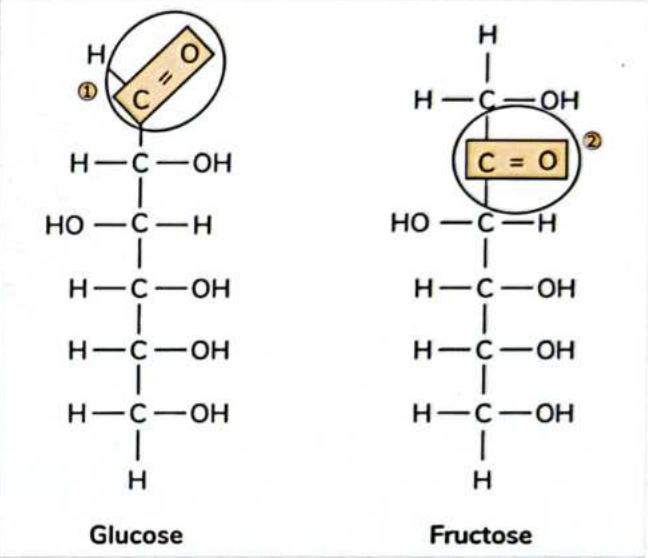
Linkages

Basic thumb rule

- All the carbohydrates preferred to link with α (1,4) linkage along straight chains. And at branch points, they have α (1,6) linkage.



Sucrose



Q. Why are glucose and fructose known as reducing sugars?

Ans.

- Both are reducing sugars because both have free carbonyl groups.
- Glucose - free carbonyl group in first carbon atom
- Fructose - free carbonyl group in second carbon atom

Q. Why is sucrose a non-reducing?

Ans. Because we get sucrose by linking 1st carbon atom of glucose and 2nd carbon atom of fructose neither the carbonyl group of glucose nor that of fructose is free. Linkage presents in sucrose α(1,2)

Lactose

- Linkage in lactose is β(1,4).
- As we can not digest cellulose, on ingestion of plant diet, undigested food reaches colon.

- Most human digestive enzymes cannot attack β linkages – indirectly tells us linkage present in cellulose, which is made up of multiple glucose residues linked multiple β(1,4) linkage.
- The only human digestive enzyme which can attack beta linkage is lactase - lactose.

Lactose Intolerance 00:21:00

- Lactose intolerance is a digestion defect, while other disorders - fructose intolerance and galactosemia are metabolism defects.
- Lactose intolerance is called a digestion defect. Because caused by a defect of lactase in the intestinal villi–when lactase is absent in intestinal villi - any lactose present in the diet cannot be digested to give galactose and glucose.
- Not able to break disaccharides into monosaccharides - disaccharides cannot be absorbed; only monosaccharides will be absorbed.
- Disaccharides will stay back in the lumen - disaccharides are osmotically active, attracting water and causing osmotic diarrhea.
- Predominant clinical presentation of lactose intolerance is it always present with osmotic diarrhea - due to the absence of lactase in intentional villi - lactose cannot be digested, causing osmotic diarrhea. - undigested substances reach the colon.
- Colon has microorganisms that utilize lactose, converting lactose into hydrogen and methane.
- Both these are gasses that accumulate in the colon - other manifestations of lactose intolerance– it presents with flatulence, frothy stools, and bloating.
- Same hydrogen and methane generate the explanation for the methane breath test and hydrogen breath test. - done to detect lactose intolerant.
- IOC for lactose intolerance is methane breath test and hydrogen breath test.

How are these tests done?

- The patient needs to fast overnight.
- Early morning breath sample is taken.
- Amount of hydrogen or methane is measured based on laboratory preference.
- Measured qty of lactose is given to the patient - periodic interval breath samples are collected.
- Estimate the hydrogen or methane level in the sample.
- If these hydrogen or methane concentrations increase beyond the physiological - it is diagnostic of lactose intolerance.
- Additionally, colonic microorganisms act on this undigested lactose to form acids are responsible for acidic ph in stools.
- This pH acidic in stools is responsible for perianal excoriation – features of lactose intolerance.

Classification of Polysaccharides

- Have Eleven or more numbers of units.

Polysaccharides

Homopolysaccharides	Heteropolysaccharides
• Individual units are the same. • They are made up of repetitive units of one sugar moiety	• Individual units are different. • Mucopolysaccharides /glycosaminoglycans GAG: Best Example

Homopolysaccharides

Homopolysaccharides	
Structural homopolysaccharides	**Storage homopolysaccharides**
• It acts as a structural component of the cell.	• Storage forms of carbohydrates
• **Examples** ○ Cellulose - is a component of plant cell wall - made up of multiple glucose residues - linked with β(1,4) linkages. ○ Inulin - used to measuring glomerular filtration rate - present in plant tubes - made up of fructose. ○ Chitin - exoskeleton scales of snake and cockroaches - made up of N-acetyl glucosamine (NAG)	• **Examples** ○ Storage form of glucose in plants: Starch ○ Storage form of glucose in animals: Glycogen

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Q. Which one will be the branch among these two?

Ans.

- Storage will be branched - then only it will accommodate more molecules in limited space.
- Structural homopolysaccharides will be unbranched.

Starch

- Storage form of glucose in plants
- Porridge and starch - resemblance
- Porridge two components - solid and liquid components
- **Starch has 2 components.**
  - Highly branched amylopectin: it has multiple glucose residues which are linked by α (1,4) linkage along straight chain and α(1,6) at branch points.

→ Sequential linkages of α(1,4) - Amylopectin.
→ It is very stable and its melting temp. Is high — 50° - solid part of starch - amylopectin.

- Unbranched Amylose - multiple glucose residues linked by α(1,4) linkages along straight chains
  → Not that stable.
  → Less melting temperature - room temp it is molten - liquid part of starch.

Glycogen

- Storage form of glucose in animals.
- Two tissues store glycogen in the human body are Liver and Muscle.
- Glycogen is the Highly branched carbohydrate structure.
- It is a Spherical molecule - center protein called as Glycogenin - every glucose residue is attached directly or indirectly
  - Straight chain - it is directly attached to glycogenin
  - Branch point - indirectly attached to glycogenin
    → Straight chain has 11-13 glucose residues - linked by α(1,4) linkages and branch point α(1,6) linkage alone
    → Entire structure is arranged in 12 concentric layers to enable compactness - glucose residues in straight chain will form 1 concentric layer hence 12 layers.

Q. In such a structure, where will you find a greater number of branch points Towards the center or Towards the periphery?

Ans. Towards the center you will find more branch points. As it moves towards the periphery, branch points decrease.

- Because you start synthesizing glycogen from the center and you proceed towards the periphery.
- Start this synthesis, you have more no. Of glucose molecules - you will introduce many possible branch points.
- Don't have many no. Glucose molecule - stop creating branch points.

One Liners

- Glucose and fructose are functional isomers.
- Inulin is a homopolysaccharide made up of fructose.
- Chitin is made up of N-Acetyl Glucosamine
- Non-reducing disaccharides are sucrose and trehalose.
- In starch, amylose is unbranched, and amylopectin is branched.

MCQS

Q. All the following are trioses except
A. Maltotriose,
B. Glycerose,
C. Dihydroxyacetone
D. Glyceraldehyde

Q. The linkage present in lactose is:
A. α(1,4)
B. β(1,4)
C. α(1,2)
D. β(1,2)

Q. the present linkage in isomaltose is:
A. α(1,4)
B. β(1,4)
C. α(1,6)
D. β(1,6)

Q. all the following are aldose except
A. Glucose
B. Galactose
C. Ribose
D. Ribulose

Integrated Clinical Case-Based MCQS

Q. A 21-year-old man presents with diarrhea, bloating, flatulence, and frothy stools every time he consumes milk and ghee butter. The probable enzyme detects in this condition is.
A. Aldolase B
B. Fructokinase
C. Galactose 1 phosphate uridyl Transferase
D. Lactase

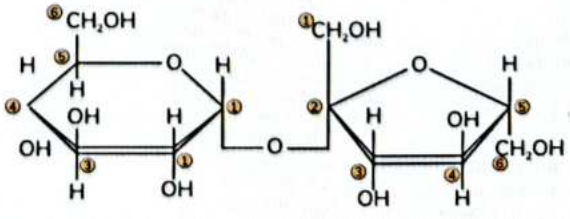
Q. A neonate presents with diarrhea, incessant cry, and frothy stools. Perianal rashes are prominent. The neonate responded well when the child was given rice porridge. What is the test that can be performed to diagnose this condition?
A. Schilling's test
B. Guthrie's test
C. Methane Breath test
D. Stool acidity

Important Information

- Schilling's test is done for B12 malabsorption.
- Guthrie test for phenylketonuria
- Methane breathe test for adults for lactose intolerant.
- Stool acidity for neonates for lactose intolerance.

Image-Based Question

Q. Identify the Structure



A. Sucrose
B. Lactose
C. Maltose
D. isomaltose

Explanation

- How many carbon atoms in the ring?
  - 4 carbon atoms: Fructose
  - 5 carbon atoms: Glucose /mannose /Galactose

- How to know which out of the three it is?
  - Check 2nd and 4th atoms.
    - → If in both 2nd and 4th atoms, OH lies below the plane it is glucose.
    - → If in 2nd carbon atoms, if OH lies above the plane of the ring it is mannose.
    - → If in 4th carbon atoms, if OH lies above the plane of the ring it is Galactose.

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3 MUCOPOLYSACCHARIDOSIS

Mucopolysaccharides

Definitions

- Mucopolysaccharidosis, also known as glycosaminoglycans.
- Long, straight unbranched chains containing repetitive units of uronic acid and amino sugar arranged alternatively.
- Additionally, they get sulphated or acetylated (-ve charges)

Exception

1. **Keratan Sulphate:**
   - Instead of uronic acid, it has Galactose (repetitive units of Galactose and amino sugar arranged alternatively).
2. **Uronic acid:**
   - Mostly Glucuronic acid.
   - Instead, Iduronic acid is present in Heparin, Heparan sulphate and Dermatan sulphate.
   - Both Glucuronic and Iduronic acid are epimers. They change only in the orientation of carboxyl growth.
3. **Amino sugar:**
   - Mostly Glucosamine.
   - Instead, Galactosamine is present in Chondroitin sulphate and Dermatan sulphate.

Additionally, gets sulphated or acetylated

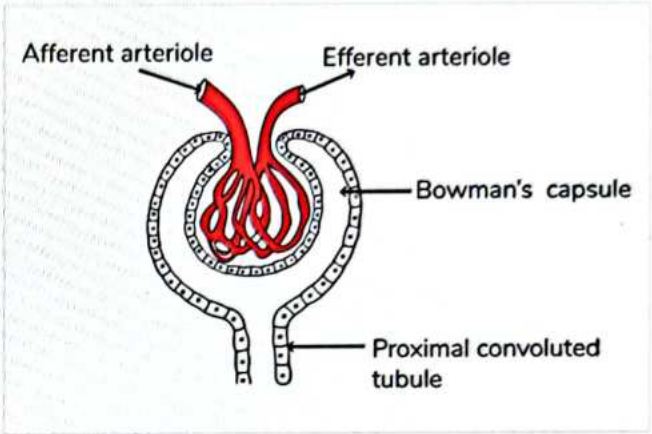
- Mucopolysaccharides are sulphated or acetylated based on their location.
- All Mucopolysaccharides are glycosaminoglycans, components of the Extracellular matrix.
- In the Extracellular matrix, negative charges provide them 2 advantages:
  - Provides gel-like viscous consistency tear matrix.
  - It is responsible for the selectivity of the Glomerular basement membrane.

Gel-like viscose matrix

- Matrix should be gel-like viscous and not rigid rod-like.
- If it is rigid rod-like, then, under pressure, the matrix will break.
- All matrices have an array of Mucopolysaccharides, which are negatively charged.
- The extracellular matrix is present in between cellular and vascular compartments of the body.
- The extracellular matrix contains negatively charged mucopolysaccharides; eventually, they attract positively charged sodium ions from the circulation.
- Sodium ions are osmotically active; they drag along the water, and therefore the matrix gets overloaded with water.
- Thus, the water provides the viscous consistency to the extracellular matrix.
- The extracellular matrix is hygroscopic; they attract more water.
- Hyaluronic acid is used as a moisturizing agent and lubricant.

Selectivity to GBM

- No. of layers in filtration surface of Glomeruli



- **Three layers of Glomeruli basement membrane**
  1. Glomerular capillary endothelial cells:
     a. Fenestrated capillaries, known for their pores.
     b. The diameter of the pores is adequate to allow any molecule with a molecular weight of less than 70 k Da.
  2. Basement membrane
  3. Bowman's capsule epithelium

Albuminuria

- At a physiological pH of 7.4, all the plasma proteins are negatively charged.
- Even if some protein escapes through the pores, the basement membrane contains heparan sulfate, negatively charged, repels the Albumin (69 k Da) back into circulation.
- Due to this phenomenon, we escape from albuminuria (excretion of albumin through urine)

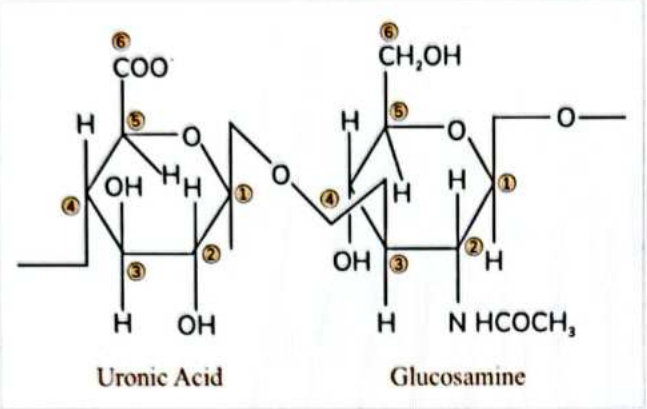
Hyaluronic acid

- Neither sulphated nor acetylated.
- Made up of Glucuronic acid and Glucosamine.
- It follows all thumb rules.

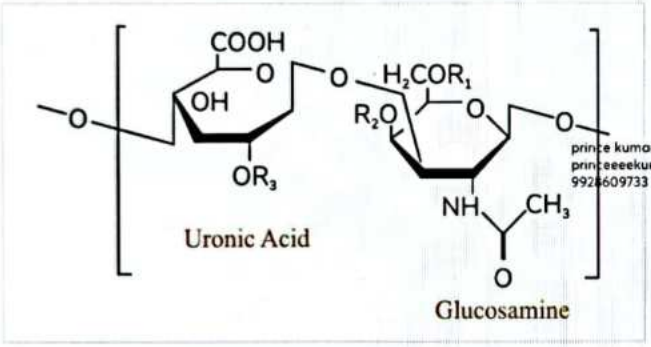
Dermatan sulfate

- Fits into all the exceptions and follows none of the thumb rules.
- Made up of Iduronic acid and Galactosamine.

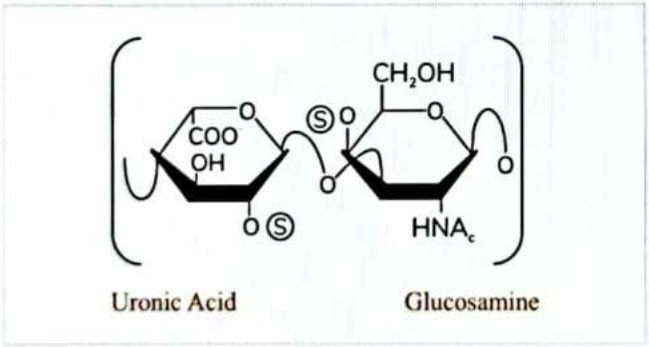
Identify the Structure of Mucopolysaccharides



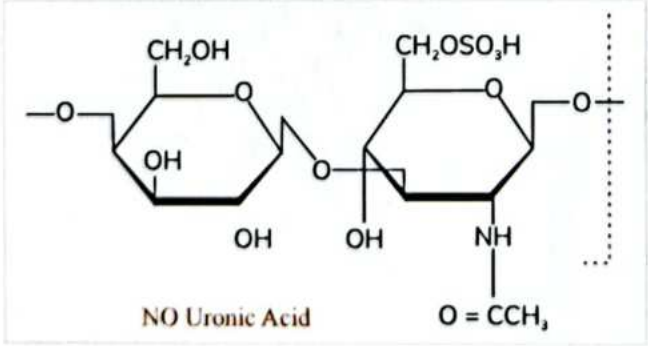
- Mucopolysaccharides which has Glucuronic acid, and Glucosamine is Hyaluronic acid.



- Mucopolysaccharides which has Glucuronic acid, and galactosamine is Chondroitin sulfate.



- Mucopolysaccharides with Iduronic acid and galactosamine, are Dermatan sulfate.



- Mucopolysaccharides which has no acid part, and it has galactose, therefore, it is Keratan sulphate.

Tips to Identify the Structure

- The uronic acid part has a carboxyl group (COO-)
- The amino sugar part has N.

Step 1: Check if there is no acid part,

- It has galactose.
- It is Keratan sulphate.

Step 2: In the uronic acid part, find if it is Glucuronic or Iduronic acid:

- If the carboxyl group is present above the plane of the ring- Glucuronic acid
- If the carboxyl group is present below the plane of the ring- Iduronic acid.

Step 3: In the amino sugar part, find if it is Glucosamine or galactosamine. In the 4th carbon atom (Glucose and galactose are epimers at the 4th carbon position).

- If OH lays below the plane of the ring- Glucosamine
- If OH lays above the plane of the ring- Galactosamine.

Important Information

- If the structure has Glucuronic acid + Glucosamine, it is Hyaluronic acid (follows the thumb rules)
- If the structure has Glucuronic acid + galactosamine, it is Chondroitin sulfate.
- If the structure has Iduronic acid + galactosamine, it is Dermatan sulfate (with exceptions).
- If the structure has no uronic acid part, it is Keratan sulfate.

Location of MPS

S.No.	Mucopolysaccharide	Location
1.	Hyaluronic acid	Synovium, Vitreous
2.	Keratan sulfate type 1	Cornea
3.	Keratan sulfate type 2	Loose connective tissue
4.	Heparin (Natural anticoagulant)	Mast cells
5.	Heparin Sulfate (Holds the enzyme within the vascular tree)	GBM, Large vessel wall like Aortic wall
6.	Chondroitin sulfate	Cartilages and bones
7.	Dermatan sulfate (widest distribution)	Skin, present in every tissue's extracellular matrix

Classes of Enzymes

- **Digestive enzymes** (Lactose) - Present in digestive duct
- **Metabolic enzymes/ Glycolytic enzyme** (within the cell)
  - Hexogines, Oligogines - Present in cytoplasm
  - Citric acid cycle enzymes - Present in mitochondria
- **Enzymes within the vascular tree:**
  - Lipoprotein Lipase: act on lipoproteins
  - Lipoprotein are transport forms of lipids in the blood.
  - VLDL, LDL, IDL, and HDL present in the blood.
  - For an enzyme to act on the blood component, it should be on the vessel wall, held in place by heparan Sulfate.

The Function of MPS

S.No.	Mucopolysaccharide	Function
1.	Due to negative charge	• Gel-like viscose consistency to the matrix • Responsible selectivity of glomerular basement membrane
2.	Heparin	• Natural anticoagulant
3.	Heparin Sulphate	• Presents along the vessel wall and holds enzymes along the vascular tree
4.	Hyaluronic acid	• Neither sulphated nor acetylated (no negative charge) • Not attached to any protein (exception) • It has no steric hindrance, so it can act as a scaffold, based on which cells can move. • Cells must move during inflammation (cells move damages region) and morphogenesis (development stems cell to move from bone marrow to the proposed hepatic region)
5.	Dermatan sulphate	• Widest distribution and present in sclera • Sclera provides additional support to the shape of the eyeball (Visco elastic tissue, Vitreous humor majorly contributed to the shape of the eyeball)
6.	Keratan sulphate type I	• Regularly arranged in the cornea such that it does not refract/reflect/diffract light rays. • Responsible for the transparency of the cornea. • When a scar replaces the cornea, it doesn't have KSI instead, it has collagen. • Collagens refract/reflect/diffract light rays and is responsible for the cornea's opacity of the scar.

- All Mucopolysaccharides are covalently attached to one core protein.
- The structure in which the central core protein and to which Mucopolysaccharide is attached on either side are called Proteoglycan.

One-liners

1. The Mucopolysaccharide with galactose is **Keratan sulphate**
2. Mucopolysaccharide with Iduronic acid are **Heparan sulphate and Dermatan sulphate.**
3. Mucopolysaccharides with galactosamine are **chondroitin sulphate and Dermatan sulphate.**
4. The Mucopolysaccharide present in the Glomerular Basement membrane is **Heparan sulphate.**

MCQs

Q. All the following are components of mucopolysaccharides except?
A. Uronic acid
B. Amino sugar
C. Sulphate
D. NANA

- NANA (N-Acetylneuraminic acid) is a Component of Glycolipid, specifically ganglioside.

Q. The mucopolysaccharide with galactose is?
A. Hyaluronic acid
B. Heparin
C. Heparan sulphate
D. Keratan sulphate

Q. The mucopolysaccharide with glucuronic acid is?
A. Hyaluronic acid
B. Heparin
C. Deramatan sulphate
D. Heparan sulphate

Q. The mucopolysaccharide with glucosamine acid is?
A. Hyaluronic acid
B. Chondroitin sulphate
C. Dermatan sulphate
D. Keratan sulphate

Q. The mucopolysaccharide present in the cornea is?
A. Keratan sulphate II
B. Heparan sulphate
C. Dermatan sulphate
D. Keratan sulphate I

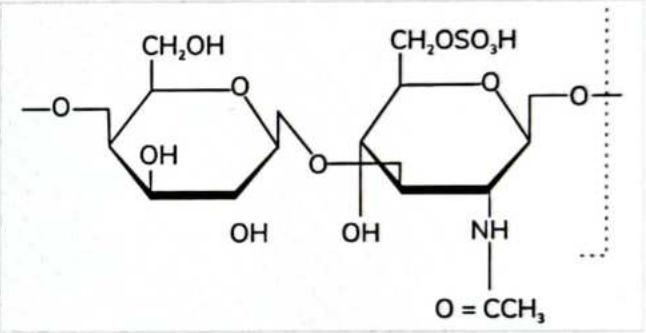
Q. An 18-month-old female child presented with a flat nasal bridge, big lips, macroglossia, gingival hypertrophy, abdominal distension, short stature, and delayed milestones. On the examination, hepatosplenomegaly and an inguinal hernia were observed. Chest X-ray shows cardiomegaly, and Urinary MPS were elevated. WBC L-iduronidase activity was low. Which of the following would accumulate?
A. Heparan sulphate
B. Keratan sulphate
C. Hyaluronic acid
D. Chondroitin sulphate

Hunter's and Hurler's Syndrome

S.no.	Property	Hurler's syndrome	Hunter's syndrome
1.	**Type**	Mucopolysaccharidosis I	Mucopolysaccharidosis II
2.	**Enzyme defect**	L-iduronidase	Iduronate sulphatase
3.	**Severity**	Severe	Relatively Mild
4.	**Mucopolysaccharides accumulation**	Heparan sulphate, Dermatan sulphate (universal)	Heparan sulphate, Dermatan sulphate (Universal)
5.	**Clinical features**	**In subcutaneous tissue:** • Macroglossia • Gingival hypertrophy **In cartilage** • Cartilage destruction (flat nasal Bridge, short stature) **In organs:** • Organomegaly • Hepatosplenomegaly (abdominal distension) • Cardiomegaly • Inguinal hernia **In nervous system:** • Mental retardation • Retinal degeneration **In cornea:** • Corneal clouding	No Corneal clouding, and no Inguinal hernia
6.	**Inheritance**	Autosomal Recessive	X linked recessive

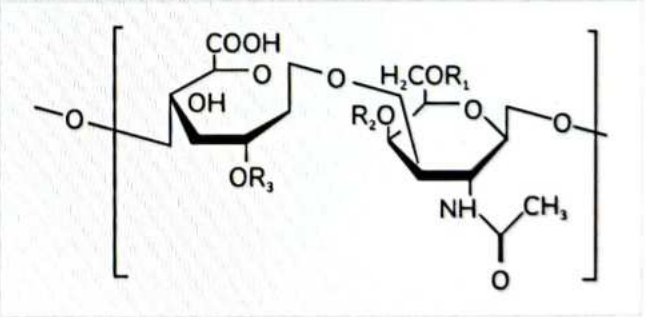
Image Based MCQ

Q. Identify the structure.



A. Heparan sulphate
B. Chondroitin sulphate
C. Dermatan sulphate
D. Keratan sulphate

Q. Identify the structure.



A. Heparan sulphate
B. Chondroitin sulphate
C. Dermatan sulphate
D. Keratan sulphate

PREVIOUS YEAR QUESTIONS

Q. Mucopolysaccharidosis holds less amount of water- false statement.
Which Mucopolysaccharides are present in the glomerular basement membrane?

Ans: Heparan Sulphate

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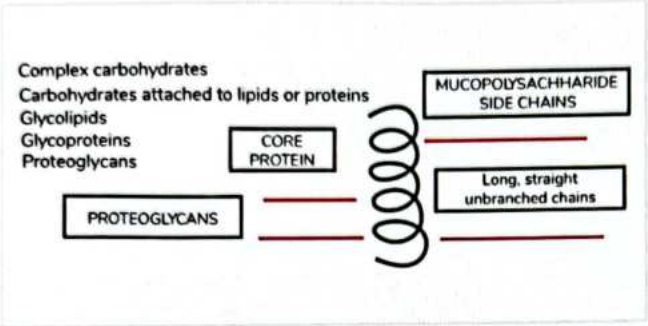
4 COMPLEX CARBOHYDRATES

Recap

Carbohydrates	
Simple	**Complex**
	• **Glycoprotein:** Carbohydrates attached with protein. • **Proteoglycans** • **Glycolipid** carbohydrate is attached to lipid

Glycoproteins & Proteoglycans



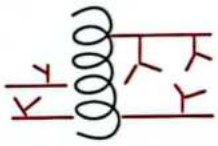
- Complex carbohydrates
- Carbohydrates attached to proteins.
- Core proteins attached to carbohydrate side chains - how to know whether it is glycoproteins and proteoglycans.
- If the carbohydrates side chain is attached is mucopolysaccharides - proteoglycans.
- Mucopolysaccharides– long straight unbranched chains containing uronic acid and amino sugar.
- If the side chain is short highly branched oligosaccharides chain - glycoproteins.
- Higher carbohydrate content - mucopolysaccharides present in Proteoglycans.
- Negatively charged because of side chains – proteoglycans - it is coated with sulfated or acetylated.
- **Where to find proteoglycans and glycoproteins?**
  - Extracellular matrix - proteoglycans are present

Three compartments

- Cellular: Glycoproteins
- ECM: Proteoglycans
- Vascular: Glycoproteins
- Any proteins present in the blood are called plasma proteins are glycoproteins except Albumin.

Property	Glycoproteins	Proteoglycans
Basic difference	Oligosaccharides chain	Mucopolysaccharides chain
Higher carbohydrates content	Lower carbohydrates	Higher Because of Mucopolysaccharides chain
Negatively charged	Not negatively charged	Mucoploysaccharides are sulphated or acetylated and hence proteoglycans are negatively charged
Location	plasma proteins except albumin and receptor proteins are glycoproteins Why are receptor glycoproteins not simple proteins? • Because receptors with simple proteins 20 choices amino acids - one linkage peptide linkage	ECM - extracellular matrix

3 Types of Glycoproteins



O- linked Glycoproteins (post-translation modification)	If side chain gets attached to serine and threonine and residue of core proteins – OH group
N- linked Glycoproteins	If side chain gets Attached to asparagine residue - NH_2 group. Synthesis is complex
GPI anchored Glycoproteins	Carbohydrates are not attached to any amino acid, attached to the carboxyl-terminal end - first amino acid is the one attached to amino end of core proteins and last amino acid is the carboxyl-terminal end protein

- When a Sidechain gets attached to an asparagine residue of the core proteins -it needs a carrier - DOLICHOL- it is the property of endoplasmic reticulum membrane - protein is synthesized in rough endoplasmic reticulum i.e., ER studded with ribosomes.
- When Ribosomes reach mRNA, the polypeptide chain will be growing into the vesicles of ER, now the ER provides DOLICHOL. It has the carbohydrate sidechain which attaches to the polypeptide chain.
- n-link glycoprotein synthesis occurs within ER
- Dolichol -polypeptide chain - endoplasmic reticulum - translation happens – modification happens-- Core translation modification n-link glycoprotein synthesis
- Core translation modification means when the polypeptide chain grows the dolichol transfer the oligosaccharide side chain attaches to asparagine residue.
- If the carbohydrate side chain attaches to serine or threonine residue, the translation gets over. Now the polypeptide chain leaves ER and reaches the Golgi complex. There it gets modified to form O-link glycoprotein synthesis.
- O-linked glycoprotein synthesis is a post-translational modification
- Dolichol is not required in O-link glycoprotein synthesis
- Carbohydrate side chain is transferred from dolichol to polypeptide chain inhibited by spermine - how does it act - spermine inhibit n-link glycoprotein synthesis.
- Spermine inhibits the dolichol oligosaccharide transferase enzyme, which transfers the carbohydrate side chain from dolichol to asparagine residue.

Paroxysmal Nocturnal Hemoglobinuria

- Acquired stem cell defect.
- Mutation of PIGA gene, it codes for Phosphatidyl inositol glycan A.
- Function of phosphatidyl inositol glycan A is to synthesise GPi anchored glycoprotein
- GPi anchored glycoprotein has carbohydrate side chain which on one hand attached to carboxyl terminal of amino acid of core protein and on other hand the carbohydrate side chain attached to phosphatidylinositol of the cell membrane. (Cell membrane is made of phospholipids, one of them is phosphatidyl inositol). Therefore, it is k/as Glycosylated phosphatidylinositol anchored glycoprotein.
  - Advantage: Glycosylated phosphatidylinositol anchored glycoprotein provides a long arm of mobility to proteins. So, they can encounter any toxic substance before they encounter any cell membrane
  - Example of GPi anchored glycoprotein – DAF/CD 55 and CD 59(proteins)
  - To accelerate the decay of C3 convertase. And C3 convertase function helps in prevents the formation of membrane attack complex.
- Membrane attack complex: It can be formed as a result of Complement pathway activation.
  - Classical Complement pathway: Initiated when there is antigen-antibody complex formation.
  - Alternate Complement pathway: Switched on for many reasons like ACIDIC pH & C3 convertase gets formed and it helps in the formation of membrane attack complex and they can attack the membrane of any cells.
  - When you sleep, respiration is not good leads to acidic pH and the C3 convertase gets activated. It forms a membrane attack complex and results in RBCs hemolysis.
  - In normal individuals, the cells are not susceptible to membrane attack complex because we have functionally active DAF/CD55 and CD59 which accelerate the decay of C3convertase, therefore, RBCs are protective.
  - In PAROXYSMAL NOCTURNAL HEMOGLOBINURIA, because of defective PIGA gene the Gpi anchorage between CD55 & CD59 is defective and C3 convertase is not decayed resulting in RBC lysis in acidic PHin the night hemoglobinuria at night.
  - PIGA gene is mutated - Phosphatidyl glycan A– a function of PIGA enzyme is to properly anchor two proteins - DAF/CD 55 and CD 59(proteins)

One Liner

1. The carrier required for N-linked glycoprotein synthesis is Dolichol.
2. Spermine inhibits the synthesis of N-linked Glycoprotein.
3. N-linked glycoproteins are attached to asparagine residues of core proteins.
4. Paroxysmal Nocturnal Hemoglobinuria is caused by a defect of the PIGA gene.

MCQ

Q. In O-linked glycoproteins, the carbohydrate side chains are attached to which amino acids of the core proteins?

A. Serine
B. Cysteine
C. Asparagine
D. Proline

Q. Which of the following is true about proteoglycans?
A. They are receptor proteins
B. They are uncharged
C. They hold excess water
D. They have lesser carbohydrate content than glycoproteins

Q. Which of the following is true about glycoproteins?
A. The side chains provide them negative charges.
B. They are present in extracellular membrane.
C. They hold excess water.
D. Plasma proteins are glycoproteins.

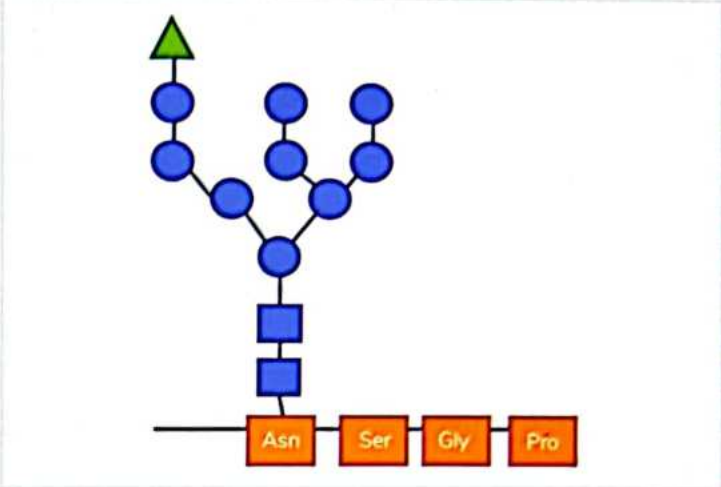
Integrated Case Based MCQ

Q. A young girl presented with abdominal pain, and distension with a history of headache and jaundice. On evaluation, we found bi-cytopenia with evidence of hemolytic anaemia and venous thrombosis of cerebral sinuses, hepatic veins and the intrahepatic portion of IVC. with these clinical features. We suspected paroxysmal nocturnal hemoglobinuria, which was later confirmed by flow cytometry. The most common gene mutated in paroxysmal nocturnal hemoglobinuria is ,

A. Phosphatidylinositol glycan A,
B. CD 55,
C. CD 59,
D. Dolichol

Image Based Question

Q. Identify the Image



A. N-linked Glycoprotein
B. O-linked Glycoprotein
C. Proteoglycan
D. GPI anchored Glycoprotein

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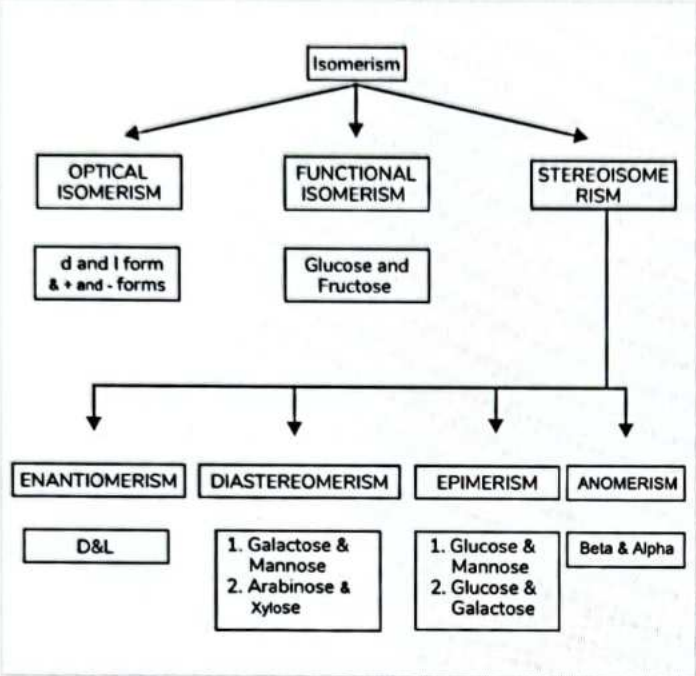
5 ISOMERISM

Isomerism

- A property by which two or more molecules have the same molecular formula.
- But either different structural formulas or They differ in the spatial orientation of their groups'.

Classification of Isomerism



Optical Isomerism

- Here the two molecules have the same molecular formula, but it differs in the way they turn the plain polarized lights.
- The isomer that turns the light to the right side is called dextrorotatory (d/+)
- The isomer that turns the light to the right side is called Levorotatory (l/-)

Functional Isomerism

- Here, the two molecules have the same molecular formula, but they have different functional groups.
- Some common examples are glucose and fructose.

Stereoisomerism

- Here, the two molecules have the same molecular formula and the same structural formula but differ in the spatial orientation of their groups.

Structure of Glucose and Fructose

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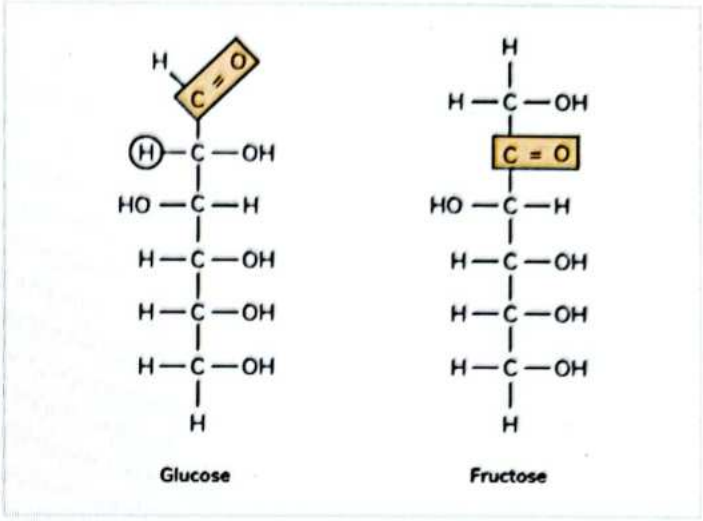
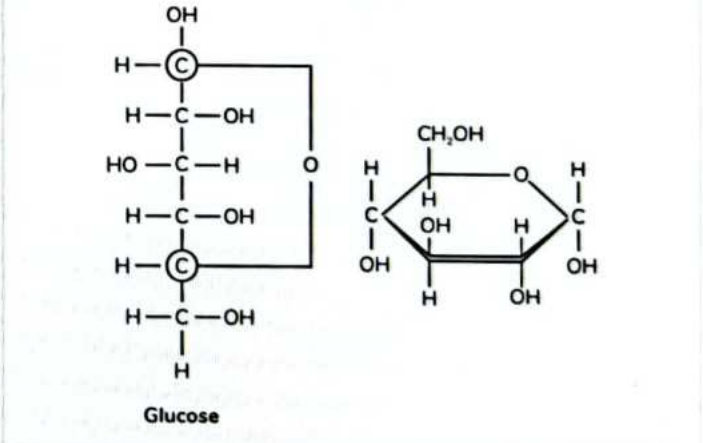


- In glucose, if you change the H and OH orientation with respect to the second carbon atom, it will be called mannose.
- In glucose, if you change the H and OH orientation with respect to the fourth carbon atom, it will be called galactose.
- In glucose, excluding the first and the last carbon atoms, the rest are asymmetric carbon atoms.
- In fructose, excluding the first, last, and functional carbon atoms, the rest are asymmetric carbon atoms.
- The total number of Stereoisomerism in a molecule with n numbers of asymmetric carbon atoms is
- The total number of Stereoisomerism of fructose is 8 and for glucose its 16.

Closed Ring Form

00:08:10



- The first carbon atom of the glucose and the second carbon atom of the fructose are highly reactive at pH 7.4.

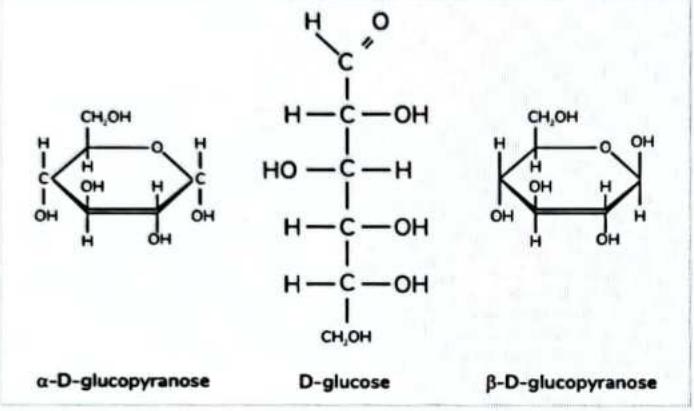
- They react with their Penultimate carbon atom, i.e., the 5th carbon atom, to form a closed ring.
- Here, one valency is satisfied by H, the second valency by OH, the third valency by OCHCH2OH, and the 4th valency by the rest of the group.
- At the physiological pH value, the glucose will have 5 asymmetric carbon atoms.
- The total number of Stereoisomerism for glucose and fructose is 32 and 16 respectively due to the formation of the closed rings.

Important Information

- The furanose rings include 4 carbon atoms. Fructose has furanose rings.
- The pyranose rings include 5 carbon atoms. 99% of glucose has pyranose rings.

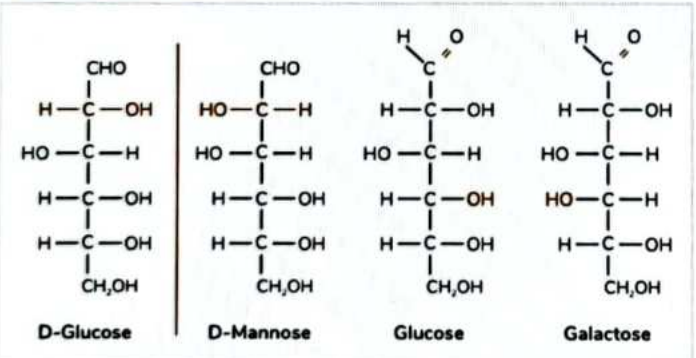
Stereoisomerism

Anomerism

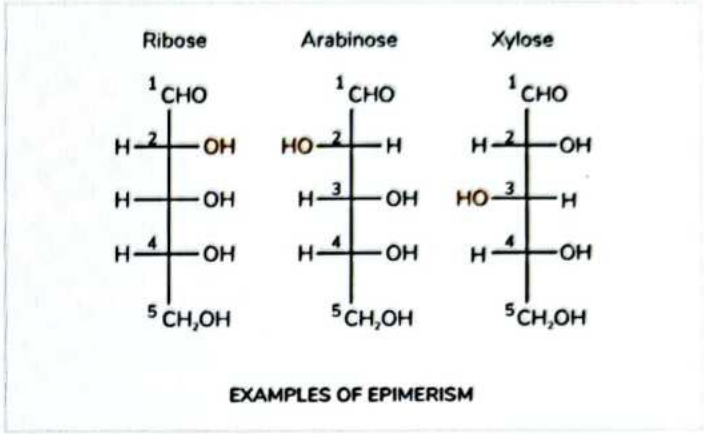


- If OH lies above the plane of the ring, it is called beta form.
- If OH lies below the plane of the ring, it is called alpha form.
- Alpha and beta form are anomers.

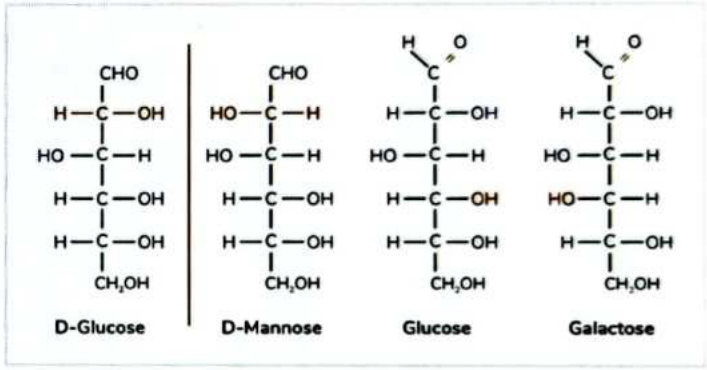
Epimerism



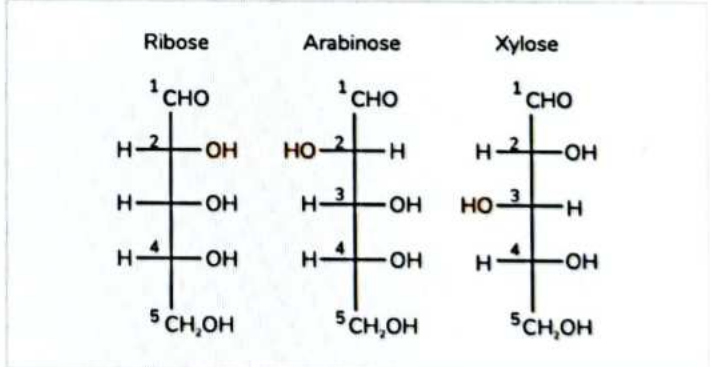
- The molecule should differ in spatial orientation with respect to one of the one asymmetric carbon atom other than the penultimate carbon atom.
- To call a molecule to be an epimer of glucose, they need to differ with respect to the 2, 3, and 4 carbon atoms. For example, D-Mannose, Galactose, etc.
- Glucose and mannose are epimers at 2nd position.
- Glucose and galactose are epimers at 4th position.
- In Ribose, the first and the last carbon atom are symmetric, and the fourth carbon atom is the penultimate carbon atom. Therefore, the 2nd and 3rd carbon atom differences can provide the epimers of ribose. They are Arabinose and Xylose.
- Ribose and arabinose are epimers at 2nd position.
- Ribose and xylose are epimers at 3rd position.



Disateriomerism



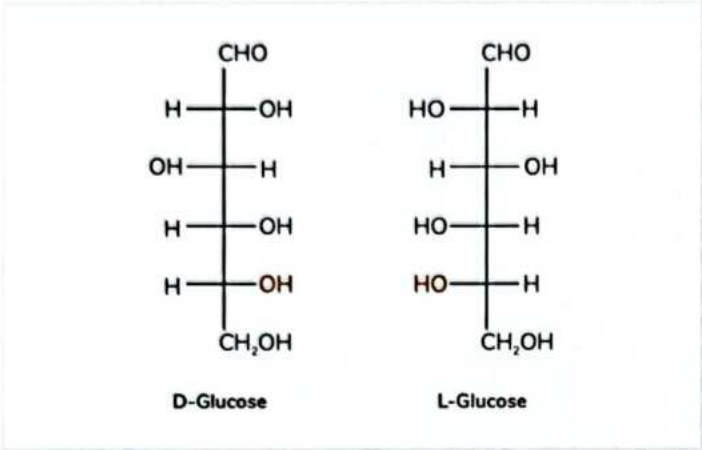
- The molecule should differ in spatial orientation with respect to one or more asymmetric carbon atoms other than the penultimate carbon atom.
- D-Mannose and Galactose differ from each other with two carbon atoms; therefore it is diastereoisomerism.



- In ribose if we change the orientation of 2nd carbon atom, we get arabinose.
- In ribose if we change the orientation of 3rd carbon atom we get xylose.
- While comparing xylose and arabinose they differ with both 2nd and 3rd carbon atoms. Therefore, it is diastereoisomerism
- Similarly, in Ribose, if the orientation is changed with respect to both second and third carbon atoms, giving Lyxose. Therefore, it is diastereoisomerism.

Enantiomerism

- Enantio means mirror. Therefore, they are the exact image of the carbon atoms.
- Other names for enantiomerism are Racemism.



- Racemase Enzymes are the ones that inter-convert the D and L forms.
- However, the Racemic Mixtures are the which have an equal composition of D and L form; there will be an equal composition of d and l.
- Due to the equal proportion of D: L, a racemic mixture is optically inactive.
- The molecule should differ in spatial orientation with respect to all the carbon atoms.
- In the case of enantiomer, if the OH in the penultimate carbon atom lies to the right, they are denoted with D, and if OH in the penultimate carbon atom lies to the left, they are denoted with L. For example, D-Glucose and L-Glucose.

Important Information

- D and L are not synonymous with d and l, but they complement each other.
- If D is dextrorotatory, then L needs to be Levorotatory. However, if D is Levorotatory, then L needs to be dextrorotatory.
- The most common physiological form of glucose is βD Glucopyranose.

Glucose Solution

- Dissolving the glucose in distilled water.
- Initially, it will be in Alpha form.
- Within 12 hours, mutarotation happens and an equilibrium is reached with 2/3rd being beta forms and 1/3rd being alpha forms
- The method used to estimate the glucose is the glucose oxidase peroxidase method (GODPOD method.)
- It only acts on the Beta Form.
- Glucose oxidase peroxidase enzyme cannot act on alpha form of glucose. Therefore when we do test immediately after preparing the solution it gives the false low value.

One-Liners

- The most common physiological form of glucose is D glucopyranose.
- d and l forms are examples of Optical Isomers
- D and L forms are examples of enantiomers
- Total number of stereoisomers possible in a molecule containing n number of asymmetric carbon atoms is 2^n.
- Glucose, and mannose are epimers at C2.
- Glucose, and galactose are epimers at C4.

MCQS

Q. Glucose and fructose are examples of.
A. Optical isomerism
B. Functional isomerism
C. Stereoisomerism
D. Epimerism

Q. Ribose and arabinose are examples of.
A. Optical isomerism
B. Diastereoisomerism
C. Enantiomerism
D. Epimerism

Q. Ribose and Xylose are examples of?
A. Optical isomerism
B. Diastereoisomerism
C. Enantiomerism
D. Epimerism

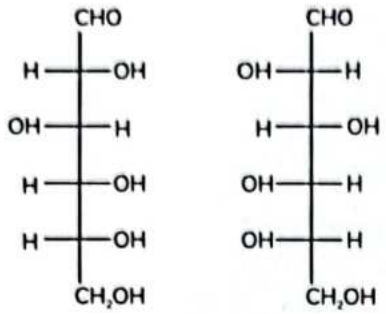
Q. D- Glucose and L- Glucose are examples of?
A. Optical isomerism
B. Diastereoisomerism
C. Enantiomerism
D. Epimerism

Q. An intern lab technician prepared a fresh glucose solution (100mg/dL). She estimated the Q concentration of the same using the glucose oxidase peroxidase method and found the concentration to be 10mg/dL. The probable cause is:

A. The glucose oxidase used was ineffective.
B. The weighing balance is defective.
C. Glucose oxidase acts only on the beta form.
D. Glucose oxidase acts only on the alpha form.

A. Anomerism
B. Epimerism
C. Diastereoisomerism
D. Enantiomerism

Q. What kind of isomerism is observed?

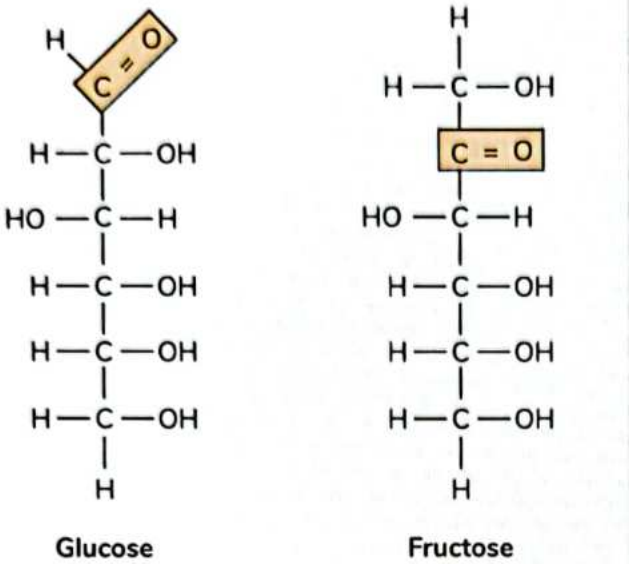


6 CARBOHYDRATES - URINE INVESTIGATIONS AND CLINICAL DIAGNOSIS

Classification of Carbohydrates

Based on reducing property

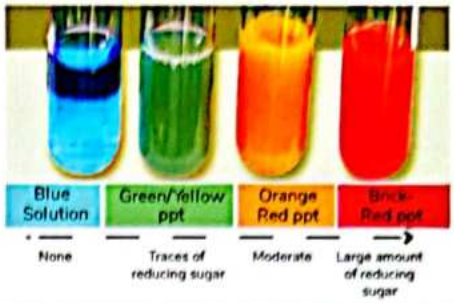
- **Reducing sugars:** Free carbonyl groups (--C=O)



  - E.g., Glucose, Fructose
  - All monosaccharides are reducing sugars, as every monosaccharide has either an aldehyde or ketone group (free carbonyl group).
  - Most disaccharides are reducing sugars, except sucrose and Trehalose.
  - Sucrose is a disaccharide made from 2 reducing monosaccharides, glucose, and fructose. But it is formed when the first carbon atom of glucose is linked with the second carbon atom of fructose (alpha 1-2 linkage); therefore, there is no free carbonyl group.
  - Trehalose is made up of 2 glucose residues with alpha 1-1 linkage (no free carbonyl)
- **Non-reducing sugars:** Does not have a free carbonyl group (--C=O)
  - E.g., Sucrose, Trehalose
  - All oligosaccharides and polysaccharides have a maximum of one free carbonyl group, which is insufficient to bring about the reduction property, as they have 3 or more sugar residues.

Benedict's Test

- Detects the presence/absence of reducing substance in a given solution.



Steps

- 5 ml of Benedict's solution (Blue) + 8 drops of solution to be tested
- Heat the mixture.
- Colour Change from blue to green to yellow/orange to red → Reducing sugar.
- The resulting colour depends on the concentration of the reducing sugar in the solution. Therefore, it is called a semi-quantitative test.

Composition of Benedict's solution

- Copper sulphate (blue colour)
- Sodium carbonate (Alkaline medium)
- Sodium citrate (Stabilizes the solution)

Compare and Contrast of Benedict's and Fehling's Solution

Benedict's Solution	Fehling's Solution
• Detects the presence /absence of reducing substance in a given solution	• Detects the presence/absence of reducing substance in a given solution
• Copper sulphate (blue colour) • Sodium carbonate (Alkaline medium). • Sodium citrate (Stabilizes the solution).	• **Fehling's A:** Copper sulphate • **Fehling's B:** Sodium hydroxide (Stronger alkaline medium- facilitates reduction property more) • **Fehling's C:** Sodium Potassium Tartrate.
• No auto-reduction	• Fehling's B is a stronger alkaline medium; it causes a reduction of $CuSO_4$ to give red color. This process is called auto-reduction. • Therefore, we keep 3 solutions separately to prevent auto reduction.
• Simpler and easier	• Practically difficult to prevent auto reduction

Interpretation of Benedict's Test

Benedict's Test	
Positive	**Negative**
Monosaccharides	Sucrose, Trehalose Oligosaccharides, Polysaccharides
Most Disaccharides	
Antioxidants- reduce the oxidant species, e.g. Vitamin A, C, E, and GSH A person who intakes antioxidant tablets probably shows a False positive for reducing sugar	
Physiologically excreted substances: Excess Uric acid in renal tubules (Hyperuricemia),	
Homogentisic acid (Alkaptonuria- Homogentisate oxidase defect) **Clinical features of Alkaptonuria** • Middle aged man with multiple intervertebral disc bulges or prolapses (Joint pains) • Pigmentation of the pinna, thenar, and hypothenar eminence and the tip of the nose • Patient reports the urine turns dark on standing. The screen test for inborn errors in amino acid metabolism is HPLC. (high-performance chromatography with tandem mass spectrometry) Not advisable to prescribe an HPLC tandem mass spectrometry test directly due to expense. So, ask for Benedict's test for primary confirmation, then proceed with HPLC	

Drugs metabolized by glucuronidation.
E.g.: Aspirin

- Xenobiotic chemical reactions happen in the liver.
- Xenobiotic chemicals convert non-polar substances into polar substances.
- Only converted polar substances can be excreted via urine.
- Phase I and II reactions carry out the conversion.
- Phase II reactions are all conjugate reactions.
- One of the conjugating agents that the liver uses is Glucuronic acid.
- When a person is on a drug that gets metabolized by glucuronidation (reducing sugar), then benedict's show positive.
- Benedict's test is not a correct indicator in this case.

Barfoed's Test

To distinguish between monosaccharides and reducing disaccharides

Composition

- Copper acetate
- Glacial acetic acid (acidic medium. Whereas Benedict's test is carried out in Alkaline medium)
- Due to acidic medium, only strongly reducing monosaccharide is answered.
- It differentiates monosaccharides from reducing disaccharides.
  - If Barfoed is positive → monosaccharides (strongly reducing - fructose, glucose, galactose, pentose)
  - If Barfoed is negative and Benedict's test is positive → Reducing disaccharides (maltose, lactose)
- Barfoed's test is positive if there is a red precipitate.

Clinical Cause of Reducing Sugars in Urine

Physiological

Reducing sugars	Causes
• Lactose is a reducing disaccharide made up of Galactose and Glucose.	• Female-Pregnancy and lactation • Prolactin excess

Pathological

Reducing Sugars	Causes

Glucose
Renal threshold of glucose= 180 mg/dL
	• Diabetes Mellitus
	• Renal glycosuria
	  ○ Damage in proximal convoluted tubules (PCT)- Reabsorb all glucose filtered by glomeruli.
	  ○ Eg. Fanconi syndrome
	• Alimentary glycosuria
	  ○ Increase in rate of absorption of glucose along the intestine.
	  ○ In the immediate postprandial state, there will be a steep increase in plasma glucose.
	  ○ Beta cells release insulin due to the steep increase.
	  ○ When insulin is released, there is not much of an increase in plasma glucose because all the intestinal glucose is already entered into the plasma.
	  ○ This leads to a steep fall in glucose levels.
	  ○ In GTT (glucose tolerance test), when there is a steep rise (hyperglycemia) and steep fall (hypoglycemia) and in an immediate postprandial state, a steep rise crosses the renal threshold found in urine, indicating alimentary glycosuria.
	  ○ Causes: Thyrotoxicosis (thyroxine causes upregulation of Glut transporters across enterocytes).

Fructose	• Essential fructosuria
	  ○ Defect of fructokinase enzyme
	• Hereditary fructose intolerance
	  ○ Caused by aldolase D defect

Galactose	• Galactosemia
	  ○ Deficiency in galactokinase
	• Classical Galactosemia
	  ○ Defect of GalPUT (galactose 1 phosphate uridylyl transferase.

Pentose	• Essential Pentosuria

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Tests to find the clinical condition because of which urine answers Benedict's test.

- Barfoed's Test helps differentiate a physiological cause (disaccharide) and a pathological cause (monosaccharides).
- If Barfoed's Test is negative, it is a physiological cause (lactose).
- If Barfoed's Test is positive, it is a pathological cause. Now we should find out which monosaccharide is present.
  - Glucose Test (aldose): Glucose Oxidase peroxidase Test (GOD-POD Strip test)
  - Fructose Test (Ketos)
    - → Seliwanoff Test: Resorcinol and concentrated HCl
      - Cherry red color → Fructose/ketoses
    - → Foulger Test
      - Urea and stannous chloride in concentrated sulphuric acid
        - Deep blue color → Fructose/ketoses
  - Galactose Test: Mucic acid test (crystallization)
    - → White crystals → galactose
  - Pentose Test- Bial's Test

Osazone Test

- Based on crystal shape

Composition

- 10 ml of sugar solution
- 1 spatula of Phenyl hydrazine hydrochloride
- 2 spatula of sodium acetate
- 1 ml of glacial acetic acid
- Filtered
- Filtrate is boiled.

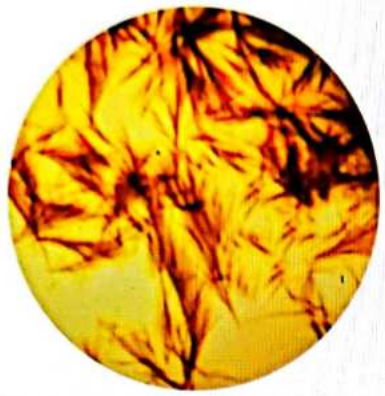
2 aspects

- **Time taken for crystallization.**
  - Monosaccharide reacts more quickly than disaccharide.
  - Among monosaccharides, ketosis reacts the fastest.

Reducing sugar	Time taken
Fructose	7 min
Glucose	10 mins
Lactosazone	20 mins
Maltosazone	30 mins

- **Shape of crystal**
  - Ozosone reaction takes place only in the 1st and 2nd carbon atoms.
  - Glucose and fructose differ only in the first and second atoms.
  - Osazone reaction will mask the difference, and Glucosazone and Fructosazone become indistinguishable.

- Glucosazone and Fructosazone: needle /broomstick shaped.



- Lactosazone: White powder puff/ hedgehog appearance

- Maltosazone: Sunflower-shaped crystals

Algorithm/Summary

1. Benedict's test is positive
2. Check History of patient
   - Antioxidant intake
   - Intake of drugs metabolized by glucuronic acid.
   - Clinical history, which resembles alkaptonuria.
   - And exclude them
3. Cause
   a. Physiological causes
      i. Pregnancy/lactation
      ii. Lactose
   b. Pathological cause

Monosaccharides	Clinical condition
Glucose	Diabetes Mellitus Renal Glycosuria Alimentary Glycosuria
Fructose	Essential fructosuria Fructose Intolerance
Galactose	Classical Galactosemia Galactosemia
Pentose	Essential Pentosuria

Test

1. Benedict test positive
2. Barfoed's test
   a. Negative-Physiological cause
      i. Lactose during pregnancy and lactation.
   b. Positive-Pathological causes
      i. Glucose: Glucose Oxidase peroxidase Test (GOD POD)
      ii. Fructose: Seliwanoff test and Foulger test
      iii. Galactose: Mucic acid test (crystallization)
   iv. Test for pentose- Bial's Test

One Liners

1. The urine test to detect the presence of a reducing substance is **Benedict's test.**
2. The test done to detect the presence of reducing monosaccharides is **Barfoed's test.**
3. Galactose can be detected by the **Mucic Acid test**.
4. Seliwanoff's test is answered by **ketoses** (urine sugar-fructose).
5. Bial's test is answered by **pentoses.**

MCQ

Q. All the following answer Benedict's test positively except?
A. Ribose
B. Vitamin C
C. Homogentisic acid
D. Trehalose

Explanation: Ribose is a pentose, Vitamin C is an antioxidant, and Homogentisic acid is a reducing substance. All three answer Benedict's test, whereas Trehalose is a non-reducing disaccharide made of 2 residues of Glucose (alpha11 linkage).

Q. Barfoed's test is not answered by?
A. Maltose
B. Glucose

C. Fructose
D. Ribose

Explanation: Maltose is a disaccharide made of 2 glucose residues with alpha 1-4 linkage.

Q. Foulger's test is not answered by?
A. Erythrose
B. Ribulose
C. Fructose
D. Sedoheptulose

Explanation: Foulger's test is positive for ketose, whereas erythrose is an aldose.

Integrated Case Based MCQs

Q. A 45-year-old man presents with multiple joints pain, intervertebral disc bulges, and prolapses. On enquiry, he gives a history of urine turning dark on standing. Which of the following screening tests will be positive in the condition?
A. Ferric Chloride test
B. Guthrie's test
C. Cyanide nitroprusside test
D. Benedict's test

Explanation

- Urine turning dark indicates alkaptonuria (alkapton means black)
- Ferric chloride test and Guthrie's test is a screen test for Phenylketonuria.
- Cyanide nitroprusside test is done to detect Homocystinuria.

Alkaptonuria

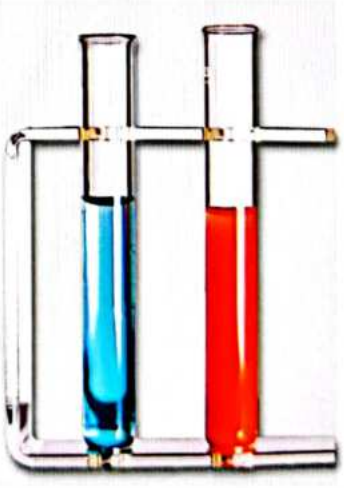
- Inborn error of tyrosine metabolism.
- Defect in homogentisate oxidase enzyme.
- Leads to homogentisic acid accumulation.
- On accumulation, homogentisic acid gets oxidized to form benzoquinone acetate.
- The benzoquinone acetate undergoes polymerization to form melanin like fibres.
- All the clinical features are caused by this Melanin like fibres accumulation.
  - **In cartilages**
    → Cartilage destruction → Ochronosis.
    → Multiple intervertebral disc bulges and prolapses.
    → Joint pains.
  - **In skin**
    → Pigmentation of the pinna, nose tip, thenar, and hypothenar eminence.
  - **In mucus membrane**
    → Osler' sign (the first sign of Alkaptonuria)- brownish pigmentation of sclera along the line of attachment of both medial and lateral rectus.

  - **In urine**
    → Urine turning dark on standing.
- Screening test is Benedict's test. Then if it is positive, prescribe HPLC with tandem mass spectrometry.
- To prevent the oxidation of homogentisic acid from forming benzoquinone acetate, Vitamin C antioxidants are prescribed in large doses.

Q. A 41-year-old attends a master health check-up. His urine answers Benedict's test. His fasting and postprandial plasma glucose are normal. His HbA1C is normal, and the person is asymptomatic. The most probable cause is.
A. Renal glycosuria
B. Diabetes mellitus
C. Hereditary fructose intolerance
D. Essential Fructosuria

Image Based MCQS

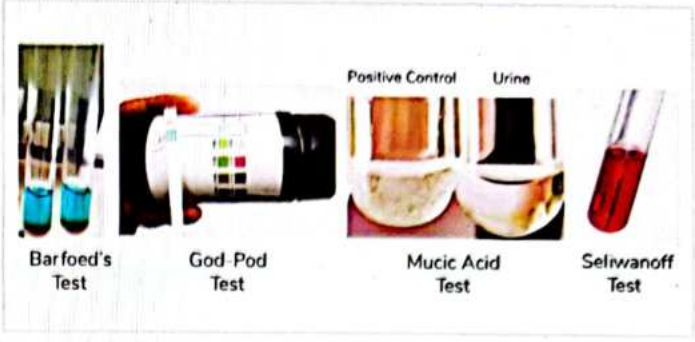
Q. A person gives her urine sample for the routine screening test. Benedict's test result is as follows. All of the following can be a cause except.



A. Multiple Myeloma
B. Pregnancy
C. Fanconi's syndrome
D. Galactosemia

Explanation: Multiple Myeloma is plasma cell neoplasia, the monoclonal proliferation of plasma cells and excess production of antibodies, a disproportionate product of lynch. In this case, lynch will be excreted through urine, called Bence Jones protein (non-reducing)

Q. A person's urine answers Benedict's test. The biochemist runs a battery of urine tests to detect the reducing sugar present in urine. Based on the images provided below find out the substance found in urine. Which of the following is the most probable cause?



A. Diabetes Mellitus
B. Fructosuria
C. Fanconi's syndrome
D. Galactosemia

7 GLUT TRANSPORTERS

Glut transporters

- It comes under facilitated Passive Diffusion.
- They transfer the glucose from the higher concentration area to the lower concentration area.
- Glut transporters are the carrier proteins.

Questions

Q. In what form is the glucose trapped in the cell?
Ans. In the form of Glucose 6 phosphates.

Q. What is the most active form of glucose?
Ans. It is UDP glucose.

Q. Which is the most common physiological form of glucose?
Ans. Beta D glucopyranose.

Types of Glut Transporters

00:04:06

S. No.	Glut Transporters	Locations
1.	GLUT 1	Neurons, RBCs, and Placentas
2.	GLUT 2	Enterocytes, Hepatocytes, cells of pancreas, proximal convoluted tubule cell
3.	GLUT 3	Neurons, RBCs, and Placentas
4.	GLUT 4	Insulin Dependent Glucose Uptake-, Skeletal Muscles, Cardiac Muscles, Adipose Tissues
5.	GLUT 5	Fructose Absorption along the intestine.

Enterocytes Glucose Uptake

- First, glucose should pass the apical sides and move forward to the basolateral sides.
- On the epical side, the Sodium-Glucose co-transporter 1 is present.
- On the basolateral side, GLUT 2 is present.
- Once the glucose is absorbed, the sodium is also absorbed. That's why we add sugar to the ORS solution.
- Sodium-Glucose co-transporter 1 related to Secondary Active Transport.
  - Galactose also follows the same process.
  - However, the fructose gets absorbed through the GLUT 5 transporter present at the apical side.
  - All the pentoses get absorbed by simple passive diffusion.

Insulin Dependent Glucose Uptake

The dependent muscles are

- Skeletal Muscles,
- Cardiac Muscles
- Adipose Tissues
- Hepatocytes by Induction of Glucokinase

Q. Insulin Dependent Glucose Uptake takes place in all except?
A. Skeletal Muscles
B. Cardiac Muscles
C. Hepatocytes
D. Endothelial

Important Information

- In hepatocytes, the glucose uptake is dependent on insulin indirectly.
- Glucokinase in the liver is stimulated by insulin and it keeps low glucose concentration within the liver, thereby facilitates further uptake of glucose.

Tissues Independent of the insulin

Lens

- In diabetes, when glucose can not enter into tissues which are dependent of insulin for glucose uptake, it causes hyperglycemia. The excess glucose enters into tissues which are independent of insulin for glucose uptake and cause glucose toxicity.
- Lens absorbs excess glucose.
- However, as long as glucose is in its original state, it doesn't cause any issues.
- As lens has aldose reductase, glucose is converted to an alcohol, sorbitol. Sorbitol gets trapped in lens causing osmotic movement of water
- Causing the cataract.

Endothelium

- Excess glucose uptake causes microvascular and

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macrovascular complications of diabetes.

- All diabetes complication is caused by glucotoxicity.

Neurons

- Excess glucose causes Neuropathy.

Kidney

- Excess glucose causes Nephropathy.

One Liner

- The transporter responsible for Insulin mediated glucose uptake is GLUT4
- Glucose uptake along the apical side of the enterocyte is by Secondary active transport or by Sodium-Glucose Cotransporter I
- The alcohol responsible for diabetic cataracts is Sorbitol

MCQs

Q. GLUT transporter present in skeletal muscle is:
A. GLUT 1
B. GLUT 2
C. GLUT 3
D. GLUT 4

Q. Fructose absorption along the apical side of enterocytes is by:
A. Simple passive diffusion
B. Facilitated passive diffusion.
C. Primary active transport
D. Secondary active transport

Q. Pentose absorption along the apical side of enterocytes is by:
A. Simple passive diffusion
B. Facilitated passive diffusion.
C. Primary active transport
D. Secondary active transport

Q. Glucose is trapped inside cells in the form of:
A. D glucopyranose
B. UDP glucose
C. Glucose 6 phosphate
D. Fructose 6 phosphate

Q. Following an early morning run, a 29-year-old man consumes a carbohydrate-rich south Indian breakfast. Which of the following will most likely be activated in his liver after breakfast?
A. Cytoplasmic PEPCK
B. Membrane GLUT4 transporter
C. Cytoplasmic PFK2
D. Cytoplasmic Glycogen Phosphorylase

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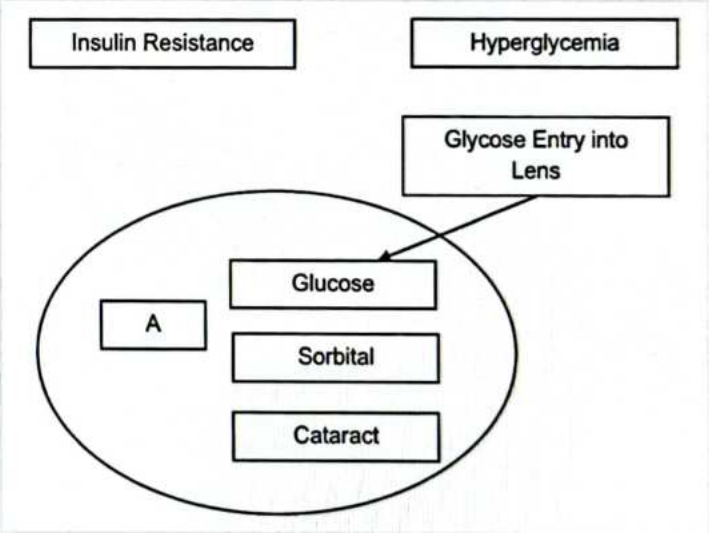
Q. After a 5-month-old baby girl failed to respond well to breastfeeding, she was switched to a cow's milk formula at the age of four weeks. She was taken to the hospital several times over the next three weeks for symptoms of screaming after eating, but she was released since no specific diagnosis was made. Suspecting it to be lactose intolerance, her parents tried to eliminate milk from her diet, but it did not improve her condition. It was then found that screaming bouts were initiated after the child drank juice which caused the formation of gas and a distended abdomen. No enzyme deficiencies were found in the intestinal needle biopsy, and no abnormal growth findings were reported on physical examination. The biopsy of intestinal tissue from this patient would most likely reveal deficiencies or defective.

A. GLUT 2
B. Lactase
C. Sucrase
D. GLUT 5

Q. Identify enzyme A



A. Sorbitol dehydrogenase
B. Sorbitol reductase
C. Ketose dehydrogenase
D. Aldose reductase

8 GLYCOLYSIS

What is Glycolysis? 00:01:12

- Splitting of 6-carbon compound glucose to form two products depending upon the process occurs aerobically and anaerobically.
- 2 Pyruvates and 7 ATPs are formed as a result of aerobic glycolysis.
- 2 Lactates and 2 ATPs are formed as a result of anaerobic glycolysis.

Glycolysis - Products

Every glucose molecule generates

- Aerobic glycolysis: 2 Pyruvates and 7 ATPs.
- Anaerobic glycolysis: 2 Lactate and 2 ATPs.

Significance of Glycolysis

- Glycolysis is the only pathway that generates ATPs even in the absence of oxygen.

Glycolysis - Cells Dependent 00:04:04

- Physiologically, the following cells are dependent on glycolysis for ATP production as they lack mitochondria.
  - RBCs
  - Retinal cells
  - Corneal cells
- White muscle fibres due to
  - Lack of fresh blood supply during isometric contractions.
    - → In isometric contractions, the muscle length should be maintained constant during contraction between two fixed points.
    - → The tone of the muscle increases enormously.
    - → Any blood vessel entering the muscle gets constricted due to muscular contraction.
    - → They won't get a fresh supply of oxygen, nor does it have myoglobin. Thus, they depend on anaerobic glycolysis.
  - Lack of myoglobin (Storage form of oxygen)
- Renal medulla
  - In ischaemic or hypoxic damage to the kidney, the renal medulla is affected the most as it receives blood from end arteries.
  - Receives very less blood supply necessary for
    - → Concentrating the urine.
    - → Maintaining interstitial medullary hyperosmolarity.

Preferred Fuels 00:07:27

- **Preferred fuel for Anaerobic cells:** Glucose
- **Preferred fuel for Aerobic cells:** They use both glucose and fatty acid. But fatty Acids over glucose, as fatty acids produce more ATPs.
  - **Examples of aerobic cells**
    - → Cardiac muscle fibres.
    - → Red muscle fibres
  - **Examples of fatty acids**
    - → Palmitic acid: Generates 106 ATPs.
    - → Stearic acid: Generates 120 ATPs.
  - Exception: Neuron Cells - use glucose aerobically
    - → Uses Glucose for ATP production.
    - → Any fatty acids (nonpolar) present in the blood are conjugated with albumin to make them soluble.
    - → Fatty acids conjugated with albumin cannot penetrate BBB.

Difference of Glycolysis in Anaerobic Cells (RBCs) and Aerobic Cells (Neurons)

Glycolysis in Anaerobic Cells (RBCs)	Glycolysis in Aerobic Cells Neurons
Anaerobic glycolysis.	Aerobic glycolysis.
2 Lactates are formed.	2 Pyruvates are formed.
2 ATPs are generated.	32 ATPs are generated on the complete oxidation of glucose in neurons. • 7 ATPs from Glycolysis. • 2 NADH × 2.5 ATP = 5 ATPs on conversion of 2 Pyruvates → 2 Acetyl CoA. With the help of PDH complex. • Acetyl CoA enters the TCA cycle to produce 10 ATPs per molecule. • 2 Acetyl CoA × 10 ATPs = 20 ATPs.

Important Information

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- Pyruvate Dehydrogenase acts on Pyruvates, converting them into Acetyl CoA.
- The role of Pyruvate Dehydrogenase is
  - Conversation of pyruvates into acetyl CoA via dehydrogenation.
  - This removes two hydrogens and gives them to its coenzyme NAD to form NADH.
  - Ultimate products obtained from the citric acid cycle are carbon dioxide.

Steps of Glycolysis 00:13:39

Importance of acknowledging steps of glycolysis

- To know about multiple inborn errors of metabolism related to glycolysis directly or indirectly.

Step-01: Glucose → Glucose 6 Phosphate.

- Glucose enters the cell.
- First enzyme to act on is Glucokinase or Hexokinase.
- This enzyme utilises 1 high-energy phosphate (ATP) for conversion.
- **Purpose of Step 01**
  - To trap glucose.
  - To trans pass the GLUT transporters, as they can cause efflux glucose out to the circulation due to an increase in glucose concentration inside the cell.
- All GLUT transporters belong to Facilitated Passive Diffusion.

Q. In what form is glucose trapped inside the cell?
Ans. Glucose 6 Phosphate.

Q. What is the purpose of hexokinase or glucokinase?
Ans. To trap the glucose inside the cell.

Step-02: Glucose 6 Phosphate (G6P) ⇄ Fructose 6 Phosphate (F6P).

- G6P and F6P
  - Both have phosphate groups and are hexoses and isomers.
  - Interconvertible.
- Phosphate Hexose Isomerase enzyme is involved.

Step-03: Fructose 6 Phosphate (F6P) → Fructose 1,6 bisphosphate.

- Phosphofructokinase I use one ATP for the conversion.
- Steps 01 to 03 are named the investment phase because 2 ATPs are utilised. Further steps are considered as the Harvest phase (ATPs are generated).

Step-04: Fructose 1,6 bisphosphate → Glyceraldehyde 3 Phosphate + Dihydroxyacetone Phosphate.

- This reaction yields two different trioses.
- Aldolase A mediates the reaction and is important in glucose glycolysis.
- Aldolase B is important in fructose glycolysis.

Step-05: Glyceraldehyde 3 Phosphate ⇄ Dihydroxyacetone Phosphate.

- G3P and DHAP are.
  - Trioses and isomers.
  - Interconvertible.
- Enzyme involved is Phospho Triose Isomerase.

Step-06: 2 (Glyceraldehyde 3 Phosphate) → 2 (1,3 bisphosphoglycerate.)

- Glyceraldehyde 3 Phosphate dehydrogenase enzyme is involved.
- This reaction is an example of oxidative phosphorylation.
- Gives two hydrogens to NAD to form 2 NAD → 2 NADH.
- Each NADH enters the ETC and gets oxidised to give 2.5 ATPs.
- 2 NADH × 2.5 ATP = 5 ATPs.

Step-07: 2 (1,3 Bisphosphoglycerate) → 2 (3-Phosphoglycerate.)

- An example of substrate-level phosphorylation.
- Enzyme - Phosphoglycerate kinase.
- 2 ATPs are generated directly.

Step-08: 2 (3-Phosphoglycerate) → 2 (2-Phosphoglycerate).

- Positional isomerism is seen in the phosphate group of the above compounds.
- Mutases always mediate this type of isomerism.
- Enzyme: Phosphoglycerate mutase.

Step-09: 2 (2-Phosphoglycerate) → 2 (Phosphoenolpyruvate).

- Enolase acts by removing a water molecule.

Step-10: 2 (Phosphoenolpyruvate) → 2 (Pyruvate).

- Catalysed by pyruvate kinase.
- An example of substrate-level phosphorylation.
- 2 ATPs are generated directly.
- Total ATPs generated = 9.
- Total ATPs invested = 2.
- Net ATP generated = 7.

Q. In which step do you observe oxidative phosphorylation?
Ans. it is catalysed by Glyceraldehyde 3 phosphate dehydrogenase enzyme.

Q. What are the two steps when you see substrate-level phosphorylation?

- Phosphoglycerate kinase step.
- Pyruvate kinase step.

Q. What happens when the same process occurs anaerobically?

- Oxidative phosphorylation step is affected.
- It has two impacts.
  - NADH cannot enter ETC; thus, the process lacks 5 generated ATPs.
  - NAD cannot be regenerated, causing NAD deficiency, and making Glyceraldehyde 3 Phosphate dehydrogenase enzyme inactive; further glycolysis is arrested.
- To counteract these limitations, in anaerobic cells,

- Lactate dehydrogenase converts 2 Pyruvates into 2 Lactates by regenerating NAD.
- Lactate is produced for the regeneration of NAD.

Facts About Glycolysis 00:28:57

- Aerobic glycolysis: 2 Pyruvates and 7 ATPs.
- Anaerobic glycolysis: 2 Lactate and 2 ATPs.
- The Hexokinase or Glucokinase step traps the glucose inside the cell.
- Irreversible steps of glycolysis, marking them can make gluconeogenesis (Reversal of Glycolysis) easy.
  - Hexokinase or Glucokinase
  - Phosphofructokinase I (rate-limiting step of glycolysis)
  - Pyruvate Kinase
- Substrate level phosphorylation steps are catalysed by phosphoglycerate kinase and pyruvate kinase enzymes.
- Oxidative phosphorylation step is catalysed by glyceraldehyde 3 phosphate dehydrogenase.
- Lactate is formed in anaerobic glycolysis for the regeneration of NAD.

Hexokinase and Glucokinase 00:30:44

Sr. No	Property	Hexokinase	Glucokinase
1.	**Location**	All cells.	• Liver cells. • Beta cells of the pancreas.
2.	**Km and affinity**	High Affinity and low Km.	Low Affinity and High Km.
3.	**Inhibition by its products, Glucose 6 Phosphate**	Yes	No
4.	**Induction by Insulin**	No	Yes

- Glucokinase is present in the liver cells and beta cells of the pancreas and exhibits low affinity because.
  - Liver is the 1st organ to meet dietary glucose.
  - If it has a higher affinity, it may consume all for itself, and this may cause the peripheral tissues to suffer from hypoglycaemia.
  - Glucose enters the pancreatic beta cells and goes through glycolysis and then to the TCA Cycle.
  - The cell gets the ATP and closes ATP-sensitive potassium channels.
  - This causes depolarisation and action potential, causing the release of insulin.
  - If pancreatic beta cells were provided with more glucose, they'll release more insulin that may cause hypoglycaemia.

Km and Affinity 00:32:00

- Km stands for Michaelis Constant.
  Km = [S - Substrate concentration] at ½ maximal velocity (Vmax).
- When the enzyme affinity for the substrate is low, more substrate is given to achieve the ½ maximal velocity.
- Example.
  - If Km = 100 µ mol, then [S] = 100 µ mol.
  - If Km = 200 µ mol, then [S] = 200 µ mol.
- Km is inversely proportional to Affinity.

Inhibitors of Glycolysis 00:39:46

Sr. No	Enzyme	Inhibitor
1.	**Glyceraldehyde 3 phosphate Dehydrogenase (Oxidative phosphorylation step)**	Iodoacetate
2.	**Phosphoglycerate kinase**	Arsenate
3.	**Enolase**	Fluoride

- Sodium fluoride + potassium oxalate (anticoagulant) comes in a grey top tube and is used for plasma glucose estimation.
- To avoid false glucose estimation as there will be a window period (1 and a half - 2 hours) between sample collection and sample analysis.

Facts About Rapaport Leubering Shunt or 2,3 BPG shunt 00:42:27

- Phosphoglycerate kinase converts 2(1,3 BPG) is converted to 2(3 PG) usually we get 2 ATPs.
- In RBC, it needs 2,3 BPG. It helps in unloading Oxygen to tissues.
- It bypasses the phosphoglycerate kinase step to deliver oxygen to the tissues by decreasing the affinity of haemoglobin.
- 2(1,3 BPG) is converted to 2(2,3 BPG) (positional isomerism) catalysed by Bis Phosphoglycerate mutase, and this molecule is useful in delivering oxygen.
- 2(2,3 BPG) is converted into 2 (3-PG) mediated by bisphosphoglycerate phosphatase.
- 2(3-PG) finally gets converted into 2 (2-PG) via phosphoglycerate mutase..

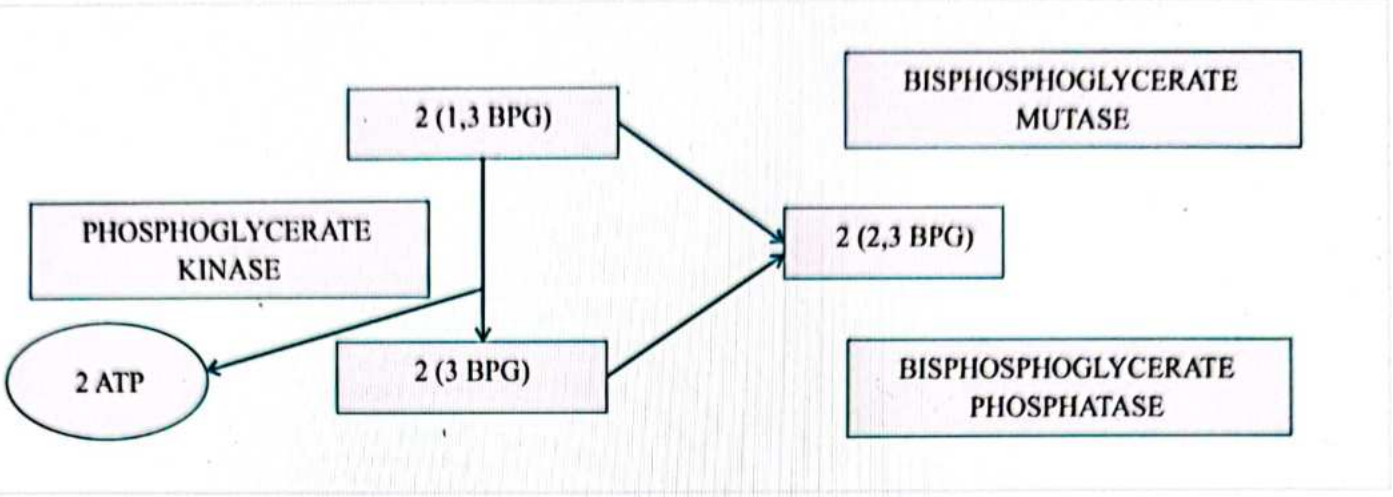
Advantages

- Acts as a source of 2,3-BPG.

Disadvantages

- Zero ATP production.

Facts about Rapaport leubering shunt



One Liners

00:47:33

- The products of aerobic glycolysis are **2 Pyruvate and 7 ATPs**.
- The products of anaerobic glycolysis are **2 Lactate and 2 ATPs**.
- The rate-limiting enzyme of glycolysis is **Phosphofructokinase I**.
- Phosphoglycerate kinase is inhibited by **Arsenate**
- Glyceraldehyde 3 phosphate dehydrogenase is inhibited by **iodoacetate**.
- In neonates, the diagnostic character to study white muscle fibres suspecting haemolytic anaemia. Estimate CK MM levels.
  - Increased CK MM levels and hemolytic anemia S/O glycolytic enzyme defective.
  - E.g., PFK 1 defect - Tarui's disease.
  - CK has got 3 isoforms.
    - → MM is present in skeletal muscles.
    - → MB is present in cardiac muscles.
    - → BB is present in the brain.

MCQs

00:48:45

Q. Which of the following is a product of anaerobic glycolysis?
A. 2 Pyruvate 10
B. 2NAD
C. 7ATP
D. 2ATP

Q. All of the following cells are dependent on glycolysis except?
A. Neurons
B. RBC
C. Renal medulla
D. Red muscle fibres

Explanation
Inborn Error: Phosphofructokinase I defect - Tarui's disease.
- RBCs are affected.
- It has Na+/K+ pumps and transports 3 Na+ and 2K+ across the membrane against a concentration gradient using an ATP.
- Na+/K+ pumps get disabled.
- This causes the accumulation of Na+ in the RBCs.
- As a result of osmosis, water enters the RBCs, slowly swells, and ruptures.
- Biochemical manifestation - Haemolytic Anaemia.
- White muscle fibres - Exercising Intolerance, particularly to isometric exercises.

Q. Glucose is trapped inside the cells as
A. D glucopyranose (The most common physiological form of glucose).
B. UDP glucose
C. Glucose 6 phosphate
D. Fructose 6 phosphate

Q. The irreversible steps of glycolysis are all except
A. Pyruvate kinase
B. Phosphoglycerate kinase
C. Hexokinase
D. Phosphofructokinase

Q. The steps in which oxidative phosphorylation occurs in glycolysis are:
A. Pyruvate kinase
B. Phosphoglycerate kinase
C. PFK
D. Glyceraldehyde 3 Phosphate Dehydrogenase

Q. In anaerobic glycolysis, lactate is formed.
A. For the generation of ATP
B. For the regeneration of lactate
C. For the regeneration of pyruvate
D. For the regeneration of NAD

Q. The significance of Rapoport Luebering Shunt is
A. ATP production
B. Lactate formation
C. Source of fatty acids
D. 2,3 BPG production

Q. The enzyme involved in Rapoport Leubering Shunt is
A. Phosphoglycerate kinase
B. Bisphosphoglycerate kinase
C. Bisphosphoglycerate mutase
D. Phosphoglycerate mutase

Integrated Case-Based MCQ

00:55:34

Q. A neonate presented with hemolytic anaemia. A peripheral smear revealed that it's a case of non-spherocytic hemolytic anaemia. Pyruvate kinase activity was remarkably low (0.675 U/g Hb). Which of the following explains haemolytic anaemia in this condition?
A. High 2,3 BPG production
B. Low 2,3 BPG production
C. High ATP production
D. Failure of Sodium Potassium ATPase pump

Explanation

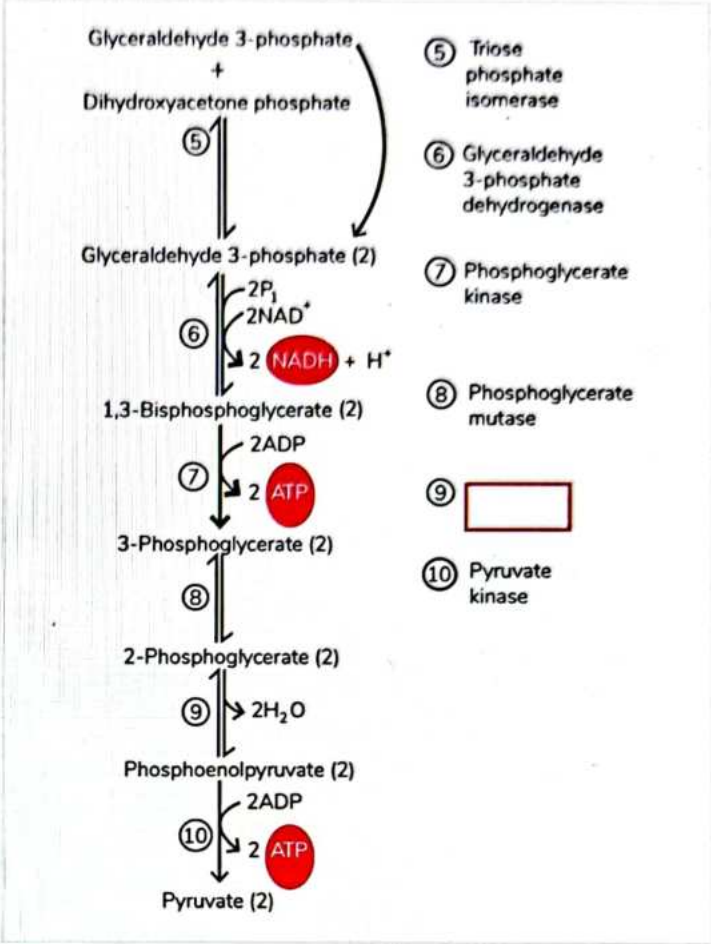
Hemolytic Anaemia falls into

- **G3PD deficiency (classical history)**
  - Hemolytic anaemia following exposure to oxidative stress.
  - Examples
    → After intake of Primaquine.
    → After intake of fava beans.
- **Glycolytic enzyme defect**
  - Skeletal muscles are involved.
  - Represented by.
    → Increased levels of CK MM.
    → Intolerance to anaerobic exercises.

Image-based MCQs

00:57:42

Q. Name a chemical that inhibits the enzyme which is shown in the red square in the given picture.



A. Iodoacetate
B. Arsenate
C. Arsenite
D. Fluoride

Explanation

- It is related to the 9th step of glycolysis.
- This step is catalysed by enolase to remove water molecules.
- Fluoride inhibits enolase.

9 CITRIC ACID CYCLE

Significance of Citric Acid Cycle

- The Citric Acid Cycle is a final furnace where every fuel comes and gets oxidized. Though the Citric Acid Cycle is discussed along with carbohydrate metabolic pathways, the citric acid cycle is not exclusively for carbohydrate metabolism. It's a common pathway for carbohydrates, lipids, and protein.

Facts about TCA Cycle

- It is a mitochondrial process.
- The cycle is strictly Aerobic. It can never happen in the absence of oxygen. Because by the time a few citric cycles get over, many NAD would convert to NADH, and many FAD would convert to $FADH_2$. Without oxygen, NAD and FAD cannot be generated. And without NAD and FAD, many enzymes of the citric acid cycle will stop.
  - Glycolysis can happen anaerobically because, in glycolysis, there is Lactate Dehydrogenase which can regenerate NAD even in the absence of oxygen.
- Every citric acid cycle gives 10 ATP.

Steps of Citric Acid Cycle

- Citrate Synthase is the first enzyme which condenses Acetyl CoA & Oxaloacetate to form Citrate
- The next enzyme is Aconitase which converts Citrate to Isocitrate.
- The next enzyme is Isocitrate Dehydrogenase which converts Isocitrate into Alpha Ketoglutarate (Isocitrate Dehydrogenase catalyzes an oxidative Decarboxylation step)
  - In this step, the enzyme Isocitrate Dehydrogenase simultaneously oxidizes and decarboxylates Isocitrate. When it oxidizes Isocitrate, it removes hydrogen from Isocitrate and gives the hydrogen to the coenzyme & because its coenzyme is NAD, NADH is formed. And when NADH enters into ETC, it gives rise to 2.5 ATPs. When it decarboxylates Isocitrate, it gives CO_2 and this is how Alpha Ketoglutarate is formed.
- The next enzyme is Alpha Ketoglutarate Dehydrogenase converts Alpha Ketoglutarate to Succinyl CoA. This enzyme also catalyzes an oxidative decarboxylation step.
  - In this catalyzation, when it oxidizes Alpha Ketoglutarate, it removes hydrogen from Alpha Ketoglutarate and gives the hydrogen to the coenzyme & because its coenzyme is NAD, NADH is formed. And when NADH enters into ETP, it gives rise to 2.5 ATP. Therefore, now there are a total of 5 ATP generated. When it decarboxylates Alpha Ketoglutarate, it gives CO_2 and this is how Succinyl CoA is formed.
- The next enzyme is Succinyl Thio Kinase (STK), which is an example of a Substrate level Phosphorylation step-catalyzing enzyme. And because this is Substrate level Phosphorylation here, 1 ATP is generated directly.
- The next enzyme is Succinate Dehydrogenase which converts Succinate to Fumarate. Succinate Dehydrogenase uses FAD as its coenzyme. So, in this step, $FADH_2$ is formed, and it is equal to 1.5 ATP. So, a total of 7.5 ATP is generated.
- The last enzyme of the cycle is Malate Dehydrogenase which converts Malate to Oxaloacetate. The same process goes as the first time, and 2.5 ATP is generated. So, a total of 10 ATP is generated.
- The Oxaloacetate used at the start of the cycle is regenerated again. And it is considered a catalyst for the Citric Acid Cycle.
- The formula for Acetyl CoA is CH_3COOH. There are 2 carbon atoms which are removed as carbon dioxide during the process. So, the product of the Citric Acid Cycle is Carbon dioxide and Water. Because there is no hydrogen left, there is no scope for this product to get oxidized further. And that is the reason why Citric Acid Cycle is the final furnace where every fuel comes and gets oxidized.

Aconitase Enzyme

- Aconitase is inhibited by fluoroacetate. Fluoroacetate inhibiting aconitase is an example of Suicidal inhibition.
- When Fluoroacetate gets into a cell, it becomes Fluoroacetyl CoA, and it reacts with Oxaloacetate to form Fluorocitrate, which is a structural analog of Citrate. Fluorocitrate goes and acts on Aconitase, and this Fluorocitrate is converted to a reactive intermediate, and that reactive intermediate inhabits the same aconitase enzyme, which facilitates the conversion of Fluorocitrate to the reactive intermediate.

When Will You Call Something a Suicidal Inhibitor?

- It has to be an irreversible inhibitor of the enzyme.
- The inhibitor should be a substrate analog of the enzyme, and it should compete with the substrate to go and bind to the enzyme. As it goes and binds to the enzyme, the enzyme catalyzes the conversion of inhibitors to a reactive intermediate, and that reactive intermediate inhibits the same enzyme which catalyzes this conversion.
- Fluoroacetate satisfies all the requirements of being called a suicidal inhibitor, as explained above.

Difference between Competitive and Suicidal Inhibition

- Competitive is a reversible inhibitor of enzymes, while suicidal is an irreversible inhibitor of enzymes.
- Suicidal inhibitor on its own it won't inhibit the enzyme. The enzyme catalyzes the conversion of the suicidal inhibitor to the reactive intermediate. And the reactive intermediate covalently binds to the enzyme and inhibits the enzyme.

Facts about Isocitrate Dehydrogenase

- It is an example of oxidative decarboxylation.
- It is an example of oxidative Phosphorylation (Because NADH is there, which has to go through the chain and get oxidizes for Phosphorylation)
- Isocitrate Dehydrogenase is one of the three sources of NADPH (A minor source as compared to HMP Shunt, which is a major source).

Facts about alpha Ketoglutarate Dehydrogenase

- Located in mitochondria.
- Catalyzing oxidative decarboxylation
- Examples of oxidative Phosphorylation
- Enzyme complexes involving 3 subunits and 5 coenzymes.

Facts about Succinyl Thiokinase

- It is an example of Substrate level Phosphorylation.
- Directly ATP is formed.
- There are two isoforms of Succinyl Thiokinase
  - One form uses ADP and forms ATP.
  - The second form uses GDP and forms GTP (Present only in GNG tissues- in the liver and kidney).
- Odd chain fatty acid, on oxidation, gives rise to Propionyl CoA, which enters the cycle by converting to Succinyl CoA. For instance: 7C is oxidized by beta oxidation. In the first cycle, the last 2C atoms come out, and the remaining is 5C. Again, the last 2C will come out. And the rest of 3C is called Propionyl CoA.
- The propionyl CoA formed also gets into the cycle by converting into Succinyl CoA. The first enzyme is Propionyl CoA carboxylase, which converts Propionyl CoA to D methyl malonyl CoA. Then this D methyl malonyl is converted to L methyl malonyl by the enzyme MNR. Then the L methyl malonyl CoA Mutase (B12 dependent enzyme) enzyme converts L methyl malonyl to Succinyl CoA. And then, Succinyl CoA gets into the citric acid cycle. When there is a B12 deficiency, methylmalonic acid levels are measured in urine.
  - Even chain fatty acid on oxidation gives rise to only Acetyl CoA molecules, whereas odd chain fatty acid on oxidation, the majority product is Acetyl CoA, but there is the formation of 1 Propionyl CoA.

Facts about the Succinate Dehydrogenase Step

- It is an example of Oxidative Phosphorylation because FADH, is formed. And then, it's dependent on the electron transport chain to convert it into ATP.
- Unlike other dehydrogenases, succinate dehydrogenase uses FAD as its coenzyme.
- It is inhibited by malonate.

Inhibitors in the Citric Acid Cycle

- Fluoroacetate inhibits aconitase (Suicidal Inhibitor)
- Arsenite inhibits both PDH complex and Alpha Ketoglutarate Dehydrogenase complex.
- Malonate inhibits succinate dehydrogenase.

Few Essential facts about Citric Acid Cycle

Example of Oxidative Phosphorylation steps

- Isocitrate Dehydrogenase
- Alpha Ketoglutarate Dehydrogenase
- Succinate Dehydrogenase
- Malate Dehydrogenase

Example of Substrate level Phosphorylation step

- Succinyl Thiokinase step

Examples of Oxidative Decarboxylation steps

- Isocitrate Dehydrogenase
- Alpha Ketoglutarate Dehydrogenase

One FAD-linked Dehydrogenase

- Succinate Dehydrogenase

Oxidative Decarboxylation Steps

- PDH (Pyruvate Dehydrogenase)
- ICDH (Isocitrate Dehydrogenase)
- Alpha KGDH (Alpha Ketoglutarate Dehydrogenase)
- 6 PGDH of HMP Shunt (6 Phosphogluconate Dehydrogenase)
- BCKADH (Branched-chain Keto acids Dehydrogenase)

FAD-linked Dehydrogenase

- Succinate Dehydrogenase (SDH) of citric Acid Cycle
- Acyl CoA Dehydrogenase is an enzyme of fatty acid oxidation (Dehydrogenation means oxidation)
- Mitochondrial Glycerol 3 Phosphate Dehydrogenase (G3PDH)
- In these 3 the FAD becomes FADH2

Anaplerotic Reaction (Filling Up Reactions)

- If Isocitrate Dehydrogenase is inhibited, alpha Ketoglutarate should deplete, but this does not happen because of

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Anaplerotic reactions: Glutamate and amino acids can form Alpha Ketoglutarate. Glutamine can form Glutamate, and it can form alpha Ketoglutarate. Histidine, proline, and Arginine can form Glutamate, and it can again form Alpha Ketoglutarate.

- Similarly, when alpha Ketoglutarate Dehydrogenase inhibits Succinyl CoA should deplete, but it accumulates. It accumulates because of VIM Amino acids – Valine, isoleucine and methionine (Anaplerotic reaction). These amino acids can be used in catabolism and can give rise to Succinyl CoA. And this Succinyl CoA will then be used for Heme Synthesis.
- The best Anaplerotic reaction is catalyzed by the Malic enzyme, which converts Malate to pyruvate. This is called the best because once pyruvate is formed, it can form both Acetyl CoA and Oxaloacetate. Pyruvate Dehydrogenase complex can convert pyruvate into acetyl CoA. Pyruvate carboxylase can convert pyruvate into Oxaloacetate. Once they are formed, they can join the Citric cycle.
- **Significance of malic enzyme**
  - Best Anaplerotic reaction
  - One of the three sources of NADPH

Amphibolic Pathway

- The citric acid cycle is an Amphibolic pathway (a pathway which can run either in a catabolic or anabolic direction). The choice of direction depends on the energy status of the cell. When the energy status of the cell is low, the pathway will be used in the catabolic direction generating ATP. When the energy status of the cell is high, the same pathway will be used for anabolism.
- The Citric Acid Cycle is called an Amphibolic pathway because when the energy status of the cell is low, the low energy status indicators (NAD, FAD, ADP) stimulate every enzyme of the cycle, and the cycle will happen in a clockwise direction generating 10 ATP per cycle, and that is catabolism. When the energy status of a cell is high, the high energy status indicators (NADH, $FADH_2$, ATP) will inhibit all enzymes of the pathway, and the precursors will be used for Anabolism.

Example

- In the first step of the cycle, citrate Synthase is inhibited by high energy, and acetyl CoA accumulates. Then it is used for fatty acids synthesis and cholesterol synthesis, both of which are anabolic.
- Similarly, when there is high energy status, alpha Ketoglutarate Dehydrogenase inhibits, and alpha Ketoglutarate accumulates. And this is used for glutamate synthesis, which is anabolism.
- Similarly, when there is a high energy, Succinyl Thiokinase inhibits, and Succinyl CoA accumulates. Then it is used for Heme synthesis, which is anabolic.

Regulation of the TCA Cycle

- In the citric acid cycle, it gets regulated at almost all steps. All enzymes of the cycle get stimulated by low energy status indicators such as high ADP, high NAD, and high FAD. And they are all inhibited by high-energy status indicators such as ATP, NADH, and FADH2.
- In addition, all dehydrogenases of the citric acid cycle are stimulated by calcium (particularly in muscle). Calcium stimulates dehydrogenases of the Citric Acid Cycle so that the final furnace, where every fuel comes and gets oxidized, gets stimulated.
- And enough ATP is there for excitation-contraction coupling. If there are four choices of citric acid cycle enzymes and one needs to be chosen, then the most appropriate one is ICDH (Isocitrate Dehydrogenase) because the narrowest bottleneck is at this level.

One-liners

- GTP is produced by: Succinyl Thiokinase
- Odd chain fatty acids on oxidation forms: Succinyl CoA
- The rate-limiting enzyme of the Citric Acid Cycle is: Isocitrate Dehydrogenase
- The number of ATPs produced in the TCA cycle is: 10.
- Glucose on complete oxidation forms: 32 ATPs

MCQ

Q. All the following are examples of oxidative phosphorylation step enzymes except:

A. Isocitrate Dehydrogenase
B. Alpha Ketoglutarate Dehydrogenase
C. Succinyl Thiokinase
D. Succinate Dehydrogenase

Q. The rate-limiting enzyme of the Citric acid cycle is:

A. Isocitrate Dehydrogenase
B. Alpha Ketoglutarate Dehydrogenase
C. Succinyl Thiokinase
D. Succinate Dehydrogenase

Q. All the following are stimulators of Isocitrate Dehydrogenase except:

A. NAD
B. FAD
C. ATP
D. Calcium

Q. Oxidative decarboxylation reactions are catalyzed by all, except:

A. PDH

B. Isocitrate Dehydrogenase

C. Alpha Ketoglutarate Dehydrogenase

D. Malate Dehydrogenase

Q. The total number of ATPs produced by the complete oxidation of Pyruvate through the Citric acid cycle:

A. 15

B. 8

C. 12.5

D. 32

Q. The total number of ATPs generated during one full cycle of the TCA cycle is:

A. 10

B. 15

C. 8

D. 2

Q. The number of ATPs produced by the complete oxidation of 1 molecule of glucose is:

A. 7

B. 14

C. 22

D. 32

Q. In 1970, fluoroacetate mixed meat bait was used as a rodent poison in a field, and it was accidentally consumed by a flock of birds. Fluoroacetate inhibits which of the following enzymes:

A. Enolase

B. Pyruvate Dehydrogenase

C. Alpha Ketoglutarate Dehydrogenase

D. Aconitase

Q. In 1970, fluoroacetate mixed meat bait was used as a rodent poison in a field and it was accidentally consumed by a flock of birds. Which of the following statements is true about fluoroacetate's inhibition of aconitase:

A. It is an example of reversible inhibition.

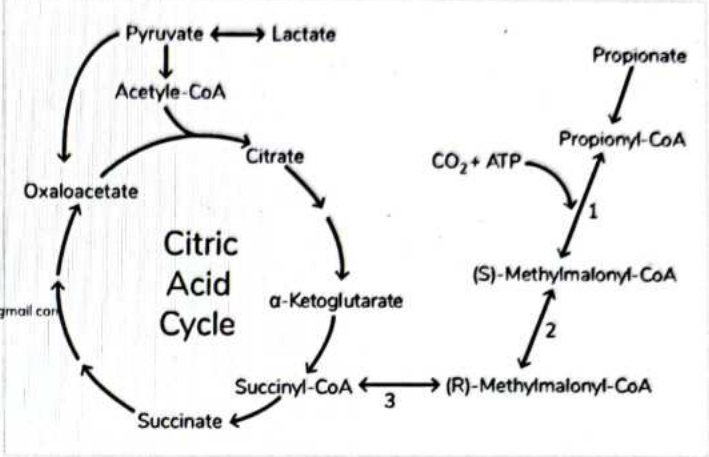
B. Fluoroacetate is a substrate analog of aconitase

C. Fluoroacetate interacts with aconitase through non-covalent linkages.

D. It is an example of competitive Inhibition.

Image Questions

Q. Enzymes 1,2,3 are



A. Propionyl CoA carboxylase, Methyl Malonyl CoA Mutase, Methyl Malonyl CoA racemase

B. Propionyl CoA Mutase, Methyl Malonyl CoA Mutase, Methyl Malonyl CoA racemase

C. Propionyl CoA Mutase, Methyl Malonyl CoA racemase, Methyl Malonyl CoA Mutase

D. Propionyl CoA carboxylase, Methyl malonyl CoA racemase, Methyl malonyl CoA mutase

10 GLYCOGEN METABOLISM

Facts About Glycogen 00:00:45

Structure of Glycogen

- Glycogen is a spherical molecule with a protein called Glycogenin in the center, to which all the glucose residue gets attached directly or indirectly.
- Every straight chain has got 11-13 glucose residue connected by α (1,4) linkage and at branched points by α (1,6) linkage.
- The entire structure is arranged in 12 concentric layers.

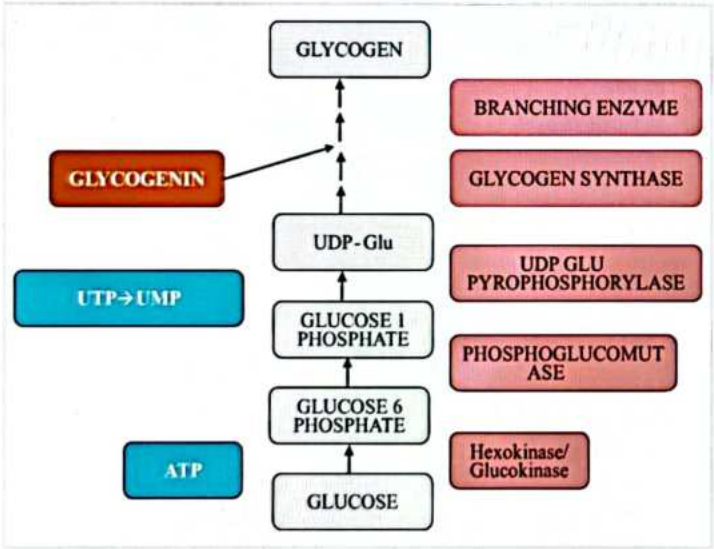
Storage

- Two tissues where glycogen is stored are liver and muscle.

Q. Between liver and muscle, which has the highest glycogen content/concentration?
Ans. Liver (expressed in g/kg).

Q. Which tissue has the highest glycogen mass?
Ans. Muscle (due to high skeletal muscle mass).

Glycogen Synthesis 00:03:35



1st step

- Glucose enters into the cell and gets converted to Glucose 6-phosphate by the enzyme Hexokinase/Glucokinase.
- Hexokinase is present in all tissues like muscle and Glucokinase is present in Liver and pancreatic ß cells.
- In the case of muscle it's Hexokinase and in the case of liver it's Glucokinase.
- Both these enzymes use 1 high energy phosphates.

2nd step

- Glucose 6-phosphate is converted to Glucose 1 phosphate by the enzyme Phosphoglucomutase. This is called Positional isomerism.

3rd step

- Glucose 1-phosphate is about to enter into anabolic pathway, Glycogen synthesis.
- Any fuel to enter into any anabolic pathway, it should get activated by attaching to one or the other nucleotide.
- Glucose 1-phosphate is converted to UDP glucose by the enzyme UDP glucose pyrophosphorylase.
- In this step, 1 UTP is used which gets converted to UMP PPi.
- Here 2 high energy phosphates are used.

Important Information

- Most physiological form of glucose: ß D-Glucopyranose.
- Form in which glucose is trapped in a cell: Glucose 6-phosphate
- Active form of glucose: UDP glucose
- Nucleotide used to activate carbohydrates: UTP.

4th step

- One UDP glucose is attached to Glycogenin by the enzyme Glycogen synthase.
- Glycogen synthase acts on UDP glucose and converts it into Glycogen primer (Glycogen with only one glucose residue).
- Glycogen synthase is the rate limiting enzyme of Glycogen synthesis.
- By the time one glycogen primer is formed, another glucose enters the cell, gets trapped and undergoes the same process again until a straight chain of 11-13 glucose residues are formed.

5th step

- Now, Branching enzyme removes the last glucose residue with α (1,4) linkage and attaches this hexasaccharide together to another chain forming α (1,6) linkage.
- Branching enzyme is also called α (1,4) to α (1,6) glucan transferase.
- Now this chain will continue to grow until 11-13 glucose residues are attached and again the last 6 glucose residues will be cleaved by the branching enzyme until 12 concentric layers are formed.

Facts about Glycogen Synthesis 00:12:30

- Glycogen is synthesized in the liver and muscle.
- Active form of glucose is UDP glucose.
- 3 high energy phosphates are used for attaching 1 glucose to a growing glycogen.
- Rate limiting enzyme of glycogen synthesis is Glycogen synthase.

- Glycogen synthase is activated by dephosphorylation.
- Branching enzyme is called α (1,4) → α (1,6) glucan transferase.

One Liner 00:15:05

- The rate limiting enzyme of Glycogen synthesis is Glycogen synthase.
- The active form of Glucose is UDP glucose.
- Number of high energy phosphates required for attaching a molecule of glucose is 3.
- Branching enzyme is α (1,4) to α (1,6) glucan transferase.

MCQs 00:19:40

Q. The organ with maximum glycogen concentration:
A. Liver
B. Skeletal muscle
C. Cardiac muscle
D. Neuron

Q. The active form of glucose is:
A. ß D-Glucopyranose
B. α D-Glucopyranose
C. UDP glucose
D. Glucose 6-phosphate

Q. The branching enzyme in glycogen synthesis is:
A. α (1,4) → α (1,4) glucan transferase
B. α (1,4) → α (1,6) glucan transferase
C. α (1,6) → α (1,4) glucan transferase
D. α (1,4) → ß (1,4) glucan transferase

Q. The enzyme which gets activated by dephosphorylation is:
A. Glycogen synthase
B. Glycogen phosphorylase
C. Fructose 1,6-bisphosphatase
D. Hormone sensitive lipase

Enzymes Activated by Phosphorylation 00:17:00

- Any enzyme that increases the blood glucose levels gets activated by phosphorylation.
  - **Glycogenolysis**
    - → Glycogen phosphorylase
    - → Debranching enzyme
  - **Gluconeogenesis**
    - → Pyruvate carboxylase.
    - → PEP CK (Phosphoenolpyruvate carboxykinase).
    - → Fructose 1,6-bisphosphatase.
    - → Fructose 2,6-bisphosphatase (indirectly stimulates blood glucose).
    - → Glucose 6-phosphatase.
    - → Hormone sensitive lipase.

Integrated MCQs 00:19:35

Q. A 4-month-old baby visited the OPD with abnormal LFTs. There were no neurologic symptoms or signs. Abdominal ultrasound showed hepatosplenomegaly with periportal thickening and ascites. Transthoracic echocardiogram revealed no abnormal findings. A liver biopsy was performed, revealing micronodular cirrhosis with marked intracytoplasmic glycogen deposits. He was diagnosed with a defect of branching enzymes. The name of the disorder is:
A. Cori's disease
B. Anderson's disease
C. McArdle's disease
D. Tarui's disease

Image Based MCQs

Q. Identify the enzyme A marked in the image:

Refer Image 10.1

A. Hexokinase
B. Glucokinase
C. Glycogen synthase
D. Glycogen phosphorylase

Glycogenolysis 00:22:50

- Glycogenolysis is done by Glycogen phosphorylase which acts on glycogen with 'n' no. of glucose residues.
- It's called phosphorylase because it mediates phosphorolysis.
- Glycogen phosphorylase adds inorganic phosphate to cleave glycogen with n no. of glucose residues to form Glycogen n - 1.
- The last glucose that is removed out of glycogen will be added to the phosphate that is used for cleaving and forms Glucose 1-phosphate.
- Glucose 1-phosphate is converted to Glucose 6-phosphate by a mutase.
- Until Glucose 6-phosphate is formed, steps involved in liver and muscle glycogenolysis are the same.
- **In liver**
  - Liver is a gluconeogenic organ with Glucose 6-phosphatase.
  - Glucose 6-phosphatase in the liver converts Glucose 6-phosphate to glucose.
  - This glucose crosses cell membranes and increases blood glucose.
- **In muscle**
  - Muscle lacks Glucose 6-phosphatase enzyme.
  - So, the muscle glycogenolysis produced Glucose 6-phosphate cannot increase blood glucose.
  - This Glucose 6-phosphate gets into muscle anaerobic glycolysis giving rise to ATP.

Important Information

- Glucose 6-phosphatase is not only an enzyme of gluconeogenesis, but also an enzyme that is necessary for glycogenolysis to increase blood glucose.
- Defect of Glucose 6-phosphatase (von Gierke disease): Both gluconeogenesis and glycogenolysis cannot increase blood glucose leading to severe hypoglycemia.

- Glycogen phosphorylase keeps removing peripheral glucose residues until there are only 4 residues in a chain because glycogen phosphorylase can break only α (1,4) linkage.
- At this point, it recruits Debranching enzyme which have two enzymatic activities.
  - Breaks the last 3 α (1,4) linkages and attaches this trisaccharide to some other chain.
  - Debranching enzyme is also called α (1,4) → α (1,4) glucan transferase.
  - The exposed α (1,6) linkage is removed by the debranching enzyme with its α (1,6) glucosidase activity.

Facts abouts Glycogenolysis 00:32:40

- Glycogenolysis happens in the liver and muscle.
- Since the liver has Glucose 6-phosphatase, the glucose formed in the liver increases the blood glucose.
- But, the muscle lacks Glucose 6-phosphatase and the glucose enters the anaerobic glycolysis to give rise to ATP.
- Glucose produced by glycogenolysis in muscle produces 1 ATP more.
- Rate limiting enzyme of glycogenolysis is glycogen phosphorylase.
- Glycogen phosphorylase is activated by phosphorylation.

Important Information

- Aerobic glycolysis gives 7 ATPs (Plasma free glucose).
- Aerobic glycogenolysis glucose gives 8 ATPs.
- Anaerobic glycolysis gives 2 ATPs.
- Anaerobic glycogenolysis glucose gives 3 ATPs.
- Complete oxidation of 1 molecule of glucose gives 32 ATPs.
- Complete oxidation of 1 molecule of glucose in muscle glycogenolysis gives 33 ATPs.

Difference Between Liver and Muscle Glycogenolysis 00:36:40

S. no	Property	Liver glycogen phosphorylase	Muscle glycogen phosphorylase
1.	Products	Glucose 6-phosphate, Glucose, ATP	Glucose 6-phosphate, ATP
2.	Inhibitor	Inhibited by all of it products	Inhibited by all its products. Hence not inhibited by Glucose
3.	Stimulated by	Glucagon and Epinephrine	Epinephrine and calcium

Glycogen Phosphorylase 00:44:42

- Both liver and muscle glycogen phosphorylase act in 2 forms.
  - a- active form (phosphorylated form)
  - b- inactive form (dephosphorylated form)
- Inactive form is converted to active form by phosphorylation by the enzyme Phosphorylase kinase.
- Phosphorylase kinase is activated by cAMP and Calcium dependent kinase.

Debranching Enzyme

- Debranching enzyme is also called α (1,4) → α (1,4) glucan transferase.
- Defective debranching enzyme cause Cori's disease.
- If the debranching enzyme is defective, liver glycogenolysis is affected which causes hypoglycemia and muscle glycogenolysis is also affected which causes exercise intolerance.
- So, Cori's disease presents with hypoglycemia and exercise intolerance.

One Liner

- The rate limiting enzyme of glycogenolysis is Glycogen phosphorylase.
- Glycogen phosphorylase activated by Phosphorylation.
- The immediate product of glycogenolysis is Glucose 1-phosphate.
- Liver glycogenolysis is stimulated by Glucagon and Epinephrine.
- Muscle glycogenolysis is stimulated by Epinephrine and Calcium.

MCQs

Q. **Assertion:** Muscle glycogenolysis cannot increase blood glucose
Reason: Muscle lacks glucose 6-phosphatase

A. **Both assertion and reason are true, and reason is the correct explanation for assertion.**
B. Both assertion and reason are true, and reason is not the correct explanation for assertion.
C. Assertion is true, but the reason is false.
D. Assertion is false, but the reason is true.

Q. Glucagon stimulates:
A. Liver glycogen phosphorylase
B. Muscle glycogen phosphorylase
C. Both
D. None

Q. Epinephrine stimulates:
A. Liver glycogen phosphorylase
B. Muscle glycogen phosphorylase
C. Both
D. None

Q. Glycogen phosphorylase a is converted to b by:
A. Insulin
B. Calcium
C. cAMP
D. Phosphorylase kinase a

Q. The no. of ATP produced from complete oxidation of glucose obtained from muscle glycogenolysis is:
A. 32
B. 33
C. 7
D. 8

Integrated MCQs

Q. Neonate presents with hypoglycemia and elevation of CK MM. Hence the IEM suspects debranching enzyme defect. The name of the disorder is:
A. Cori's disease
B. Anderson's disease
C. McArdle's disease
D. Tarui's disease

Important Information

- Creatine kinase has three isoforms:
  - CK MB is present in the cardiac muscle
  - CK BB is present in the brain.
  - CK MM is present in the skeletal muscle.

Image MCQs

Q. Identify the enzyme A marked in the image:

Refer Image 10.2

A. α(1,4) → α(1,6) glucan transferase
B. α(1,4) → α(1,4) glucan transferase
C. α(1,6) → α(1,4) glucan transferase
D. α(1,6) → α(1,6) glucan transferase

Image 10.1

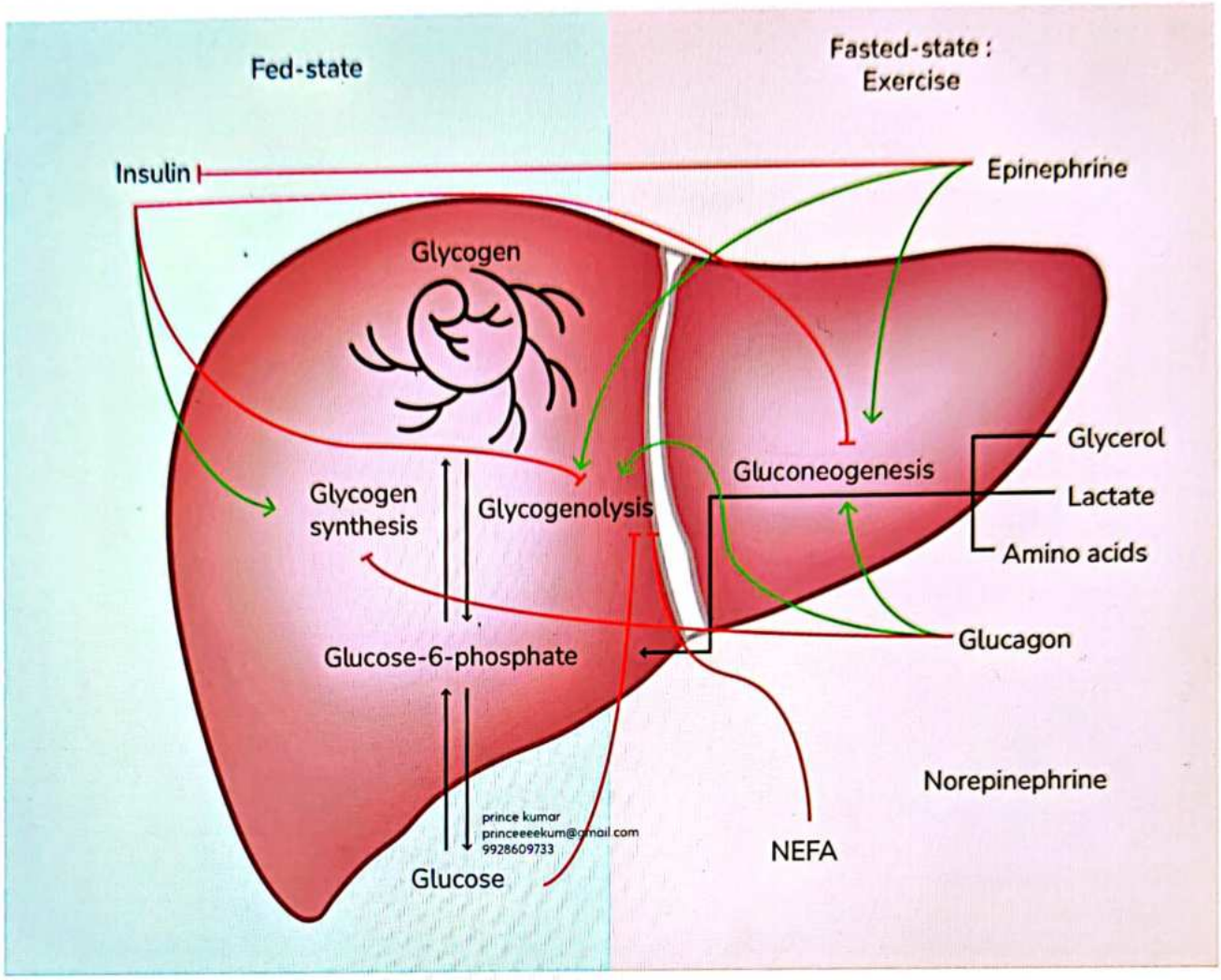
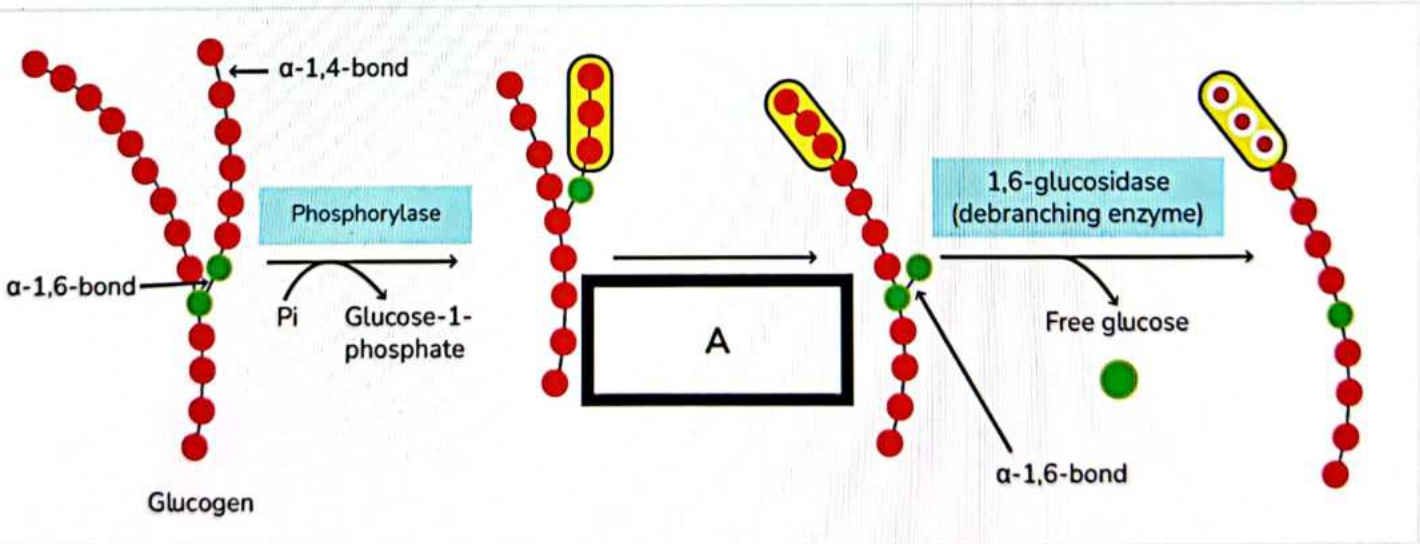


Image 10.2



11 GLUCONEOGENESIS

Gluconeogenesis
- Reversal of glycolysis

Facts About Gluconeogenesis 00:01:22
- **Location:** Liver and Kidney
- In this process, two molecules of pyruvate are combined to form glucose (irreversible steps of glycolysis).
- **Enzymes of irreversible steps of glycolysis:** Hexokinase, Phosphofructokinase-1 (PFK-1), Pyruvate kinase (involved in irreversible steps of glycolysis).
- In gluconeogenesis, the last irreversible step of glycolysis will be reversed. Hence the first step to be reversed in gluconeogeneis is Pyruvate kinase step.

Steps Involved in Gluconeogenesis 00:03:00
- There are four steps with three enzymes.

Reversal of Pyruvate kinase: It is a two-step process.

Step- 1	Step- 2
• Pyruvate to oxaloacetate (OAA) in presence of pyruvate carboxylase.	• Oxaloacetate is converted to Phosphoenolpyruvate in the presence of PEPCK (phosphoenolpyruvate carboxykinase)

- PEPCK uses GTP as its coenzyme.
- GTP comes from succinyl thiokinase (STK) synthesized in Liver and Kidney.

PFK-1: Step-3

Irreversible step (Glycolysis)	Enzyme of Gluconeogenesis
• Fructose-6-phosphate is converted to fructose 1,6 bisphosphate.	• Fructose-1,6-bisphosphate is converted to fructose 6 phosphate by the enzyme fructose 1,6-bisphosphatase (rate limiting step).

Hexokinase/ Glucokinase: Step 4

Irreversible step (Glycolysis)	Enzymes of Gluconeogenesis
• hexokinase/ glucokinase to Glucose 6 phosphate by enzyme hexokinase	• Glucose-6-phosphate to glucose by enzyme glucose-6-phosphatase.

Enzymes Involved in Gluconeogenesis

There are four enzymes involved in gluconeogenesis.
- Pyruvate carboxylase (pyr C)
- PEPCK
- Fructose-1,6-bisphosphatase
- Glucose-6-phosphatase: Liver and Kidney
- First three enzymes are present in all the tissues.
- Conversion of two molecules of pyruvate to one molecule of glucose: 11 ATP is used.

Q. Location of Glucose 6 Phosphatase?
Ans. Liver and Kidney

Location of Enzymes of Gluconeogenesis

Sr. No.	Enzyme	Location
01.	Pyruvate carboxylase	Mitochondria
02.	PEPCK	Cytoplasm
03.	Fructose 1,6 bisphosphatase	Cytoplasm
04.	Glucose 6 phosphatase	Endoplasmic reticulum

- Biomarker of ER: Glucose 6 phosphatase

Significance of malate shuttle
- Pyruvate is converted to OAA in mitochondria.
- OAA is further converted to malate in presence of Mitochondrial malate dehydrogenase (malate shuttle)
- Now, this malate is transported to cytoplasm (malate transporter).
- Malate is converted to OAA by cytoplasmic malate dehydrogenase.
- Finally, OAA is converted to PEP by PEPCK (phosphoenolpyruvate carboxykinase).

Uses of Malate Shuttle 00:09:45

- To transport oxaloacetate from mitochondria to cytoplasm in gluconeogenesis.
- To transfer 2 molecules of NADH from cytoplasm to mitochondria (in aerobic glycolysis).
- Therefore, malate shuttle is required in both gluconeogenesis and glycolysis.
- But glycolysis is not always dependent on malate shuttle.

Explanation

- Glyceraldehyde 3 phosphate dehydrogenase (source for 2 NADH) converts G-3-P to 1, 3 BPG.
- Now, 2 NADH should enter the electron transport chain for converting into ATP.
- ETC is in the inner mitochondrial membrane.
- To achieve this malate shuttle is used.

Cytoplasmic Malate Dehydrogenase

This enzyme acts on 2 molecules of NADH and collects electrons from it.

↓

These electrons are transported to OAA. It accepts the electrons, and it further converts to malate.

↓

Finally, malate is entered into mitochondria along with the electrons.

Mitochondrial Malate Dehydrogenase (MMD)

MMD acts on malate and converts it into OAA.

↓

While conversation electrons are transported to coenzyme NAD

↓

This NAD is converted into NADH. Now, this NADH enters ETC

Q. In which pathway Malate shuttle is used?

A. Gluconeogenesis

B. Glycolysis

Ans. Glycolysis is not preferred because in case of anaerobic glycolysis NADH is not entered into ETC whereas in case of aerobic glycolysis: Malate shuttle and glycerol phosphate shuttle is used.

Regulation of Glycolysis and Gluconeogenesis 00:17:52

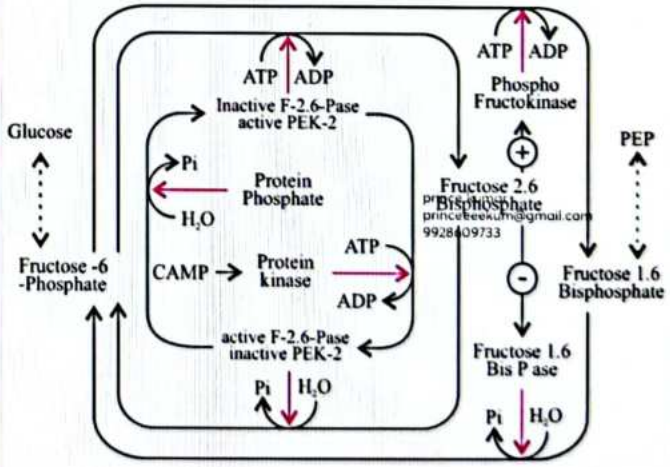
- Both are reversal to each other.
- Regulated in such a way that one pathway is activated and another one is inactivated (allosteric regulator).
- Otherwise, they end up in a futile cycle.
- **Example:** A person in starvation has used 11 high energy phosphates and has converted 2 pyruvate molecules to 1 fructose 6 phosphate.
- If PFK1 is not inhibited, then fructose-6-phosphate is converted to fructose 1,6 bisphosphate, then it enters into glycolysis.
- This process is called a futile or waste cycle. To avoid this, the two pathways have to be regulated in such a way that, when one pathway is active, the other pathway has to be inactive. Such a regulation is possible only in the presence of a common allosteric regulator. This common allosteric regulator should be a stimulator of one pathway and an inhibitor of the other pathway.

Facts for the Regulation of Glycolysis and Gluconeogenesis

- Common Allosteric regulator (fructose 2,6 bisphosphate).
- Fructose 2,6 bisphosphate is a stimulator of glycolysis (PFK-1).
- Fructose 2,6 bisphosphate is an inhibitor of gluconeogenesis (Fructose 1,6 bisphosphatase).
- Fructose 2,6 bisphosphate is the product of a tandem enzyme - PFK2.

Important Information

- A tandem enzyme is a bifunctional enzyme i.e., it is one protein with two exactly opposite enzymatic activities.
- One enzymatic activity synthesises a compound and the other activity breaksdown the same compound.
- One enzymatic activity is expressed when the protein is phosphorylated and the other activity is expressed when the protein is dephosphorylated.
- PFK-2 has both PFK-2 activity and fructose 2,6 bisphosphatase activity.
- Reversible conversion of fructose 6 phosphate to fructose 2,6 bisphosphate (PFK-2)
- Fructose 2,6 bisphosphatase activity is stimulated by phosphorylation.
- On dephosphorylation, it exhibits its PFK2 activity.



Important Information

- **Starvation:** Blood glucose is decreased and release of glucagon into the blood occurs.
  - Then glucagon acts on the GS receptor (S stands for the stimulation of adenylyl cyclase) produce cyclic AMP.
  - It activates protein kinase A that phosphorylates tandem enzymes.
  - This tandem enzyme acts as Fructose 2,6 bisphosphatase cleaves all the Fructose 2,6 bisphosphate.
  - Now, there is no fructose 2,6 bisphosphate to stimulate glycolysis (stops).
  - As there is no fructose 2,6 bisphosphate to inhibit gluconeogenesis. Therefore, gluconeogenesis is stimulated.

    Finally, plasma glucose levels are increased during starvation.

One Liners

00:30:44

- Gluconeogenesis occurs in **Liver and Kidney**
- The rate limiting enzyme of gluconeogenesis is **Fructose 1,6 bisphosphatase**
- Pyruvate carboxylase is present in **Mitochondria**
- Microsomal enzyme marker is **Glucose 6 phosphatase**
- The common allosteric regulator which regulates glycolysis and gluconeogenesis is **Fructose 2.6 bisphosphate**
- Fructose 2, 6 bisphosphate is a stimulator of **PFK-1 (Glycolysis)**
- Fructose 2,6 bisphosphate is an inhibitor of **Fructose 1,6 bisphosphatase (Gluconeogenesis)**
- PFK2 activity is stimulated by **Dephosphorylation.**

Multiple Choice Questions

00:32:36

Q. Fructose 1, 6 bisphosphatase is seen in:

A. Liver
B. Skeletal muscle
C. a & b
D. none

Q. Fructose 1, 6 bisphosphatase is seen in:

A. Liver
B. Skeletal muscle
C. a & b
D. None

Q. Malate shuttle is used in:

A. Glycolysis
B. Gluconeogenesis
C. Citric acid cycle
D. None of the above

Q. The tandem enzyme which regulates glycolysis and gluconeogenesis is.

A. PFK1
B. PFK2
C. Fructose 1,6 bisphosphatase
D. Pyruvate carboxylase

Q. In a well-fed state which of the following enzyme activities will be high?

A. Pyruvate carboxylase
B. PFK2
C. Fructose 1,6 bisphosphatase
D. Fructose 2,6 bisphosphatase

Integrated Case Based MCQs

Q. A 19 year old boy is influenced by Sylvester Stallone's "Rocky" body and consumes 6 raw eggs every day. On one of those days, he suddenly complained of giddiness, palpitation and sweating and was taken to the ER. His capillary plasma glucose was 50 mg/dL. Name the enzyme, low activity of which is the explanation for hypoglycemia in this condition. What would be expected to be elevated in this person's blood?

A. Acetyl CoA carboxylase, methyl malonyl CoA
B. Acyl CoA dehydrogenase, Propionyl CoA
C. Pyruvate Carboxylase, Methyl Malonyl CoA
D. Pyruvate carboxylase, Propionyl CoA

Explanation

- Person is suffering with hypoglycemia (50 mg/dL) (counter regulatory hormones released: glucagon, growth hormone, norepinephrine, cortisol)
- Raw egg has avidin that has more affinity for biotin (biotin malabsorption)
- Biotin is a coenzyme for carboxylases (pyruvate carboxylase)
- In biotin deficiency pyruvate carboxylase will be inactive and results in hypoglycemia.
- Other enzymes dependent on biotin: propionyl carboxylase. Accumulation of propionyl CoA in case of deficiency.

Q. A genetic mutation of Fructose 1,6 bisphosphatase enzyme gene has resulted in reduced responsiveness of the enzyme to Fructose 2, 6 bisphosphates. This results in which of the following conditions?

A. No change in glucose homeostasis
B. Hypoglycemia
C. Increased lactate
D. Hyperglycemia

Explanation

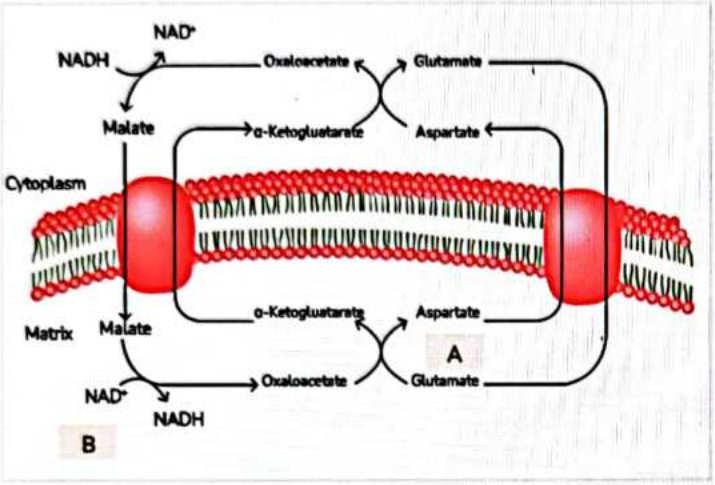
- Fructose 2, 6 bisphosphate is a stimulator of glycolysis and

inhibitor of gluconeogenesis.

- It usually Inhibits fructose 1,6 bisphosphatase.
- As it is mutated, there is no inhibition of fructose 1,6 bisphosphatase
- Hyperactivity of this enzyme leads to increased synthesis of glucose (gluconeogenesis)
- This leads to hyperglycemia.

Image based MCQs

Q. Identify enzyme A in the image.



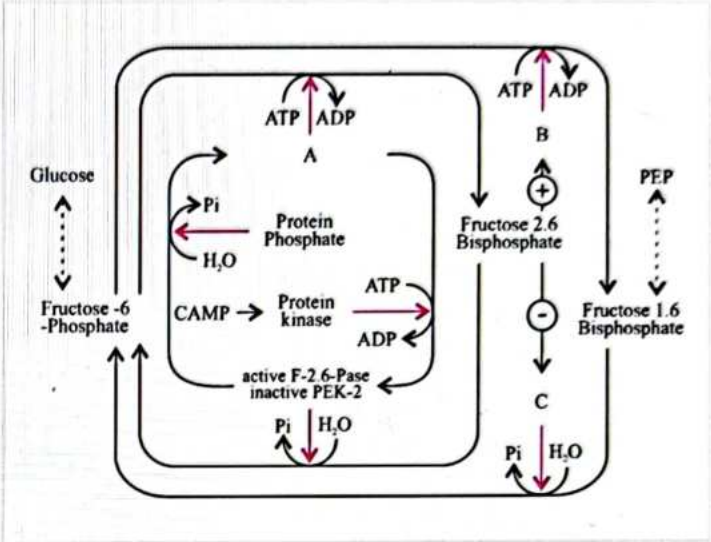
A. Serum Glutamate Pyruvate Transaminase
B. Serum Glutamate Oxaloacetate Transaminase
C. Alanine Transaminase
D. Malate Dehydrogenase

Explanation

- Transaminases: AST (SGOT), ALT (SGPT)
- Aspartate on transamination from oxalo acteate (reversible process)

- This enzyme acts on aspartate and oxaloacetate and allows it to react with keto acid (alpha-ketoglutarate). Then aspartate donate amino groups to alpha-ketoglutarate to form glutamate.
- Alanine reacts with alpha-ketoglutarate. Then alanine donates amino group to alpha-ketoglutarate to form glutamate (pyruvate).

Q. Identify enzymes A, B and C in the image.



A. Fructose 1, 6 bisphosphatase, PFK-1 and PFK2
B. PFK-1, PFK2 and Fructose 1, 6 bisphosphatase
C. PFK-2, PFK1 and Fructose 1, 6 bisphosphatase
D. PFK-2, PFK1 and Fructose 2, 6 bisphosphatase

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12 GLYCOGEN STORAGE DISORDERS

Disorder and Enzyme Defect Table

Sl. No	Type	Name	Enzyme Defect
1	Type 1	Von Gierke's Disease	Glucose-6-phosphatase
2	Type 2	Pompe's Disease	Acid maltase/ alpha glucosidase
3	Type 3	Cori's Disease	Debranching enzyme
4	Type 4	Anderson's Disease	Branching enzyme
5	Type 5	McArdle's Disease	Muscle Phosphorylase
6	Type 6	Hers Disease	Liver phosphorylase
7	Type 7	Tarui's Disease	Phosphofructokinase-1

Type-2 Pompe's Disease

- This is the only lysosomal storage disorder along with glycogen storage disorder.
- Acid maltase or alpha glucosidase defect.
- 2% glycogen is metabolized using the enzyme by lysosomes.
- Occurs in cardiac and skeletal muscle.
- Acid maltase or alpha glucosidase defect causes:
  - Cardiomegaly
  - Cardiomyopathy
  - Hypotonia/ floppy baby
  - Respiratory distress
  - Failure to thrive
- Enzyme replacement therapy is available.

Q. How to identify the floppy baby?

- Parachute reflex will be missing.
- The upper and lower limbs will be hanging down.
- Lack of tone in the neck when you pull the baby by holding their hands.

Categories of Glycogen Storage Disorders

There are 4 categories:

1. Those which present with only hypoglycemia
2. Those which present with only exercise intolerance
3. Those which present with both hypoglycemia and exercise intolerance
4. Those which present with neither hypoglycemia nor exercise intolerance

Glycogen Storage Disorders with Hypoglycemia

- **Includes**
  - Type 1
  - Type 3
  - Type 6
  - Fanconi Bickel syndrome

Points to remember:

- **Two pathways:** Gluconeogenesis and glycogenolysis are important to maintain plasma glucose concentration.
- Glucose-6-phosphatase is common to both pathways.
- G6P deficiency causes Type-1 glycogen storage disorder.
- Leading to severe hypoglycemia.
- Doesn't respond to counter regulatory hormones like:
  - Glucagon
  - Growth hormone
  - Nor-epinephrine
  - Cortisol
- **Muscle glycogenolysis:** Can't increase glucose levels - lacks G6P.
- **Liver glycogenolysis:** Can increase glucose levels - contains G6P.

Counter Regulatory Hormones

- Acts by increasing glycogenolysis pathway if gluconeogenesis doesn't work.
- Vice Versa.
- If both pathways are inhibited, CR hormones don't work.

S.no	Type	Name	Feature
1	Type 1	Von Gierke's Disease	• Hypoglycemia • Doesn't response to counter regulatory hormones
2	Type 3	Cori's Disease	• Hypoglycemia • Exercise intolerance
3	Type 6	Hers Disease	• Hypoglycemia (response to counter regulatory hormones)
4	Other	Fanconi Bickel syndrome (defect in GLUT 2)	• Hypoglycemia • Glycosuria • Polyuria • Polydipsia • Short stature

Fanconi Bickel Syndrome

- Result of GLUT2 transporter defect.
- **GLUT 2 present in:**
  - Enterocytes - deficiency causes Hypoglycemia.
  - Hepatocytes - Hypoglycemia
  - Pancreatic beta cells
  - PCT of kidneys – glycosuria
  - PCT failure leads to glycosuria, amino aciduria, bicarbonate Uria. Polyuria and polydipsia.

Diagnosis of Glycogen Storage Disorders with Hypoglycemia

- If a patient has glycosuria, polyuria, polydipsia and short stature: Fanconi Bickel syndrome.
- Hypoglycemia + Exercise intolerance: Cori's Disease.
- No other features
  - No response to CR hormones: Von Gierke's Disease.
  - Response to CR hormones: Hers Disease.

Von Gierke's Disease

- Defect of glucose 6 phosphatase.

Glucose-6-phosphatase not able to convert to glucose.
↓
G6P enters glycolysis.
↓
Converts to pyruvate and then Acetyl coA
↓
Leads to fatty acids and cholesterol synthesis.
↓
Excess levels stored as triglycerides and cholesterol esters. And excess triglycerides accumulate in adipose tissue leading to Doll like facies
↓
TAG accumulation causes hepatomegaly and renomegaly
↓
Cholesterol and cholesterol esters accumulation causes xanthoma.
↓
Sometimes acetyl coA accumulates - causing ketosis.
↓
Inhibition of acetyl coA inhibits PDH complex.
↓
Leads to accumulation of pyruvate.
↓
Pyruvate converts to lactate - causing lactic acidosis.
↓
Therefore, G6P Enters HMP shunt (source of R5P)
↓
Synthesis of purines and pyrimidines
↓
Excess purine is metabolized to uric acid - causing hyperuricemia

Doll Like Faces

- Clinical features of Von Gierke's Disease.
- Occurs due to accumulation of TG and CE in adipose tissues.

Clinical Presentations of Von Gierke's Disease

- Hypoglycemia
- Doll like faces
- Xanthomas
- Ketosis
- Lactate acidosis
- hyperuricemia

Glycogen Storage Disorders with Exercise Intolerance

- **Includes**
  - Type 3
  - Type 5
  - Type 7
- Glycogenolysis and anaerobic glycolysis is necessary for anaerobic exercise by muscle.
- Decrease in glycogenolysis and anaerobic glycolysis - exercise intolerance.

S.no	Type	Name	Feature
1	Type-3	Cori's Disease	• Hypoglycemia • Exercise intolerance
2	Type-5	Mc Ardle's Disease	Exercise intolerance (particularly to anaerobic exercise)
3	Type-7	Tarui's Disease	• Hemolytic anemia • Exercise intolerance

Diagnosis of Glycogen Storage Disorders with Exercise Intolerance

- If a patient has hypoglycemia: Cori's Disease.
- Hemolytic anemia: Tarui's Disease.
- No hypoglycemia and no hemolytic anemia: McArdle's Disease.

Category	Diseases
Hypoglycemia	Type-1, type-6, and Fanconi Bickel syndrome
Exercise intolerance	Type-5, type-7
Both	Cori's Disease
Neither hypoglycemia nor with exercise intolerance	Type-4 (Anderson's Disease)

Anderson's Disease

- Abnormal glycogen without branch points gets accumulated because of branching enzyme deficiency.
- Presents with hepatomegaly and then progress to liver cirrhosis.
- Accumulation of Glycogen in the liver without branches.

Both Hypoglycemia and exercise intolerance:

1. Cori's disease
2. Glycogen storage disorder Type 0 or Glycogen synthase defect

These two conditions can be differentiated by accumulation of amylopectin or alpha dextrin in Type 3 or Cori's and complete absence of glycogen in Type 0

One Liners

- The Glycogen storage disorder that presents with hypoglycemia and exercise intolerance is Cori's disease.
- The Glycogen storage disorder that presents with neither hypoglycemia nor exercise intolerance is Anderson's disease.
- The glycogen storage disorder that is also a lysosomal storage disorder is Pompe's disease.

MCQs

Q. The glycogen storage disorder which presents with hypoglycemia, which does not respond to CR hormone administration is?

A. Cori's Disease
B. Fanconi Bickel's syndrome
C. Mc Ardle's disease
D. Von Gierke's disease

Q. The glycogen storage disorder caused by the defect of muscle phosphorylase is?

A. Pompe's disease
B. Mc Ardle's disease
C. Hers disease
D. Tarui's disease

Q. The glycogen storage disorder which does not present with hypoglycemia is?

A. Cori's Disease
B. Mc Ardle's disease
C. Hers disease
D. Von Gierke's disease

Q. The glycogen storage disorder which does not present with hypoglycemia nor exercise intolerance is?

A. Cori's Disease
B. Anderson's disease
C. Mc Ardle's disease
D. Von Gierke's disease

Case Based MCQs

Q. A 8 month old infant presents with hypoglycemia. On examination, hepatomegaly was observed. Blood investigations revealed lactic acidosis. Ketosis and Xanthomas on the buttocks. What is the most probable enzyme deficiency?

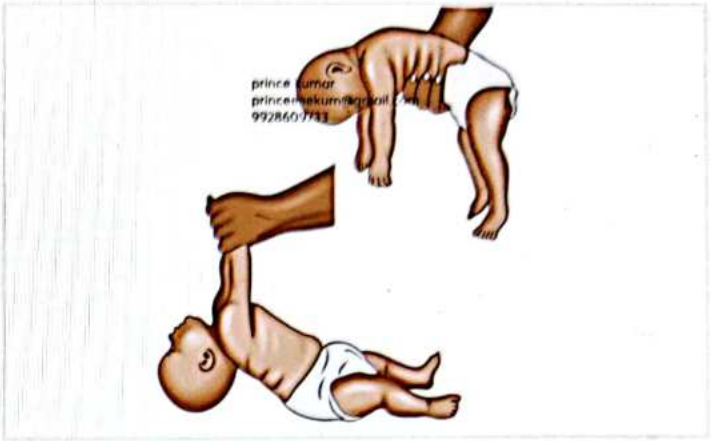
A. Branching Enzyme
B. Glycogen synthase
C. Acid maltase
D. Glucose-6-phosphatase

Q. A person presents with intolerance to anaerobic exercises and he says he experiences pain just following 5 minutes of anaerobic exercises. His plasma glucose is normal. LDH is normal. The most probable cause is?

A. Cori's Disease
B. Mc Ardle's disease
C. Hers disease
D. Tarui's disease

Image Based MCQs

Q. Which of the following glycogen storage disorders can cause the condition provided in the image?



A. Cori's Disease
B. Mc Ardle's disease
C. Tarui's disease
D. Pompe's disease

13 FRUCTOSE AND GALACTOSE METABOLISM

Normal Galactose Metabolism

00:01:20

- Glucose and galactose are hexoses.
- Glucose and galactose are epimers which means that the initial few steps of galactose metabolism are the same as that of the glucose.

1st STEP OF GLUCOSE METABOLISM:

- As soon as glucose enters any cell, irrespective of the final fate of glucose, hexokinase or glucokinase will act upon glucose and convert it into glucose-6-phosphate.
- This step aims to trap glucose in the cell; otherwise, the galactose will be effluxed back to the circulation through the same transporter through which it enters a cell.
- Analogous to that, galactokinase is the first step in galactose metabolism. Galactokinase converts galactose into galactose 1 phosphate.
- After galactose-1-phosphate is Synthesised, galactose is ready to get into many anabolic pathways because there are few structures in our body which need galactose for getting synthesised. Example: keratan sulphate, glycolipid and myelin
- For any fuel to enter into anabolic pathways, it should be activated first.
- UDP glucose is the active form of glucose, and the active form of galactose is UDP galactose.
- Galput (Galactose-1-phosphate Uridylyltransferase) is the enzyme that will convert galactose-1-phosphate into UDP galactose.
- Then this UDP galactose is used for keratan sulfate, myelin, and glycolipid synthesis.
- When keratan sulfate, myelin, and glycolipid need not to be synthesized then this UDP galactose is converted into UDP glucose by epimerase enzyme.
- This epimerase enzyme also converts UDP galactose to UDP glucose.
- If both epimerase and Galput are be defective then UDP galactose can not be synthesised and keratan sulfate, myelin, and ganglioside will also be not synthesized. This will cause the nervous system to be involved and mental retardation and hypotonia will occur.
- UDP glucose either enters glycogen synthesis or it enters the uronic acid pathway.

Galactose metabolism disorders:

1. Galactokinase deficiency

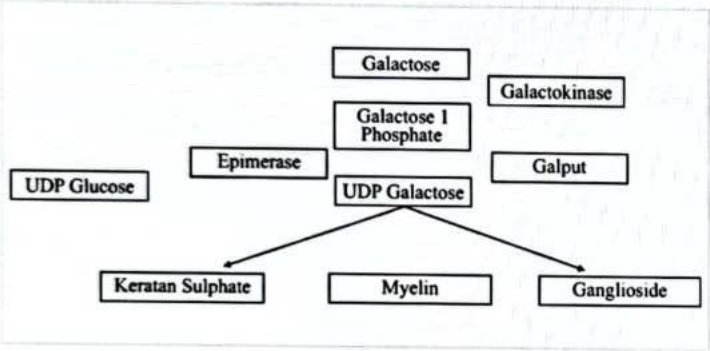
- When galactokinase is defective then galactose will not be trapped inside any cell and when this galactose enters the cell, it will be effluxed back into the circulation. In this condition the galactose level in the blood gets high and it is called galactosemia.
- Whenever something is high in blood it gets reflected in urine because anything in blood other than plasma proteins get filtered along the glomeruli.
- Galactose is reducing sugar so the urine will start answering Benedict's test.
- The excess galactose in circulation gets into lens fibers. Lens has got an enzyme named aldose reductase.
- Aldose reductase acts upon galactose and converts this aldehyde into alcohol. This alcohol obtained from galactose is known as galactitol or dulcitol.
- Once it becomes dulcitol it cannot come out of lens fiber and get trapped in the lens.
- Galactitol or dulcitol are osmotically active so that they attract water, which causes swelling in lens fiber and that will begin cataracts changes.

Classical galactosemia or galput deficiency:

- When Galput (Galactose-1-phosphate Uridyl transferase) is defective then in this condition, initially galactose-1-phosphate accumulates but this galactose-1-phosphate in feedback mechanism inhibits galactokinase.
- So, galactose accumulates in the cell that gets reflected back into the circulation. Even in this condition, the galactose level in the blood gets high. And galactosemia occurs in this too but this time this is classical galactosemia. It will also start answering Benedict's test.
- Excess galactose gets into lens fiber, aldose reductase converts galactose to galactitol and dulcitol and that causes cataract changes.
- Additionally in this condition, Galactose-1-phosphate accumulates, here galactose getting trapped into the cell is not something to be worried about but phosphate getting trapped in the cell is. Because Galput is active, this UDP galactose will enter into the metabolic pathway and the phosphate gets recycled back and forth. Because the enzyme Galput is defective, all galactoses will be trapped as galactose-1-phosphate. It will result in low phosphate level, or it causes hypophosphatemia.
- Hypophosphatemia inactivates glycogen phosphorylase (Phosphorylase needs inorganic phosphate to be active)and this causes hypoglycemia.
- So, when this hypophosphatemia occurs glycogen phosphorylase becomes inactive glycogenolysis will be impaired and that causes hypoglycemia.
- ADP +pi gives rise to ATP. Now there is hypophosphatemia, ADP can not be converted into ATP. So, all cells concerned

with galactose metabolism will suffer from ATP deficiency.

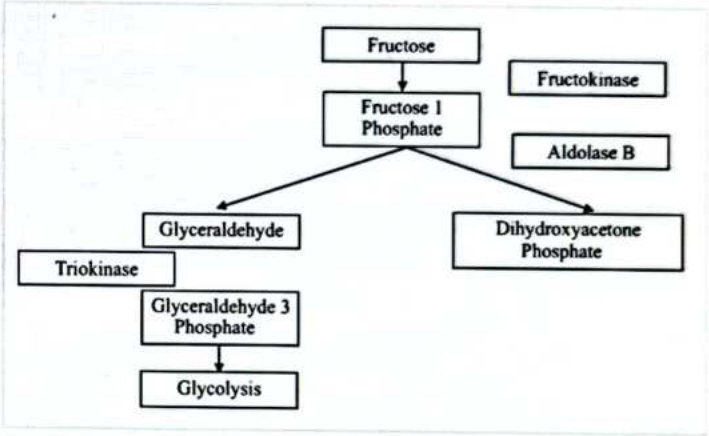
- Galactose metabolism happens in hepatocytes and in proximal convoluted tubules.
- So, hepatocytes will also suffer from ATP deficiency and will cause jaundice and hepatomegaly.
- Proximal convoluted tubules when suffering from ATP deficiency will cause Fanconi syndrome. Proximal convoluted tubules reabsorb the 60 to 70% of solutes and for this proximal convoluted tubule is dependent on secondary active transport such as sodium glucose cotransporter, sodium amino acid cotransporter. All these secondary active transport mechanisms need ATP. In classical galactosemia because there is deficiency of ATP, PCT does not have ATP so it cannot reabsorb any of this solute and will result in glucosuria, phosphaturia, bicarbonaturia, polyuria polydipsia collectively known as fanconi syndrome.
- Now this unwanted ADP will be catabolized to form uric acid, and this will cause hyperuricemia.
- Classical galactosemia neonate is susceptible to E. coli neonatal sepsis and female children of classical galactosemia will suffer from premature ovarian failure (menopause at early age)



Normal fructose metabolism

00:20:32

- Fructokinase will act upon fructose and convert it into fructose-1-phosphate.
- The purpose of this step is to trap fructose into the cell otherwise the fructose will reflect back to the circulation through the same transporter through which it enters into a cell.
- This fructose-1-phosphate will immediately be acted upon by aldolase B to form 2 triose, dihydroxyacetone phosphate, and glyceraldehyde. Only dihydroxyacetone phosphate has phosphate attached to it.
- This glyceraldehyde will further be acted upon by glyceraldehyde kinase or triokinase. That will convert it into glyceraldehyde-3-phosphate.
- Now glyceraldehyde-3-phosphate and dihydroxyacetone phosphate go through usual glycolytic steps. Every fructose gives 2 pyruvates, and 2 pyruvates gives 2 acetyls CoA.
- When fructose goes to glycolysis, it skips the rate limiting enzyme phosphofructokinase
  - Fructose has skipped the rate limiting enzyme completely which means fructose glycolysis is unregulated.
- For example, suppose that you are taking 100 molecules of glucose, the initial 50 molecules of glucose will go through glycolysis, and you will get 100 pyruvate. 100 pyruvate will give 100 acetyl CoA. Once you have enough products, all these products in a feedback mechanism will inhibit glycolysis. At one point glucose stops getting into glycolysis and glucose gets into alternate pathways. It starts to get into glycogen synthesis or it gets into mutations.
- But if you consume 100 molecules of fructose, all 100 molecules will go through glycolysis because it is not regulated. All 100 molecules will give 200 pyruvates and 200 pyruvates will give 200 acetyls CoA. Acetyl CoA is the building block of fatty acids and cholesterol. So, all 200 molecules of acetyl CoA will enter into fatty acids synthesis and cholesterol synthesis. So, the person will end up getting hyperlipidemia and hypercholesterolemia. Fructose based diet is more lipogenic and dangerous than glucose-based diet.



Fructose metabolism disorders:

1. Fructokinase deficiency

- When fructokinase is defective, fructose cannot be trapped in any cell. And fructose level in blood gets high and it will be reflected in urine. Fructose is a reducing sugar, so urine answers Benedict's test. This condition is called essential fructosuria because there is no clinical feature other than urine answering Benedict's test.
- Other than this there are no such abnormalities found in this. When fructose accumulates in the blood and gets into the lens, it doesn't get trapped into this because aldolase reductase cannot act upon fructose. Because fructose is a ketose. That is why fructose metabolism disorder is not that commonly present in cataracts.
- But sometimes, sorbitol dehydrogenase can act on fructose and can convert it into sorbitol. The affinity of this enzyme for fructose is very low such that it does not occur unless the person consumes excess fructose.

- When aldolase B is defective, this condition is called hereditary fructose intolerance. In this fructose will be trapped inside the cell in the form of fructose-1-phosphate then fructose-1-phosphate accumulates in a feedback mechanism that inhibits glucokinase. So, fructose accumulates in the cell and reflects back in the circulation. So, fructose level in blood gets high and it will be reflected in urine and urine will answer benedict's test.
- Here fructose getting trapped into the cell is not something to be worried about but phosphate getting trapped in the cell is. It will result in low phosphate level, or it causes hypophosphatemia.
- When hypophosphatemia occurs glycogen phosphorylase activity will decrease, and glycogenolysis is impaired and causes hypoglycemia.
- At low phosphate level ADP cannot be converted into ATP and tissues which metabolise fructose suffer from ATP deficiency. Fructose metabolism happens to a greater extent in liver.
- Liver suffers from ATP deficiency. This causes jaundice and hepatomegaly
- Unwanted ADP will be catabolized to form uric acid, and this will cause hyperuricemia that is about hereditary fructose intolerance.
- The classical clinical picture of fructose intolerance is that a child will be developing normally until the child's mother fed. Once there is a transition of mother's milk to infant food, all these features will start appearing. Because mother's milk has lactose and infant food is known to be rich in sucrose. Sucrose gets digested to give glucose and fructose. When fructose gets into the system then all these features start appearing. These features are hypoglycemic episodes, jaundice, hepatomegaly, hyperuricemia, lactic acidosis. If all these are found after the transition of mother's milk to infant food, then hereditary fructose intolerance will be suspected. If all these features are found since birth with cataractous changes, it is probably classical galactosemia.
- The common features between hereditary fructose intolerance and classical galactosemia are hypoglycemia, urine will give benedict's test, hypophosphatemia, jaundice, hepatomegaly, hyperuricemia, and lactic acidosis.

Differences between classical galactosemia and fructose intolerance:

1. Galactosemia features appear since birth, fructose intolerance presents after transition from mother's milk to infant food
2. Galactosemia presents with cataractous changes, fructose metabolism disorders do not that commonly present with cataract
3. E.coli neonatal sepsis is common in classical galactosemia

One liners

1. Classical Galactosemia is caused by the defect of Galactose 1 Phosphate Uridyl Transferase
2. Essential Fructosuria is caused by the defect of Fructokinase.
3. Hereditary Fructose Intolerance is caused by the defect of Aldolase B

MCQ's

Q. All the following are features of classical Galactosemia except

A. Hypoglycemia
B. Low lactate
C. High Uric acid
D. E. coli sepsis

Q. All the following are features of hereditary fructose intolerance except

A. Diarrhea
B. Hypoglycemia
C. Urine answers benedict' test
D. Lactic acidosis

Q. Which of the following is a feature of Fructosuria

A. Diarrhea
B. Hypoglycemia
C. Urine answers benedict's test
D. Lactic acidosis

Integrated Case Based MCQS

Q. A neonate presents with fever, jaundice, diarrhea, hypotension, and features of sepsis. History gives multiple episodes of admissions before for loss of consciousness, which were later diagnosed to be due to hypoglycemia. Blood investigations reveal hypoglycemia, hypophosphatemia, and lactic acidosis. Urine answers Benedict's test. The most probable enzyme defect is

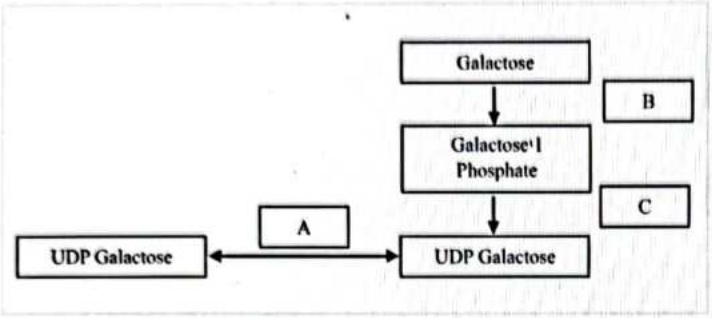
A. Galactokinase
B. Galactose 1 phosphate uridyl transferase
C. Epimerase
D. Glucose 6 phosphatase

Q. A young child was developing normally until he began to make the transition from breastfeeding to infant foods. During this time, he had frequent but intermittent bouts of vomiting and intestinal distress, and slept poorly at night. He was brought to the doctors office one afternoon about an hour following a severe reaction to his food. His blood sugar was 40 mg/dl, blood lactate was 4mM (normal is less than 1mM) and serum phosphate was 2 mg/dl (normal is 4-7). There was

mild evidence of jaundice and hepatomegaly. The probable diagnosis is

A. Galactosemia
B. Fructose intolerance
C. Lactose intolerance
D. Essential fructosuria

Q. Identify enzyme A?



A. Galactokinase
B. Galactose 1 Phosphate Uridyl Transferase
C. Epimerase
D. Aldose reductase

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14 HMP SHUNT

Facts about HMP Shunt (PPP)

- It is a pathway where ATP is neither generated nor utilized.
- Acts as a source of NADPH.
- It acts as a source of Ribose 5 Phosphate. Ribose 5 Phosphate is like a foundation on which every nucleotide is built. (Ribose 5 Phosphate is necessary for purine and pyrimidine nucleotide synthesis.

Two phases of hmp shunt:
1. Oxidative phase
2. Non oxidative phase

Oxidative phase/ irreversibe phase: Involves Glucose 6 phosphate dehydrogenase and it acts as a source of NADPH

Nonoxidative phase/ reversible phase:
Acts as a source of Ribose 5 Phosphate

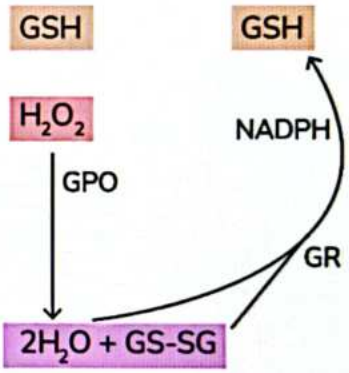
Interesting about hmp shunt in muscle:

- Muscle Lacks Glucose 6 Phosphate Dehydrogenase. Hence the oxidative phase is not operative in muscle. Skeletal muscle has the gene (Glucose 6 Phosphate Dehydrogenase), but it's not active in skeletal muscle. And skeletal muscle does not require much NADPH. So, no oxidative phase happens in Skeletal muscles. But skeletal muscle requires Ribose 5 Phosphate. As the result of the reversible phase, 2½ Glucose 6 Phosphate molecules, it is reversed (as it is a reversible reaction), and Ribose 5 Phosphate is generated.
- Skeletal muscle Lacks Glucose 6 Phosphate Dehydrogenase, so the significance of the HMP Shunt in muscle is that it generates only Ribose 5 Phosphate in skeletal muscle.

NADPH and NADH

- NADH can enter the Electron Transport chain, ETC (ETC=2.5ATP), while NADPH Cannot enter ETC & Cannot act as a source of ATP.
- NADH source is Glycolysis, Citric acid cycle, and Fatty Acid oxidation.
- All the paths which generate ATP, most of them will have NADH.
- NADPH Is necessary as a coenzyme for the reductive biosynthesis of all lipids.
  - Lipids are more reduced than carbohydrates, and lipids give more energy than carbohydrates. (1g lipid = 9 calories while 1g of carbohydrates= 4 calories). The reason that lipids give more energy is that they are reduced. Anything that is reduced will give more energy. If lipids are reduced, it means synthesizing lipids; there will be multiple reduction steps. And for all steps hydrogen source is needed. And NADPH will act as a hydrogen source.
  - In Fatty acid synthesis, Cholesterol synthesis, Bile acid synthesis, etc. It is seen that NADPH is used as a coenzyme.
- NADPH is necessary for regenerating GSH (an effective antioxidant in RBCs). Moreover, RBCs act as a major source of Oxidative stress in the body. RBCs also carry oxygen in which there is a proficiency that oxygen is converted to superoxide radical, which initiates Oxidative stress. The major source of Oxidative stress in the body is RBCs. One of the antioxidant mechanisms which RBCs use is Glutathione (GSH).
- NADPH is necessary as a coenzyme for Ribonucleotide Reductase. RNAs get converted to DNAs in the presence of Ribonucleotide Reductase, and for that hydrogen, a source is needed NADPH. (RNAs – DNAs).

Q. How GSH (Glutathione) acts as an antioxidant?



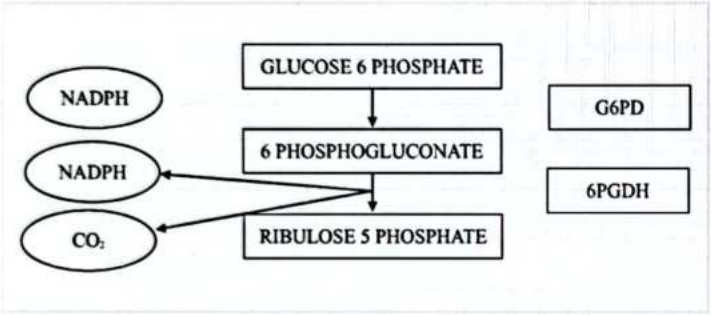
- Suppose Hydrogen Peroxide(H_2O_2) is generated in RBCs. To detoxify these H_2O_2 (Hydrogen Peroxide)
  - 2 molecules of GSH react (Glutathione). And both give 1 Hydrogen to H_2O_2 (Hydrogen Peroxide) and convert it into H_2O_2 or $2H_2O$ (2 molecules of water). Therefore, Hydrogen Peroxide is detoxified into Nontoxic water with the help of Glutathione.
  - GSH (Glutathione) Peroxidase (GPO) is the catalyst.
- Now, it's needed to regenerate Glutathione back. Only then will this glutathione be ready to detoxify another Hydrogen Peroxide.

- For regenerating Glutathione, Glutathione needs to be reduced. By giving Hydrogen, two molecules of GSH (Glutathione) become GS-SG.
- Now, they need to be reconverted into GSH (Glutathione) by reducing them.
- For reducing Glutathione Hydrogen source is needed, which is NADPH.
- The enzyme is GR (Glutathione Reductase).
- NADPH is necessary for regenerating Glutathione in RBCs, an effective antioxidant mechanism.

Steps of HMP Shunt: Two Phases

Oxidative/Irreversible Phase: Acts as a source NADPH

- This is initiated by Glucose 6 Phosphate Dehydrogenase (G6PD), which acts as Glucose 6 Phosphate. This is the first enzyme in HMP Shunt. G6PD removes hydrogen from Glucose 6 Phosphate and gives that hydrogen to its Coenzyme NADP, forming NADPH. And the Glucose 6 Phosphate becomes 6 Phosphogluconate. The next enzyme is 6 PGDH (Phosphate Gluconate Dehydrogenase).



- **Examples of enzymes that catalyze oxidative decarboxylation steps:**
  - PDH (Pyruvate Dehydrogenase) converts pyruvate to Acetyl coA that catalyzes oxidative Decarboxylation steps.
  - Alpha KGDH (Alpha-Ketoglutarate Dehydrogenase)
  - ICDH (Isocitrate Dehydrogenase)
  - 6PGDH (6 Phosphogluconate Dehydrogenase)
  - BCKADH of Branched chain Amino Acid Metabolism- The defect of the enzyme which causes MSUD (maple syrup urine disease).
- Now 6 Phosphogluconate Dehydrogenase catalyzes an oxidative decarboxylation step. So, hydrogen is removed from 6 Phosphogluconate and again given to NADP, forming NADPH. So, for every glucose 6 Phosphate that gets into HMP Shunt, 2 NADPH is formed. When it decarboxylates, CO^2 is removed. From a Hexose, because one carbon atom is removed, the product has to be a Pentose, and that Pentose is Ribulose 5 Phosphate. With this, the first phase is over. Both the reactions of this phase are irreversible.

Non-Oxidative/ Reversible Phase: Acts as a source of Ribose 5 Phosphate

- The Ribulose 5 Phosphate formed in the first phase needs to be converted to Ribose 5 Phosphate. The difference between Ribulose and Ribose is that Ribulose is a Ketose while Ribose is an Aldose. The next enzyme is Keto Isomerase which converts Ribulose 5 Phosphate to Ribose 5 Phosphate.
- Then Ribose 5 Phosphate will be used for purine and pyrimidine nucleotide synthesis. Not always Purine and Pyrimidine nucleotide synthesis. So excess or multiple Ribose 5 Phosphate would accumulate when there is no active Purine and Pyrimidine Nucleotide synthesis.
- So, these Ribose 5 Phosphate molecules will react with each other in the presence of Transketolase and Trans aldolase (they go through a series of Transketolase and Trans aldolase chemical reactions). And they try to regenerate all the Glucose 6 Phosphate back. But they won't be able to regenerate all the Glucose 6 Phosphate back as there is the absence of carbon. Simultaneously 3 Glucose and 6 Phosphate molecules react.
- When 3 Glucose 6 Phosphate molecules react, at starting, there would be 18 carbon atoms, and every Glucose 6 Phosphate will lose one carbon as Carbon Dioxide. So, from there, 3 Glucose and 6 Phosphate, three carbon atoms lose. Then at the end, 15 Carbon atoms are left, which is 15C = 2½ Glucose 6 Phosphate. But half is not possible; it means whole. Therefore, the trio we get is G3P (Glyceraldehyde 3 Phosphate).
- **Transketolase**- This enzyme needs Thiamine as its coenzyme. If there is a Thiamine deficiency, it means RBC Transketolase activity is low.
- Simultaneously in HMP Shunt $3G6P \rightarrow 3CO2 + 2G6P + G3P + 6\,NADPH + 6H+$

Source of NADPH

1. HMP Shunt (The major source)
2. Cytoplasmic Isocitrate Dehydrogenase
3. Malic Enzyme (converts Malate to Pyruvate)

HMP Shunt Defects

Glucose 6 Phosphate Dehydrogenase Deficiency

- NADPH will not be generated when it has a defect. If NADPH is not generated, then GSH cannot be regenerated. So, without GSH, the antioxidant mechanism is missing in RBCs. RBCs become susceptible to lysis following exposure to oxidative stress.

Hemolytic Anemia following exposure to oxidative stress

- In the absence of GSH, the antioxidant mechanism is missing in RBCs. RBCs become susceptible to lysis following exposure to oxidative stress. The history would be classical. An 11-year-old boy presented with hemolytic anemia after

intake of primaquine (a prophylactic drug for malaria). Primaquine induces oxidative stress. So, until the person is very normal as per exposure to Oxidative stress because RBCs cannot regenerate Glutathione quickly, they become susceptible to Lysis following exposure to Oxidative stress (for example: after intake of Primaquine or intake of Fava Bean).

- Even normally, there is oxidative stress. So, there is a need for GSH to be regenerated in RBCs even normally, but that is met by two minor sources of NADPH: Cytoplasmic Isocitrate Dehydrogenase and Malic Enzyme will help in the regeneration of glutathione usually. Still, they have slowed up the pace, and when they get exposed to oxidative stress, there is a rapid rate at which hydrogen peroxide gets generated.
- So, there should be a rapid pace at which Glutathione should be regenerated, and for it, a major NADPH source is needed, which is missing. So, they present with Hemolytic anemia following exposure to Oxidative stress.
- It is X-LINKED RECESSIVE DISORDER.

X Linked recessive

5 defects which are X linked

- Carbohydrate metabolism defect is G6PD deficiency.
- Lipid metabolism defect is Fabry's disease caused by Alpha Galactosidase that is x linked recessively inherited.
- Amino acid metabolism defect is Type 2 Hyperammonemia by OTC
- Puri Salvage metabolism is HGPRTase defect, which is an enzyme of Purine Salvage pathway, and the Defect of this enzyme causes Lesch Nyhan Syndrome.
- MPS (Mucopolysaccharidosis) is Hunter's, caused by defects of Iduronate sulfatase.

One Liners

A. Products of HMP Shunt are NADPH **and Ribose 5 Phosphate**

B. The coenzyme required for Transketolase is **Thiamine Pyrophosphate**

C. Thiamine Deficiency is diagnosed by estimating **RBC Transketolase activity**.

D. The significance of the HMP Shunt in skeletal muscle is the synthesis of **Ribose 5 Phosphate formation**.

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MCQ

Q. The number of NADPH synthesized, CO2 liberated, and Glucose 6 Phosphate regenerated from 3 Glucose 6 Phosphate are

A. 3,3,3
B. 6,3,3
C. 6,3,2.5
D. 6,0,2.5

Q. The main function of the HMP Shunt in skeletal muscle is

A. Synthesis of NADPH
B. Synthesis of Ribose 5 Phosphate
C. Both
D. None

Q. Thiamine acts as a cofactor for all except

A. PDH
B. Isocitrate Dehydrogenase
C. Transketolase
D. Alpha ketoglutarate Dehydrogenase

Q. Which of the following increases 2,3 BPG in stored blood:

A. IMP
B. hypoxanthine
C. Phosphate
D. Citrate

Q. Why should you provide a source of 2,3 BPG in stored blood?

- It is because stored blood in a bag does not have a continuous supply of glucose. And this would result in less glycolysis rate.
- When the glycolysis rate is less, 2,3 BPG production is less, which is a by-product of glycolysis. When there is no 2,3 BPG, the affinity of hemoglobin for oxygen is high. RBCs' affinity for oxygen will be high, and when affinity for oxygen is high, these RBCs will unload oxygen to tissues. And tissues would not get oxygen (suffer from Hypoxia). So, the entire purpose of blood transfusion will not be served if there is no source of 2,3 BPG. That is why in all the stored blood, there should be a source of 2,3 BPG, and that is inosine Monophosphate (IMP).
- Inosine Monophosphate is Hypoxanthine + Ribose 5 Phosphate. So, if you add IMP to stored blood, it enters into RBCs, and within RBCs, IMP will be split into Hypoxanthine and Ribose 5 Phosphate. Then Ribose 5 Phosphate gets into HMP Shunt.
- As Ribose 5 Phosphate is an intermediate of HMP Shunt. And as a result of HMP Shunt, we get 2½ Glucose 6 Phosphate, which is Glyceraldehyde 3 Phosphate. This G3P then becomes 1,3 BPG in the presence of Glyceraldehyde 3

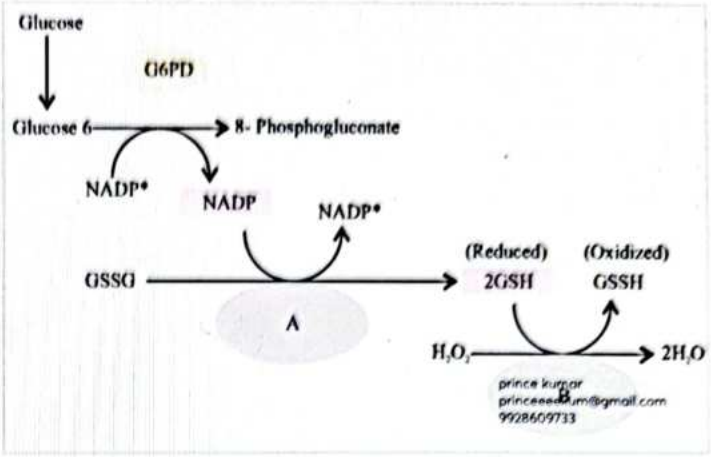
Phosphate Dehydrogenase. Then 1,3 BPG becomes 2,3 BPG which helps in unloading oxygen to tissues.

Q. Just before a planned departure to the tropics, a patient visits his physician complaining of weakness and noticing that his urine has recently become dark. Physical examination revealed a slightly jaundiced yellow-colored sclera. His MpMf was negative. His CKMM level was normal; he says this is the second visit, and he had some prophylactic measures during the previous visit. The probable enzyme Defect is.

A. Pyruvate kinase
B. Phosphofructokinase 1
C. Glucose 6 Phosphatase
D. Glucose 6 Phosphate Dehydrogenase

Image-based MCQ

Q. Enzyme A and Enzyme B, respectively, are



A. Glutathione Peroxidase Glutathione Reductase
B. Glutathione Reductase and Glutathione Peroxidase
C. Glucose 6 Phosphate Dehydrogenase and 6 Phosphogluconate Dehydrogenase
D. 6 Phosphogluconate Dehydrogenase Glucose 6 Phosphate Dehydrogenase

15 ELECTRON TRANSPORT CHAIN

Location

- All complexes of ETC are present along the Inner side of the inner mitochondrial membrane.

Tool of Oxidative Phosphorylation

- It is a tool of Oxidative Phosphorylation, that is a type of catabolism.
- In catabolism, the breakdown of biomolecules into simpler substances occurs by breaking covalent linkages. Whenever covalent linkages get broken, energy gets liberated.
- In the case of oxidative phosphorylation, the liberated energy does not get trapped directly in the form of ATP. Rather it is trapped in the form of reducing equivalents like NADH or FADH 2.
- When NADH and FADH 2 oxidized, they lose electrons, and these electrons then get collected and transported along complexes of the electron transport chain. Electron transport chains in a complex way liberates energy. Then this energy is used for phosphorylating ADP to form ATP.
- Hence, electron transport chain acts as a tool for oxidative phosphorylation.
- The fact is that for something to get into the electron transport chain it must be either NADH or FADH 2 and not NADPH. Because NADPH cannot give rise to energy.
- Glycolysis happens in cytoplasm and in glycolysis, for every molecule that gets into this pathway, 2 NADH get generated.
  - Those 2 NADH will have to be transported from cytoplasm to mitochondria. NADH cannot cross the mitochondrial membrane so a shuttle is going to be used. There will be two shuttles- Malate shuttle and glycerol phosphate shuttle.

Malate shuttle

- In malate shuttle NADH is transported in the form of malate. For this purpose, two enzymes will be used- cytoplasmic malate dehydrogenase and mitochondrial malate dehydrogenase.
- Cytoplasmic malate dehydrogenase acts upon NADH, gets electrons from it, and uses that electron to reduce its substrates.
- The substrate is oxaloacetate, it accepts electrons and converts them to malate. Electrons get hidden in the form of malate.
- Now the mitochondrial membrane will transport malate from cytoplasm to mitochondria.
- Once malate reaches the mitochondria, mitochondrial dehydrogenase acts upon malate and converted it into oxaloacetate. During this conversion of malate to oxaloacetate, electrons are taken out again and that electron is given to its coenzyme. Its coenzyme is NAD and after getting this it will become NADH.
- This malate shuttle is present all the cells, but 2 exceptions are there. They are white muscle fiber and neurons. They both have glycerol phosphate shuttle.

Glycerol Phosphate Shuttle

- In glycerol phosphate shuttle NADH is transported in the form of glycerol. For this purpose, two enzymes will be used- cytoplasmic glycerol-3-phosphate dehydrogenase and mitochondrial glycerol-3-phosphate dehydrogenase.
- Glycerol-3-phosphate dehydrogenase acts upon NADH. It will get electrons from it and give these electrons to its substrates.
- Here the substrate is dihydroxyacetone phosphate which accepts the electron and converts it to glycerol-3-phosphate. Now the electron is hidden in the form of glycerol-3-phosphate.
- Carrying electron glycerol-3-phosphate will enter mitochondria. Mitochondrial glycerol-3-phosphate dehydrogenase converts this into dihydroxyacetone phosphate. During this conversion electrons are taken out again and that electron is given to its coenzyme. Its coenzyme is FAD and after getting this it will become FADH2.
- This FADH2 will enter the electron transport chain.
- The major difference between malate shuttle and glycerol phosphate shuttle is that in malate shuttle, cytoplasmic NADH will become mitochondrial NADH and in glycerol phosphate shuttle cytoplasmic NADH will become mitochondrial FADH2.
- So, there will be loss of ATP because NADH would give 2.5 ATPs and if it is going to become FADH2 it will give only 1.5 ATPs. There is a difference of 1 ATP for every NADH that is getting transported this way.
- In glycolysis, from every molecule of glucose, 2 NADH is obtained. To transport both these NADH, two ATPs will be missing.

Differences between malate shuttle and glycerol phosphate shuttle

Sr no.	Property	Malate shuttle	Glycerol phosphate shuttle
1	Location	All cells	White muscle fiber and neurons
2	Energy	No loss of energy	Loss of 2 ATPs/ glucose

- **Example:** Aerobic glycolysis gives 7 ATP but if cell uses glycerol phosphate shuttle it will give 5 ATPs, Complete oxidation of 1 molecule gives 32 ATPs and if the cell uses glycerol phosphate shuttle it will give 30 ATPs, irrespective of the shuttles anaerobic glycolysis always gives 2 ATPs.

Complexes of electron transport chain

- All complexes of the electron transport chain are made up of cytochrome heme proteins.
- Heme proteins have heme in the center and are surrounded by protein or polypeptide genes.
- Every heme will have metal in its center surrounded by 4 pyrrole rings. This central metal can be the ion found in hemoglobin or myoglobin or it can be magnesium found in chlorophyll.
- Non-cytochrome heme proteins: the central atom cannot shift between their states of oxidation. For example: hemoglobin and myoglobin. In hemoglobin, for transformation of oxygen the central atom must be Fe+2. When this Fe+2 becomes Fe+3 it becomes methemoglobin. Hemoglobin is a non-cytochrome heme protein.
- When the metal in the center can shift between its various oxidation states, it will become cytochrome heme proteins.
- If the metal in the center can shift between its various oxidation states, then only it can help in electron transport.
- For example, complex 1 is the cytochrome heme protein with the central atom Fe+2. But this atom can shift to Fe+3 and then come back to Fe+2.
- When complex 1 accepts electrons from NADH, Fe3+ will become Fe2+. When this complex donates its electron to another complex, it will be back to its Fe3+ form.
- All complexes have ions in the center, only one exception is there, that is complex 4. Complex 4 is cytochrome a and a3 and has copper in the center.
- There is one complex that does not have cytochrome heme proteins is the mobile complex Q.

There are 5 stationary complexes and 2 mobile complexes

- 5 stationary complexes are named in Roman letters:

Complex I

- It is NADH linked dehydrogenase.
- Electrons from NADH will enter into electron transport chain through complex I

Complex II

- Long back we used to call complex II as FADH2 linked dehydrogenase and electrons from FADH2 will enter into the electron transport chain through complex II.
- Now we know that complex II is a succinate dehydrogenase.

Complex III

- Complex III is cytochrome b and c1

Complex IV

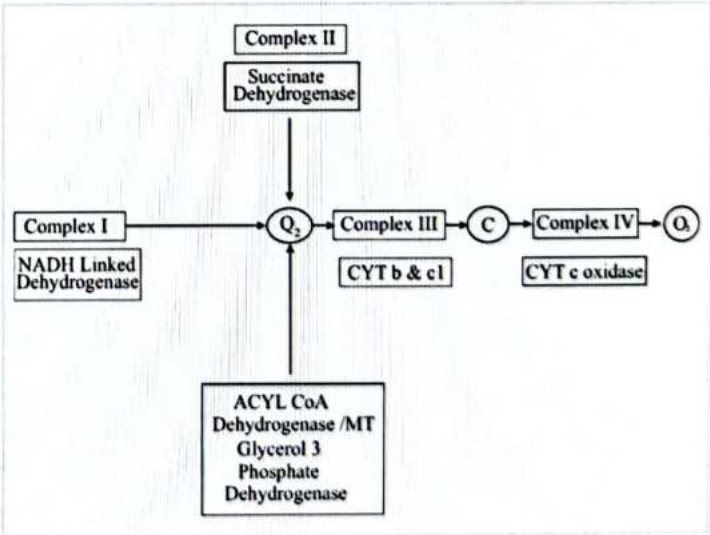
- Complex IV is cytochrome a and a3

Complex V

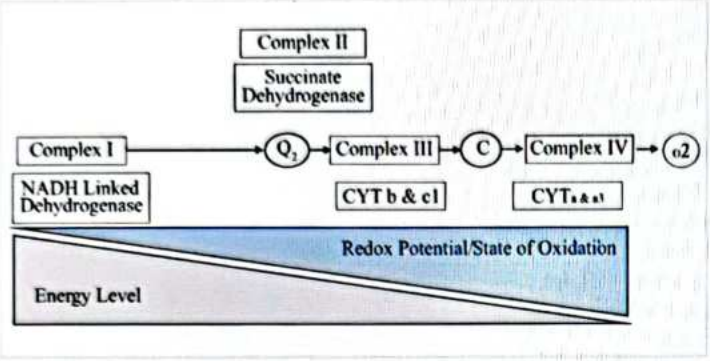
- Complex V is ATP synthase complex.

2 mobile complexes

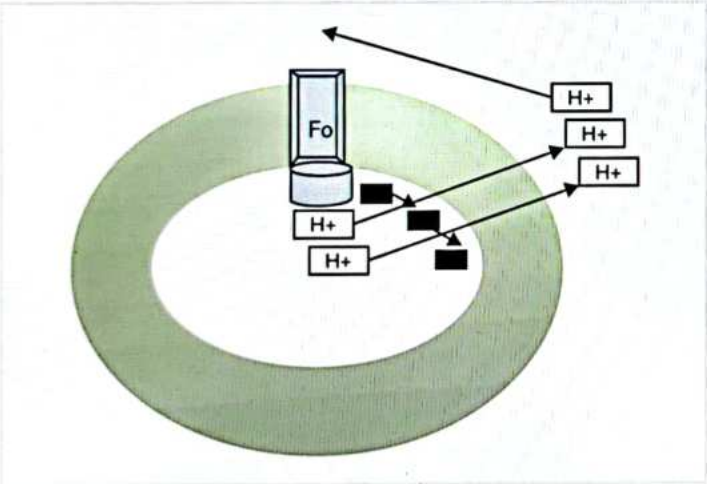
- First stationary complex is the ubiquinone/ Q placed between complex I and complex III.
- The second one is cytochrome C placed between complex III and complex IV.
- Stationary complexes are placed between mobile complexes because it helps in electron transport.
- Complex Q takes electrons from complex I and gives them to complex III.
- Cytochrome C goes to complex III, takes electrons and gives it to complex IV.
- Direction of transport of electron is, if it is an NADH electron then it will enter from complex I, from complex I it will go to complex Q, from complex Q it will go to complex III, from complex III it will go to cytochrome C, from this it will go to complex IV and finally will be accepted by O2.
- The final acceptor of the NADH electron is O2.
- If is an FADH2 electron, it will enter into electron transport chain through complex II, from complex II it goes to complex Q, from complex Q it will go to complex III, from complex III it will go to cytochrome C, from this it will go to complex IV and finally will be accepted by O2.
- Apart from succinate dehydrogenase, there are few other FADH2 linked dehydrogenase such as Acyl COA dehydrogenase (enzyme of fatty acid oxidation), mitochondrial glycerol-3-phosphate dehydrogenase,
- Other than succinate dehydrogenase, the other 2 dehydrogenases will directly enter from complex Q and will follow the same direction.



- Production of ATP from ETC complexes was given by mitchell chemiosmotic theory.
- According to this theory, all these complexes are arranged in an increasing order of redox potential (measure of ability to reduce).
  - Redox potential means Ability to get reduced is directly proportional to state of oxidation.
  - More the substance is reduced, the higher the energy it contains.
  - Oxidation and energy levels will be inversely proportional.
  - Such that these complexes are arranged in a decreasing order of energy levels.



- The energy that is liberated during the electron transport from higher energy level to lower energy level is used to pump hydrogen atoms from the matrix to outside the inner mitochondrial membrane.



- This green ring is the inner mitochondrial membrane. All these complexes are arranged in the inner side of the inner mitochondrial membrane.
- After the hydrogen atom accumulates outside the Inner mitochondrial membrane, these hydrogen ions will find their way through the Fo component of ATP synthase complex.
- This complex V has two subunits, one is Fo subunit and the other one F1 subunit.
- Fo is an ion channel through which hydrogen ions can move and F1 is actual ATP synthase enzyme.
- Hydrogen ions move from the higher concentration region to lower concentration region and that will liberate energy.
- When hydrogen ions move through the Fo component of ATP synthase complex, energy is liberated, and that energy is finally used for phosphorylating ADP to form ATP. This is how ETC helps in ATP production.

Q. Why is 2.5 ATP for NADH and 1.5 for FADH2? Why does this discrepancy occur?

Ans. It is because NADH enters ETC through Complex I and there is an energy difference between complex I and Q. When an electron moves from complex I to complex Q, 4 hydrogen atoms can be translocated. The number of electrons that can move depends upon the energy that is liberated. The energy that is liberated will depend upon the energy difference. The energy difference between the complex I and Q is such that when electrons move from I to Q, 4 hydrogen ions get translocated. And the energy difference between the complex Q and III is such that when electrons move from Q to III, again 4 hydrogen ions get translocated. But the energy difference between the complex III and IV is such that when electrons move from III to IV, only 2 hydrogen ions get translocated. Totally 10 hydrogen ions get translocated when the NADH electron gets into the ETC chain. ATP synthase complex works in such a way that when 4 hydrogen ions go through the Fo component, 1 ATP generated. 4 ions help in generating 1 ATP and 10 ions help in generating 2.5 ATP. That is why it is 2.5 ATP for NADH.

- FADH2 enters ETC either through to complex II or they directly get into through complex Q. In either case, Complex II and Q have the same energy level. Because they have the same energy level, no hydrogen atom will translocate. From Q to III, 4 hydrogen ions will translocate. From III to C, 2 hydrogen ions will translocate. Totally 6 hydrogen ions get translocated when the FADH2 electron gets into the ETC chain. 4 ions help in generating 1 ATP and 6 ions help in generating 1.5 ATP. That is why it is 1.5 ATP for FADH2.

Inhibitors of ETC

- The electron transport between complex I and complex Q is inhibited by amobarbital, piericidin A, rotenone.
- Complex II or Q is inhibited by malonate.
- The electron transport between complex II and complex Q is inhibited by TTFA and carboxin.
- The electron transport between complex III and complex C is inhibited by BAL and antimycin.
- The electron transport between complex IV and oxygen is inhibited by hydrogen sulfide, cyanide, and carbon monoxide. Cyanide causes histotoxic hypoxia, does not matter how much oxygen is available, tissues will not be able to extract oxygen because electron transport is inhibited by cyanide. The major mechanism by which carbon monoxide

exhibits toxicity is that carbon monoxide exhibits very high affinity to Fe2+ of hemoglobin so it displaces oxygen. The oxygen carrying capacity of hemoglobin reduces and this is called anemia. So, carbon monoxide causes anemic hypoxia.

- Any electron transport inhibitor will cause histotoxic hypoxia.

Uncouplers

- The major function of ETC is that it couples oxidation of fuel with phosphorylation of ADP to form ATP. The uncoupler uncouples oxidation from phosphorylation. It means oxidation of fuel will be done continuously without phosphorylation.

Effects of uncouplers

1. The first effect of uncoupler will be that there will be no ATP production.
2. Increase in the rate of heat production.
3. Increase in the rate of oxidation of all the fuels so it will increase basal metabolic rate.

Definition of uncouplers

- All uncouplers are ion channels and they get inserted into the inner mitochondrial membrane. Through these ion channels, hydrogen ions will bypass. Because hydrogen ions do not go through Fo components, ATP will not be produced.
- Here also hydrogen ions move from regions of higher concentration to regions of lower concentration, so the energy will be liberated out as heat.

Types of Uncouplers

- Two types of uncouplers are there, physiological uncouplers and artificial uncouplers.
- **Physiological uncouplers:** They include thyroxine and brown adipose tissue. Thyroxine causes upregulation of uncoupler proteins in the inner mitochondrial membrane through which hydrogen ions by pass, no ATP, no heat production occurs that is why thyrotoxicosis presents with heat intolerance and that is why hypothyroidism presents with cold intolerance. For want of ATP, more and more fuels will be oxidized in the presence of thyroxine. Brown adipose tissue is of brown color because it has got numerous mitochondria and in all these inner mitochondrial membrane, uncoupler proteins are inserted. Through these uncouplers, hydrogen ions by pass that is why no ATP production and only heat production will be there. Brown adipose tissue helps in heat production and they are considered as a mechanism for non-shivering thermogenesis neonates and hibernating animal. Neonates do not exhibit shivering, but they have to generate heat. At the time of birth, we all born with adequate amount of brown adipose tissue. For example, the interscapular region has lots of brown adipose tissue, in the neonatal period that helps in heat production. As age progresses, the brown adipose tissue regress. In some people, this brown adipose tissue is retained and that is quite fortunate. That means any fuel they will intake will be oxidized, and energy will be liberated as heat. It doesn't couple to ATP production and there will be no anabolism and wright gains.
- **Artificial Uncouplers:** Artificial uncouplers include 2,4 Dinitrophenol, valinomycin, nalinomycin, and nijaricin. These 4 were once tried as anti-obesity drugs. In the presence of these uncouplers, all the fuels will be oxidized, and energy will be liberated as heat, no ATP production, and no anabolism. Most of them are presented as increased heat production and many of them are posterior subcapsular cataracts, they all withdrew from the market.

Oligomycin

- Oligomycin acts by inhibiting the Fo component of ATP synthase complex. It is denoted by Fo to show that it is inhibited by oligomycin. In the presence of oligomycin, hydrogen atoms will not enter into mitochondria through ATP synthase complex. So there will be no ATP synthesis. The difference between this and other uncouplers is that In other uncouplers hydrogen a ions somehow manage to get into the matrix. As long as hydrogen ions stay within the matrix, oxidation of fuel will happen continuously. In the presence of uncoupler, only phosphorylation is arrested but oxidation happens continuously. In the presence of oligomycin which inhibits the Fo component of ATP synthase complex, there is no way hydrogen ions can get into the matrix. Without hydrogen ions, electron transport will stop. When electron transport stops, oxidation of fuel will also stop. The only difference between uncouplers and oligomycin is that in oligomycin not only phosphorylation but also oxidation are arrested.

Atractyloside

- It inhibits ATP ADP translocator of mitochondrial membrane. ATP ADP translocator takes an ADP and gives out ATP that is inhibited by Atractyloside.

One Liners

1. The ETC Complex with copper is Complex IV
2. Mobile complexes are Q and cytochrome C.
3. The complex in ETC without Heme is Q
4. The final acceptor of electron in ETC is Oxygen
5. Complex II is Succinate dehydrogenase.

MCQ's

Q. According to Mitchelle's chemiosmotic theory, which of the following is true?

A. They are arranged in a decreasing order of redox potential.

B. They are arranged in a decreasing order of ability to get reduced.

C. They are arranged in a decreasing order of energy level.

D. They are randomly arranged.

Q. The number of H+ translocated, when FADH2 enters ETC:

A. 10

B. 6

C. 3

D. 2

Q. Antimycin inhibits the electron transport at which level:

A. Complex I to Q

B. Complex II to Q

C. Complex III to C

D. Complex IV to Oxygen

Integrated Case Based MCQ

Q. An obese woman, who is desperate to lose weight takes an online weight reduction pill and ends up in hyperthermia. A paramedic checks the content of the pill and finds valinomycin as one of the components. Mechanism of action of valinomycin is

A. It is an uncoupler

B. It inhibits ATP synthase

C. It inhibits ATP/ADP translocator

D. It inhibits electron transport between Complex I and Q

Q. Marilyn Manroe, the famous American model, actress and singer was suffering from insomnia. Her friend introduced her to a street drug "Lilly 33s.", which is amobarbital!! She died at the age of 36, following intake of few "Lillies"!! Amobarbital acts by inhibiting electron transport from

A. Complex I to Q

B. Complex II to Q

C. Complex III to C

D. Complex IV to Oxygen

Q. A compound was added to isolated mitochondria in a medium containing succinate, fumarate, ADP and Pi and oxidative phosphorylation was decreased. Name the compound.

A. Oligomycin

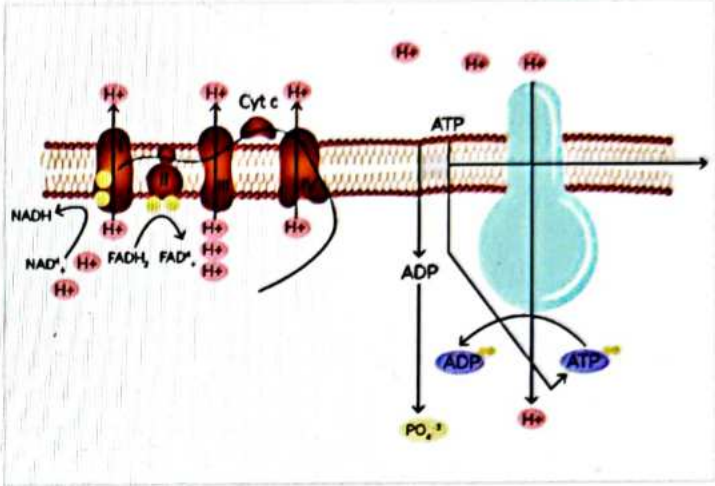
B. 2,4 dinitrophenol

C. Antimycin

D. Rotenone

Image Based MCQS

Q. INHIBITOR X acts on a transporter marked in the image. Inhibitor A is



A. Hydrogen Sulphide

B. Oligomycin

C. 2,4 Dinitrophenol

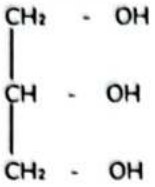
D. Atractyloside

16 LIPID CHEMISTRY AND COMPLEX LIPIDS

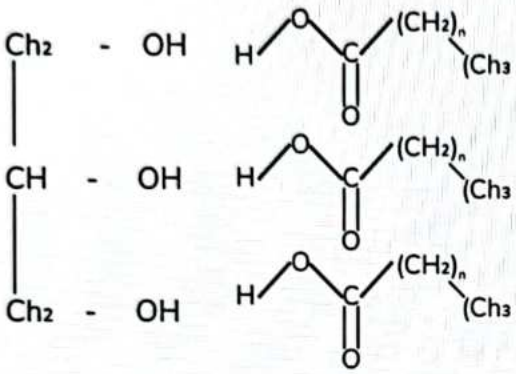
Lipids 00:01:00

- Lipids are a group of heterogeneous substances.
- Solubilized in non-polar solvents like chloroform and ether.
- Insoluble in polar solvents like water.
- **Ex:** Triacylglycerol = Glycerol + 3 fatty acids

Glycerol



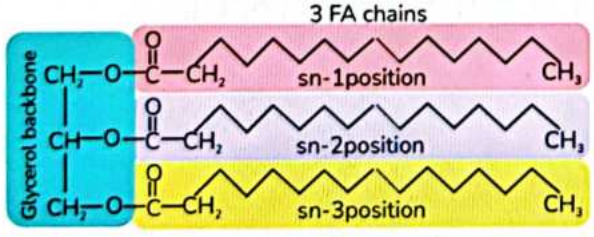
- The 3 OH of glycerol get attached to fatty acid to form triacylglycerol.



- The H of glycerol and OH of fatty acid is removed as water molecules.
- 3 water molecules are removed.
- The linkage now formed is Ester linkage - formed between an alcohol and an acid group.

Important Information

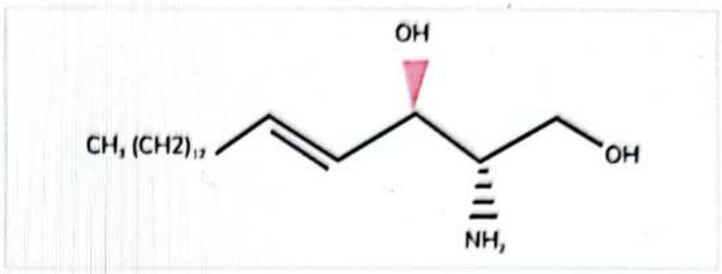
- OH + OH → Ether linkage.
- OH + COOH → Ester linkage.



- Most of the Lipids are esters containing fatty acids and alcohol.
- Sometimes instead of glycerol sphingosine is found in a lipid as alcohol.

Sphingosine 00:04:45

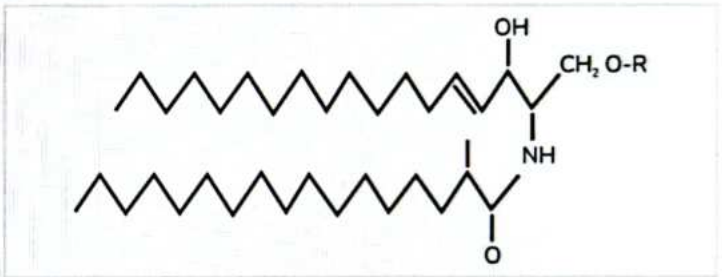
- It is an amino alcohol.
  - **Functional groups:** Amino and alcohol.



- **Precursors:** Amino acid having alcohol side chain.
  - Serine - used for sphingosine synthesis.
  - Threonine
  - Tyrosine
- Serine + Palmitoyl CoA → Sphingosine
- The sphingosine now attaches to fatty acid.
- The acid group of fatty acids binds to the NH_2 group of sphingosine.
- Removes H_2O and forms an amide linkage - Ceramide.

Ceramide 00:07:55

- Sphingosine + fatty acid



- **If alcohol is glycerol** - Lipid formed is triacylglycerol.
- **If alcohol is sphingosine** - Lipid formed is ceramide.

Classification of Lipids 00:09:15

- Simple Lipids
- Complex Lipids
- Precursor/ Derived lipids

Simple Lipids

- These are the esters containing fatty acid and alcohol.
- E.g. Triacylglycerol
- Depending on alcohol present, simple lipids are classified into two types:
  - Fat
  - Wax

Fat	Wax
• Low molecular weight alcohol like glycerol. • Ex: Triacylglycerol.	• High molecular weight alcohol like sphingosine as alcohol. • Ex: Ceramide.

Complex Lipids 00:12:10

- These are the esters containing some additional groups along with fatty acid and alcohol.
- Based on additional group present, complex lipids are classified as:
  - **Phosphate** - Phospholipids.
  - **Carbohydrate** - Glycolipids.
  - **Amino** - Amino Lipids.
  - **Sulfate** - Sulpholipids (sulfated derivatives of either phospholipid or glycolipid).
    → **E.g.** Sulfogalactosyl ceramide
    → Major chemical composition of myelin.

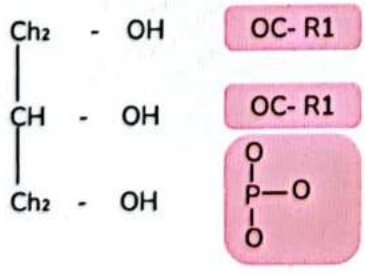
PHOSPHOLIPIDS 00:15:25

- These are esters containing phosphate groups with fatty acid and alcohol.
- Based on alcohol present, phospholipids are classified as:
  - **Glycerol** - Glycerophospholipids
  - **Sphingosine** - Sphingophospholipids

Glycerophospholipids	Sphingophospholipids
• Esters - containing fatty acid, phosphate and alcohol. • Derivatives of phosphatidate.	• Esters - containing fatty acid, phosphate and sphingosine. • Fatty acid + sphingosine → ceramide. • Derivatives of ceramides. • Ceramide has a free OH group - attached to the phosphate group. o Phosphate + choline → sphingomyelin → Major composition of myelin.

Phosphatidate

- Glycerol has 3 OH groups.
- 2 OH groups get attached to fatty acid.
- 1 OH group gets attached to the phosphate group.
- This is called phosphatidate.
- Precursor of every glycerophospholipid.



- The phosphate group is free.
  - Free Phosphate group + Choline→ Phosphatidyl Choline (Lecithin)
    → If 1st 2 fatty acids are palmitic acid (Dipalmitoyl phosphatidyl choline) - Major chemical component of surfactant.
    a. Produced by type 2 pneumocytes.
    b. Not matured till late trimester.
    c. In premature neonates - No surfactant produced.
  - Free phosphate group + Serine→Phosphatidyl serine
  - Free phosphate group + Inositol →Phosphatidyl inositol
    → If inositol is attached to 2 phosphate groups → Phosphatidyl inositol diphosphate (PIP2).
  - Free phosphate group + Ethanolamine →Cephalin

Free phosphate group + Diphosphatidyl glycerol→ Cardiolipin.

Important Information

Infant Respiratory Syndrome

- Lack of surfactant - Dipalmitoyl phosphatidyl choline.
- No reduction in surface area of lungs.

Plasmalogens

- Glycerophospholipid having ether linkage at 1st carbon alone.
- Usually, Ester linkages are present.
- The OH of 1st carbon attaches to alcohol but not the fatty acid.

Composition of myelin

- Sulphogalactosyl ceramide.
- Sphingomyelin.

Precursor Or Derived Lipids 00:29:00

- Anything else which is non-polar and not included so far.
- Acts as precursor for other Lipids or can be derived from other Lipids.
- Examples:
  - **Fatty acid** - Precursor
  - **Cholesterol** - Derived lipid
  - **Bile acids** - Derived lipid (derived from cholesterol)
  - **Steroid hormones** - Derived lipids.

One Liners 00:30:45

- Sphingosine and fatty acids are called **ceramide.**
- Amino acid precursor of sphingosine **serine.**
- Phosphatidylcholine is **Lecithin.**
- Phosphatidyl ethanolamine is **cephaline.**
- Diphosphatidyl glycerol is **cardiolipin.**

MCQs 00:31:33

Q. Which of the following is not a lipid?
A. Glycerol
B. Palmitic acid
C. Triacylglycerol
D. Cholesterol

Q. All of the following are components of triacylglycerol except?
A. Glycerol
B. Palmitic acid
C. Sphingosine
D. Stearic acid

Q. Which of the following is a wax?
A. Triacylglycerol
B. Ceramide
C. Sphingomyelin
D. Lecithin

Q. Which of the following is a derived lipid?
A. Triacylglycerol
B. Fatty acid
C. Sphingomyelin
D. Cholesterol

Q. All of the following are components of Sphingomyelin except?
A. Sphingosine
B. Fatty acid
C. Choline
D. Ethanolamine

Explanation
- Sphingosine + fatty acid - ceramide.
- OH binds with phosphate.

Phosphate binds with choline - forms Sphingomyelin.

Integrated Case Based Mcqs 00:35:45

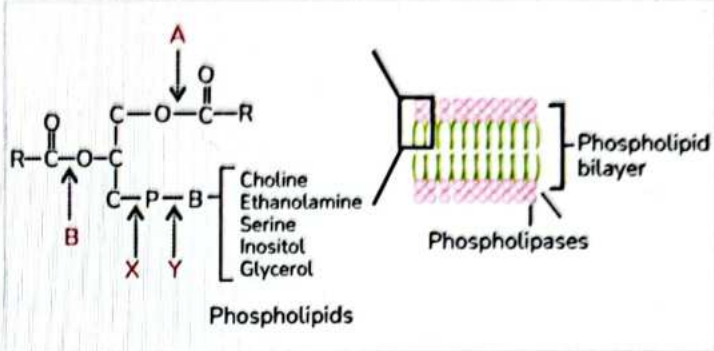
Q. A premature child presence with infant respiratory distress syndrome. The neonatologist says the neonate is yet to produce lecithin adequately. All of the following are components of Lecithin except?
A. Glycerol
B. Choline
C. Phosphatidate
D. Ceramide

Explanation
- Glycerol - has 3 OH groups.
- 1st 2 OH groups should attach to 2 fatty acids.
  - In a surfactant the fatty acid is dipalmitoyl phosphatidyl choline.
- 3rd OH attached to the phosphate group.
  - Gets attached to choline.
- This structure is now called dipalmitoyl phosphatidyl choline.
- Therefore, there are glycerol, Palmitic acid, phosphate group and choline.

Ceramide is not found - component of sphingomyelin.

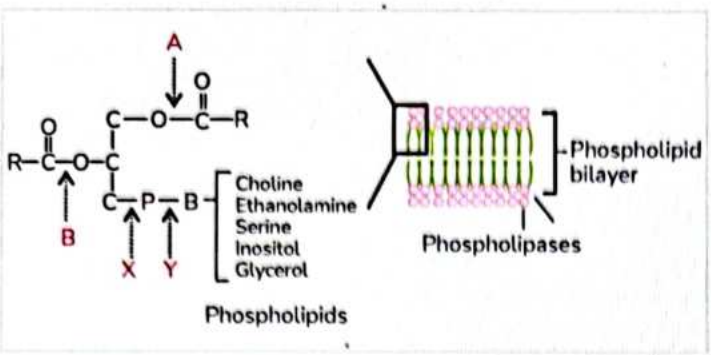
Image Based Mcqs 00:38:00

Q. Viper venom has phospholipase A2. Phospholipase A2 activity is shown in the image



a. A
b. B
c. X
d. Y

Explanation
- A, B, X, Y in the image are the sites where various enzymes can act.
- Various enzymes are:
  - Phospholipase A1
  - Phospholipase A2
  - Phospholipase B
  - Phospholipase C
  - Phospholipase D
- Phospholipase A1 acts at the fatty acid attached to the 1st carbon of glycerol.
  - **Location A in image** - phospholipase A1.
  - Gives rise to lysophospholipid and fatty acid.
- Phospholipase A2 acts at the 2nd carbon atom.
  - **Cleaves the linkage**-makes the fatty acid (arachidonic acid) free.
  - **Location B in image** - phospholipase A2.
- Phospholipase B acts at both the 1st and 2nd carbon atoms.
  - Converts them to 2 fatty acids and a lysophospholipid
- Phospholipase C and D - both act at 3rd carbon.
  - Phospholipase C acts before the phosphate group.
    → Forms diacylglycerol and inositol triphosphate by action on inositol diphosphate.
    → **Location X in image** - phospholipase C
  - Phospholipase D acts after the phosphate group.
    → Forms phosphatidate and free alcohol.
    → **Location Y in image** - phospholipase D.



Explanation

- Enzymes X acts before the phosphate group of the 3rd carbon atom.
- Enzyme X is phospholipase C.
- The products formed are Inositol trisphosphate and diacylglycerol

Q. Products on the enzyme X are:

A. Lysophospholipid and fatty acid

B. Inositol trisphosphate and diacylglycerol

C. Phosphatidic acid and a nitrogenous base

D. Inositol triphosphate and phosphatidic acid

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17 FATTY ACID, CHOLESTEROL AND GLYCOLIPIDS

Recap of Lipid Chemistry Part 1

- Lipids are Nonpolar substances
- **Classification of Lipids** (Based on Structure)
  - Simple
    - → Fats
    - → Wax
  - Complex (additional components)
    - → Phospholipids
    - → Glycolipids
    - → Aminolipids
    - → Sulpholipids
  - Precursor/ Derived

Glycolipids (Complex) 00:01:55

- Esters containing Carbohydrate group, in addition to fatty acid and alcohol.
- Alcohol in Glycolipids is Sphingosine,
- Glycolipids are known as Glycosphingolipids.
- Fatty acid + Sphingosine= Ceramide
- All Glycolipids are derivatives of ceramide.

Three Types of glycolipids are:

I. Cerebroside:
Ceramide + Monosaccharide
Example: Glucosyl ceramide or galactosyl ceramide

II. Globoside:
Ceramide + Oligosaccharde
Example: Cer- Glu - Gal or Lactosyl Ceramide

III. Ganglioside:
Ceramide + Oligosaccharide - NANA
Example:
a. GM3 Ganglioside = Cer-Glu-Gal - NANA
b. If N Acetyl Galactosamine is atteched, it is GM2 Ganaglioside
c. If galactose is attached, it is GM1 Ganglioside

Disorders (Glycosphingolipidosis) 00:07:00

- Beta Galactosidase, removes Galactose in GM1 Ganglioside and converts to GM2 Ganglioside.
  - Absence of Beta Galactosidase causes GM1 Gangliosidosis.
- Hexosaminidase, removes N-Acetyl Gal NH2 in GM2 Ganglioside and converts to GM3 Ganglioside.
  - Absence of Hexosaminidase leads to GM2 Gangliosidosis.
- Neuraminidase, removes NANA in GM3 Ganglioside and converts to Globoside.
  - Absence of Neuraminidase leads to Gm3 Gangliosidosis or Sialidosis.

Important Information

- Galactose in GM1 Ganglioside is in Beta form
- Galactose in Globoside is in alpha form

- Alpha Galactosidase, removes Galactose in Globoside and converts to Cerebroside.
  - Absence of Alpha Galactosidase leads to Fabry's disease.

Fabry's disease

- X-linked recessive.
- Defective Alpha Galactosidase.
- Accumulates Globoside.
- **Triad**
  - Keratoangioma or Angiokeratoma, Purplish red spots (macuoles) in the skin.
  - Kidney affected- proteinuria.
  - Early Myocardial infarction.
- Beta Glucosidase removes Glucose from Cerebroside and converts to Ceramide.
  - Absence of Beta Glucosidase causes Gaucher's disease.

Gaucher's disease

- Defective Beta Glucosidase.
- Accumulates Cerebroside (GlucosylCerebroside) in RBCs and platelets membranes.
- Abnormal RBCs and platelets are engulfed by macrophages that lead to Anemia and Thrombocytopenia.
- Accumulation of GlucosylCerebroside membrane inside macrophages appears as crumpled tissue paper.
- Diagnosis, Erlenmeyer flask deformity.
- Enzyme replacement therapy is available.
- Ceramidase splits Sphingosine and Fatty acids
  - Absence of Ceramidase causes Farber's disease

Farber's disease

- Granulomatous disease.
- Painful subcutaneous nodules.
- Renal failure.

S. no	Enzyme defect	Disease
1.	Beta Galactosidase	GM1 Gangliosidosis
2.	Hexosaminidase	GM2 Gangliosidosis
3.	Neuraminidase	Gm3 Gangliosidosis/ Sialidosis
4.	Alpha Galactosidase	Fabry's disease
5.	Beta Glucosidase	Gaucher's disease
6.	Ceramidase	Farber's disease

Important Information

- Alpha Glucosidase/ Acid maltase defect leads to Pompe's disease

Q. Beta Galactosidase defect causes all except
A. Morquio B disease
B. Krabbe disease
C. GM1 Gangliosidosis
D. **Gaucher's disease**

Explanation

- Galactose is a component of GM1 Ganglioside, Keratan sulfate, Myelin.
- Galactose has two isoforms, Galactose-I and Galactose-II.
- Galactose-I produces GM1 Ganglioside.
- Galactose-II produces:
  - Keratin sulfate (Morquio B disease).
  - Myelin (Krabbe disease).

Important Information

- Keratin sulfate has Galactose instead of Neuraminic acid
- Important component of Myelin is Sulphogalactosyl Ceramide

Facts About Hexosaminidase 00:20:45

- Two subunits
  - Alpha (α)
  - Beta (β)
- Genes
  - Hex A
  - Hex B
- Enzymes
  - Hex A enzyme has α β subunits
  - Hex B has β β subunits
- Diseases
  - Hex A gene mutation
    → Tay Sachs disease
  - Hex B gene mutation
    → Sandhoff disease
- Defective enzymes
  - Tay Sachs disease
    → Hex A enzyme
  - Sandhoff disease
    → Hex A enzyme
    → Hex B enzyme

	Tay Sachs disease	Sandhoff disease
Cause	Hex A	Hex B
Subunit	α	β
Defective enzymes	Hex A	Hex A and Hex B
Defective Metabolism (accumulation)	Gm2 Ganglioside	GM2 Ganglioside, Globoside

- Cherry red spots with only GM2 Ganglioside accumulated in Tay Sachs disease.
- Cherry red spots with both GM2 Ganglioside and Globoside accumulated in Sandhoff disease.
- Both Tay Sachs and Sandhoff diseases present with neurological manifestations.
- GM1 Gangliosidosis, caused by Beta Galactosidase defect present with both Neurological and systemic manifestations.

Facts About Glycosphingolipidosis 00:26:45

- **No mental retardation in**
  - Gaucher's disease
  - Fabry's disease
- **No cherry red spots in**
  - Gaucher's disease
  - Fabry's disease
  - Krabbe's disease
- **No organomegaly in**
  - GM2 Gangliosidosis
  - Krabbe's disease

Krabbe's disease

- Defects in Beta Galactosidase and Myelin Metabolism get affected predominantly.
- No hepatosplenomegaly.
- Neurological manifestations are predominant, cranial nerve involvement.
  - Deafness
  - Blindness

Precursor/ Derived Lipids 00:29:10

- Examples
  - Fatty acids
  - Cholesterol
  - Bile acids
  - Steroid hormones

Fatty Acids

- **Examples**
  - Palmitic acid
  - Stearic acid
  - Oleic acid
  - Acetic acid
  - Propionic acid
  - Butyric acid
- All fatty acids have carboxyl group attached to an aliphatic side chain:
  - $CH_3-CH_2-CH_2-COOH$
- **Numbering of carbon atoms**
  - Delta numbering
  - Omega numbering
  - Alpha, Beta, Gamma numbering

- **Delta numbering**
  - Carbon in Carboxyl group is given D1.
  - Adjacent carbon is D2, D3, D4.
  - Numbering starts from the Carboxy end.

- **Omega numbering**
  - Methyl group is given ω1.
  - Adjacent carbon is given ω2, ω3.
  - Numbering starts from the Methyl end.

Important Information

- ωn = N-Dn
- ωn-omega numbering
- N-total number of carbons
- Dn-Delta numbering

- **Alpha, Beta, Gamma numbering**
  - Carbon attached to Carboxyl group is α
  - Carbon attached to α carbon is β
  - Carbon attached to β is γ

Important Information

- Based on the presence of double bonds, omega numbering changes.
- Omega 9 fatty acid has double bond after 9th carbon from Methyl end.

Q. What are the two nutritionally essential fatty acids?
Ans. linolenic acid and Alpha linolenic acid

Explanation

- linolenic acid
  - 18 carbons
  - Omega 6 type
- Alpha linolenic acid
  - 18 carbons
  - Omega 3 type
- These fatty acids are not synthesized by metabolic pathways
- Saturated fatty acids are synthesized
- Unsaturated fatty acids maintain membrane integrity
- Saturated fatty acids are synthesized and double bonds are introduced using Human desaturase enzyme system
- This enzyme removes hydrogen atoms at specific location and introduce double bonds
- Human desaturase enzymes cannot desaturate beyond D9.
- Introduction of 18 carbon linolenic acid (omega6) and Alpha linolenic acid (omega3)
- These fatty acids have chain elongase system, add carbon atoms to the chain

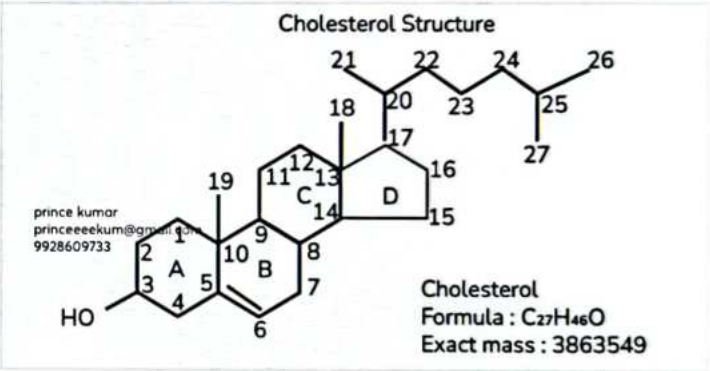
Important Information

- Two nutritionally essential fatty acids
  - linolenic acid (omega6)
  - Alpha linolenic acid (omega3)
- Human enzymes cannot desaturate beyond omega 9, these fatty acids are essential
- Arachidonic acid is nutritionally not essential, it is semi nutritionally essential
  - 20 carbons
  - Omega 6 type
- If diet lacks linolenic acid, then arachidonic acid becomes essential

Cholesterol 00:38:30

Cholesterol structure

- Steroid nucleus is described as, CPPP (CycloPentano Perhydro Phenanthrene) ring
- All steroids have 4 rings
- D-ring:
  - 5 sides
  - Cyclopentano
- A, B, C rings, Phenanthrene ring.
- Per hydro
  - One hydroxy ring at 3rd position
  - Two methyl groups at 10, 13 positions
- Side chain at 17th position



Important Information

- Bile acids are produced by modifying the side chain
- Steroid nucleus remains same
- Cholesterol has hydroxyl group at 3rd position, Amphipathic lipid.
- It is both polar and nonpolar
  - Hydroxyl group-polar
- CPPP ring non-polar

Classification of Lipids (Based on Solubility) 00:42:00

- Lipids are generally Non-polar
  - Amphipathic lipids
    → Lipid with an additional free polar group
  - Neutral lipid
    → Lipid with no polar groups

- Cholesterol is Amphipathic, both polar and non-polar groups are present
- Hydroxyl group in cholesterol is added with fatty acid to produce Cholesterol Ester
  - Cholesterol ester is completely Non-polar
- Triacylglycerol, glycerol with 3 hydroxyl groups with attached fatty acids
  - TAG is neutral lipid
- Phospholipids, phosphate is negatively charged (Polar)
  - Phospholipids have additional polar group, Amphipathic
- Amphipathic lipids
  - Cholesterol
  - Phospholipids
- Neutral lipids
  - Cholesterol ester
  - TAG

One Liners

00:44:40

Q. Glycolipids are derivatives of
Ans. Ceramide

Q. Nutritionally essential fatty acids are
Ans. Linoleic acids and Alpha Linolenic acid

Q. Amphipathic lipids are
Ans. Phospholipids, Cholesterol

Q. Non-polar lipids are
Ans. Cholesterol ester, Triacylglycerol

Q. Lipid which accumulates in Gaucher's disease
Ans. Cerebroside

Q. Lipid which accumulates in Fabry's disease
Ans. Globoside

Q. Globosides accumulates in
Ans. Fabry's and Sandhoff diseases

MCQ's

00:45:55

Q. Hexosaminidase A defect causes,
A. Cerebrosidosis
B. GM3 Gangliosidosis
C. GM2 Gangliosidosis
D. GM1 Gangliosidosis

Q. Beta Galactosidase defect causes all except:
A. Krabbe's disease
B. GM3 Gangliosidosis
C. Morquio B disease
D. Gm1 Gangliosidosis

Explanation
- GM3 Gangliosidosis is caused by Neuraminidase
- Also known as Sialidosis

Q. All the following are true about cholesterol except:
A. Hydroxyl group at 3rd position
B. Methyl groups at 10, 13th positions
C. Side chain at 17 position
D. Has benzene ring

Explanation: Cholesterol has Phenanthrene ring.

Q. Which of the following is a Neutral lipid?
A. Phospholipid
B. Monoacyl glycerol
C. Diacylglycerol
D. Cholesterol ester

Explanation
- Neutral lipids are Nonpolar
- Glycerol has 3 Hydroxyl groups
- Diacylglycerol, Amphipathic
  - Two groups are attached fatty acids
  - One group is polar
- Monoacyl glycerol, Amphipathic
  - One group is attached fatty acids
  - Two group polar

Q. Arachidonic acid is a fatty acid of which type?
A. **20, omega 6**
B. 20, omega 3
C. 18, omega 6
D. 22, omega 3

Explanation
- 20, omega 3, Timnodonic acid
- 18, omega 6, Linoleic acids
- 22, omega 3, Cervonic acid (DHA)
- DHA (Docosahexaenoic acid)
  - 22 carbons
  - 6 double bonds
  - Omega 3 type
  - Essential for myelination
  - Supplements in children diet

Q. A 46-year-old male was referred for dermatological evaluation of purpura. He was already getting treated for proteinuria and a recent episode of MI. Clinical biochemists suspected Fabry's disease. The lipid which accumulates in Fabry's disease is?
A. Cerebroside
B. **Globoside**
C. Ganglioside
D. Ceramide

Explanation

- Triad of Fabry's disease:
  - Angiokeratoma, reddish color lesions
  - Proteinuria
  - Recent episode of MI

Q. For the first few months, an eight-month-old child's growth and development were normal, then symptoms such as deafness, blindness, atrophied muscle, inability to swallow, and convulsions began to appear. During the fundus inspection, a cherry red macula was also seen in both eyes. Suspecting sphingolipidosis, the physician tested for globosides and gangliosides levels and found that both were increased. Based on this finding, what is the most accurate diagnosis for this patient?

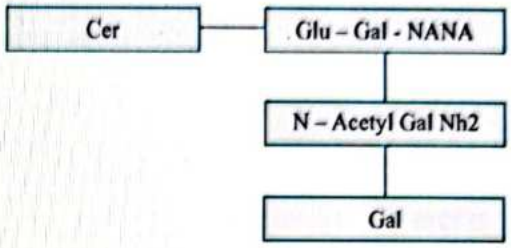
A. Sandhoff disease
B. Tay Sachs disease
C. Gaucher's disease
D. Fabry's disease

Explanation

Sandhoff disease

- Neurological manifestations:
  - deafness
  - blindness
  - atrophied muscle
  - inability to swallow
  - convulsions
- Cherry red appearance
  - No cherry red spots in
    - → Krabbe's
    - → Gaucher's
    - → Fabry's
- Elevated Globoside and gangliosides
  - Sandhoff disease
- In Gaucher's Cerebroside is accumulated
- In Fabry's Globoside is accumulated

Q. Identify the structure provided above



A. Cerebroside
B. Globoside
C. GM1 Ganglioside
D. GM2 Ganglioside

PREVIOUS YEAR QUESTIONS

Q. Which is the lipid that accumulates in Fabry's disease?
Ans: Globoside

Q. Enzymes which are defective in Fabry's disease?
Ans: Alpha Galactosidase

18 FATTY ACID SYNTHESIS

Outline of the Session
- Location of Fatty acid synthesis
- What is the building block of fatty acid?
- Prerequisite for fatty acid synthesis
- Steps involved in fatty acid synthesis.
- Rate limiting enzyme of this pathway.
- Rate limiting pathways for all pathways in lipid metabolism.
- Regulation of the pathway

Facts Related to Fatty Acid Synthesis
- Where does it happen?
  - Fatty acid synthesis - Cytoplasm
  - Fatty acid oxidation - Mitochondria
- The building block of fatty acid and cholesterol is acetyl CoA.
- Two acetyl molecules combine to give - butyryl CoA, which has four carbon atoms.
- Three acetyl molecules combine to give - Hexanoyl CoA, which has 6 carbon atoms.

Sources of acetyl CoA
- **Carbohydrate metabolism**
  - It is a pyruvate dehydrogenase complex.
  - Pyruvate dehydrogenase complex converts pyruvate into acetyl CoA.
  - Pyruvate dehydrogenase complex is an enzyme of mitochondria.
- **Lipid Source**
  - N carbon atoms containing fatty acids undergo beta-oxidation to give n/2 acetyl CoA.
  - Fatty acid oxidation happens in mitochondria.
  - Glycolysis, which is anaerobic oxidation, happens in the cytoplasm.
- **Protein Source**
  - All ketogenic amino acids on oxidation give acetyl CoA.
  - Oxidation happens in mitochondria.
- Three sources of acetyl CoA are stored in mitochondria.
- **Three requisites for fatty acid synthesis**
  - Transport all acetyl CoA to the cytoplasm from mitochondria.
  - As acetyl CoA cannot cross the mitochondria membrane, we use the citric acid cycle.
  - Acetyl CoA reacts with oxaloacetate to form citrate.
  - Tricarboxylate is a transporter in the mitochondria membrane that transports citrate from mitochondria to the cytoplasm.
  - An enzyme called ATP-citrate lyase in the cytoplasm produces acetyl CoA and oxaloacetate.
  - Acetyl CoA is then ready to get into fatty acid synthesis.
  - Oxaloacetate also is a part of fatty acid synthesis.
  - Malate dehydrogenase converts Oxaloacetate to malate.
  - The malic enzyme converts malate to pyruvate.
  - The malic enzyme is one of the sources of NADPH.

Recollecting Facts of NADPH
3 sources of NADPH
- HMP shunt
- Cytoplasmic Isocitrate dehydrogenase
- Malic enzyme

Significance of NADPH
- Reductive biosynthesis of all the lipids.
  - Fatty acid synthesis
  - Bile acid synthesis
  - Cholesterol synthesis
  - Steroid hormones synthesis
- NADPH is needed in all the reduction steps.
- The malic enzyme converts malate to pyruvate and forms NADPH.
- That NADPH is used for fatty acid synthesis.

Fate of Acetyl CoA
- Acetyl CoA reacts with oxaloacetate and enters the citric acid cycle.
- Unwanted Acetyl CoA will condense to form ketone bodies.
- Acetyl CoA can be a building block of fatty acid.
- Acetyl CoA can be a building block of cholesterol.
- If Acetyl CoA carboxylase reacts, then converts to malonyl CoA, it gets committed to fatty acid synthesis.
- Acetyl CoA carboxylase is a rate-limiting enzyme of fatty acid synthesis.

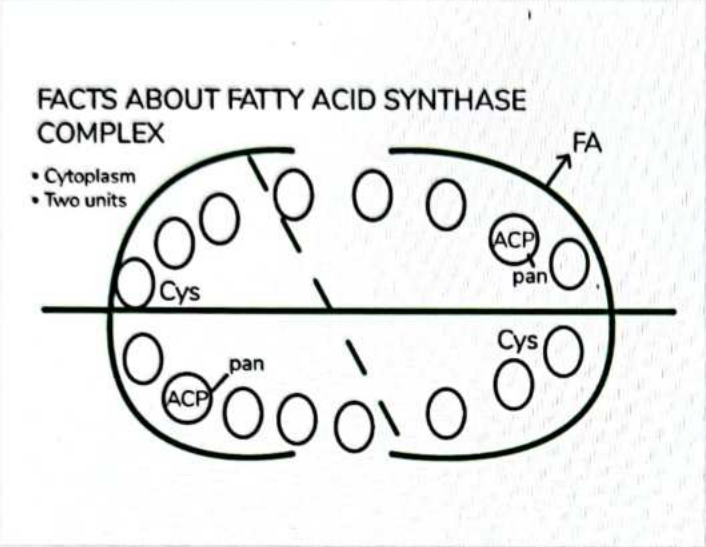
Three requirements of carboxylase
- Bicarbonate
- Biotin
- ATP is used as a source of energy.

Rate Limiting Enzyme

Sr. No	Pathway	Rate Limiting Enzyme
1	**Fatty acid synthesis**	Acetyl CoA carboxylase
2	**Fatty acid oxidation**	Carnitine Acyl transferase I
3	**Cholesterol synthesis**	HMG CoA reductase
4	**Ketone body synthesis**	HMG CoA synthase
5	**Bile acid synthesis**	7 Alpha Hydroxylase

Facts About Fatty Acid Synthase Complex

- Where is the fatty acid synthase complex present? - cytoplasm
- Fatty acid synthase is a dimer.
- Two units, and every unit has 8 subunits but only 7 enzymatic activities.
- The 8th unit is acyl carrier protein which carries fatty acid getting synthesized.
- Every unit has a cysteine end, and four phosphopantothiene ends. Two fatty acids get synthesized.
- Simultaneously, two fatty acids get synthesized.



- Subunit division is not the same as functional division.
- Because 1 half of every unit work together to synthesis 1 fatty acid and the other one another fatty acid simultaneously.

Steps Involved in the Fatty Acid Synthesis

- Acetyl CoA converts to malonyl CoA.
- One molecule of Acetyl CoA and one molecule of malonyl CoA will attach to the fatty acid synthase complex.
- Acetyl CoA is transferred by the enzyme Acetyl transacylase and gets attached to the cysteine end.
- Melanyl transacylase will transfer the melanyl group to the four phosphopantetheine ends.
- The Malonyl group is another carboxyl group.
- The 3rd subunit transfers the acetyl group to many groups.
- The other carbo group is released as carbon dioxide.
- The left out of the four phosphopantetheine ends is the carboxyl group.
- This acid has a ketone group, so the subunit is keto acyl synthase.
- Ketoacyl reductase reduces to form CHOH. Hydrogen source: NADPH
- 4 phosphopantetheine end has - hydroxy acyl enzyme.
- Enzyme to remove hydroxyl group - hydratase.
  - The product on the four phosphopantetheine end is - the unsaturated acyl-enzyme.
- The enzyme is enoyl reductase to reduce. Hydrogen source: NADPH
  - The product on the four phosphopantetheine ends is - butyric acid.
- Melanoyl CoA tries to get attached to the four phosphopantetheine ends, and the fatty acid synthesis gets shifted to the cysteine end.
- Cysteine end - Butyric acid, four phosphopantetheine end - Malonyl group
- During the transfer, additional carboxyl gets removed as carbon di-oxide.
- The keto acyl-enzyme causes the four phosphopantetheine ends to have a keto acyl group.
- Hydratase removes OH, and the double bond is reduced with the help of enoyl reductase to give hexanol reductase. This keeps going to give palmitic acid.
- Palmitic acid has 16 carbon atoms.
- The most common fatty acid by fatty acid synthase is palmitic acid.

Stoichiometry of Palmitic Acid Synthesis

- In the first cycle, one acetyl CoA and one malonyl CoA. Just by the end, four carbon atoms.
- Every cycle, we add 2 carbon atoms.
- Fatty acid synthesis goes through an n/2 - 1 cycle to get n - carbon atoms containing fatty acid.
- So, palmitic acid can go through 7 cycles.
- So, you need one acetyl CoA and 7 malonyl CoA.
- In 7 cycles, 14 NADPH and 14 H+.
- To form 7 malonyl CoA, you use 7 ATPs and 7 Carbon Dioxide.
- All are synthesized to give palmitic acid.
- Thioesterase makes this fatty acid-free and is released from the fatty acid synthase complex.
- N carbon atom containing fatty acid - n/2-1 cycles
- n/2-1 malonyl molecule and acetyl CoA molecules + 2x(n/2-1) NADPH + H+ + To form n/2 - 1 malonyl CoA, you need n/2-1 ATP.
- $\text{acetyl coA} = \left(\frac{n}{2} - 1\right) \text{malonyl} + 2x\left(\frac{n}{2} - 1\right) \text{NADPH} + \left(\frac{n}{2} - 1\right) ATP$
- Put together the synthesis of n carbon atom containing palmitic acid.
  - Cycles: 7
  - Malonyl coA: 7
  - Acetyl coA: 1
  - NADPH: 14
  - Carbon dioxide: 7
  - ATP: 7

Regulation of Acetyl Coa Carboxylase

Subunits of fatty acid synthase complex

- First subunit - Acetyl transacylase
- Second subunit - Malonyl transacylase
- Third subunit - Keto acyl synthase
- Fourth subunit - Ketoacyl reductase
- Fifth subunit - Hydratase
- Sixth subunit - Enoyl reductase
- Seventh subunit - Thioesterase
- Eight subunit - Acyl carrier protein

Acetyl CoA carboxylase - anabolic enzyme

- **Stimulate by high energy status indicators.**
  - NADH
  - FADH2
  - ATP
  - Citrate
  - Insulin
    - → Stimulates glycolysis to facilitate the conversion of glucose to pyruvate.
    - → Stimulates pyruvate dehydrogenase complex to convert pyruvate to acetyl CoA.
    - → Induces acetyl CoA carboxylase to help enter acetyl CoA to fatty acid synthesis.
- **Inhibited by low energy status indicators.**
  - ADP
  - NAD
  - FAD
  - Glucagon
  - Acyl CoA
- Carbohydrate rich diet is a lipogenic whereas lipid-rich diet suppresses lipogenesis.

Q. Why do we say a carbohydrate-rich diet is lipogenic?

- Carbohydrate-rich diet will provide the fuel that will get converted to fat.
- Insulin is released and stimulates all the phases of fatty acid synthesis.

Q. What is the basis of a ketogenic diet?

- It is based on a zero-carbohydrate high-fatty diet. You do not have glucose which is a precursor and without insulin no anabolism.
- A high-fat diet causes high availability of acyl coA, and this endogenous fatty acid synthesis is suppressed. So, taking in polyunsaturated fatty acid suppresses it.

Q. Is there any inborn error of metabolism related to fatty acid synthesis?

- None

Important Information

Refsum's Disease

- Refsum's disease is caused by the defect of alpha oxidation of branched-chain fatty acid, which happens in the peroxisome. It is a defect of fatty acid oxidation.

One-Liners

1. The building block of fatty acid **Acetyl CoA**
2. Rate limiting of fatty acid synthesis Acetyl **CoA Carboxylase**
3. The number of units in fatty acid synthase complex is **- 8.**
4. The most commonly synthesized fatty acid is **palmitic acid.**
5. Number of NADPH required for synthesizing palmitic acid **14.**
6. Number of ATPs required to synthesize palmitic acid **7.**

MCQ's

Q. The precursor of fatty acid is
A. Propionyl CoA
B. Malonyl CoA
C. Acetyl CoA
D. Methyl malonyl CoA

Q. All the following are fates of acetyl CoA except
A. Co2
B. Ketone body
C. Cholesterol
D. Glucose

Q. The rate-limiting enzyme of fatty acid synthesis is
A. HMG CoA reductase
B. HMG CoA Lyase
C. Acetyl CoA carboxylase
D. 7 alpha hydroxylases

Q. All the following are requirements of acetyl CoA carboxylase except
A. Bicarbonate
B. Biotin
C. ATP
D. NADH

Q. All are true about fatty acid synthesis except
A. It is dimer.
B. Acetyl CoA carboxylase is the first enzyme of the complex.
C. Pantothenic acid is a component of the enzyme.
D. It has 7 enzymes in each unit.

Q. The most synthesized fatty acid by fatty acid synthase complex is

A. Linoleic acid
b. Palmitic acid
C. Arachidoic acid
D. Stearic acid

Q. Lipogenic is stimulated by all except.

A. High fatty diet
B. High glucose diet
C. High fructose-based diet
D. Insulin

Case-Based MCQ

Q. A 19-year-old moderately obese girl presents with menstrual irregularities. She is diagnosed with polycystic ovarian syndrome and is prescribed metformin. Metformin helps in avoiding weight gain because it inhibits.

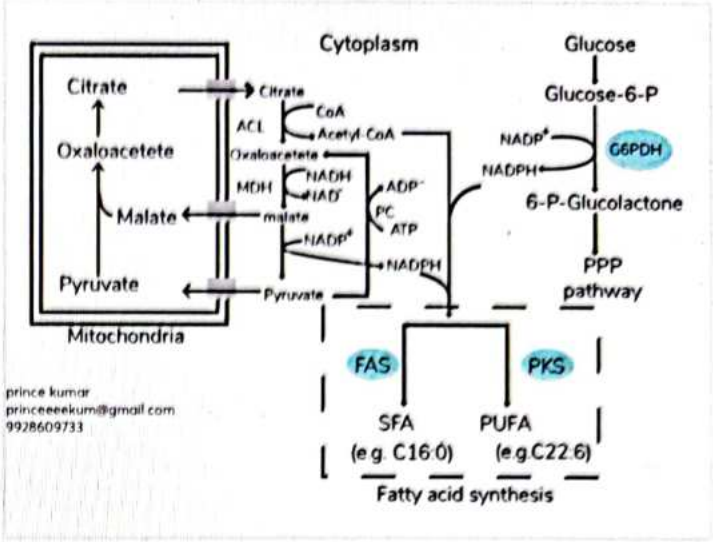
A. Complex II
B. ATP synthesis
C. Acetyl CoA carboxylase
D. Insulin

Important Information

Mechanism of Action of Metformin

1. Stimulates AMP kinase pathway - increases insulin sensitivity.
2. Inhibits glucagon receptor.
3. Amp kinase pathway inhibits fatty acid synthesis.
4. Inhibits complex of etc. - lactic acidosis.

Q. Identify the enzyme A.



A. Malate dehydrogenase
B. SGOT
C. SGPT
D. Malic enzyme

19 FATTY ACID OXIDATION

Fatty acid oxidation

Q. Where does fatty acid get oxidized? 00:01:05

A. Mitochondria

B. Nucleus

C. Endoplasmic reticulum

Facts About Fatty Acid Oxidation

- Fatty acids also get oxidised in peroxisomes.
- Very long-chain fatty acid gets oxidized in peroxisome.
- Very short-chain fatty acid gets oxidized in Mitochondria.
- Short-chain, medium-chain, and long-chain fatty acids get oxidized in both Mitochondria, and peroxisome.

Functions of Peroxisome 00:02:00

- They are concerned with very long chain fatty acid oxidation.
- They are concerned with ether lipid (plasmalogen) synthesis.
- They are concerned with α oxidation of branched-chain fatty acids.

Oxidation 00:03:16

Fatty Acid

CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOH

- The carbon atom just before alpha carbon atom is β carbon atom. When the β carbon atom is oxidized, it is no more CH2. It becomes COOH (carboxyl group), I.e., we have pre-positioned the functional grp. Which will give us

CH_3-CH_2-CH_2-COOH + CH_3 - COOH
(Acetic acid).

- When the acetic acid gets attached to coA, we will call it acetyl CoA.

Q. How many carbon atoms are there in acetyl CoA.

Ans. There are two carbon atoms.

- If there is n carbon containing fatty acid which undergoes β oxidation will always give n/2 Acetyl CoA because acetyl CoA contains 2 Carbon atoms
- Product of β oxidation of fatty acid is always acetyl-CoA molecules
- Peroxisome - the hydrogen peroxide generation due to β oxidation is peroxisome

Property	Mitochondria	Peroxisome
Fatty acids	Very short chain, short chain, medium chain and long chain fatty acids	Short chain, medium chain, long chain and Very long chain fatty acids
Type of Oxidation	β	β
Products	n/2 acetyl CoA	n/2 acetyl CoA
Energy	Yes	No

Important Information

- Defect of alpha oxidation of branched chain fatty acid in peroxisome is Refsum's disease

Q. The difference between mitochondrial oxidation and peroxisomal oxidation.

Ans.

- When oxidation happens in Mitochondria, we remove the hydrogen atom from β carbon atom and give it to NAD and FAD, which further forms NADH and FADH2.
- When NADH and FADH2 go through an electron transfer chain, they give rise to ATP.
- When oxidation happens in the peroxisome, we remove the hydrogen atom from β carbon atom and give it to the oxygen molecule.
- This then forms hydrogen peroxide (H_2O_2).
- To detoxify hydrogen peroxide, the peroxisome is equipped with catalase enzyme.

Fate of Fatty Acid in a Cell

- As soon as fatty acid gets into any cell, irrespective of the final fate of fatty acid the 1st enzyme to act on this fatty acid will be acyl CoA synthetase.
- This acyl CoA synthetase uses 1 ATP which gets converted to AMP + PPI.
- This step uses two high energy phosphate and successfully converts fatty acid into acyl CoA
- The purpose of acyl CoA synthetase is to trap fatty acid within the cell
- Fatty acids are nonpolar substances. It can easily cross a cell membrane and get into cytoplasm.
- When entered in the cytoplasm, if we don't convert the fatty acid into any another form, the fatty acid concentration inside the cell becomes higher starts getting reflex across the membrane back into the circulation

Q. Will you oxidize the fatty acid if the cell's energy status is already high?

Ans. No we will store the fatty acid in Triacylglycerol or cholesterol ester for further use.

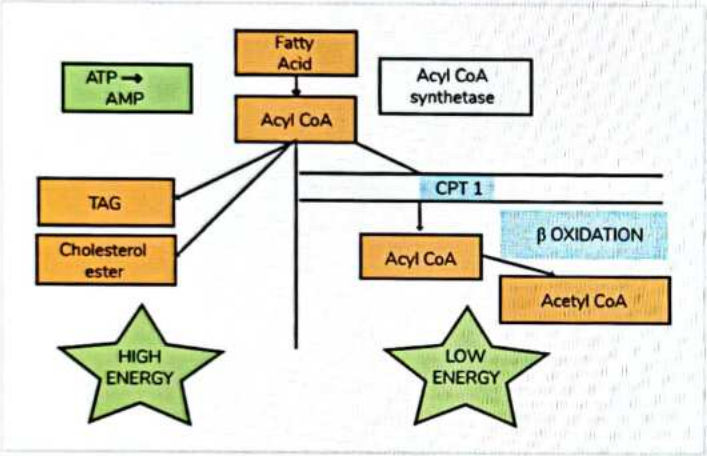
Q. Will you oxidize the fatty acid if the cell's energy status is low?

Ans. Yes, then the fatty acid must be oxidized.

Q. Which carrier Carries fatty acid across the inner mitochondrial membrane?

Ans. Carnitine

- Acyl CoA with carnitine gives acylcarnitine, and the enzyme is carnitine acyl transferase 1 (rate-limiting enzyme)
- Acyl carnitine crosses the inner mitochondrial membrane and enters the matrix.
- Once the acylcarnitine has entered the matrix if you retain the carrier the same carrier will the acylcarnitine back into cytoplasm.
- Carnitine acyl transferase 2 releases the carnitine back and converts acylcarnitine to acyl CoA.



Phases of Fatty Acid Oxidation 00:17:04

- **1st phase**: Is n carbon fatty acids goes under β oxidation to form n/2 acetyl CoA
- **2nd phase**: Every acetyl CoA Enters into citric acid Cycle every acetyl CoA molecule will come out as carbon dioxide, and every acetyl CoA will give you 10 ATPs.

Steps of Phase I

Details of Phase 1 - Every Cycle

Enzymes involved	Energetics	Product
Acyl CoA Dehydrogenase (co- enzyme: FAD) Hydratase β Hydroxyacyl CoA dehydrogenase (co- enzyme: NAD) Thiolase	4 ATPs	Acetyl CoA

- n carbon atoms containing fatty acids on β oxidation gives you n/2 acetyl CoA.
- For n/2 acetyl CoA, we should go for (n/2 cycles - 1)

Energetics of Complete Oxidation of Fatty Acids 00:33:00

- Formula for Total ATP is
  $= \{(n/2-1)\times 4\} + \{(n/2)\times 10\}$
- Formula for Net ATP
  $= \{(n/2-1)\times 4\} + \{(n/2)\times 10\} - 2$
- To convert the fatty acid into acyl CoA we use two high energy phosphates.

Example

Q. Total and Net ATPs in Palmitic acid (16c)

Solution

- Total ATP $= \{(n/2-1)\times 4\} + \{(n/2)\times 10\}$
  $= 7\times 4 + 8\times 10$
  $= 108$ ATPs
- Net ATP $= \{(n/2-1)\times 4\} + \{(n/2)\times 10\} - 2$
  $= \{7\times 4 + 8\times 10\} - 2$
  $= 106$ ATPs

- Palmitic acid on complete oxidation provides 106 ATPs

Q. Net ATPs in stearic acid (18 c)

Solution

- Net ATP $= \{(n/2-1)\times 4\} + \{(n/2)\times 10\} - 2$
  $= \{8\times 4 + 9\times 10\} - 2$
  $= 120$ ATPs

- Stearic acid on complete oxidation provides 120 ATPs.

Regulation of Fatty Acid Oxidation 00:37:25

- The rate limiting enzyme of fatty acid oxidation is CPT1.

Regulation of CPT1

- **Stimulators**: CPT1 gets stimulated by low energy status indicators (ADP, NAD, FAD, Acyl CoA)
- It gets stimulated by catabolic enzyme, and catabolic enzyme gets stimulated by glucagon.
- **Inhibitors**: CPT1 gets inhibited by high energy status indicators (ATP, NADH, FADH2, INSULIN, MALONYL COA)
- Malonyl CoA is an intermediate of fatty acid synthesis. if it is present in the cell, then this means that the cell is striving hard for fatty acid synthesis, which means it is rich in energy.

Q. How is carnitine acyl transferase 1 linked to increasing in blood glucose?

Ans: Gluconeogenesis increases blood glucose. Fatty acid oxidation is mandatory for gluconeogenesis, which is why glucagon stimulates carnitine acyl transferase 1.

Fatty Acid Oxidation Defects 00:43:20

Congenital

- Mutation in the genes for any of the enzymes which are involved in fatty acid oxidation.
  - Carnitine acyl transferase 1 (CPT I)
  - Carnitine acyltransferase 2 (CPT II)
  - Acyl CoA dehydrogenase
  - Hydrates
  - β hydroxy acyl CoA dehydrogenase
  - Thiolase

Acquired

- Jamaican vomiting sickness which is caused by intake of unripe Ackee fruit.
- The toxic principle which is present in unripe ackee fruit is called Hypoglycin.
- Hypoglycin acts by inhibiting medium chain acyl CoA dehydrogenase enzyme.

Fatty Acid Oxidation Defects – Features 00:46:22

- Congenital or acquired are always present with Hypoglycemia because whenever the fatty acid oxidation is defective, energy cannot be produced. Without energy, gluconeogenesis is impaired, and that causes hypoglycemia.
- Fatty acid gets oxidized to form Acetyl CoA. All the Acetyl CoA molecules go into the citric acid cycle by reacting with oxaloacetate and will give rise to 2 carbon-di-oxide.
- If the person is in starvation, all the Oxaloacetate will be used for gluconeogenesis.
- When acetyl CoA accumulates, it condenses to form ketosis.
- N no. of possibilities are for the formation of hypoglycemia in a neonate that will be accompanied by ketosis.
- If hypoglycemia is because of a fatty acid oxidation defect, there will be no Acetyl CoA and no Ketosis. Therefore, it will be nonketotic hypoglycemia.
- To support fatty acid synthesis, amino acid oxidation takes place, and wherever amino acid gets oxidized, the amino group will be released as ammonia. So, there will be access to ammonia generation in fatty acid oxidation defects, so they Represent Hyperammonemia.
- When peroxisome starts mediating beta-oxidation, they can oxidize all these fatty acids only till very short-chain fatty acid. They start meditating omega oxidation of very short chain fatty acids, forming a dicarboxylic aciduria.

Odd Chain Fatty Acid Oxidation 00:54:00

- When 7 carbon-containing fatty acids are beta oxidized then on the first Cycle, the last two carbon atoms will come out as Acetyl CoA.
- The five carbon-containing fatty acids go to the second Cycle of β oxidation, where the last two carbon atoms form acetyl CoA.
- Then the remaining three carbon atoms are called propionyl CoA.
- The majority of the products that we get are acetyl CoA molecules.
- Propionyl acid also goes into the citric acid Cycle. It manages to go into the citric acid cycle only when converted to succinyl CoA.
- Propionyl CoA and its enzyme carboxylase convert propionyl CoA to D methyl malonyl CoA. Then this gets converted to L-methyl malonyl CoA with the help of methyl malonyl CoA Racemase. Methyl malonyl mutase (B 12 dependent enzyme) converts it to succinyl CoA.
- B 12. Deficiency causes methylmalonic aciduria. The methylmalonic acid gets incorporated into myelin which causes demyelination. That's why B12 Deficiency presents with neurological manifestation.

Hypoglycemia 01:00:31

- To maintain blood glucose, we must Stimulate gluconeogenesis to support gluconeogenesis; there should be peripheral lipolysis.
- In starvation, where there is no insulin when there are excess counter-regulatory hormones, they stimulate hormone-sensitive lipase.
- During starvation, there is an increase in free fatty acid levels in the serum.
- The free fatty acid and glycerol, when in the liver, the glycerol will be used as a substrate for gluconeogenesis, and the fatty acid will be oxidized to provide the necessary energy for gluconeogenesis.
- During starvation, the Acetyl CoA produced can't go under the citric cycle because it requires oxaloacetate, but all the oxaloacetate is used to produce gluconeogenesis. Then the accumulated Acetyl CoA condenses to form ketone bodies.

Q. When are ketone bodies produced?
Ans. During starvation or hypoglycemia

Q. Why is ketosis in starvation or hypoglycemia? Give any three reasons.
Ans.

- Excessive peripheral lipolysis
- Excessive fatty acid oxidation
- Low availability of oxaloacetate

Diabetes Mellitus 01:04:06

- In diabetics, there is absolute insulin deficiency there is peripheral lipolysis. Lipolysis is inevitable by insulin.
- In diabetes, there is excessive peripheral lipolysis, leading to the formation of glycerol and fatty acids. Therefore, a person loses weight wherever there is the aggression of insulin resistance.

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- The fatty acid and glycerol, when in the liver the glycerol, will be used as a substrate for gluconeogenesis. Gluconeogenesis is possible in insulin resistance because glucagon is hyperactive, and the fatty acid will be oxidized to provide the necessary energy for gluconeogenesis. The Acetyl CoA produced can't go under the citric cycle because it requires oxaloacetate, but all the oxaloacetate is used to produce gluconeogenesis. Then the accumulated Acetyl CoA condenses to form ketone bodies
- **Steps involved in conversion of acetyl CoA to ketone bodies.**
  - Whenever acetyl CoA accumulates, two molecules of acetyl CoA condenses each other in the presence of thiolase to form Acetoacetyl CoA.
  - Then this Acetoacetyl CoA reacts with another molecule of acetyl CoA in the presence of HMG CoA synthase to form HMG CoA
  - Then the enzyme HMG CoA lyase takes of one acetyl CoA which gets recycled back, and that is how HMG CoA then it gets into first ketone body which is Acetoacetate.
  - Acetoacetate On spontaneous decarboxylation forms acetone
  - In the presence of β hydroxybutyrate dehydrogenase forms β hydroxybutyrate using 1 NADH

Q. What is the primary ketone body?
Ans. Acetoacetate

Q. What is the rate-limiting enzyme of ketone body synthesis?
Ans. HMG CoA lyase and HMG CoA Synthase

Facts About Ketone Body Synthesis 01:10:20

- Starvation or Hypoglycemia
  - Excessive peripheral lipolysis
  - Excessive fatty acid oxidation
  - Low availability of oxaloacetate
- Diabetes
- HMG CoA lyase > HMG CoA Synthase Are rate-limiting enzymes
- Acetoacetate- primary ketone body
- Ketone bodies synthesis is in Liver Mitochondria
- They get utilized in extrahepatic tissues.

Q. Why can't liver use ketone bodies?
Ans. Because it lacks an enzyme which is thiophorase.

Q. Why does ketone body synthesis?
Ans. Because acetyl CoA cannot go into the citric acid cycle.

- Odd chain fatty acid oxidation will give rise to propionyl CoA then, which will get converted into succinyl CoA then, which will go into the citric acid cycle and then it forms oxaloacetate with the help of succinyl thiokinase.
- Succinate is not formed from succinyl because succinyl thiokinase is suppressed because of high energy; thus, we require a bypass to form Succinate. We use CoA transferase, which allows succinyl CoA to react with a ketone body(acetoacetate) which forms aceto acetyl CoA.
- The enzyme thus formed is a thiophorase called succinyl CoA acetoacetate CoA transferase.

Fact About Ketone Body Utilisation 01:18:37

- Ketone body gets utilized by only extrahepatic tissues.
- Liver cannot utilize ketone body.

Summary 01:19:18

- Fatty acid gets oxidized in both Mitochondria and peroxisome.
- Formula for Net ATP
  {(n/2-1) x4} + {(n/2) x10}-2
- Ketone bodies get generated in diabetes and starvation.
- Fatty acid oxidation defect causes hypoglycemia (non ketotic) there will be hyper ammonia and dicarboxylic aciduria.
- MC cause of sudden infant death syndrome is medium chain acyl coA dehydrogenase deficiency.

One Liner 01:23:22

- Very long chain fatty acid oxidation takes place in **peroxisome**.
- Very short Chain fatty acid oxidation takes place in **mitochondria**.
- Is the rate limiting enzyme of fatty acid oxidation is **CPT I**.
- Even chain fatty acids on oxidation give rise to **acetyl CoA**.
- Odd chain Fatty acids on oxidation gives rise to **propionyl CoA**.

MCQ's 01:24:05

Q. Fatty acid oxidation occurs in
A. Mitochondria
B. Peroxisome
C. Both
D. None

Q. Very short chain fatty acid oxidation occurs in
A. Mitochondria
B. Peroxisome
C. Both
D. None

Q. Very long chain fatty acids undergo.
A. β oxidation
B. ω oxidation
C. α oxidation
D. Gamma oxidation

Q. Rate limiting enzyme of fatty acid oxidation.
A. Carnitine acyl transferase I
B. Carnitine acyltransferase II
C. Acyl CoA dehydrogenase
D. Thiolase

Q. How many cycles of β oxidation does palmitic acid go through
A. 7
B. 8
C. 9
D. 16

Q. CPT1 is activated by all,except
A. Acyl CoA
B. Malonyl CoA
C. High ADP/ATP ratio
D. Glucagon

Q. Number of ATP generated in the liver by complete oxidation of palmitate
A. 106
B. 33
C. 26
D. 16

Q. Number of ATP generated in the liver by complete oxidation of stearic acid.
A. 106
B. 120
C. 26
D. 16

Q. Ketosis is observed in diabetes because of:
A. Low availability of oxaloacetate
B. Excess oxaloacetate
C. Low energy
D. Low fatty acid oxidation

Q. Fatty acid oxidation defects present with all except:
A. Hypoglycemia
B. Ketosis
C. Hyperammonemia
D. Dicarboxylic aciduria

Case Based MCQ

01:27:07

Q. A group of children from Muzaffarpur district presented with convulsions and, following intake of litchi fruits. The medical activity informed the press that litchi fruit has a toxin, and the enzyme inhabited by the toxin are
A. β N-oxalyl L-Amino alanine, Lysyl oxidase
B. β aminopropionitrile, Lysyl oxidase
C. Methyl cyclo propionyl glycine, Acyl CoA dehydrogenase
D. Methyl cyclo propionyl glycine, beta hydroxy acyl CoA dehydrogenase

Image Based MCQ

01:31:04

Q. Identify Enzyme A and mention the organs which lacks the enzyme.

Refer Image19.1

A. Thiolase, Liver
B. Thiolase, Brain
C. Thiophorase, Liver
D. Thiophorase, Brain

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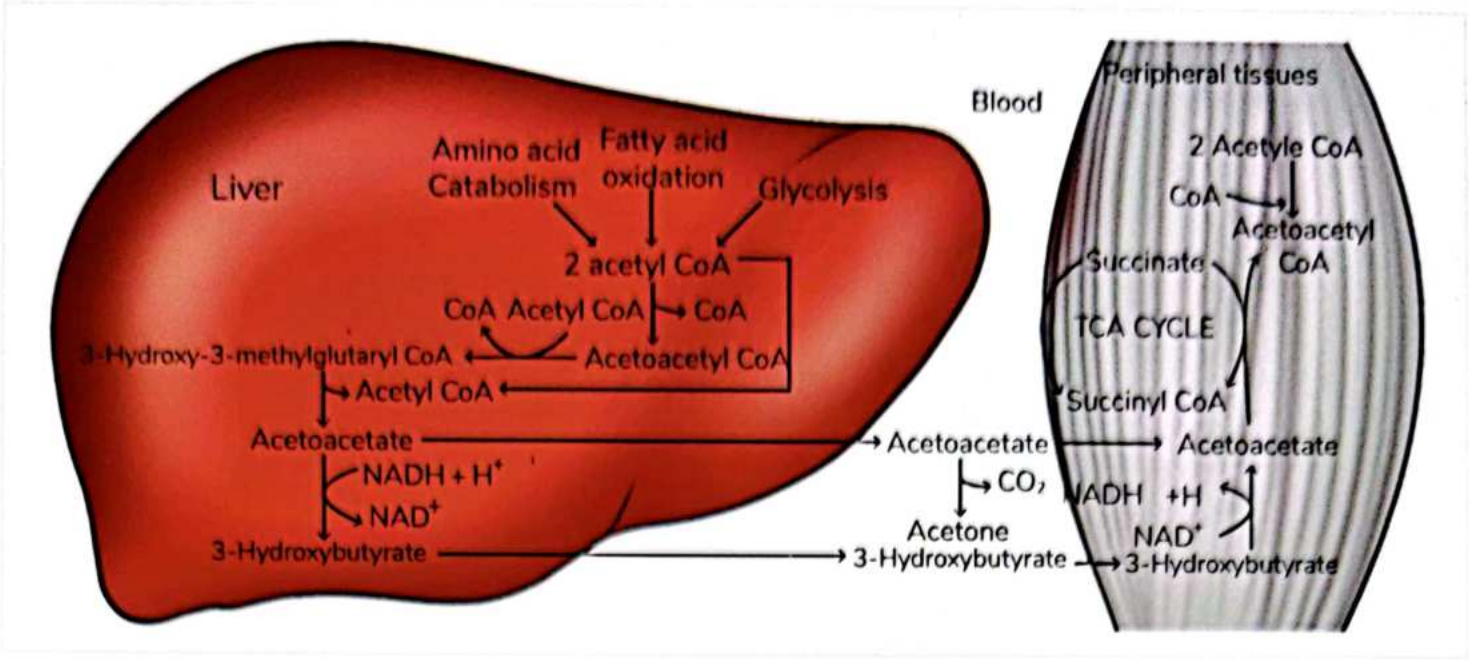
Image19.1



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20 CHOLESTEROL AND BILE ACID SYNTHESIS

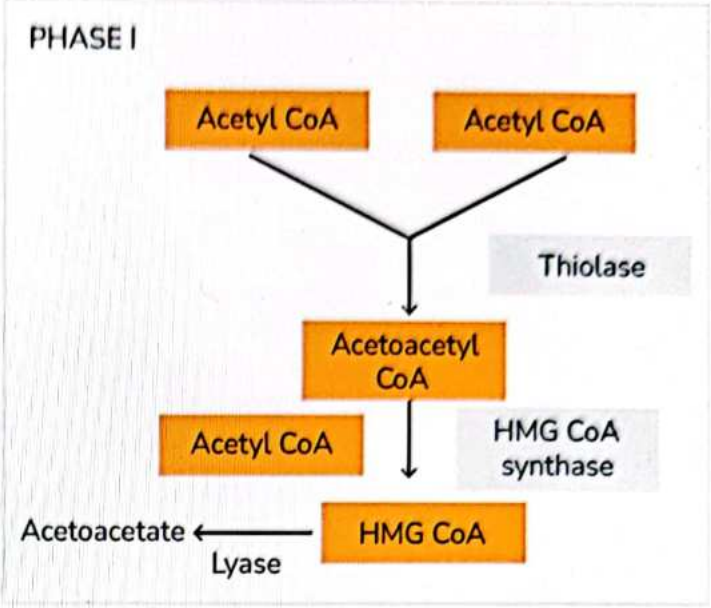
Cholesterol Synthesis Pathways

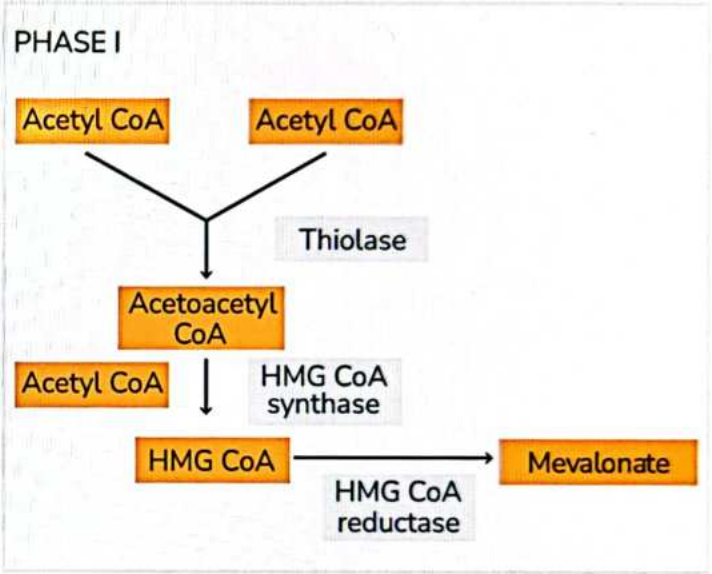
- Cholesterol is a steroid, and it is synthesized in the smooth endoplasmic reticulum.
- The rough endoplasmic reticulum is related to protein synthesis and smooth endoplasmic reticulum is related to steroid synthesis
- Cholesterol synthesis partly happens in the smooth endoplasmic reticulum and partly in the cytoplasm.
- The building block of cholesterol is Acetyl-CoA (Acetyl coenzyme A).
- Acetyl Co A is a common building block for both cholesterol and fatty acid synthesis.
- There are 5 phases for Cholesterol synthesis.

Phase I

00:02:19

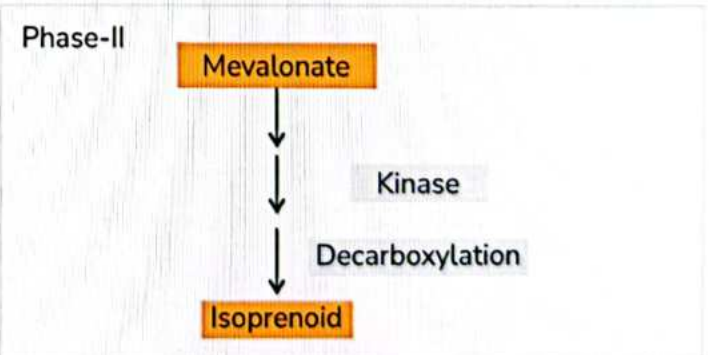
- Precursor Acetyl-CoA gets converted to Mevalonate.
- In both ketone body synthesis and cholesterol synthesis, Acetyl-CoA first gets converted into β-Hydroxy β-methylglutaryl-CoA (HMG-CoA).
  - When Acetyl-CoA gets accumulated, two molecules of Acetyl-CoA condense in the presence of Thiolase to form acetoacetyl CoA.
  - The third molecule of Acetyl-CoA condenses in the presence of HMG-CoA synthase to form HMG CoA.
  - Until the HMG CoA is formed steps involved in ketone body synthesis and cholesterol synthesis are same.
  - In ketone body synthesis, the HMG CoA gets converted to HMG CoA lyase, and this HMG CoA lyase converts HMG CoA into the first ketone body i.e., acetoacetate.
    - → HMG CoA lyase commits acetyl CoA to ketone body synthesis
    - → So it is the rate-limiting enzyme for ketone body synthesis
  - In cholesterol synthesis, the next enzyme is the HMG CoA reductase which acts on HMG CoA and converts it into Mevalonate.
    - → HMG CoA reductase commits acetyl CoA to cholesterol synthesis
    - → Therefore, HMG CoA reductase is the rate-limiting enzyme of the cholesterol synthesis pathway.
    - → NADPH acts as a hydrogen source for the reductase.
  - NADPH is a necessary co-enzyme for the reductive biosynthesis of all the lipids.





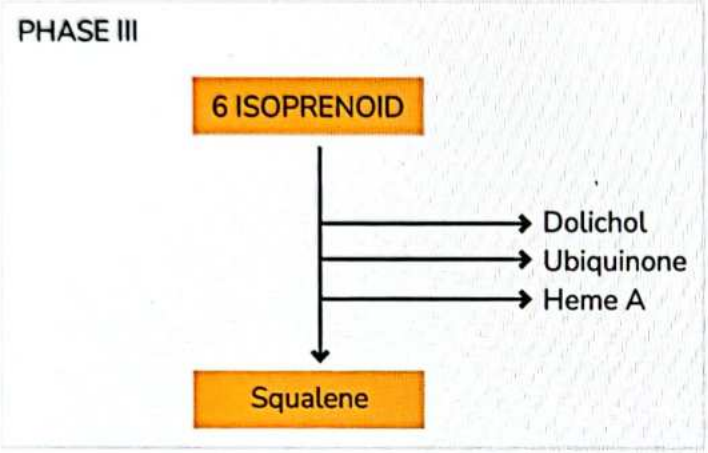
Phase II

- Mevalonate undergoes three kinase reactions or phosphorylation thrice in the presence of kinase which is followed by Decarboxylation to form Isoprenoid units.



Phase III

- Six isoprenoid units condense together to form Squalene.
  - During this conversion, there are few byproducts
    - → D – Dolichol
      - – Related to glycoprotein synthesis
      - – Property of endoplasmic reticulum membrane
    - → U – Ubiquinone
      - – Also called Complex Q
      - – It is one of the mobile complexes of the electron transport chain
      - – Ubiquinone is the ubiquitin proteosome pathway involved in protein degradation (short lived proteins).
    - → H - Heme A



Phase IV

- Squalene epoxidase
  - It is present only in the endoplasmic reticulum.
  - After squalene formation which happens in the cytoplasm, it gets into the endoplasmic reticulum.
- In the endoplasmic reticulum, Squalene epoxidase acts on Squalene to convert it into Lanosterol.

Phase V

- Lanosterol → Zymosterol → Desmosterol → Cholesterol
- The HMG CoA reductase is inhibited by statins; therefore, statins are recommended to reduce the blood cholesterol level.
- HMG CoA reductase exhibit diurnal variation.
- Statins result in myopathy because it inhibits HMG CoA reductase, and without mevalonate, the squalene cannot be synthesised hence the by-product of cholesterol synthesis cannot be formed.
- In the presence of statin, Ubiquinone cannot be synthesised which is mobile complexes of the electron transport chain.
- Thus, oxidative phosphorylation does not happen in muscle which is indispensable for the working of muscle.
- This is one of the causes of myopathy in the presence of statins.

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- So, without Ubiquinone, without oxidative phosphorylation muscle gets affected there in statin prescription.

Regulation of Cholesterol Synthesis 00:09:56

- Rate limiting enzyme of cholesterol synthesis is HMG CoA reductase
- Every enzyme gets regulated by one of the three ways -
  - Allosteric regulation
  - Covalent modification
  - Induction or repression

Allosteric Regulation

- It is products inhibiting an enzyme.
- HMG CoA reductase is inhibited by all its product i.e., it gets inhibited by
  - Its immediate product i.e., Mevalonate
  - Its final product - cholesterol
  - By the byproduct of its product - bile acid (synthesised from cholesterol which undergoes hydroxylation and is converted into bile acid)

Covalent Modification

- Enzymes get regulated by Covalent modification, either by phosphorylation or dephosphorylation.
  - To find out whether an enzyme gets activated by phosphorylation or dephosphorylation, the effect of insulin on that enzyme should be understood.
- HMG CoA reductase is an anabolic enzyme.
  - And all anabolic enzymes get stimulated by insulin or glucagon
- Insulin acts on anabolic enzyme by Dephosphorylation
  - Insulin acts through a phosphatase.

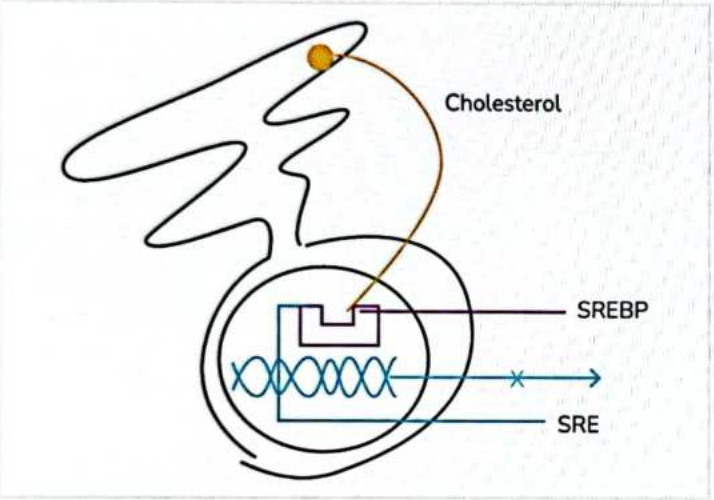
Important Information

- If an enzyme decreases blood glucose, it is stimulated by insulin by Dephosphorylation.
- If it is an anabolic enzyme, it is stimulated by insulin by Dephosphorylation.

Induction or Repression

- Allosteric regulation and Covalent modification will modify an enzyme's activity by acting at the protein level.
- Whereas induction and repression act at the transcription level.
  - Anything that can increase the number of times of transcription of a gene, increasing the number of mRNA produced and increasing the number of enzyme molecules that get synthesised out of them is called induction and the opposite is that is repression.
- HMG CoA reductase is chiefly regulated by repression through cholesterol.

- When an enzyme's product is a steroid (like cholesterol), then it is chiefly regulated by repression.
  - These steroids are synthesised in the smooth endoplasmic reticulum E.g., cholesterol is synthesised in the smooth endoplasmic reticulum then it crosses all the membranes (endoplasmic and nuclear membrane) and gets into the nucleus.
  - In the nucleus, it binds to steroid response element binding protein
    → The steroid response element is a sequence.
  - Within the genes present in chromosome in nucleus, one of the genes is HMG CoA reductase and steroid response element is present near to it.
  - This protein binds with the steroid response element following which the HMG CoA reductase gene will not be transcribed.
    → This prevents further cholesterol
- HMG CoA reductase gets chiefly regulated by repression through cholesterol through steroid response element binding protein.



- HMG CoA reductase exhibits diurnal variation.
  - It is active in the night.
    → That is why short-acting statins are prescribed at night.
  - To have a better effect, enzyme should be inhibited when it is active.
  - And HMG CoA reductase is active at night and that is when it is inhibited effectively.
  - This is why one should have a dinner like a pauper (minimal diet) and breakfast like a price/princess.
  - If one eats heavy dinner, every carbohydrate that is present in diet will enter glycolysis and all these carbohydrate gets converted to pyruvate which then gets converted to Acetyl CoA.
  - When Acetyl CoA is formed at night it directs HMG CoA reductase to be active at night.
  - Hence, every Acetyl CoA formed will be diverted to cholesterol synthesis causing hypercholesterolemia.

Bile Acid Synthesis

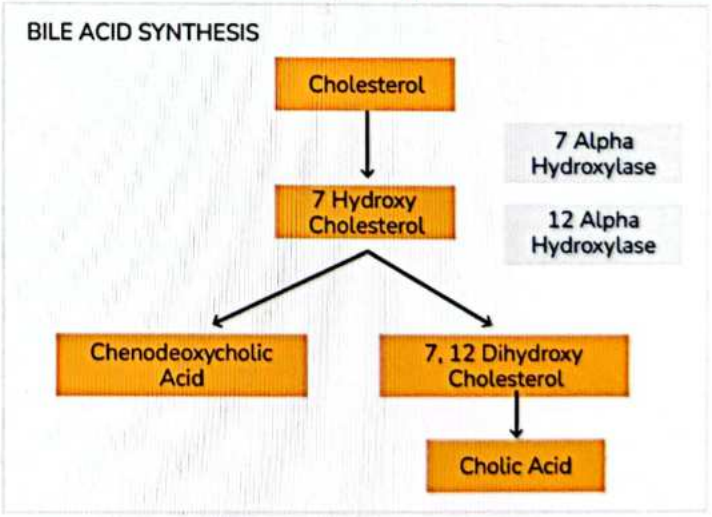
00:18:46

- Bile acids become bile salts, and these help in fat digestion and absorption.
- Bile acids are formed from cholesterol, and thus, it is also a steroid.
- Cholesterol undergoes hydroxylation, and it undergoes modification to form all these bile acids.
- The first enzyme to begin the conversion is 7 alpha-hydroxylase.
- The first step in bile acid synthesis is catalysed by 7 alpha-hydroxylase which converts cholesterol to 7 hydroxy cholesterol.
  - If 7 hydroxy cholesterol goes into modification immediately in the side chain at position 17 (after hydroxylation) it forms the first bile acid called chenodeoxycholic acid.

Important Information

- Structure of cholesterol is cyclopentane per hydro phenanthrene ring.
  - This ring has a side chain in position 17.

- The 7 hydroxy cholesterol goes another hydroxylation at the 12th position by the 7 alpha-hydroxylase and gets converted in the 7,12 Dihydroxy cholesterol.
  - Then 7,12 Dihydroxy cholesterol undergoes modification in the side chain at position 17 it gives Cholic acid (2nd bile acid)
- Primary bile acid
  - Obtained directly from cholesterol
    → Chenodeoxycholic acid
    → Cholic acid
- Both Chenodeoxycholic acid and Cholic acid undergo conjugation with glycine and taurine in a ratio of 3:1.
- Such glycine and taurine conjugated Chenodeoxycholic acid and Cholic acid are called primary bile acids.

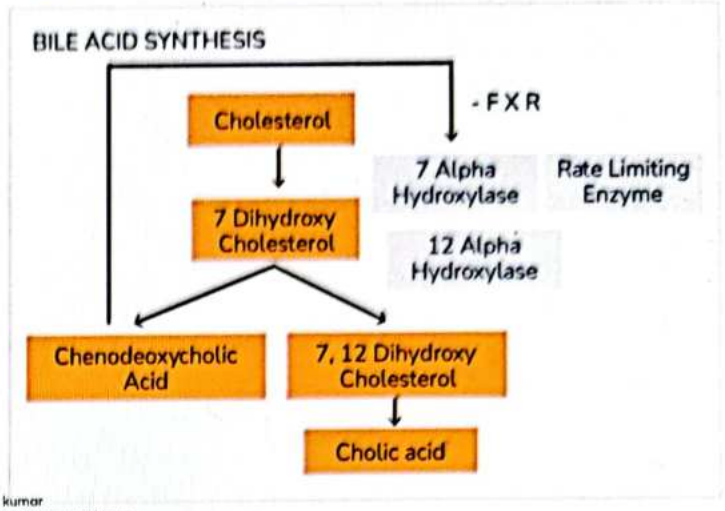


- When the bile acids are added to bile whose pH is alkaline.

- These acids will try to neutralise the alkaline pH in the medium for which they give off their H^+ and become negatively charged.
- Thus they form salts with sodium and potassium.

- Sodium and potassium salts of glycine and taurine conjugated Chenodeoxycholic acid, and Cholic acid are called bile salts.
- All these bile components (bile acids and salts) are present in the bile and this bile reach the second part of the duodenum after which it proceeds further to reach the colon.
- The microorganism in colon (colonic microorganism) act on these components
  - First, they deconjugate them (glycine and taurine conjugation are removed) and then these undergo dehydroxylation at the 7th position.
- Deconjugated and Dehydroxylated primary bile acids are called secondary bile acids.
- Coholic acid is converted into deoxycholic acid
- And Chenodeoxycholic acid gets converted to lithocholic acid.
- Then these bile components undergo enterohepatic circulation
  - These bile components are present in the colon
  - From colon they get absorbed into one of the radicals of veins
  - Finally they combine to form the portal vein
  - Through this vein, they reach the liver
  - In the liver, the portal vein breaks into hepatic sinusoids.
  - From hepatic sinusoids, they are taken up by hepatocytes
- From hepatocytes, they are dropped into intrahepatic bile ductule
  - Then they get into the
- Extrahepatic bile ductule → Hepatic ducts → Common bile duct.
  - And Through the common bile duct it manages to get into the duodenum again.
- The enterohepatic circulation helps in maintaining the bile salt pool size
  - It is maintained as bile salts are essential for lipid digestion and absorption
- But in the presence of microorganisms, these microorganisms try to convert all these primary bile components into secondary bile components.
- Out of these secondary bile components, Lithocholic acid cannot enter the enterohepatic circulation.
  - In the case of blind loop syndrome, most of the primary bile acid becomes secondary bile acid and one of the secondary bile acid is lithocholic acid which cannot enter into enterohepatic circulation and gets lost in the faeces.
  - Thus, it reduces the bile salt pool size due to which lipid digestion absorption gets affected causing steatorrhea.

Regulation of Bile Acid Synthesis 00:26:39



- The rate limit enzyme of bile acid synthesis is 7 Alpha hydroxylase.
  - It commits cholesterol to bile acid synthesis.
  - 7 Alpha hydroxylase gets chiefly regulated by the first bile acid that is synthesised i,e, chenodeoxycholic acid (by repression as these are steroid)
  - Chenodeoxycholic acid is a chief negative regulator for 7 Alpha hydroxylase.
  - Chenodeoxycholic acid represses 7 Alpha hydroxylase by acting through the Farnesoid X receptor.
  - Chenodeoxycholic acid also gets synthesized in smooth endoplasmic reticulum from where it crosses all the membrane and gets into the nucleus where it binds with Farnesoid X receptor.
  - This Farnesoid X receptor represses 7 Alpha hydroxylase.

Facts about Bile acid synthesis

- Bile acids are by product of cholesterol.
- The rate limit enzyme of bile acid synthesis is 7 Alpha hydroxylase
- This enzyme gets chiefly regulated by chenodeoxycholic acid acting through the Farnesoid X receptor.
- Primary bile acids
  - Chenodeoxycholic acid
  - Cholic acid
- Secondary bile acids
  - Deoxycholic acid
  - Lithocholic acid.
- Colonic microorganisms Deconjugate them and Dehydroxylate primary bile acids to form secondary bile acids
- Lithocholic acid cannot enter into the enterohepatic circulation
  - Blind loop syndrome results in steatorrhea/fat malabsorption.

One Liner

Q. Rate limiting enzyme of cholesterol synthesis is

_____

Ans. HMG CoA reductase

Q. Rate limiting enzyme of bile acid synthesis is

_____

Ans. 7 Alpha hydroxylase

Q. HMG CoA reductase is chiefly regulated by _____
Ans. Repression

Q. the chief regulator of 7 Alpha hydroxylase is _____
Ans. Chenodeoxycholic acid

MCQs

Q. Cholesterol synthesis takes place in
A. Cytoplasm
B. Nucleus
C. Endoplasmic reticulum
D. a&c

Q. Statins act by inhibiting:
A. HMG CoA reductase
B. HMG CoA Lyase
C. Acetyl CoA carboxylase
D. 7 alpha-hydroxylase

Description:

- HMG CoA Lyase is the rate-limiting enzyme of ketone body synthesis
- Acetyl CoA carboxylase is the rate-limiting enzyme of fatty acid synthesis
- 7 alpha-hydroxylase is the rate-limiting enzyme of bile acid synthesis

Q. All the following are byproducts of cholesterol synthesis except
A. Dolichol
B. Ubiquitin
C. Heme A
D. Cholesterol

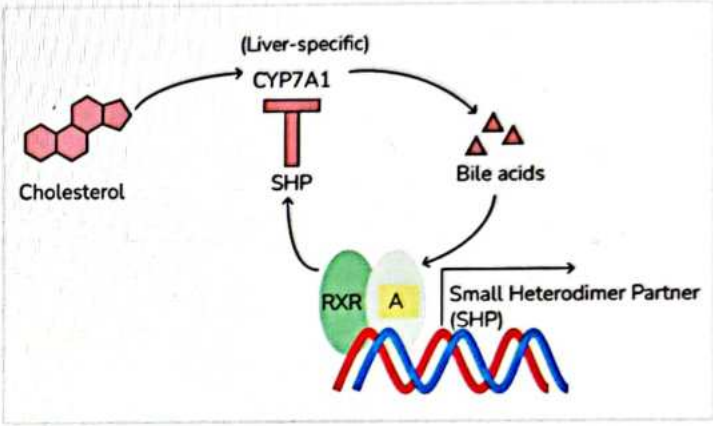
- It is ubiquinol
- Mnemonic – 'DUH'
- D – Dolichol
- U – Ubiquinone
- H – Heme A

Q. Primary bile acids are all except:
A. Cholic acid
B. Glycocholate
C. Chenodeoxycholic acid
D. Lithocholic

Q. A patient with a family history of coronary artery disease and high cholesterol is prescribed statins. He complains of myopathy. The possible explanation for the same is?
A. Cholesterol is necessary for normal muscle metabolism
B. Hypocholesterolemia causes myopathy
C. Ubiquinone is a part of electron transport chain
D. Dolichol is a part of Electron Transport Chain

Image-based MCQ

Q. Identify the receptor A in the image, which is capable of inhibiting 7 alpha-hydroxylase?



A. Retinoic Acid A receptor
B. Retinoic Acid X receptor
C. Steroid Response element
D. Farnesoid X rec

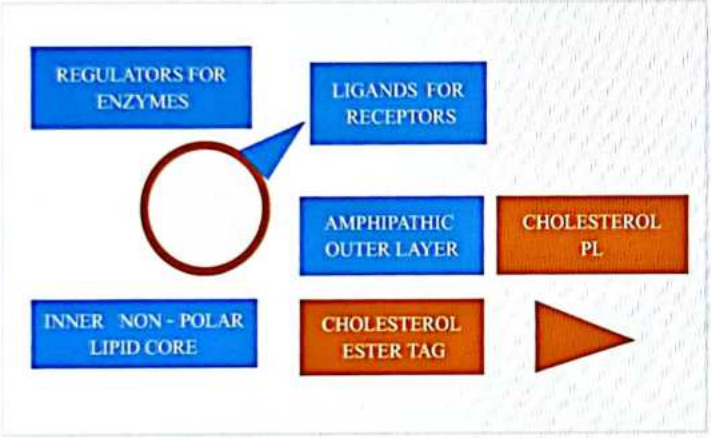
21 LIPOPROTEIN METABOLISM

Functions of Lipoprotein

- Lipoproteins help lipids to transport in the blood.

Q. Do we want a special transport system for lipids alone?

- Lipids are nonpolar and immiscible in blood.
- Lipids float in blood and coalesce to form very large drops.
- Thereby, these large drops clog to small capillaries
- Amphipathic substance is used to transport any non-polar substance in a polar medium.
- Amphipathic substances contain both polar and nonpolar groups.
- Polar groups face to the exterior and help to be miscible in the polar medium.
- While nonpolar groups face the interior and help in holding the non-polar lipids within themselves.



- Lipid particle is attached to the apoprotein.
- The lipid part of the lipoprotein will have an Amphipathic outer layer and encloses a nonpolar lipid core in the centre.
- Cholesterol and phospholipids are the examples of Amphipathic lipids.
- Cholesterol has a polar OH group and complex non-steroidal structure.
- The Phosphate group in phospholipids is negatively charged, the charged part is considered polar.
- Cholesterol and phospholipids will be on the outer portion whereas the inner portion is covered with cholesterol ester and Triacylglycerol.

Q. What are the functions of apoproteins?

Ans. They act as ligands for receptors.

Example

- Apo E which is accepted by remnant receptors.
- ApoB 100 is accepted by LDL receptors.

Important Information

- Remnant receptors are very specific and accept only lipoproteins with apoE as an apoprotein.
- LDL receptors accept either apo E or apo B100 as apoprotein.

Functions of Apoproteins

- They act as regulators for the enzymes which are concerned with metabolism.
- For example, apo c2 activates lipoprotein lipase and apo a1 activates LCAT.
- Apo c3 inhibits lipoprotein lipase.

Functions of Chylomicrons

- Chylomicrons carry exogenous or dietary triacylglycerols from the intestine to extrahepatic tissues.
- It can't get to the liver due to its larger size and is drained by lymphatics.
- From the lymphatics it gets entered to the thoracic duct, then to the left subclavian vein and finally to the systemic circulation.

Metabolism of Chylomicrons

Chylomicrons are formed in enterocytes.

↓

Apo B48 is attached to the Chylomicrons and called as nascent chylomicron.

↓

After acquiring Apo c2 and Apo E from HDL, nascent Chylomicron gets converted to functional chylomicron.

Major Functions of HDL

- Reverse cholesterol transport as it collects phospholipids and cholesterol ester from extrahepatic tissues and dumps it in the liver.
- It also acts as a repository for all the apoprotein and helps in recycling.
- Lipoprotein lipase is clinging to the vessel wall held in place with heparin sulphate (present in large aortic valves).
- Lipoprotein lipase acts on chylomicrons triacylglycerol to produce fatty acid and glycerol.
- Fatty acid crosses vessel wall endothelium and membranes of extrahepatic tissues.
- Liver is the organ which can use glycerol because liver has glycerol 3 kinase.

- Chylomicron never retains its apo c2 and gives back to HDL.
- All remnants together reach the liver, gets accepted by either LDL or remnant receptors.

VLDL Metabolism

- VLDL transports endogenous triacylglycerols that are synthesised by the liver.
- VLDL is formed in liver or hepatocytes.
- VLDL will be released into circulation and will have apoproteins like Apo B100, ApoC2 and Apo E.
- So, it is called nascent VLDL that is inactive.
- With the help of HDL, inactive apo CII can be converted to active apo CII. Apo CII activate lipoprotein lipase. Lipoprotein lipase acts on the triacylglycerol of VLDL , which is converted to glycerol and fatty acid.
- VLDL becomes a VLDL remnant if it gives back apoC2 immediately to HDL and contains leftover triacylglycerols.

Fate of VLDL

- If VLDL loses its apoCII immediately to HDL, it is called as VLDL remanant.
- The VLDL remnant contains apoB100 and Apo E.
- If VLDL does not give back apo CII immediately, it undergoes circulation and stimulates lipoprotein lipase and then it returns apo CII to HDL.
- Then the size gets reduced, and the density gets increased.
- Now, it gets converted to IDL.

Fate of IDL

- The apoproteins present in IDL are apoB100 and Apo E
- IDL reaches the liver after being accepted by a remnant or LDL receptor.
- Alternatively, if IDL comes across HDL, which is rich in cholesterol ester, two exchange reactions occur.
  - Cholesterol ester transfer protein transfers all cholesterol esters from HDL to IDL.
  - IDL gives back Apo E to HDL.
- Now it is rich in cholesterol ester and called LDL.

Fate of LDL

- LDL gets either accepted by LDL receptors of the liver or by LDL receptors of extra hepatic tissues.

Q. Why do we call HDL as good cholesterol and LDL as bad cholesterol?

Ans. HDL reverses atherosclerotic changes in extrahepatic tissues as it collects phospholipids and cholesterol ester from extrahepatic tissues and dumps it in the liver.

- LDL collects all cholesterol ester from HDL and it goes back to extrahepatic tissues and causes atherosclerotic changes.

Example

- Dyslipidemia Patient has very low HDL and very high LDL
- The protein that can be inhibited for reversing dyslipidemia is Cholesterol ester transfer protein (CETP).
- Cholesterol ester transfer protein transfers cholesterol ester from HDL to LDL.
- If we inhibit CETP: HDL level increases and LDL level decrease.
- E.g., Tolcetrapib (inhibits CETP and withdrawn from market because of side effect.)

HDL Metabolism

- HDL is formed in both enterocytes and hepatocytes.
- Liver HDL has its own apoproteins, but intestinal HDL gets its apoproteins from the liver.
- After HDL gets released into circulation, all the HDL particles will have only cholesterol and phospholipids.
- Thereby gets attached to Apo A1
- Both cholesterol and phospholipids are Amphipathic, thus can't form a core and hence the HDL takes a discoidal shape.
- Apo A1 activates LCAT which transfers acyl group or fatty acid from lecithin to cholesterol.
- When fatty acid gets attached to cholesterol, it becomes cholesterol ester which is a nonpolar lipid.
- This will form a central core and get surrounded by phospholipid and cholesterol.
- Finally, the discoidal HDL will get converted to spheroidal HDL.
- Therefore, LCAT converts the discoidal HDL to spheroidal HDL3.

Functions of spheroidal HDL3

- The apoprotein present is apo A1.
- Apo A1 activates ABC1, an enzyme present along the membranes of extra hepatic tissues.
- ABC1 collects cholesterol ester and phospholipids from all extra hepatic tissue membranes by using ATPs.
- Then it adds to HDL3 and their size gets increased, density gets decreased.
- This large HDL is called HDL2.

Fate of HDL2

- It reaches the liver and gets bind with scavenger receptor B1 which has hepatic lipase activity.
- It extracts the contents of HDL2.

- Therefore, the size gets decreased.
- When other remnants reach the liver, they bind with a receptor and the cell engulfs the whole remnant's lipoprotein.
- This is how the metabolism occurs.
- Coming to HDL, only some of the content gets emptied and it forms again as HDL3.

Intermediate Stage in Between the Conversion of HDL2 to HDL3

- During the conversion of HDL2 to HDL3, there is an intermediate stage with excess memnrane and the membrane gets pinched off as pre Beta HDL.
- Membrane is made up of cholesterol, phospholipids, and other lipoproteins.
- This gets attached to apo A1. This structure is called as pre beta HDL.
- Pre beta HDL becomes discoidal HDL apo A1 and activates LCAT.
- It converts cholesterol into cholesterol ester which forms spheroidal core.
- This makes discoidal HDL to spheroidal HDL3.
- It activates ABC1 and gets converted to HDL2, reaches the liver.

Causes of hypoalphalipoproteinemia

- Low HDL concentrations lead to hypo alpha proteinemia.
- LCAT deficiency leads to low HDL concentrations.
- Lipoprotein X is the feature of LCAT deficiency and obstructive jaundice formed due to the accumulation of discoidal HDL.
- Bile Salts are Amphipathic and take lamellar structures, looks like lipoprotein X

Clinical Features of LCAT Deficiency

There are 2 forms of LCAT deficiency.

- **Partial LCAT Deficiency** - Causes fish eye disease
- **Complete LCAT Deficiency** - Causes fisheye disease along with CKD (because of accumulation of lipoprotein X in mesangium), early myocardial infarction and haemolytic anaemia.

ABC a1 Mutation

- Causes tangier's disease - greyish orange tonsils, hepatosplenomegaly, mono neuritis multiplex.

One Liners

1. Chylomicron transports **exogenous/dietary triacylglycerols** from intestine to **extra hepatic tissues**.
2. Lipoprotein lipase is activated by **Apo C2**
3. Lipoprotein lipase is inhibited by **Apo C3**
4. LCAT is activated by **Apo A1**
5. The most active form of HDL is **pre beta HDL**

MCQs

Q. The main function of lipoprotein is to
A. Activate fatty acid synthesis.
B. Transport lipids to kidney for excretion
C. Stimulate lipolysis.
D. Transport lipids in blood between tissues

Q. Apo A1 activates.
A. Lipoprotein lipase
B. Lecithin Cholesterol Acyl Transferase
C. Hormone Sensitive Lipase
D. LRP

Q. Apo C2 activates.
A. Lipoprotein Lipase
B. Lecithin Cholesterol Acyl Transferase
C. Hormone Sensitive Lipase
D. LRP

Q. Chylomicron transports triacylglycerol from intestine to
A. Liver
B. Kidney
C. Extrahepatic tissues
D. Brain

Q. The apoprotein which activates Lipoprotein lipase is
A. Apo B48
B. Apo B100
C. Apo C2
D. Apo A1

Q. The apoprotein present in nascent chylomicron is
A. Apo B100
B. Apo B48
C. Apo C2
D. Apo E

Q. True regarding chylomicron are all except:
A. HDL is involved in activation of chylomicron.
B. LPL helps in the conversion on chylomicron to chylomicron remnant
C. Chylomicron remnant reaches the Extrahepatic tissues.
D. Chylomicron reaches the Extrahepatic tissues.

Q. Remnant receptor accepts lipoproteins with which of the following apoproteins?
A. Apo E

B. Apo B100
C. Both
D. None

Q. LDL receptor accepts lipoproteins with which of the following apoproteins?
A. Apo E
B. Apo B100
C. Both
D. None

Q. Which of the following helps in the conversion of discoidal to spheroidal HDL?
A. LPL
B. LCAT
C. ABCA 1
D. CETP

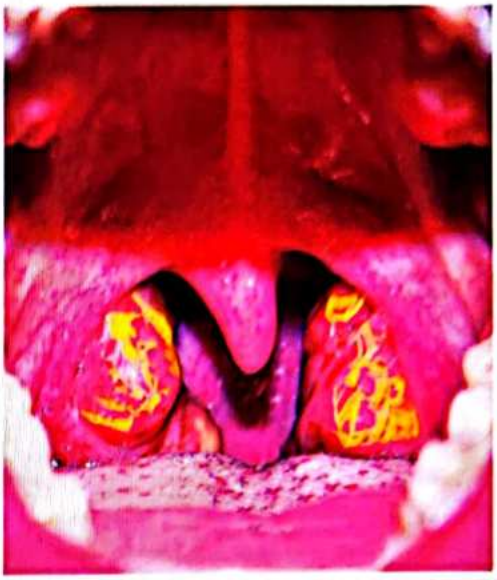
Q. Regarding HDL all are true, except:
A. HDL is synthesised by liver and intestine.
B. Pre beta HDL is the most active form
C. Mutation in ABC-1 cause Tangier's disease.
D. Both liver and intestine HDL have Apo C and Apo E

Case Based MCQs

Q. A 45-year-old man with a family history of coronary artery disease wants his master health check-up done. His biochemical values show low HDL and high LDL. Inhibition of which of the following proteins can theoretically cause a reverse of this dislipidemia
A. LPL
B. LCAT
C. ABCA1
D. CETP

Image Based MCQs

Q. Mutation of which of the following proteins can cause the characteristic tonsil shown in the image.



A. LCAT
B. Apo C2
C. ABCA1
D. Apo C3

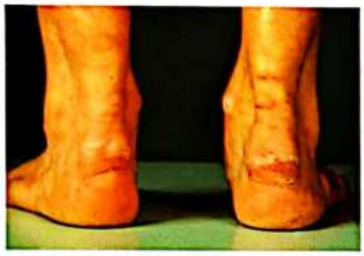
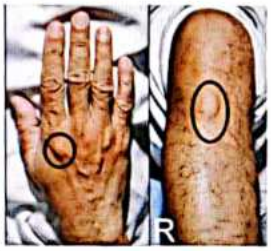
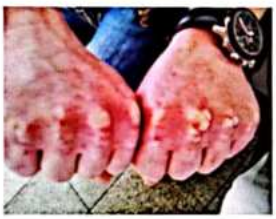
22 HYPERLIPOPROTEINEMIA & FATTY LIVER

Freidrickson's classification 00:00:30

- Six types
  - Type I
  - Type IIa
  - Type IIb
  - Type III
  - Type IV
  - Type V
- Categories
  - Hypercholesterolemia
  - Hypertriglyceridemia
  - Both

Hypercholesterolemia presents as 00:01:30

- Tendon xanthoma
- Accelerated atherosclerosis

Hypertriglyceridemia presents as

- Eruptive xanthomas
- Recurrent pancreatitis
- Lipemia retinalis

- Eruptive Xanthomas and phrynoderma are tiny papular lesions present on the extensor surfeces of limb. However, Phrynoderma can be differentiated from eruptive xanthomas by the presence of keratin plug in the tip, as phrynoderma is follicular hyperkeratosis and is caused by essential fatty acid deficiency or by Vitamin A deficiency
  - Vit A deficiency
  - Essential fatty acid deficiency
- Type IIa (only cholesterol elevated)
  - Familial hypercholesterolemia
  - Autosomal Dominant disorder with gene dose effect
  - LDL receptor defect

Important Information

- Gene dose effect
  - One allele effected: Milder
  - Both allele effected: Severe

CATEGORIES OF HYPERLIPOPROTEINEMIAS:

I. Isolated elevation of cholesterol : Type IIa
2. Isolated elevation of triglyceride: Type I, IV, V
3. Elevation of both cholesterol and triglyceride: Type IIb and Type III

Type I Hyperlipoproteinemia 00:08:02

- Familial chylomicronemia syndrome
  - Apo C II or LPL defect
  - Isolated elevation of TGL
- Eruptive xanthoma and recurrent pancreatitis
- LPL activity is low

LPL activity estimation

- Post heparinised blood sample
- If LPL activity is low
- Apo C II defect or LPL defect
  - Mixing study
    - → In Patient's pot heparinised plasma, add equal amount of pooled normal plasma (apo C II)
    - → If it normalises its is apo C II defect and it will respond to FFP administration.

Type IV and Type V Hyperlipoproteinemia's 00:17:45

- Familial Hypertriglyceridemia
- Autosomal Dominant
  - Type IIa/ FHC, IV and V
- Increase in the rate of VLDL synthesis and decrease in the rate of VLDL catabolism
- Type IV is just the genetic defect
- Type V is a combination of a primary defect and secondary

hypertriglyceridemia

Secondary causes of Hypertriglyceridemia

- Obesity
- DM
- Nephrotic syndrome
- Von Gierke's disease

Type IIb Hyperliproproteinemia 00:20:17

- Familial combined Hyperlipidemia
  - Increase in VLDL synthesis: ↑ TGL
  - Small LDL particles.

Type III Hyperlipoproteinemia 00:22:45

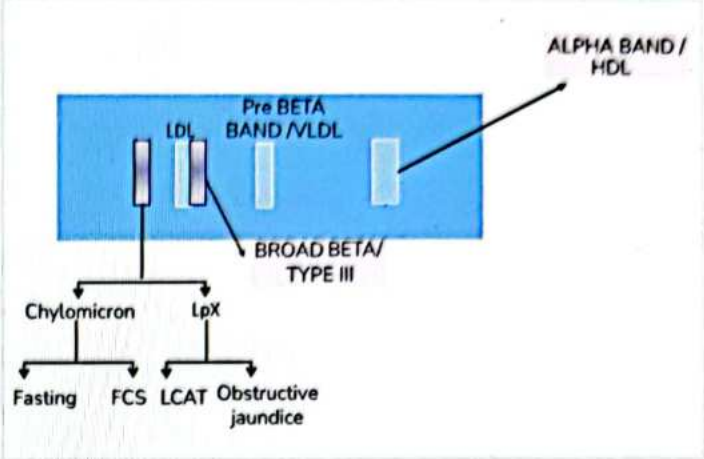
- Apo E defect
- Remnant disease
- Elevation of Both cholesterol and TGL
- Broad Beta disease
- Familial Dysbetalipoproteinemia
- Caused by Homozygous E2 mutation.

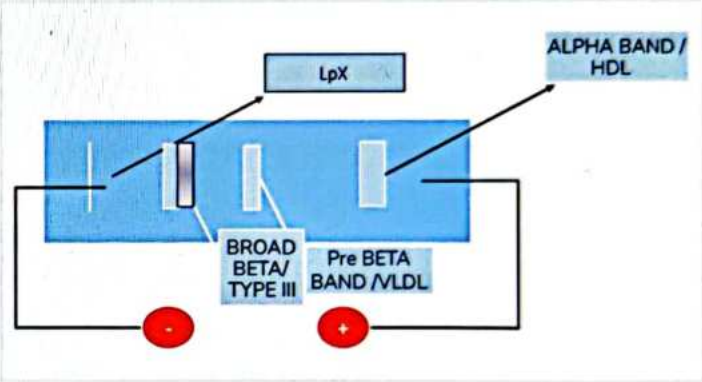
Lipoprotein electrophoresis 00:24:53

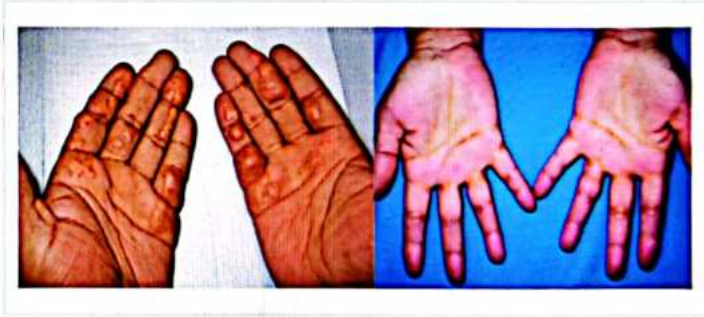
- Lipoprotein electrophoresis is performed on a glass slide with agarose as the support medium
- The electrophoretic tank is filled with an alkaline buffer to provide negative charges to all the lipoproteins
- Close to the point of application is connected to a negatively charged electrode and the opposite side is connected to positively charged electrode
- When the electric current is switched on, all lipoproteins start moving towards positively charged electrode and the migration is affected by two factors
  - Size
  - Charge
- The band that moves the farthest is the alpha band which has HDL, as HDL has the highest phospholipid and protein content
- Chylomicron has the largest size and hence stays at the point of application, if at all it is present. As a fasting sample is run for lipoprotein electrophoresis, no band is seen at the point of application. If a band is seen at the point of application, it is LpX, caused by LCAT deficiency or Obstructive jaundice
- The band nect to chylomicron band is the beta band, made up of LDL
- The lipoprotein which moves ahead of LDL is the VLDL and it forms the prebeta band
- Remnants migration is in such a way that it forms a band close to the beta band but in a normal individual, remnant concentration is so negligible that it is not seen. In type III hyperlipoproteinemia, remnants concentration increases and they form a band close to the beta band making the beta band broader. Hence, the name broad beta disease

Important Information

- CKD in LCAT deficiency due to accumulation of LpX in the mesangium







- Dermatological pathognomic features of Type III Hyperlipoproteinemia
  - Eruptive xanthomas in Palm
  - Xanthoma palmaris striae

Fatty Liver 00:33:25

Fatty liver is defined as fat accumulation in the liver. It caused by three possibilities:

I. Increased input of lipid into the liver:
This is possible by increased peripheral lipolysis. Peripheral lipolysis is caused by Hormone sensitive lipase. Hormone sensitive lipase is inhibited by Insulin. So whenever there is low insulin or low insulin activity, there is high

peripheral lipolysis and this causes fatty liver. Eg., Starvation and Diabetes Mellitus

II. Increased synthesis of lipid in the liver:
This happens in the presence of anabolism. Eg., chronic alcoholism, Obesity

III. Decreased output of lipid from the liver:
Lipid is secreted from liver in the form of VLDL. So any condition which is associated with decreased VLDL synthesis or VLDL organisation, will result in fatty liver. Eg., Choline deficieny interferes with outer amphipathic layer formation, essential fatty acid deficiency interferes with inner non polar lipid core formation, Carbon tetrachloride poisoning or puromycin interferes with apoprotein synthesis, orotic aciduria interferes with lipoprotein organisation, as it damages golgi complex.

One Liners 00:47:00

- The hyperlipoproteinemia which presents with only an elevation of cholesterol is Type IIa.
- The hyperlipoproteinemias which present with only an elevation of triglycerides are Type I, Type IV Type V
- Lipoprotein X is a feature of Obstructive jaundice and LCAT deficiency
- Brood beta band in Lipoprotein electrophoresis is a feature of Apo E defect

Q. Hypertriglyceridemia is seen in all, except:
A. Von-Gierkes disease
B. Alcoholism
C. Type IIa Hyperlipoproteinemia
D. Nephrotic syndrome

Q. Tendon Xanthomas occur in
A. Type IIa dyslipidemia
B. Type I
C. Type IV
D. Type V

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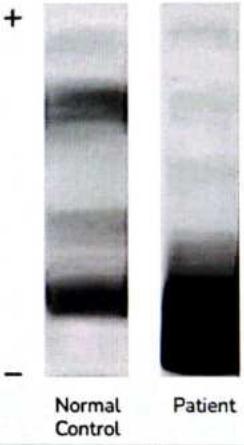
Q. All of the following are causes of fatty liver except:
A. Obesity
B. Prolonged starvation
C. Alcoholism
D. Choline excess

Q. 15 year old child presents with repeated episode of pancreatitis. Serum triglyceride concentration is 1100 mg/dL, Cholesterol is 250 mg/dL. Lipoprotein lipase activity is low. The diagnosis is:
A. Type I Hyperlipoproteinemia
B. Type IIa Hyperlipoproteinemia
C. Type III Hyperlipoproteinemia
D. Type IV Hyperlipoproteinemia

Q. A patient presents with corneal haziness, high urea, high creatinine, high potassium. Suspecting LCAT deficiency, Lipoprotein electrophoresis was performed. His HDL was undetectable. Name the abnormal lipid found in the electrophoresis?

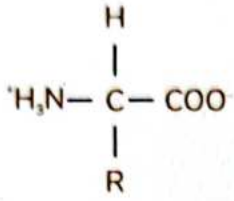


A. Spheroidal HDL3
B. LpX
C. HDL2
D. Bread Beta band

23 AMINO ACID PROTEIN CHEMISTRY

Amino Acid

- To write the amino acid, the C-H should be in the center, and they should get attached to CORN (CO is the Carboxyl group, R is the side chain, and N is the other functional group, which is NH2 group):
- At the physiological pH of 7.4, the Amino acid is present as Zwitter Ion.



- To write amino acid in the zwitter Ion form, the C and H should be in the center, and they should get attached to CORN (CO exists as COO- and N exists as Nh3+), and the R is a functional group.
- The formation of the zwitter ion depends on the pKa of the group and the pH value of the amino acids.
- The pKa of the carboxyl group is 2, and of the amino group is 9.
- In the surrounding of pH 7.4, The carboxyl group will sense the surrounding as alkaline giving the H+ to the surrounding to neutralize itself, leaving behind COO- and the amino group will sense the surrounding as acidic, and accepts the H+ to neutralize itself and become NH3+.

pKa

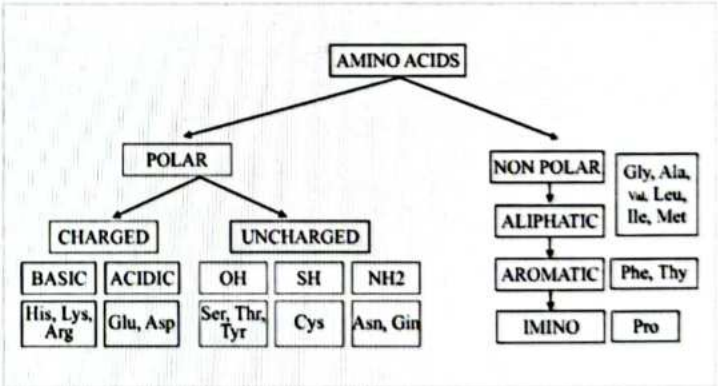
- pH at which 50% of the groups are ionized.
- Ionization or deionization of a functional group depends upon its pKa
  - If a substance has pKa of 2 and other of pKa of 4.
  - If they are put in the surrounding of pH 3.
  - Therefore, the substance with higher pKa will start accepting the H+ from the surrounding to neutralize it. Similarly, the substance with lower pKa will start releasing the H+ from the surroundings to neutralize it.
- Lower the pKa, the stronger the acidic nature of the substance.
- A substance acts as an effective buffer when dropped in a medium of pH = pKa +/- 1.
- pKa is not a constant.

Isoelectric pH

- The pH at which the net charge carried by the particle is zero.
- For a molecule with two ionizable groups,
- $Pi = \frac{Pk1 + Pk2}{2}$
- Least electric mobility
- Least solubility
- Least buffering capacity

Classification of Amino Acids:

- Based on the functional groups, they are classified into two groups:
  - **Polar**
    - → Only if it is soluble in a polar solvent like water.
    - → They need to be charged to get dissolved.
    - → Solute needs to interact with the solvent.
    - → If not charged, they must be of polar groups (OH / SH/ NH2) to bond hydrogen with water.
    - → Polar amino acid is further classified as
      - **Charged Polar amino acid**
        1. Positively charged/basic amino acids (Histidine / Lysine/arginine)
        2. Negatively charged/ acidic amino acids (Aspartic Acid / Glutamic Acid)
      - **Uncharged Polar amino acid**
        1. OH (Serine / Threonine / Tyrosine)
        2. SH (Cysteine)
        3. NH2 (Asparagine / Glutamine)
  - **Non-Polar: 3 types**
    - → Aliphatic amino acid (Glycine / Alanine / Valine / Leucine / Isoleucine)
    - → Aromatic amino acid (Phenylalanine / Tryptophan)
    - → Imino acids (Proline)
- Once you know the polar and non-polar amino acids, the protein folding will be easy to understand.
- The major function achieved by protein folding is solubility.



Important Information

- pKa of Histidine is 6.
- Aspartic Acid is called a Beta carboxyl group.
- Glutamic Acid is called a gamma carboxyl group.
- As you go down the Aliphatic amino acid groups, the non-polar nature increases.
- Aromatic amino acid absorbs UV light at 280nm.

Points to remember about Amino Acids

- Histidine is the physiological buffer as its pKa is 6.
- Glycine is the simplest amino acid.
- Glycine has no asymmetric carbon atom.
- Glycine does not exhibit stereoisomerism.
- Glycine is optically inactive.
- Aromatic amino acids absorb UV light at 280nm. (Purines and pyrimidines absorb UV light at 260nm)
- Proline is an imino acid.
- Proline and Glycine disrupt the alpha helix.

Q. DNA concentration is measured by
A. Electrophoresis
B. Chromatography.
C. Spectrophotometry

Color Reactions

S. No.	Amino Acids	Group	Colour Reactions
1.	Proteins	2 or more Peptide Linkages	Biuret Test  Blue ⇒ Negative (Proteins are absent)  Deep purple ⇒ Positive (Proteins are Present)
2.	All amino acids	Amino	Ninhydrin test
3.	Aromatic amino acids	Benzene or phenol	Xanthoproteic acid test
4.	Tyrosine	Phenol	Millon's Test
5.	Tryptophan	Indole	Aldehyde Test
6.	Histidine	Imidazole	Pauly's Test
7.	Arginine	Guanidinium	Sakaguchi Test

Biuret Reagent & Benedict's Reagent

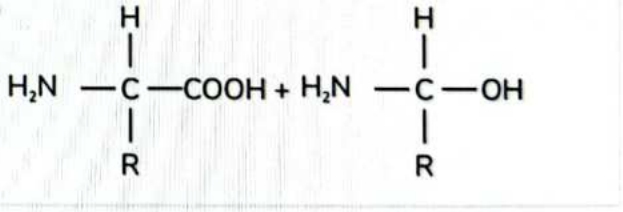
S. No.	Chemical	Benedict's reagent	Biuret reagent
1.	Copper	$CuSO_4$	$CuSO_4$
2.	Alkaline	Na_2CO_3	NaOH
3.	Stabilizing solution.	Sodium citrate	Sodium potassium tartrate

Important Information

- Urea cam answer Biuret test on heating, as it forms a compound called as Biuret, with two peptide linkages.

Peptide Linkage

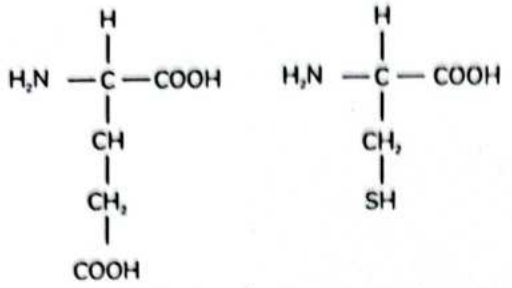
- Considering the 2 amino acids, both will have CH, a carboxyl group, an Amino Group, and the side chain.
- Once the bond occurs, the alpha carboxyl group combines with the alpha amino group to form water, leaving behind CONH and 2 C-alpha atoms.
- The CONH is the peptide linkage.
- The peptide linkage consists of 4 atoms.
- All the atoms in the peptide linkage are coplanar.
- At the physiological pH level of 7.4, the nitrogen will try to satisfy the valency of 4.
- Therefore, it gives the peptide linkage a partial double bond character. So, there is no freedom of rotation along the peptide linkage.
- However, there is a freedom of rotation among the C-alpha atoms.
- The angle at which C-alpha & C can rotate is called Ψ.
- The angle at which C-alpha & N can rotate is called ϕ.
- These angles are called the Ramachandra angle.



Glutathione

- It is an antioxidant and tri-peptide.
- It is denoted by Gamma Glutamyl cysteinyl glycine.
- Because it is GSH, with a hydrogen atom, it can be called an anti-oxidant.
- It is called a gamma because the linkage is the pseudo-linkage.

Glutamate



It is a pseudo linkage because the peptide linkage is formed between gamma carboxyl group of glutamic acid and alpha amino group of cycteine.
For a peptide linkage to be called as a true peptide linkage, it should be formed between alpha carboxyl group and alpha amino group.

Structure	Linkage	Method
Primary	Peptide	Sanger's Sequencing. Edman's Sequencing, reverse sequencing.
Secondary	Hydrogen Bond	Optical rotatory dispersion. Ocular Dichroism.
Super secondary Structure	Hydrogen Bond	X-ray Crystallography, UV spectroscopy & NMR spectroscopy
Tertiary Structure	Hydrophobic Bond	X-ray Crystallography, UV spectroscopy & NMR spectroscopy
Quaternary Structure	Disulphide Bridges	Reducing type of SDS Page

Important Information

- Primary structure of the protein is defined by the number and sequences of the amino acid, which is linked by the peptide linkage.
- Sanger was the first person to sequence protein.
- Sanger's reagent is 1 fluoro2,4 dinitrobenzene.
- The first protein that was sequenced was insulin.

Secondary Structur

- It is defined by how adjacent segments of the polypeptide chain get organized to form ordered units.
- There are 2 types of ordered units:
  - Alpha Helix
  - Beta-Pleated Sheet

Difference Between Alpha Helix and Beta Pleated Sheet

S. No.	Alpha Helix	Beta Pleated Helix
1.	Compact (1.5A)	Extended (3.5A)
2.	Intrachain hydrogen Bonding	Interchain hydrogen Bonding
3.	Only right-handed alpha Helix	Parallel and unparallel beta-pleated sheets are found
4.	Proline and glycine disrupt	-

Super secondary Structures

- These are the segments of polypeptide chains that link the adjacent secondary structures.
- **There are 2 types of super-secondary structures.**
  - Turn / Bend (If limited numbers of amino acids are used to link the adjacent structures.)
  - Loop (If more numbers of amino acids are used to link the adjacent structures.)
- These loops or turns can act as a binding domains, catalytic domains or allosteric sites. Hence super secondary structures often form functional domains.
- They have hydrogen bond linkages.
- Proline and glycine are found in a super secondary structure.

TERTIARY STRUCTURE OF PROTEIN:

It is defined by how the polypeptide chain gets folded in three dimensional configuration to form functional domains. This governs protein folding. The major driving force for protein folding is hydrophobic interactions. Based on tertiary structure, proteins are classified into two types -

1. Globular protein
2. Fibrous protein

Difference between globular and fibrous protein

S. No.	Globular protein	Fibrous protein
1.	Functional protein.	Structural protein.
2.	Compact.	Extended.
3.	All secondary and super secondary structures.	Beta pleated sheet.
4.	Axial ratio is less than 3.	Axial ratio is more than 10.

Important Information

- Tertiary Structure is defined as how the polypeptides get folded into the 3-dimensional structure to form the functional domain.

Quaternary Structure

- The protein that exists with more than one polypeptide chain can only have a quarternary structure.

Proteins that can exhibit quarternary structure include:

1. Hemoglobin
2. Insulin
3. LDH
4. CK

The linkage which stabilises quartenary structure of a protein is disulphide bridge.
The method used for studying quartenary structure of a protein is reducing type of Sodium Dodecyl Sulphonate - PolyAcrylamide Gel Electrophoresis

Difference between myoglobin and hemoglobin

- Myoglobin consists of one polypeptide chain, whereas hemoglobin consists of multiple polypeptide chains.
- Therefore, hemoglobin can exist in a quaternary structure.

Important Information

- If there is a strong interaction between the individual polypeptide chain, it is called a taut structure.
- If there is a weak interaction between the individual polypeptide chain, it is called a relaxed structure.

One Liners

- The amino acid which acts as a physiological buffer is **Histidine**.
- Aromatic amino acids are **Phenylalanine Tyrosine & Tryptophan.**
- The optically inactive amino acid is **Glycine**.
- **Tryptophan** answers the aldehyde test.
- **Arginine** answers the Sakaguchi test.
- **Tyrosine** answers Millon's test.

MCQs

Q. Ninhydrin test gives purple color with all except
A. Glycine
B. Alanine
C. Glutamine
D. Proline

Q. Biuret test gives violet color with all except
A. Albumin
B. Glycoproteins
C. Glutathione
D. Dipeptide

Q. The xanthoproteic acid test is answered by
A. Histidine
B. Arginine
C. Cysteine
D. Tyrosine

Q. Which of the following is optically inactive?
A. Tryptophan
B. Tyrosine
C. Phenylalanine
D. Glycine

Q. All of the following have hydroxyl groups except.
A. Cysteine
B. Tyrosine
C. Serine
D. Threonine

Q. All of the following are aromatic amino acids except.
A. Phenylalanine
B. Tyrosine
C. Tryptophan
D. Histidine

Q. All of the following are basic amino acids except?
A. Lysine
B. Glutamate
C. Arginine
D. Histidine

Q. Which of the following is an imino acid?
A. Lysine
B. Glutamate
C. Arginine
D. Proline

Q. Which of the following is a polar but uncharged amino acid?
A. Serine
B. Glutamate
C. Arginine
D. Tryptophan

Q. All the following are true about Glutathione except.
A. It is a tripeptide.
B. It has 3 peptide linkages.
C. It has a pseudo peptide linkage.
D. It is an antioxidant.

101

Q. The primary structure of a protein is stabilized by
A. Peptide linkage
B. Hydrogen bond
C. Hydrophobic interaction
D. Disulphide bridges

Q. The secondary structure of a protein is studied by
A. Sanger's method
B. Optical Rotatory dispersion
C. X-ray crystallography
D. NMR Spectroscopy

Q. All the following are true about Alpha helix' except
A. It is stabilized by intra-chain hydrogen bonds.
B. It is compact.
C. Proline disrupts alpha helix.
D. Left-handed alpha helices are common.

Q. All the following are true about beta pleated sheets except
A. It is stabilized by interchain hydrogen bonds.
B. It is extended.
C. Parallel beta pleated sheets are found.
D. Antiparallel beta pleated sheets are never possible.

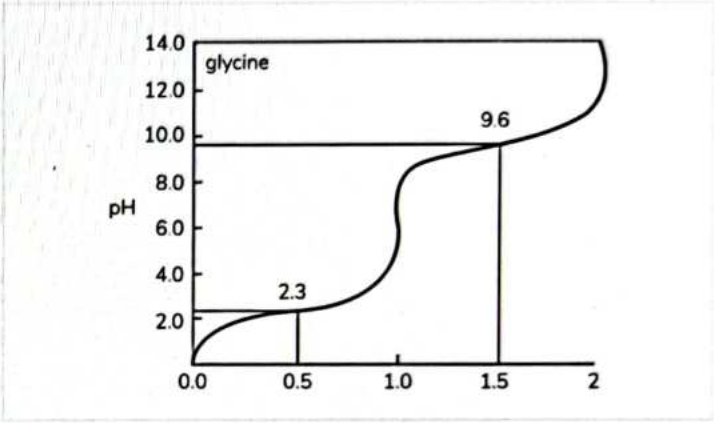
Q. Which of the following is true about super secondary structures?
A. They connect adjacent tertiary structures.
B. They are found adjacent to motifs.
C. Proline is found more among these structures.
D. They are stabilized by covalent interactions.

Q. The tertiary structure of a protein is studied by
A. Sanger's method
B. Optical Rotatory dispersion
C. X-Ray crystallography
D. NMR Spectroscopy

Q. Globular proteins true is.
A. Structural proteins
B. The axial ratio is more than 10.
C. Compact
D. More beta pleated sheets

Q. A person presented with recurrent renal stones, which were hexagonal on microscopy. He was diagnosed with cystinuria. True about Cystine is:
A. It is an alpha amino acid.
B. It is a dipeptide.
C. it has a free sulfhydryl group.
D. It is an imino acid.

Q. Calculate the isoelectric pH of glycine using the titration curve.



A. 5.9
B. 6.9
C. 7.1
D. 7.3

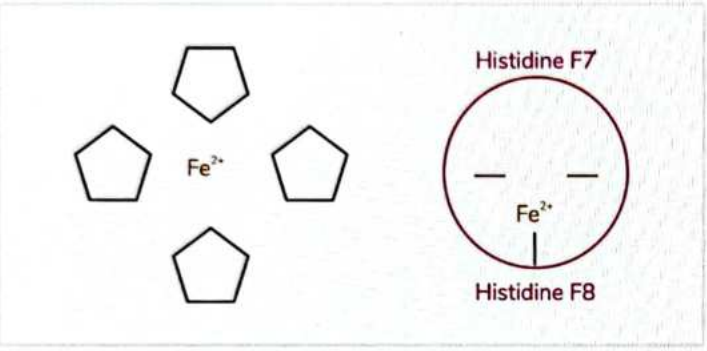
24 HEMOGLOBIN AND MYOGLOBIN

Structure of Myoglobin

- Contains one heme and one surrounding globin chain.
- Globin chain - peptide chain with 150 amino acids (Aas).
- AAs arranged in 8 alpha helices (A to H).
- Every AA in an alpha helix is numbered.
- **Example:** His F8 is the 8th amino acid of the Fth Helix.

Structure of Myoglobin - Heme

- Fe^{2+} is the metal present in the middle, below the plane of the ring.
- Surrounded by 4 pyrole rings.
- Fe^{2+} is covalently attached to an amino acid (Histidine F8) of the globin chain.
- Amino acid present on the other side is Histidine E7.



- **Histidine E7:** Provides a hindered environment and helps to escape from carbon monoxide poisoning.

Relation between carbon monoxide and heme

Carbon monoxide exhibits high affinity to Fe^{2+}

↓

Displaces oxygen away.

↓

Reduces oxygen carrying capacity of haemoglobin.

↓

Leads to anaemic hypoxia

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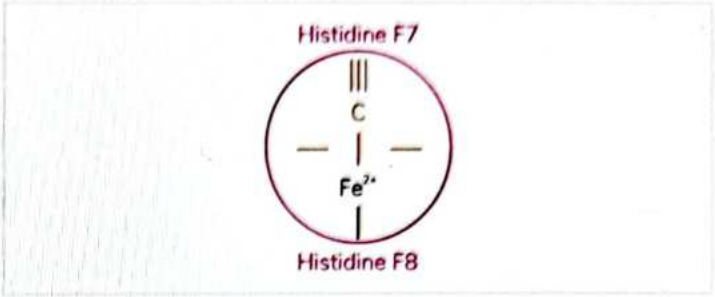
- Therefore, carbon Monoxide poisoning causes anaemic hypoxia.
- Carbon Monoxide has 2000 times higher affinity for the heme than oxygen - leading to CO poisoning.
- To escape this the heme is surrounded by the globin chain.
- Globin chain provides histidine E7 - providing a hindered environment.

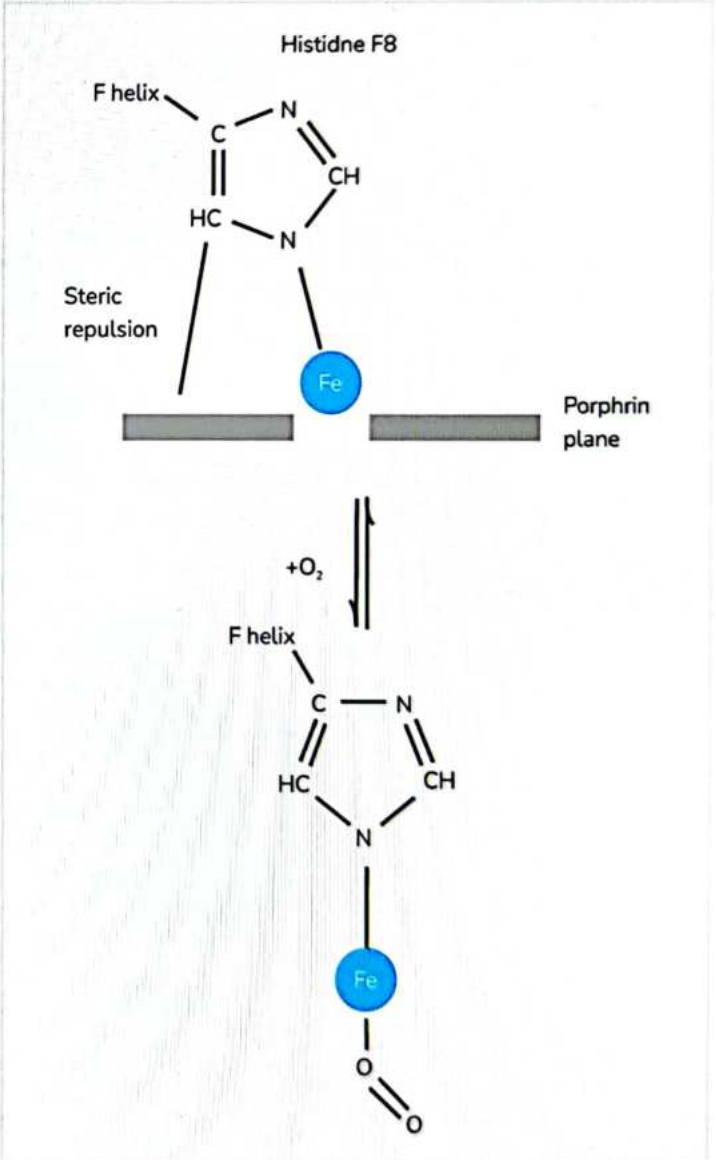
Q. How does Histidine E7 provide a hindered environment?

- Carbon monoxide has 2 atoms: C and O.
- Fe^{2+} is present below the plane of the ring.
- C binds opposite to Fe^{2+} perpendicular to the ring.
- O also binds perpendicular to C.
- But Histidine E7 present right there hinders the binding of O of CO, providing a hindered environment.



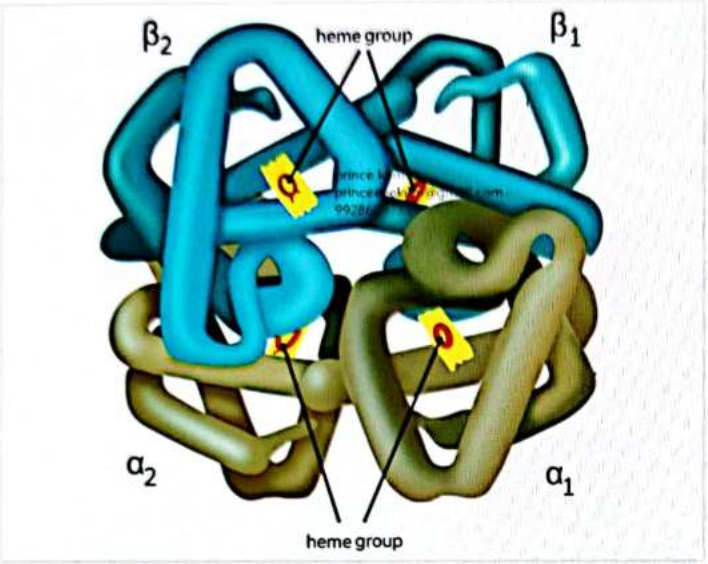
Q. Does Histidine E7 act the same way when oxygen binds?

- Oxygen also has 2 atoms: O and O.
- The first O binds perpendicular to the plane of the ring.
- Second O binds in an inclined position.
- Not hindered by Histidine E7



Structure of Haemoglobin

- Contains 4 heme molecules.
- Surrounded by globin chains.
- As it contains more than 1 peptide chain - forms a quaternary structure.
- Strong polypeptide chain interaction: Called taut structure.
- Taut structure favours deoxygenation.
- Weak polypeptide chain interaction: Called relaxed structure.
- Relaxed structure favours oxygenation.



At Tissue Side

- Has high carbon dioxide concentration.

Carbon dioxide diffuses into the RBCs.
↓
Few carbon dioxide molecules bind with amino acids of globin chains.
↓
Forms carbamino compounds - negatively charged.
↓
Reacts with nearby positively charged molecules - salt bridge formation.
↓
Forms taut structure.
↓
Leads to deoxygenation - release of oxygen.
↓
Pka of Histidine of globin chain rises to 9.
↓
To neutralise surrounding PH which is 7.4 Histidine accepts H^+
↓
The H^+ is provided as a result of some carbon dioxide combining with water to form bicarbonate and H^+
↓
The H^+ is utilised by the Histidine.
↓
Bicarbonate is exchanged with chloride through bicarbonate - chloride antiporter: Called chloride shift.
↓
More chloride in RBC attracts more water molecules.
↓
Therefore, RBCs in venular blood swells

Important Information

Haematocrit and Venular blood

- Hematocrit value is more in venular blood than in arteriolar blood.
- **Reasons are**
  - Chloride shift
  - Starling forces - plasma oozes out from arteries enters the veins in small amounts.
    → Making the venular blood more concentrated.
    → Therefore, high haematocrit value.

Important Information

Bohr Effect

- Reciprocal binding of oxygen and H^+

(Or)

- H^+ binding Reduces the affinity of heme for oxygen.

At Lung Side

- More oxygen is present.

Oxygen Enters the RBC by passive diffusion.
↓
Binds to Fe^{2+} present below the plan of rings.
↓
Oxygen pulls the Fe^{2+} towards the plane of the ring along with Histidine F8
↓
Therefore, one globin chain is pulled away from the others.
↓
Favours relaxed structure.
↓
Favours oxygenation
↓
Therefore, binding of one oxygen to Fe favours cooperative binding of other O to other Fe - allosteric property
↓
Salt bridge complexes formed at tissue site gets ruptured and COO^- released out as carbon dioxide.
↓
The H^+ and Histidine F8 complex is also broken down.
↓
The released H^+ binds with bicarbonate in the plasma
↓
Converts to water and carbon dioxide which is exhaled out

- More carbon dioxide is transported through dissolved bicarbonate in plasma.

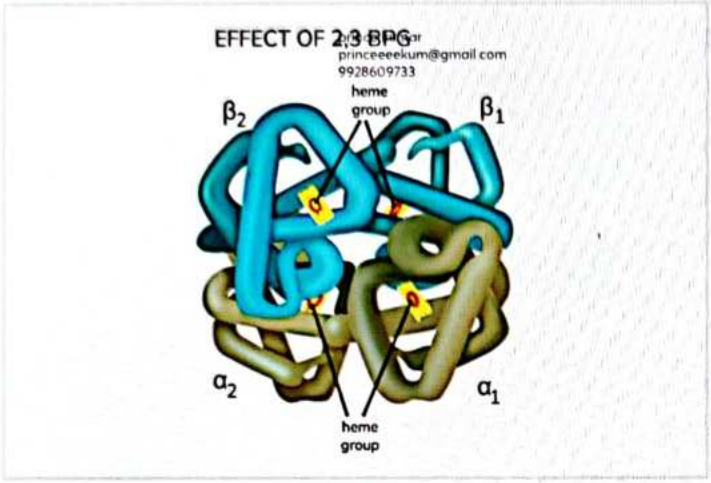
Important Information

Haldane Effect

- Reciprocal binding of oxygen and carbon dioxide.
- At the same partial pressure of carbon dioxide, its concentration of a deoxygenated blood is more than the oxygenated blood.

Effect of 2,3 BPG

- Reduces the affinity of haemoglobin for oxygen.
- Helps in unloading oxygen to tissues.
- By-product of glycolysis.



When 4 globin chains are placed together

↓

Encloses central cavity.

↓

Central cavity is surrounded with parts of Beta globin chain.

↓

Offers positively charged amino acids like Histidine H21, lysine.

↓

2,3 BPG is negatively charged, and central cavity is positively charged.

↓

The 2,3 BPG accumulates in the central cavity forming salt bridge formation.

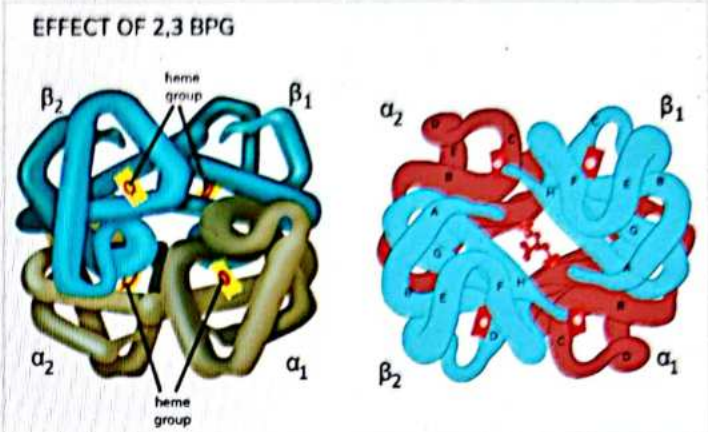
↓

Favour's taut structure and causes deoxygenation.

↓

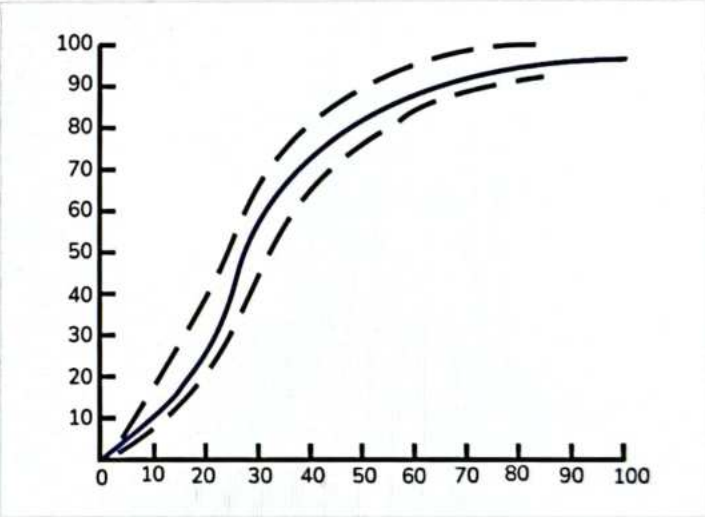
Therefore, Reduces the affinity for oxygen

- **Foetal Haemoglobin**
  - Has a high affinity for oxygen.
  - Haemoglobin in foetal circulation extracts oxygen from another circulation.
  - PO2 is less than the atmosphere Po2.
  - Globin chains of foetal haemoglobin are alpha2 and Gamma2, whereas for adults it is alpha2 and beta2.
  - Beta chain in adult haemoglobin contains - Histidine H21.
  - The Gamma chain in foetal haemoglobin contains - serine.
  - Positive charge in foetal haemoglobin is less than in adults - less affinity for 2, 3 BPG - more affinity for oxygen.



Oxyhaemoglobin Dissociation Curve

- Percentage saturation on y-axis.
- Partial pressure of oxygen on x-axis.
- Oxyhaemoglobin Curve is sigmoid.
- Oxymyoglobin Curve is hyperbolic.
- The shifting of the curve to Left or right tells about P50.



- P50: Partial pressure of oxygen at which it is 50% saturated.
- P50 for normal haemoglobin - 25mmHg
  - Shift of the curve to the right - increase in P50.
  - High P50 - low affinity.
  - Shift of the curve to the left - decrease in P50.
  - **Low P50:** Even at low P50 values the PO2 is saturated to 50%.
  - **Low P50:** High affinity.
  - Curve should she be shifted to right causing low affinity and having high P50 in conditions like:
    → Hypoxia - to supply oxygen.
    → Anaemia - to supply the oxygen.
    → Increase in body temperature - to help in metabolism.

→ Acidosis - to support aerobic metabolism.

Hypoxia, anaemia, and high body temperature

↓

Stimulates glycolysis.

↓

Releases 2,3 BPG

↓

Curve is shifted to right side

- Acidosis shifts the curve to the right side by the Bohr Effect.

- Curve shifting to Left in the following conditions:
  - → Decreased body temperature
  - → Alkalosis

Acclimatization

- Includes all adaptive changes which happen when a person slowly climbs up health.
- If hypoxia occurs suddenly it leads to the following steps.

Sudden hypoxia

↓

Patient hyperventilates - removing all carbon dioxide from the body

↓

Causes Alkalosis

↓

Curve is shifted to Left

↓

More affinity to oxygen

- The above mechanism by sudden hypoxia leads to 2 ill effects.
  - Tissue hypoxia - as there is no deoxygenation.
  - Central chemoreceptor centre stimulation decreases - Respiratory system is depressed.
- **Exception-1**
  - In case of slow hypoxia Alkalosis occurs.
  - It slowly moves the curve to Left.
  - In the meantime, glycolysis is stimulated.
  - 2,3 BPG is released.
  - Shift the curve to the right side.
- **Exception-2**
  - Anaemia shifts the curve to the right.
  - But in case of anaemia caused by carbon monoxide poisoning it shifts the curve to the left.
  - Because CO binding to heme makes the structure relaxed.
  - More oxygen is bound to haemoglobin.
  - Increase In oxygen affinity - P50 reduced - curve shifting to left side.
  - Increase Foetal haemoglobin concentration in thalassemia major also shifts the curve to Left as it has higher affinity.

- Hyperbolic oxymyoglobin Curve indicates the curve is shifted far towards the left side.
- P50 of Myoglobin - 8mmHg.
- Therefore, Myoglobin is not used for oxygen transport - due to less P50.
- Only used for oxygen storage.

One Liners

1. Fe^{2+} is attached to **His F8** of heme.
2. The amino acid, which is responsible for the hindered environment, protecting from carbon monoxide poisoning is **His E7.**
3. Carbon monoxide causes **anaemic** hypoxia.
4. 2,3 BPF facilitates **Taut** structure of haemoglobin.
5. Anaemia dissociates the oxyhaemoglobin curve to the **right.**

MCQs

Q. The amino of globin chain that provides hindered environment for carbon monoxide binding to haemoglobin is

A. His F8
B. His E7
C. HIS H21
D. Ser H11

Q. All of the following is true about Bohr Effect except

A. Deoxygenation increases the affinity of Hemoglobin for hydrogen ions
B. Decrease in the affinity of haemoglobin for Oxygen on H+ binding
C. Happens in the lung side
D. Acidosis shifts the curve to right

Q. Binding of carbon dioxide to haemoglobin causes which of the following?

A. Taut structure to relaxed structure.
B. Relaxed structure to Taut structure
C. Both
D. None

Q. All of the following cause shift of oxyhemoglobin dissociation curve to right except

A. Alkalosis
B. 2,3 BPG
C. Hypoxia
D. Anaemia

Q. All of the following cause shift of oxyhemoglobin dissociation curve to right except

A. Increase in body temperature
B. 2,3 BPG
C. Alkalosis
D. Acclimatization

Q. Foetal haemoglobin shows higher affinity for oxygen because.

A. It exhibits higher affinity for 2,3 BPG.

B. It has lower affinity for carbon monoxide.

C. It has lower affinity for 2,3 BPG.

D. It exists in taut structure.

Q. Total number of 2,3 BPG bound to one molecule of haemoglobin during conditions of hypoxia.

A. 1

B. 2

C. 3

D. 4

Case Based MCQs

Q. A person in his mid-forties wants to try skiing at 3950m height and is prescribed Acetazolamide because

A. It facilitates 2,3BPG production.

B. If shifts the oxyhaemoglobin dissociation curve to right.

C. It causes metabolic alkalosis.

D. It causes respiratory alkalosis.

Explanation: Effect of Climbing Uphill

Respiratory alkalosis

↓

Curve shifts to left.

↓

Haemoglobin can't load oxygen.

↓

Acetazolamide (carbonic anhydrase inhibitor) is given.

↓

It stops the bicarbonate reclamation at PCT.

↓

Bicarbonate stays in the PCT and removed from the body via urine.

↓

Leading to Acidosis (metabolic acidosis)

Acetazolamide cause normal anion gap metabolic acidosis.

↓

Shifts oxyhaemoglobin dissociation curve to right.

↓

Thus brings the body in equilibrium and also keeps the Respiratory centre stimulated

25 EPP CHROMATOGRAPHY

Electrophoresis

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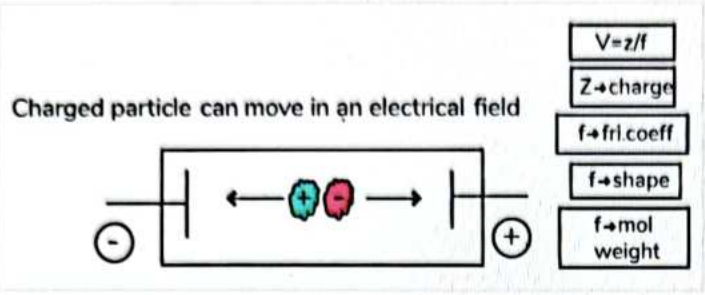
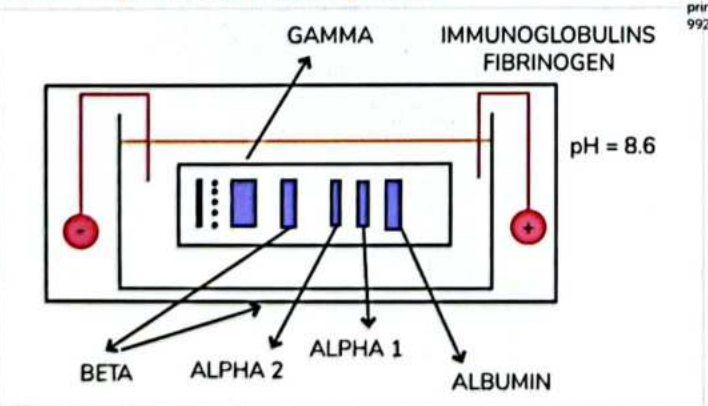


- Any charged particle moves in an electric field as an electrical field is created by combination of a negatively charged and a positively charged electrode.
- If the charge is positive, it will move quickly towards the negative charge and if the charge is negative, it will move quickly towards the positively charged side of the electrode.
- The rate of migration (velocity) of a charged particle in an electrical field is given by, V(velocity) = z/f where, z is the charge and f is the frictional co-efficient value.
- f is frictional co-efficient factor is mainly determined by two factors: molecular weight and shape.
- The number of charges carried by a particle is directly proportional to its velocity. More the number of positive charges, quicker the particles will move towards the negative charge. This is the reason why; charge is in the numerator.
- In case the particle is heavy, it cannot move. So, velocity and weight will become inversely proportional to each other. Therefore, weight is placed in the denominator.
- Thus, following are the three major factors that determine the mobility of a charge particle in an electric field
  1. Charge
  2. Molecular weight
  3. Shape
- It can be concluded that, electrophoresis separates protein based on charge molecular weight and shape.

Protein Separation by Electrophoresis



- Usually, serum protein electrophoresis is done on a glass slide.
- On one end of the glass rod, serum or plasma that is to be separated is applied using a micropipette.
- The slide is then placed into an electrophoresis tank that is filled with alkaline buffer with pH value 8.6.
- All the protein present in plasma or serum will immediately start to resist alkaline pH by giving their H+ ions.
- Now the protein present in the plasma is left with only negative charge.
- Once the protein has only negative charges left with them, the electrophoresis tank is connected with electrical supply that is close to the point of application.
- Close to the point of application is connected to negatively charged electrode and the opposite side of the electrode is connected to a positively charged.
- As soon as the electric current is switched on, all the negatively charged proteins start moving towards positive electrode.
- The migration of protein here, is affected by charge, molecular weight and shape.
- Through this process, all the proteins present in serum are separated into 5 bands.
- The band that is present farthest from the point of application is called albumin band.
- Albumin band is the farthest from the point of application due to two main reasons:
  1. It has lowest molecular weight
  2. It has maximum number of negative charges present in it.
- Five bands of protein separated from the serum in order of their closure to positive electrode are :
  1. Albumin band
  2. Alpha-1 band
  3. Alpha-2 band
  4. Beta band
  5. Gama band
- Alpha 1 band has the following proteins present in it.
  1. Alpha 1 antitrypsin
  2. Alpha fetoprotein
  3. Transthretin
- Alpha 2 band has the following proteins present in it.
  1. Alpha 2 macroglobulin
  2. Ceruloplasmin
  3. Haptoglobin
- Beta band has the following proteins present in it.
  1. Beta 2 microglobulin

2. Transferrin

- Gamma band has the following proteins present in it.
  1. Gamma globulins also known as immunoglobulins
  2. Fibrinogen

Q. Name the only diagnostic application of protein electrophoresis?

Ans. Multiple myeloma is the only diagnostic application of protein electrophoresis.

Q. What is diagnostic application?

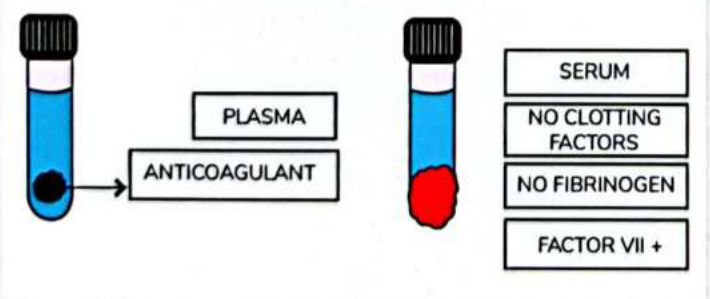
Ans. If a person is able to make a diagnosis showing an electrophoresis pattern it is called diagnostic application of electrophoresis. Multiple myeloma is the only diagnostic application of protein electrophoresis.

Multiple Myeloma

- It is monoclonal proliferation of plasma cells. This means that one clone of plasma membrane starts producing excess amount of antibodies.
- Excess amount of antibodies are usually found in the gamma region.
- Therefore, gamma band, in case of multiple myeloma will be intense.
- Gamma band will be a narrow band.
- Due to the effect of monoclonal proliferation, all of the excess antibodies produced are of the same type, having similar charge, molecular weight and shape. Hence, they move in a single line making it intense and narrow.
- This intense, narrow band present in the gamma region is known as M band of multiple myeloma.
- A broad band in gamma region would be seen in case of polyclonal proliferation.
- Any chronic infection such as tuberculosis causes polyclonal proliferation of the gamma band.
- Therefore, a band in the multiple myeloma in the gamma region should be carefully checked whether it is narrow or broad. A narrow line denotes monoclonal proliferation whereas a broad line shows polyclonal proliferation that is observed in chronic infections.

Serum and Plasma: Difference

00:11:28



- If the blood sample is collected in a tube and an anticoagulant is placed inside it, plasma is separated above them.
- On the contrary, if the blood sample is collected in a tube without anticoagulant present in it, negative charges of the tube stimulate invitro clotting after which serum is separated above them.
- Thus, serum is separated after clotting and it will not have clotting factors left inside it.
- This means serum will also not have fibrinogen.
- Factor 7 however, is present in serum after clotting because factor 7 is involved in extrinsic pathway.
- Once tissue factor is released, factor 7 becomes 7a which can stimulate extrinsic pathway.
- Invitro clotting on the other hand, involves intrinsic pathway. The negative charges of the tube transform factor 12 into 12a, 11 into 11a, 9 into 9a and so on.
- Thus, invitro clotting involves separation of serum through intrinsic pathway and extrinsic clotting factor, factor 7 is not used.

Q. Which clotting factor is found in serum?

Ans. Factor 7 is the clotting factor is found in serum.

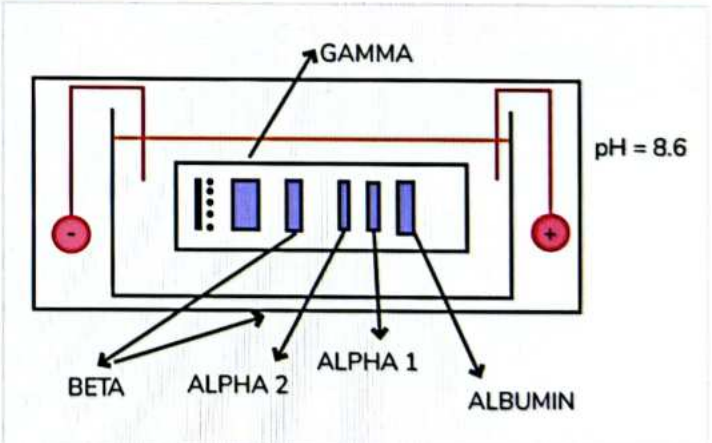
Q. Name the preferred sample for electrophoresis between serum and plasma samples.

Ans.

- The preferred sample for electrophoresis is serum due to absence of fibrinogen. Fibrinogen present in plasma forms a band in gamma region that can obscure multiple myeloma band.
- Serum is the preferred sample for electrophoresis because it does not have fibrinogen. Therefore, it is always written as serum protein electrophoresis (SPE).

Nephrotic Syndrome

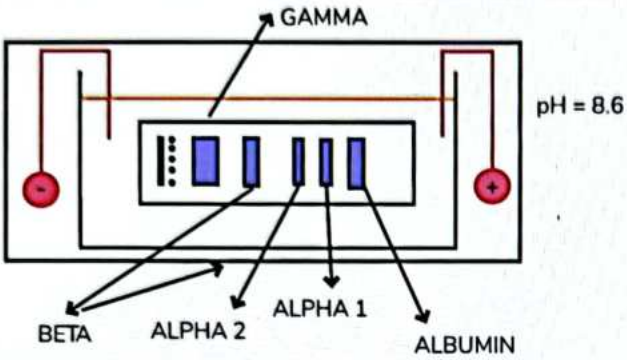
00:14:34



- Nephrotic syndrome is a leaky glomerulus from which all small proteins get dissolved in the urine.
- Albumin gets lost in the urine. As a result, albumin band in nephrotic syndrome appears very dull.

- Due to absence of albumin, the oncotic pressure decreases. This is why they have edema.
- In general, all proteins are passed in the urine but alpha 2 macroglobulin due to its large size is not filtered into the urine.
- Thus, when all the other bands including albumin or alpha 1 band are dull, alpha 2 macroglobulin remains relatively prominent.
- Therefore, in nephrotic syndrome all the bands appear dull while alpha 2 remains relatively prominent across the electrophoretic pattern.

DCLD/Decompensated Liver Disease



- Decompensated chronic liver disease (DCLD) previously known as liver cerosis means that liver has lost its function and albumin is no longer synthesized.
- Albumin band is dull.
- Plasma oncotic pressure decreases.
- Liver fails to compensate decreasing oncotic pressure and therefore plasma cells start compensation.
- Plasma cells produce more antibodies to maintain oncotic pressure.
- Decompensated liver disease is more prone to infections and excess antibodies are produced.
- Polyclonal proliferation of gamma band generates excessive multiple antibodies and its thickness increases.
- Broader gamma band fuses with beta band and beta-gamma fusion can be seen.
- Therefore, beta-gamma fusion is found in decompensated chronic liver disease due to polyclonal proliferation of antibody, gamma band generated by plasma membrane to maintain oncotic pressure.

Electrophoresis Pattern Found in Various Diseases 00:17:35

Sl NO.	Disorder	EPP Pattern
1.	Multiple Myeloma	Narrow and intense band in the gamma region
2.	Nephrotic syndrome	Relative prominence of alpha 2 band.
3.	DCLD	Beta-gamma fusion

Factors Determining EPP Mobility

1. Charge
2. Molecular weight
3. Shape

- If a protein has migrated far from the point of application, it cannot be assumed that it migrated due to minimum molecular weight as it's migration can also be due to possession of highest negative charge.
- Similarly, if the protein does not move at all from the point of application, it might not be due to absence of negative charges but its high molecular weight.
- Thus, no assumption can be made on the protein based on its mobility in electrophoresis, as migration is affected by multiple factors.
- Electrophoretic migration, therefore, cannot be used to characterise a protein.

SDS Page

- Polyanionic detergent
- Removes charge effect

- Removes shape effect
- Molecular Weight

- To characterize a protein based on its migration, all the factors determining it have to be removed. Therefore, SDS is used.
- Sodium Dodecyl Sulphate (SDS) is a polyanionic detergent that add numerous negative charges to all the proteins.
- Therefore, the difference in migration determined by the difference of number of negative charges becomes insignificant.
- During serum protein electrophoresis, albumin moves the fastest from the point of application due to highest amount of negative charge present in it.
- Albumin has six negative charges whereas the closest band to the point of application, Gamma band has only 2 negative charges present in it.
- At a pH of 8.6, albumin can have six negative charges whereas immunoglobulin has only two negative charges. Thus, albumin has three times more number of charges than immunoglobulin. Due to this reason, albumin moves the fastest.
- When the protein is treated by SDS, it uniformly ads thousands of negative charges to all the proteins.

- So, albumin has 1006 negative charges and immunoglobulins now has 1002 negative charges present in it.
- Ratio between albumin and immunoglobulins now is 1:1.
- As a result, no migration can be seen due to difference of number of charges present in various bands.
- Charge-based migration, therefore, is ruled out after treating proteins by SDS.
- A detergent removes all higher order of structure from the protein.
- As already known, every protein has its unique shape due to assistance provided by secondary, tertiary, and quaternary structure.
- Primary structure of a protein like a long thread that gets intertwined to form a ball.
- Therefore, proteins having primary structure are spherical in shape without any unique structure differentiating them.
- Now SDS as a detergent removes all the secondary and tertiary structures of protein and only the primary structure can be retained.
- Thus, all proteins become spherical in shape after being treated by SDS.
- Hence, shape-based separation is also ruled out by SDS.
- Therefore, molecular weight is the only category on which proteins can be separated after treating them with SDS.
- Thus, it can be concluded that, SDS page separates proteins purely based on molecular weight.

Purpose of SDS

- SDS page is a polyanionic detergent that removes charge and shape effects and retain only the effect of molecular weight.

Q. What is the purpose of using SDS page?

Ans. SDS page is not used for separating protein. SDS page is used for identifying the molecular weight of an unknown protein.

- Suppose a protein is extracted from protein culture.
- To identify its molecular weight, it has to undergo through SDS page along with protein ladder.
- Protein ladder is the combination of known proteins of known molecular weight. It is added in well1.
- When electrical field is applied to protein ladder, all the protein present on it will get separated based on their molecular weight.

- This ladder is usually bought from a manufacturer who can provide all the details of molecular weights of different proteins present in it.
- For example, if the topmost band does not move at all, it can range it can correspond to molecular weight of thousand kilo Dalton; the second band may correspond to 500 kilo Dalton; the third band maybe of 200; fourth of 100 and the last band may have molecular weight of 50 kg Dalton as informed by the manufacturer.
- So, if the unknown protein moves and stops at hundred kilo Dalton in the ladder, it means that this protein has molecular weight of 100 kilo Dalton.
- Hence, SDS page is used to find the molecular weight of an unknown protein.
- Further extension of SDS page is known as reducing type of SDS page.
- Quaternary structure of protein is studied by reducing type of SDS page.

Reducing Panel 00:26:30

- Reducing type of SDS page means that protein is treated not only with SDS but also with mercaptoethanol.
- Mercaptoethanol is a reducing agent.
- It reduces the disulphide bridges which bridge subunits in an oligomeric protein.

For example

- Insulin has two polypeptide chains connected by disulphide bridges.
- When functional insulin is treated with mercaptoethanol, it reduces disulphide bridges to form sulphide group.
- After being converted into sulphide group, individual subunits become free as the link is now broken. This is the effect of reducing type of SDS page.
- Thus, in reducing type of SDS page, mercaptoethanol (which is a reducing agent) reduces disulphide bridges which linked individual subunits in an oligomeric protein and frees them.
- After treating with mercaptoethanol, when it runs through SDS page, the number of units present in protein can be found by observing it the number of bands. This gives quaternary structure of the protein.
- Quaternary structure of the protein is defined by how individual subunits of polypeptide chains interact with each other.

Q. A protein was run through SDS-PAGE and gave rise to a band corresponding to 100 kilo Dalton. After the protein was treated with mercaptoethanol, it gave rise to two bands: one corresponding to 20 and the other corresponding to 30. What is your comment on the protein. Whether it is a monomer, dimer, or tetramer?

Ans. A monomer when treated with mercaptoethanol forms same band in 100 kg Dalton region. Here, it's distributed between two bands, that means it is not a monomer.

- It is also not a dimer, because it's molecular weight corresponds to 20 and 30 kg Dalton. Had it been a dimer, it's

whole molecular weight should be equal to 100 kg Dalton. Clearly, two subunits of 20 and 30 kg Dalton do not give the required molecular weight. Hence, it is not a dimer.

- Therefore, the protein is a tetramer with two subunits of molecular weight 30 kg Dalton and two subunits of molecular weight 20 kg Dalton. When all of these molecular weights are added together, they become 100 kg Dalton that is the tot molecular weight of the protein. Hence, the protein is a tetramer with two subunits of molecular weight 30 kg Dalton that form a band in 30 kg region and two subunits of molecular weight 20 kg that again form a band in the 20 kg region.
- This helps in understanding the quaternary structure of the protein.

Conclusion 00:31:30

- Electrophoresis is based on the principle that any charge particle moves inside an electrical field.
- The rate of migration of a charged particle in an electrical field depends on its charge,
- Molecular weight and shape.
- Protein when separated from serum or plasma through electrophoresis get distributed into five bands.
- Albumin band is the farthest band from the point of application. Alpha One, Alpha two, beta and gamma band follow after that.
- Proteins present in Alpha 1 band are Alpha 1 antitrypsin, alpha fetoprotein and transthretin.
- Proteins present in Alpha 2 band are Alpha 2 macroglobulin, ceruloplasmin and haptoglobin.
- Proteins present in beta band are beta 2 macroglobulin and transferrin.
- Proteins present in gamma band are gamma globulins also known as immunoglobulins and fibrinogen.
- Fibrinogen can form a band in gamma region that can overlap multiple myeloma, therefore, serum is preferred sample for protein electrophoresis.
- In nephrotic syndrome, glamorous is leaky and all proteins get dissolved in urine.
- Therefore, all the bands appear dull except alpha 2 that has macroglobulin that doesn't get filtered due to its large size. Thus, alpha 2 macroglobulin remains relatively prominent across the dull electrophoretic pattern.
- In decompensated chronic liver disease (DCLD), beta-gamma fusion is found in due to polyclonal proliferation of gamma band generated by plasma membrane to maintain oncotic pressure.
- SDS Page separates proteins based on their molecular weight.
- Reducing type of SDS page uses mercaptoethanol to identify the number of subunits present in protein. Thus, it ultimately helps in understanding and identifying quaternary structure of proteins.

Chromatography 00:34:00

- Separation technique
- Mobile phase
- Stationary phase

Q. Which is the fastest moving amino acid in paper chromatography?

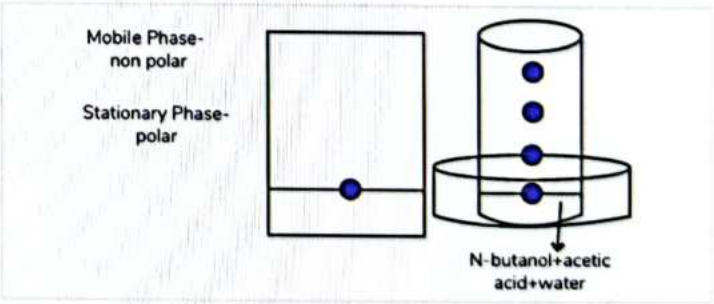
Ans. In paper chromatography, amino acid which has maximum polarity moves fastest.

Q. What is the most common technique for HbA1c estimation?

Ans. Cation exchange chromatography is the most common technique for HbA1c estimation.

- Chromatography is a separation technique.
- It separates solutes based on their distribution difference between stationery and mobile phase.
- In any chromatographic separation, a solid a stationary phase and a mobile phase is always present.
- The solute either moves fast or it gets retarded.
- Suppose a solution with two solutes S1 and S2, where S1 has affinity towards solvent A and S2 has affinity towards another solvent B.
- Construct a chromatographic separation wherein, A is mobile phase and solvent B has stationary phase.
- Clearly S1 solute will move faster as it has got affinity towards solvent A.
- As S2 has affinity towards solvent B, it will not move at all and will soon get retarded. This way S1 and S2 segregate.
- Thus, chromatography is a separation technique where solutes are separated based on their differential distribution between stationery and mobile phase.
- If a solute has affinity towards mobile phase, it moves faster but if a solute has affinity towards stationary phase, it does not move at all.
- Two or more solutes can be separated using this method.

Paper Chromatography 00:36:22



- Paper chromatography is the simplest chromatographic

separation technique.
- It is an obsolete technique.
- In this method, whatman paper number 1 is cut into exact dimension of 30×35 cm.
- Mark a line on it 5 cm from the base.
- Make a dot on this line.
- Apply amino acid mixture on this dot that is to be separated using a capillary tube.
- Once amino acid mixture is applied on this paper, it is folded from the edges and tied with help of a needle and thread.
- Now, place this paper on a petri dish and fill the dish with a solvent that is used for amino acid separation.
- The solvent which is used for separation of amino acid in paper chromatography is a combination of n-butanol, acetic acid and water.
- The paper and water used here are polar.
- As soon as the paper is placed on the solvent it will form a thin layer on the paper due to polarity.
- Although n-butanol and acetic acid are nonpolar, they also start moving on the paper due to capillary action.
- At this moment, n-butanol and acetic acids are in mobile phase. Thus, mobile phase is nonpolar.
- Paper with water is in the stationary phase. Thus, stationary phase is polar.
- When n-butanol and acetic acids move, they cross the line upon which amino acid mixture was applied while trying to take some amount of amino acid along with them.
- As both n-butanol and acetic acids are nonpolar, they can extract nonpolar amino acid only.
- Therefore, nonpolar amino acid will move faster.
- The more polarity amino acid has, the longer it will take to migrate.
- Hence, amino acid can be separated based on their polarity.
- It can be concluded that in paper chromatography, n-butanol and acetic acid are in nonpolar mobile phase. Paper and water are in polar stationary phase. Nonpolar amino acid moves faster, and polar amino acid stay close to the point of application. In this manner amino acid can be separated based on their polarity.
- This is called a planar chromatography as the process takes place on a plain surface.

Column Chromatography 00:41:44

- There are two main examples of column chromatography are:
  1. Gel filtration chromatography
  2. Ion exchange chromatography

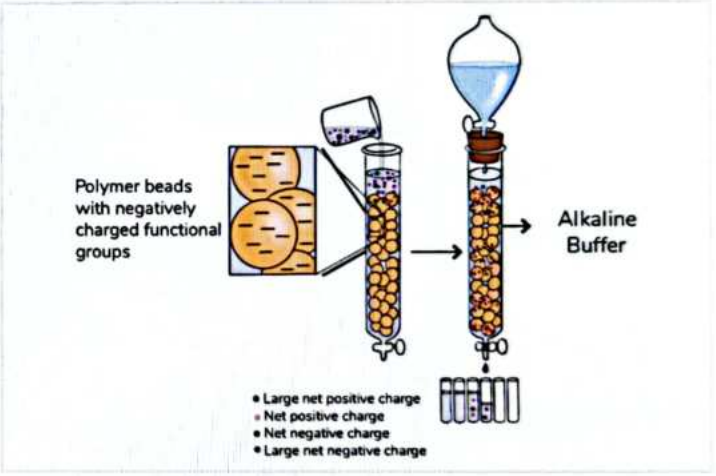
Gel Filtration Chromatography

- It is also known as size exclusion chromatography.
- In this case, a column is used as a stationary phase. This column is filled with synthetic polymer beads that have characteristic pores.
- This column is a burette filled with synthetic polymer beads having pores.
- The radius of these pores is measured as stoke radius.
- When a complex protein mixture is poured upon this column, all the proteins that are smaller than the pores get retarded inside the pores.
- As soon as they come out of one pore at a slow rate, they again get retarded in the next pore. Thus, proteins that are small in size get retarded inside the pores.
- On the other side, proteins that are larger than the pores, swiftly move between the beads.
- They can quickly come down to the bottom of the column.
- Therefore, this technique separates protein on the basis of their size. And this is why, it is known as size exclusion or gel filtration chromatography.

ION Exchange Chromatography 00:45:10

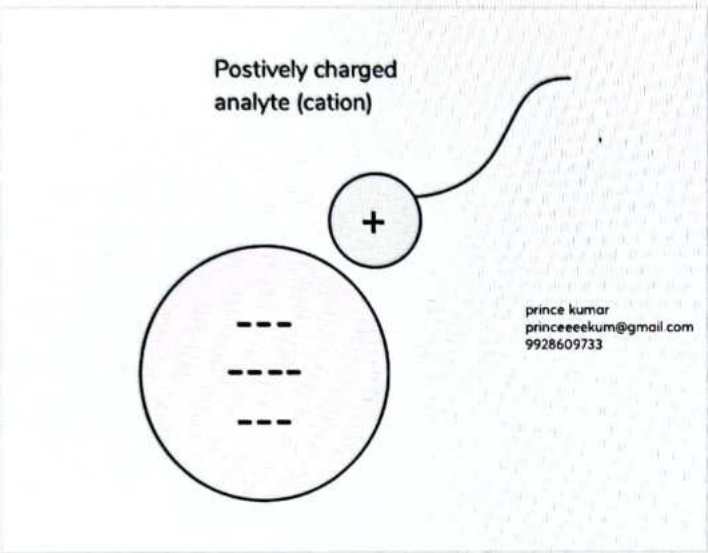
- Ion exchange chromatography separates proteins based on their charge.
- There are two types of ion exchange chromatography
  1. Cation exchange chromatography
  2. Anion exchange chromatography

Cation Exchange Chromatography



- Cation exchange chromatography is used only when the protein to be separated is a cation.
- In general application of chromatography, protein that is to be used is first held at stationary phase so that unwanted impurities can descend faster. Once all the impurities are removed, a technique known as elution is used upon stationary phase to derive desired result.
- In cation exchange chromatography, cation proteins can be retarded at stationary phase only when the stationary phase is anion.
- Thus, in cation exchange chromatography, the stationary phase is anion.

- In this method, a column filled with synthetic polymer beads that are covalently attached to negatively charged groups. In other words, anion is negatively charged.
- Carboxymethyl sepharose is an important example where a column filled with stationary polymer beads that are covalently attached to negatively charged groups such as carboxyl or any acidic group.
- When a complex protein mixture is poured upon this column, all positively charged particles will be held back onto the stationary phase and negatively charged particles will descend in the form of impurities.
- Once all the impurities are removed, technique of elution is used.
- The stationary phase or the column is filled with an alkaline buffer.



- All the protein can sense the alkaline pH and try to resist or neutralise alkaline pH by realising their H+ ions.
- As soon as the protein release their positive charge they can no longer be retarded on the stationary phase and descend along with the alkaline buffer. This is known as cation exchange chromatography.
- Hence, carboxymethyl sepharose is an important example of cation exchange chromatography.
- Cation exchange chromatography is the most widely used technique for HbA1c estimation.

Anion Exchange Chromatography 00:50:42

- Anion exchange chromatography is used when the protein to be separated is anion.
- Anion can be retarded at stationary phase only when the stationary phase is cation.
- In anion exchange chromatography, a column filled with synthetic polymer beads that are covalently attached to positively charged groups. In other words, cation is positively charged.
- In quaternary ammonium groups, a column filled with stationary polymer beads that are covalently attached to positively charged groups are used.
- When a complex protein mixture is poured upon this column, all negatively charged particles will be held back onto the stationary phase and positively charged particles will descend in the form of impurities.
- Once all the impurities are removed, technique of elution is used.
- The stationary phase or the column is filled with an acidic buffer.
- Diethylaminoethyl (DEAE) column, a quaternary ammonium group is a crucial example of anion exchange chromatography.
- Sepharose is the bead and amino is the quaternary ammonium group that helps in retarding anion.

Conclusion 00:52:31

- Chromatography is a separation technique that separates solutes based on their distribution difference between stationery and mobile phase.
- Paper chromatography which is planer in nature is the simplest form of chromatography.
- In paper chromatography, stationary phase is polar and mobile phase is nonpolar.
- Nonpolar amino acids move faster than polar amino acid.
- The solvent which is used for separation of amino acid in paper chromatography is a combination of n-butanol, acetic acid and water.
- Gel filtration chromatography separates protein on the basis of their size.
- Contrary to popular belief, proteins that are large in size move faster.
- Ion exchange chromatography separates proteins based on their charge. It is divided into two types: Cation exchange chromatography and anion exchange chromatography.
- Cation exchange chromatography is used only when the protein to be separated is a cation.
- The stationary phase should be anion to retard cation.
- Carboxymethyl Sepharose is an important example of cation exchange chromatography column.
- The most widely used technique for HbA1c estimation is cation exchange chromatography.
- Anion exchange chromatography is used when the protein to be separated is anion.
- Anion can be retarded at stationary phase only when the stationary phase is cation.
- Diethylamino ethyl (DEAE) column, a quaternary ammonium group is a crucial example of anion exchange chromatography.

One Liners and MCQ's

00:54:11

1. The preferred sample for protein electrophoresis is serum.
2. SDS page separates protein based on molecular weight.
3. The method used to study quaternary structure of protein is reducing type of SDS page. (Where mercaptoethanol is used)
4. The buffer used for elution in cation exchange chromatography is alkaline.
5. The buffer used for elution in anion exchange chromatography is acidic.
6. The most commonly used method for HbA1C estimation is cation exchange chromatography.

MCQ's

Q. Electrophoresis separtes proteins based on all except
A. charge
B. Molecular Weight
C. size
D. Shape

Q. The most widely used technique for estimating HbA1C?
A. Cation exchange chromatography
B. Anion exchange chromatorgraphy
C. Affinity chromatography
D. Electrophoresis

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Q. The clotting factor present in serum is
A. Factor XII
B. Factor X
C. Factor V
D. Factor VII

Q. Which of the following is true about Ion exchange chromatography?
A. Cation exchange chromatography uses cations in the stationary phase
B. In anion exchange chromatography, acidic buffer is used
C. DEAE column is the example of anion exchange chromatography
D. In cation exchange chromatography, alkaline buffer is used

Q. The preferred sample for electrophoresis of diagnosis of multiple myeloma is
A. Serum
B. Plasma
C. CSF
D. Whole blood

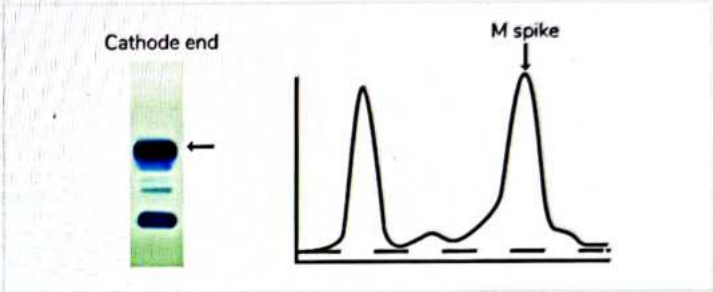
Q. SDS PAGE separates proteins based on
A. Charge
B. Molecular weight
C. Shape
D. Size

Q. The amino acid which moves the fastest in paper chromatography is
A. Glutamic acid
B. Aspartic acid
C. Histidine
D. Tryptophan

Image Based MCQ's

00:58:30

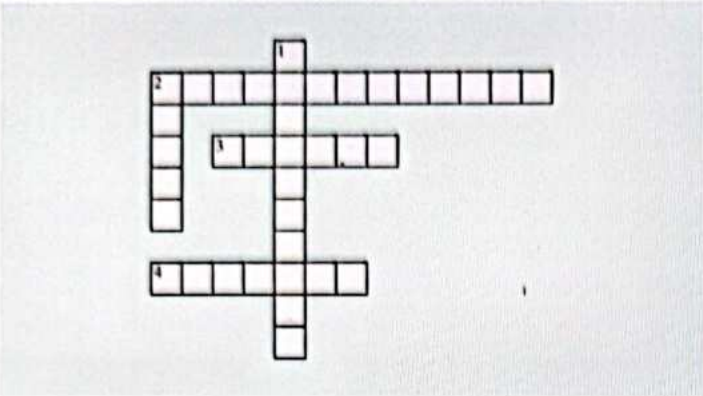
Q. What is the abnormal pattern seen in the EPP pattern provided?



A. Narrow intense band in the gamma region
B. Broad intense band in the gamma region → CHRONIC INFECTIONS
C. Relative prominence of alpha 2 bank → NEPHROTIC SYNDROME
D. Beta gamma fusion → DCLD

CROSS WORD PUZZLES

Crossword Puzzle 1



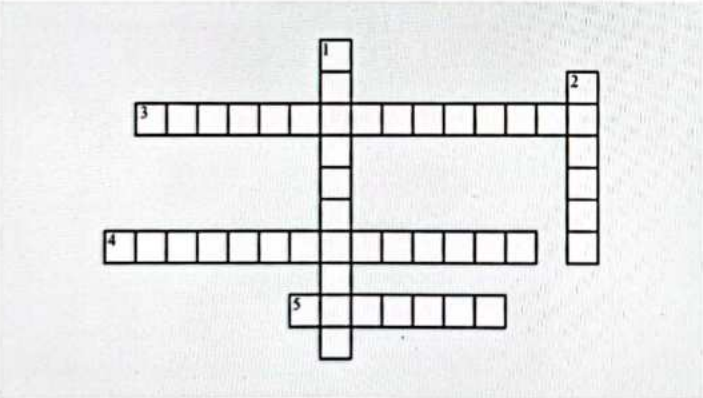
Across

2. Gel filtration chromatography is also known as --- chromatography.
3. Electrophoresis separates protein based on their ---, molecular weight and shape.
4. The band that is present farthest from the point of application is called --- band.

Down

1. In decompensated chronic liver disease ---- fusion is found in due to polyclonal proliferation.
2. Frictional co-efficient factor is determined by molecular weight and ---.

Crossword Puzzle 2



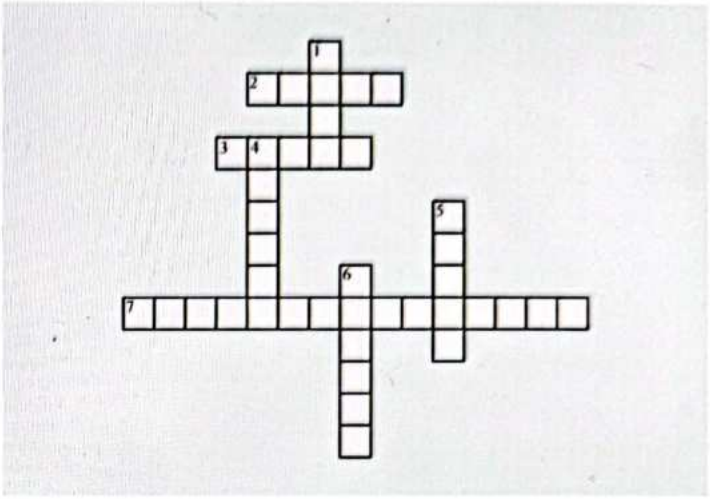
Across

3. _____ is the only diagnostic application of protein electrophoresis.
4. _____is a separation technique where solutes are separated based on their differential distribution between stationery and mobile phase.
5. _____is present in serum even after clotting because it is involved in extrinsic pathway.

Down

1. Broad line shown in gamma region is due to ------ proliferation that is observed in chronic infections.
2. Carboxymethyl sepharose is an important example of ------ exchange chromatography column.

Crossword Puzzle 3



Across

2. Anion exchange chromatography is used when the protein to be separated is -----.
3. ------ chromatography is the most simple and obselete chromatographic separation technique.
7. SDS page is used to find ------ of an unknown protein.

Down

1. Protein when separated from serum or plasma through electrophoresis get distributed into ------ bands.
4. The buffer used for elution in anion exchange chromatography is ------ in nature.
5. ----- chromatography is called a plainer chromatography as the process takes place on a plain surface.
6. Nonpolar amino acid move ------ than polar amino acid.

26 AMINO ACID CATABOLISM UREA CYCLE AND HYPERAMMONEMIA

Amino acid breakdown

- When an amino acid breaks down, most of the amino acids release the amino group first. Then, the carbon skeleton undergoes catabolism.
- Depending upon how they give off their amino group, the amino acid breakdown is of two types
  - Transamination reaction
  - Deamination reaction.

Facts about Transamination Reactions

- All transaminases need Pyridoxal phosphate as a coenzyme. The basis on which transaminase acts is that when an amino acid undergoes breakdown it acts with a keto acid (most commonly alpha ketoglutarate).
- PLP

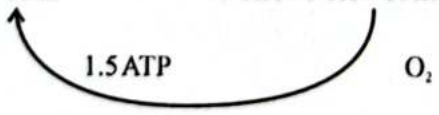
$$AA + KG \longleftrightarrow Glu + KA$$

- Amino acid gives off its amino group to alpha-ketoglutarate, converting it to glutamate. This way, the amino acid going through a breakdown becomes a keto acid. This is the basis of any transaminase.
- If Aspartate amino acid decides to undergo breakdown and reacts with a keto acid, alpha keto glutarate. It converts into glutamate. Aspartate becomes a keto acid, oxaloacetate. When the enzyme is named based on the forward reaction, it will be named aspartate transaminase. If the enzyme is named based on the reverse reaction, it will be called serum glutamate oxalacetate transaminase. Transaminase 1 is AST, otherwise called SGOT.
- PLP AST/SGOT
- Aspartate + ketoglutarate $\longleftrightarrow$ OAA + Glutamate
  AST/SGPT
- Alanine + ketoglutarate $\longleftrightarrow$ Pyruvate + Glutamate
- If Alanine decides to undergo breakdown, it reacts with alpha keto glutarate. It gets converted to glutamate and Alanine becomes a keto acid which is Pyruvate. If the enzyme is named based on a forward reaction, it is called Alanine transaminase. If this enzyme is named based on the reverse reaction, it is called serum glutamate pyruvate transaminase.
- The mnemonic here is LP. Alanine on transamination forms pyruvate and pyruvate on transamination forms Alanine.

Deamination Reaction

00:04:32

- LAA + FAD $\xrightarrow{\text{LAA oxidase}}$ KA + NH3 + FADH2



Facts about L–Aminoacid Oxidase

- Uses FAD
- L–Amino acid + FAD $\xrightarrow{\text{L-Amino acid Oxidase}}$ Ketoacid + Ammonia + $FADH_2$

- The deamination reaction is done by L Amino Acid oxidase. When an amino acid is undergoing breakdown. In the presence of L Amino Acid oxidase, it gets converted to keto acid. The amino group is released as ammonia. It is an oxidative deamination reaction. It oxidizes amino acids, removes oxygen from them, and gives that hydrogen to the enzyme FAD. That way it becomes FADH2. This FADH2 enters the electron transport chain, giving rise to 1.5 ATP. The FADH2 is not linked to the electron transport chain. To regenerate FAD, the oxygen molecule reacts with FADH2, accepts the hydrogen atom from FADH2, and oxygen becomes hydrogen peroxide.
- **Two detrimental effects of oxidative deamination reactions:**
  - Toxic ammonia is released.
  - Hydrogen peroxide (source of oxidative stress) is generated.

Exceptions

- 4 amino acids do not give off their amino group and undergo breakdown along with their amino group. These do not undergo transamination or deamination.
  - Lysine
  - Threonine
  - Proline
  - Hydroxyproline
  - These undergo breakdown along with their amino groups.

Facts about glutamate dehydrogenase

Glutamate Dehydrogenase

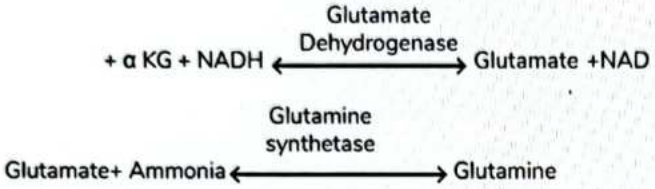
- Ammonia + KG + NADH $\longleftrightarrow$ Glutamate + NAD
- Amino acid breaks down in every tissue of the body. Ammonia is generated in all tissues of the body. Ammonia is supposed to be converted into a non-toxic form in which it is to be transported from all of these production sites to reach the liver. In the liver, Ammonia can be converted to urea. Glutamate is the most non-toxic form of ammonia. Ammonia is converted into glutamate with the help of a glutamate

dehydrogenase enzyme. Wherever ammonia is generated, it reacts with alpha keto glutarate and NADH, in the presence of glutamate dehydrogenase to form glutamate and NAD. This glutamate will be released into circulation and reach the liver. In the liver, it will be converted to urea.

- **Two exceptions to this are**
  - There are two tissues where glutamate is not formed as a non-toxic form of ammonia:
    → Neurons
    → muscles

Neurons

Facts about Glutamine Formation in Neurons



- Glutamine is formed here. Whenever Ammonia gets generated in neurons it reacts with Alpha keto glutarate and NADH, forming glutamate and NAD. When glutamate immediately reaches circulation after going through another reaction, catalyzed by glutamine synthetase. Glutamine synthetase allows glutamate to react with one more molecule of ammonia thereby converting glutamate to glutamine; glutamine will then come out of the neuron, reach the circulation, and through circulation, it reaches the liver. Glutamine is the non-toxic form of ammonia in neurons.
- Ammonia is toxic because its PK is very high. When Ammonia of very high PK is synthesized, it is released into a cell with PH 7.4. It will send this pH as acidic. To neutralize this, Ammonia will react with a hydrogen ion, forming osmotically active ammonium ions. These attract water which causes osmotic swelling of all the cells. Ammonia is therefore an osmolyte.
- **What if it sequesters hydrogen ions? What will happen to the electron transport chain?**
  - As and when electrons go through various chains of complexes, hydrogen ions will get translocated. Only these hydrogen ions go through the F2 component of the ATP synthase complex, to generate ATP. In the presence of ammonia, because it sequestrates hydrogen ions, no hydrogen ion is available for the electron transport chain to be able to generate ATP. This causes ATP depletion.
- The third reason why ammonia is toxic is, as and when Ammonia forms it immediately reacts with alpha keto glutarate. It causes alpha-keto glutarate deficiency. Alpha keto glutarates are one of the intermediates of the citric acid cycle. When there is hyper ammonia, Ammonia decreases the levels of Alpha keto glutarate, without which the citric acid cycle stops. The citric acid cycle is the final furnace where every fuel comes and gets oxidized. If the furnaces are switched off none of the fuels will be able to generate ATP. This is another reason for ATP depletion.
- One of the mechanisms by which ammonia brings about toxicity is that it uses Alpha keto glutarate in the neuron which is the principal cell of the body. One would try to optimally utilize Alpha keto glutarate. That is why in neurons first Alpha keto glutarate is used. One Ammonia gets detoxified to form glutamate which again detoxifies one more ammonia to form glutamine. This way one is only using one Alpha keto glutarate to detoxify two ammonia. This is why neurons have evolved in such a way that they form glutamine as a nontoxic form of ammonia.

Muscles

- As and when Ammonia gets generated it reacts with the Alpha keto glutarate and NADH to form glutamate and NAD to form glutamate and dehydrogenase enzyme. In muscles, this glutamate does not directly reach the circulation but goes to another transamination reaction where it reacts with pyruvate converting pyruvate to Alanine. Glutamate that way becomes Alpha keto glutarate. Alanine comes out of the muscle and reaches circulation. Ammonia is transported through the muscle in the form of alanine.
- **There are two advantages of this**
  - There is the regeneration of Alpha keto glutarate. Had it mean glutamate the nontoxic form of ammonia, the Alpha keto glutarate would become utilized. The regeneration keeps the citric acid cycle going. For muscle ATP production is essential and hence the citric acid cycle cannot be stopped. This is why regeneration is important.
  - There is detoxification of glutamate and pyruvate. If pyruvate accumulates it gets converted to lactate which is toxic to the muscles because it does not allow glycolysis to happen.
- **Ammonia is transported into its non-toxic form in the circulation in the form of**
  - Glutamate
  - Glutamine
  - Alanine
- These then successfully reach the liver and all these three forms will be successfully channelized to glutamate. After glutamate is formed in the liver, there will be a reversal of the

glutamate dehydrogenase step. Glutamate will give rise to Alpha keto glutarate, ammonia, and NADH. This Ammonia will get into the urea cycle. The urea cycle is a cycle by which ammonia is converted to urea which is excreted through the urine.

- Urea is the best non-toxic form of ammonia, and both N1 and N2 of urea are to be from ammonia.
- **What is the source of N1 and N2 of urea? MCQ**
  - N1 of Urea is from ammonia and N2 of urea is from aspartate. Urea is still the best non-toxic form of ammonia. Urea is produced in human beings in their metabolism. Human beings are highly evolved organisms on the earth.
  - Only 50% of the glutamate will go through a reversal of the glutamate dehydrogenase step. This meaning 50% will undergo a transamination reaction with oxaloacetate. Glutamate gives its amino group to oxaloacetate forming aspartate, which becomes the N2 donor.

Urea cycle

00:22:32

- The urea cycle happens in the liver, partly in mitochondria, and partly in the cytoplasm. The first two steps happen in mitochondria and the remaining steps happen in the cytoplasm.
- The first two steps are catalyzed by the enzyme Carbamoyl phosphate synthetase 1 and Ornithine transcarbamylase in the mitochondria. The remaining steps happened in the cytoplasm. The first product is Carbamoyl phosphate. For this, a carbon donor (carbon dioxide), a phosphate donor (ATP), and an amino group donor(ammonia) are required.
- **Step 1:** Carbamoyl phosphate synthetase 1 allows carbon dioxide to react with Ammonia and ATP in the presence of another ATP which acts as a source of energy to form Carbamoyl phosphate. For the first step, two high-energy phosphates are used.
- **Step 2:** Ornithine transcarbamylase will allow Ornithine to react with Carbamoyl phosphate to form citrulline.
- Citrulline should immediately leave mitochondria and reach the cytoplasm for the next set of enzymes to act on it. At one point Ornithine will enter mitochondria, and the entry and exit would be through a common transporter, the Ornithine-Citrulline transporter. Citrulline has reached the cytoplasm, and within the structure of Citrulline, the carbon, and N1 of urea are attached. N2 is yet to be attached.
- Citrulline should react with aspartate in the presence of ArginoSuccinateSyntatase, to form ArginoSuccinicAcid. This enzyme uses 1 ATP, which gets converted to AMP and PPI. This step uses high-energy phosphates. So far, four high-energy phosphates have been used.
- Argininosuccinate lyase is the next enzyme that acts on Argininosuccinate acid, converting it into Arginine and fumarate. The fumarate enters the citric acid cycle. It is converted into Malate. Malate dehydrogenase converts Malate to oxaloacetate. They remove hydrogen from Malate and give it to NAD, converting it to NADH, which gives 2.5 ATPs. Every urea cycle utilizes 1.5 ATPs to detoxify two ammonia to form urea. Oxaloacetate is formed which undergoes translation with 50% glutamate to form aspartate. This comes and takes part in the Argininosuccinate synthesis step. This acts as a source of N2. The urea cycle and the citric acid cycle or closely linked and are together called the bicycle.
- **Which amino acid is the link between the urea cycle and the citric acid cycle?**
  - Aspartate
- **Which intermediate is the link between the urea cycle and the citric acid cycle?**
  - Fumarate
- The last enzyme of the urea cycle is Arginase. This acts on arginine and releases urea and ornithine are back. Ornithine is considered to be the catalyst of the urea cycle. It closes the urea cycle. It is the same as the use of oxaloacetate in the citric acid cycle.

The difference between CPS 1 and CPS 2

S. No.	Property	CPS I	CPS II
1.	Pathway	Urea Cycle	Pyrimidine synthesis
2.	Suborganelle	Mitochondria	Cytoplasm
3.	Amino group donor	Ammonia	Glutamine
4.	Regulation	Stimulated by NAG	Inhibited by Uridine, Cytidine and Thymidine

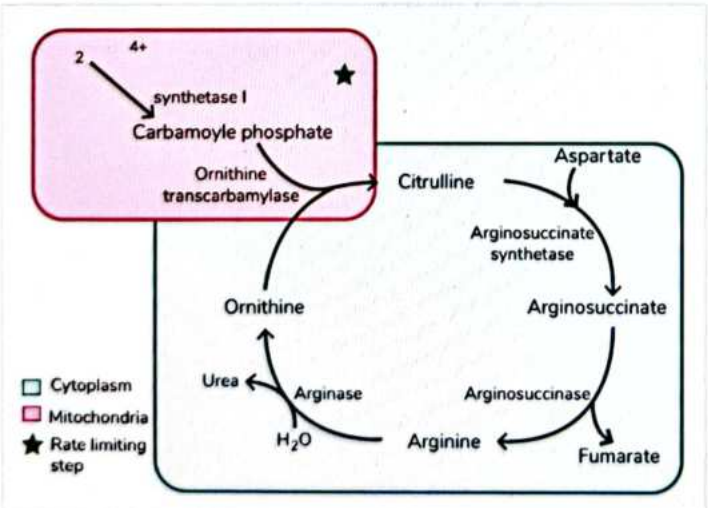
- **Regulation**
  - For CPS 1, it is aldosterically stimulated by N acetyl glutamate. The function of CPS 1 is the urea cycle. The function of the urea cycle is detoxifying ammonia. The signal for high Ammonia generation in a person is glutamate. That reacts with acetyl-CoA to form N acetyl glutamate, which stimulates CPS 1.
  - When glutamine is formed it reacts with acetyl CoA to form N acetyl glutamate which goes and stimulates CPS 1. When there is a fatty acid oxidation defect, acetyl-CoA will not be generated. N acetyl glutamate will not be formed. The urea cycle will be suppressed. Ammonia will accumulate, causing hyperammonemia.

- For CPS 2, there is no such stimulation. CPS 2 is inhibited in a feedback mechanism by all pyrimidine nucleotides (cytidine, thymidine, and uridine).

Clinical features of any Cause of Hyperammonemia 00:39:00

- It can be in a liver disorder where the urea cycle cannot work and there can be hyperammonemia. It can also be a congenital defect of any of the enzymes of the urea cycle. The clinical feature will be because of the accumulation of ammonia. There will be a combination of CNS depression and stimulation manifestations. The child will be apathy, drowsy, unconscious, or even comatose.
- Ammonia sequestrates hydrogen ions, without which the electron transport chain will be suppressed, and there will be no oxidative phosphorylation and no ATP generation in neurons. Ammonia sequester Alpha keto glutarate, no citric acid cycle, and no ATP production.
- **Examples of CNS stimulation manifestation**
  - There can be convulsions, vomiting, and respiratory centers stimulation.
  - The osmotic effect of ammonia causes neurons to swell and gets stimulated. The respiratory centre gets stimulated causing the person to hyperventilate.
  - Respiratory alkalosis is the most common acid-based disorder that is observed in a patient with hyperammonemia.
  - The other reason for CNS stimulation is, glutamate is an excitatory neurotransmitter, and excess glutamate being released into the synapse will cause convulsions and vomiting.
  - To stop these, the person has to be removed from a mixed balanced diet and has to be on a glucose-only diet. When on a mixed balanced diet, there will be a source of amino acid which gets catabolized to form ammonia.

Urea cycle disorders 00:42:32



Carbomyl Phosphate Synthetase 1 When Defective- Type 1 Hyper Ammonemia

- There will be an elevation of ammonia which gets converted to glutamate. There will be an elevation of glutamate and glutamine.

Ornithine Transcarbamylase When Defective- Type 2 Hyper Ammonemia

- This is X-linked recessively inherited, like Fabry disease and G6PD deficiency. First, Carbomyl Phosphate accumulates. This inhibits CPS 1 and ammonia accumulates. High Ammonia, glutamate, and glutamine are observed. Additionally, Carbomyl Phosphate also accumulates in this condition and overflows from mitochondria and reaches the cytoplasm.
- There it will be treated as an intermediate of pyrimidine synthesis. There will be an excess flux of intermediate through pyrimidine in synthesis. All of these will get converted into uracil. There will be high uracil. During the conversion of Carbomyl Phosphate to uracil, an intermediate orotic acid also accumulates. This is how Type 1 ammonia can be differentiated from type to ammonia.

Arginosuccinate Synthetase When Defective- Citrullinemia

- Citrulline accumulates. In a feedback mechanism, it inhibits ornithine transcarbamylase. Carbamoyl phosphate accumulates, overflows to the cytoplasm, and enters into pyrimidine synthesis, therefore there is high uracil and high orotic acid. Carbamyl phosphate inhibits CPS 1 leading to high Ammonia, high glutamate, and high glutamine.

Argininosuccinase Lyase When Defective

- Argininosuccinate acid accumulates and such a condition (Argininosuccinate aciduria, ASA). There is the elevation of citrulline, uracil, orotic acid, high Ammonia, high glutamate, and high glutamine. Argininosuccinate acid causes the tufting of hair and is known as Trichorrhexis nodosa (image-based question).

Arginase When Defective

- This condition is called Argeninemia. All other urea cycle disorders present close to 2-5 months postnatal life, Argeninemia presentation is quite delayed to 2 years of life. Even after 2 years, a classical combination of CNS stimulation and depression manifestation.
- They present with neurological deficits like spastic quadriplegia and spastic diplegia. It is because once arginine is formed, it is just like urea has not been released from it. Both N1 and N2 have been sequestrated and will not cause overt manifestations of hyperammonemia There is still

certain damage that is happening to the nervous system over quite some time. After a few years, there will be mild mental retardation.

Defect of Orthithine-Citrullin Transporter Defect 00:49:50

- There is one common transporter by which ornithine enters mitochondria and citrulline comes out of mitochondria. When this transporter is defective, HHHH syndrome (Hyperornithinemia- hyperammonemia- homocitrullinuria) is caused. Ornithine will not be able to enter into mitochondria, Ammonia will not be detoxified and cause hyperammonaemia. Carbamoyl phosphate will be formed and it will not find ornithine and starts reacting with lysin instead forming homocitrullimia.

Example Cases

- In a ward, if there is a child presenting with symptoms of CNS stimulation, or CNS depression manifestations and they are suspecting hyperammonemia, the balanced diet is stopped and the glucose diet is started. The child starts responding to the treatment. There is reinforcement that it was the correct modality of treatment. To confirm if this is hyper ammonia, blood samples can be collected and sent to the laboratory. An estimate of the ammonia levels is to be done, if it is elevated, all that is known is that this is a case of hyperammonemia. The blood and urine sample is now to be sent with the note which should state that this is a case of hyperammonemia, to a laboratory specializing in and equipped with screening for inborn errors of metabolism.
- **The laboratory will provide six parameters**
  - Ammonia
  - Glutamate
  - Uracil
  - Orotic acid
  - Citrulline
  - Argininosuccinate acid
- When suspecting our argeninemia, it should be mentioned. In that case, the arginine value will also be included. When interpreting these values interpretation should start from the last value. Arginine levels are elevated then it is argeninemia caused by arginase deficiency.
- If arginine levels are normal, Argininosuccinate acid levels are to be checked. When high, it is Argininosuccinate aciduria caused by the defect of arginosuccinate lyase.
- If these levels are normal or less than citrulline values must be looked at. If elevated, its citrullinemia caused by Argininosuccinate synthetase. If citrulline values are also normal, uracil and OA values are to be checked. If both of these are high, its Ornithine Transcarbamylase is defective- Type 2 hyperammonemia. If both of these values are also normal, only elevation of Ammonia and glutamate is there, it is type 1 hyperammonemia or liver disorder, or an inborn error of metabolism causing elevation of ammonia (fatty acid oxidation defect).

One liner 00:57:34

- The most common non-toxic form of ammonia is glutamate.
- The most common non-toxic form of ammonia formed in neurons is glutamine.
- The most common non-toxic form of ammonia formed in muscles is alanine.
- The urea Cycle and TCA cycle are linked through the intermediate, fumarate.
- The urea and TCA cycle are linked through the amino acid, aspartate.
- The step in which urea is released is catalyzed by Arginase.

MCQ'S

Q. The amino acid that does not undergo transamination is:
A. Serine
B. Cysteine
C. Threonine
D. Tryptophan

Q. Aspartate on transamination forms:
A. Pyruvate
B. Alpha Ketoglutarate
C. Alanine
D. Oxaloacetate

Q. Alanine on transamination forms:
A. Pyruvate
B. Alpha Ketoglutarate
C. Alanine
D. Oxaloacetate

Q. All are true about amino acid oxidase except:
A. FAD is used as a coenzyme
B. Linked to ATP production
C. Source of Oxidative Stress
D. Release toxic ammonia

Q. The most common non-toxic form of ammonia is
A. Alanine
B. Glutamine
C. Glutamate
D. Alpha keto glutarate

Q. The most common non-toxic form of ammonia formed in neuron is

A. Alanine
B. Glutamine
C. Glutamate
D. Alpha keto glutarate

Q. The most common non-toxic form of ammonia formed in muscle is

A. Alanine
B. Glutamine
C. Glutamate
D. Alpha keto glutarate

Q. Urea cycle and citric acid cycle are linked through.

A. Aspartate
B. Fumarate
C. Malate
D. Pyruvate.

Q. What is true about CPS 1?

A. Present in cytoplasm
B. Involved in Pyrimidine synthesis
C. Stimulated by NAG
d. Glutamine is the nitrogen source

Case-Based MCQ

Q. A 6 months old female infant began to vomit occasionally and ceased to gain weight. The child became habitually drowsy, temperature raised and her liver was enlarged. The EEG was abnormal. When the milk feeding was avoided and was started on glucose, the condition improved. Urine analysis showed abnormally high glutamine, uracil, orotic acids. Blood showed high ammonium concentration. Citrulline levels were normal. The child has defective:

A. CPS I
B. CPS II
C. Ornithine trans carbamoylase
D. Arginino succinate synthase

- In hyperammonemia there will be abnormal changes in EEG with characteristic slowing of waves. CNS depression so slowing of waves will be observed. It is not type one hyper ammonia. The defect was before citrulline. If it was CPS 1 was defective, the uracil, and orotic acid value would have been less. CPS 2 enzyme on being defective uracil, the orotic acid value would have been less. For option d, the citrulline level will be elevated. Option c. is left and is the right answer.

Image-based MCQ

Q. A 14 year old child presents with coarse, brittle hair, variable degrees of irritability and behavioral or psychomotor retardation. Previous history of lethargy, poor feeding with vomiting, hypothermia and hyperventilation have been recorded. The hair showed characteristic nodules and brittle nature as shown in the image. Blood investigation revealed high ammonia, glutamate, orotic acid, Citrulline and Argininosuccinic acid. Arginine levels were normal. The probable enzyme defect is

A. CPS I
B. OTC
C. Argininosuccinate synthetase
D. Argininosuccinate lyase

- There is tufting of hair. Blood investigation showed high ammonia, glutamate, orotic acid, citrulline, and argininosuccinic acid. When argininosuccinate lyase is defective, there is accumulation of argininosuccinic acid causing argininosuccinic aciduria. Answer is option d.

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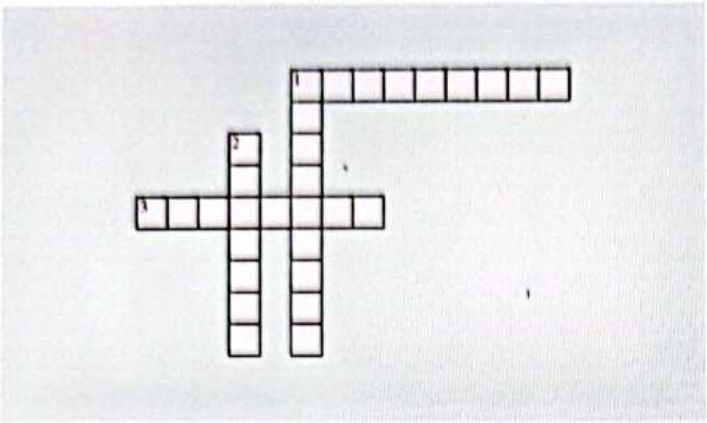
CROSS WORD PUZZLES

Crossword Puzzle 1



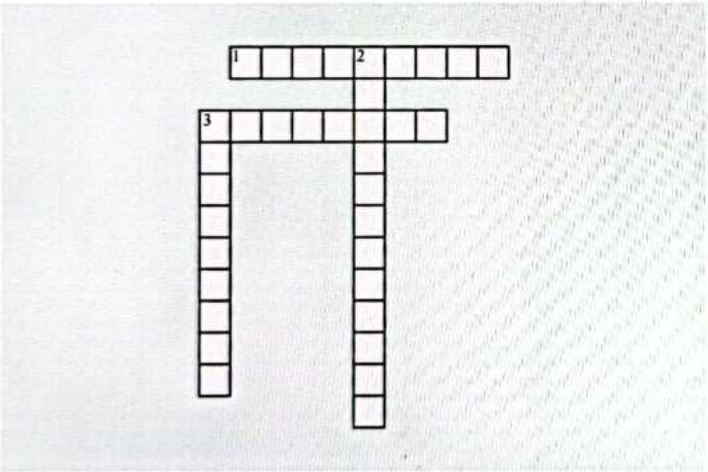
Across

1. The most common non-toxic form of ammonia formed in neurons is -----.
3. Urea Cycle and TCA cycle are linked through the intermediate, -----.

Down

1. The most common non-toxic form of ammonia is -----.
2. The most common non-toxic form of ammonia formed in muscles is -----.

Crossword puzzle 2



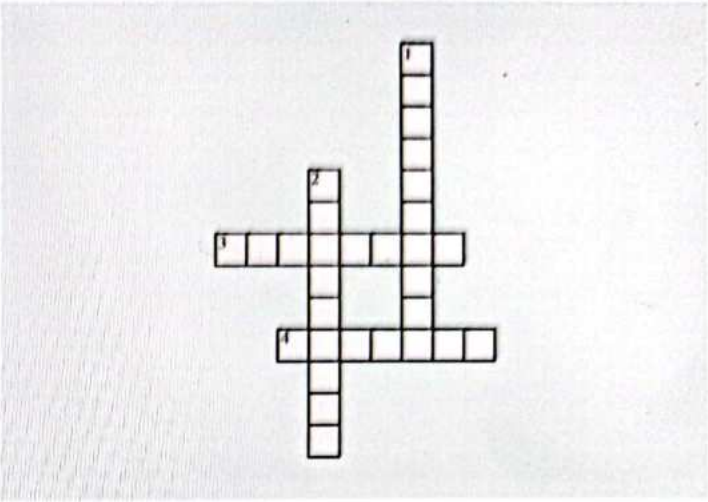
Across

1. The amino acid that does not urdergo transamination is ---- ------.
3. The step in which urea is released is catalyzed by -----.

Down

2. Aspartate on transamination forms -----.
3. Urea Cycle and TCA cycle are linked through the amino acid, -----.

Crossword puzzle 3



Across

3. Alanine on transamination forms -----.
4. The function of urea cycle is detoxifying -----.

Down

1. Amino acid oxidase is not linked to ATP -----.
2. The signal for high ammonia generation in a person is high -----.

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27 GLYCINE AND ONE CARBON POOL

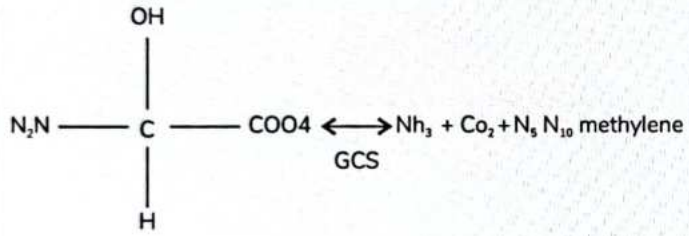
- Glycine is a nonessential amino acid.
- It can be synthesized by body through a metabolic pathway.

Source of Glycine

- They are of three types.
  - Reversal of Glycine cleavage system
  - Serine
  - Threonine
  - The above has two hydroxyl groups.

Glycine Cleavage System

- It is a simplest amino acid containing C-H in the middle.
- This C-H is attached to two functional groups (carboxyl group, amine group) and the hydrogen is attached to the R group.
- This is cleaved by the glycine cleavage system; the amine group is released as ammonia.
- Whereas the carboxyl group is released as carbon dioxide.
- The leftover CH2 is carried by tetrahydrofolate.
- CH2 is converted to N5 N10 methylene tetrahydrofolate.



Clinical Significance

- Hyperammonemia leads to metabolic encephalopathy.
- To decrease ammonia levels, use scavenging agent (sodium benzoate-nonpolar)
  - If any non-polar molecule enter into the body, it undergoes first pass metabolism (Liver)
  - Nonpolar molecules will be stored in the adipose tissue.
  - Liver uses conjugation reactions. It conjugates sodium benzoate with glycine to form hippuric acid (polar)
  - It is further excreted through urine.
  - Liver detoxifies the body by converting nonpolar molecules to polar:
    - → To convert Phase 1 and Phase 2 (conjugation) reactions are used.
    - → Conjugating agent: Glycine
  - In this condition glycine activity is reduced.
  - As it is reduced, glycine synthesis occurs by using ammonia (that reduces blood ammonia levels)
  - So, sodium benzoate is given.
- Phenyl acetate can be used as a scavenging agent.
  - Phenyl acetate is conjugated with glutamine to form phenyl acetyl glutamine (PGA)
  - It is excreted through urine.
  - Decrease in the glutamine levels.
  - To compensate this, all the ammonia is reacted with alpha ketoglutarate to form glutamate.
  - This glutamate reacts with ammonia to form glutamine (uses two molecules of ammonia)
- Glutamine is a better scavenging agent than glycine because it detoxifies two moles of ammonia for every mole of glutamine.

Ways to Reduce Blood Ammonia Levels

- There are two ways through which you can reduce the ammonia levels:
- **Arrest the source of ammonia.**
  - Amino acid breakdown (protein degradation)
    - → To avoid protein degradation, person should take adequate amount of calories (if not, negative buildup of nitrogen balance and all proteins will be broken down to increase ammonia leaves)
  - Urease producing microorganisms in the intestine.
    - → To avoid this, low dose of neomycin is suggested.
    - → Giving lactulose (when it enters into the intestines it acts as a source of acid, this acid converts ammonia to ammonium that can't be absorbed in the intestine)

When these microorganisms act on the urea,
it gets converted to ammonia within the intestinal lumen
↓
As ammonia is a non-polar molecule it enters
into the blood circulation
↓
It increases the blood ammonia levels.

- **To scavenge ammonia**
  - By starting sodium benzoate
  - By giving phenyl acetate (better scavenger)

Subunits of Glycine Cleavage System

There are four subunits

- **Mnemonic: T P L H**
- T: Tetra hydro folate dependent amino methyl transferase (THFAAMT)
- P: PLP dependent glycine decarboxylase
- L: Lipoamide dependent dehydrogenase
- H: Electron transport system or Hydrogen transport system

Coenzymes Used in Glycine Cleavage System

- Tetra hydro folate
- PLP
- Lipoamide

Serine

- It is a hydroxyl group containing amino acids.
- C-H attaches to the carboxyl group and amino group. The R group is CH2OH.
- Hydroxy methyl group is removed to get hydrogen by the enzyme serine hydroxy methyl transferase (Serine to glycine). The removed methyl group is attached to tetra hydro folate to for N5 N10 methylene tetrahydrofolate.

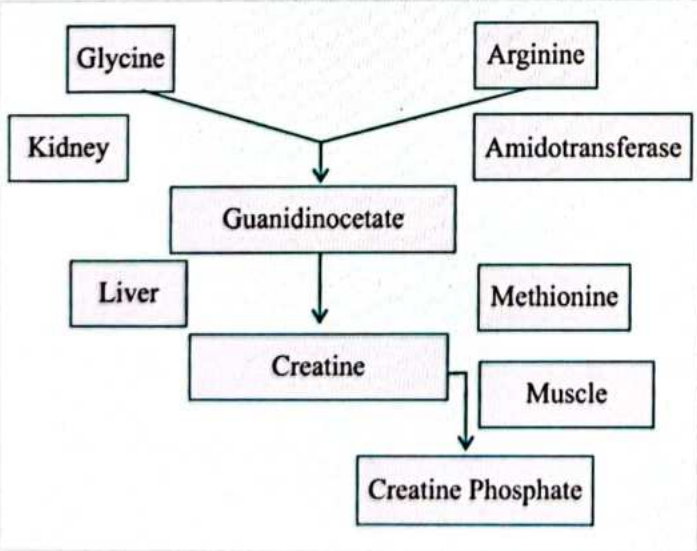
Threonine

- Threonine is converted to glycine in the presence of threonine aldolase.

Uses of Glycine

- It is a inhibitory neurotransmitter
  - For brain: GABA
  - For spinal cord: Glycine
- It is a conjugating agent.
- One of the amino acid presents in glutathione (tripeptide: Gamma Glutamyl Cysteinyl Glycine)
  - It has glutamic acid, cysteine, glycine.
  - It is a conjugating agent and antioxidant.
- It is necessary for creatine synthesis.
  - Mnemonic: GAMe Glycine, arginine, methionine)
- It is the most abundant amino acid of collagen. It is a triple helix structure made up of repetitive unit of (glycine XY) n
- Heme synthesis
- Purine ring formation- C4, C5 and N7
- One carbon pool

Creatine Synthesis



Reaction	Enzyme	Location
Glycine reacts with arginine to form guanidinoacetate	Amido transferase (Kidney)	Kidney
S adenosyl Methionine react with guanidinoacetate to form creatinine	Methionine	Liver
Creatinine to creatine phosphate	Creatinine kinase	Muscle

Important Information

Vitamin-D Activation

- First step is catalyzed by 25 alpha hydroxylases (liver)
- Second step is catalyzed by 1 alpha hydroxylase (kidney)

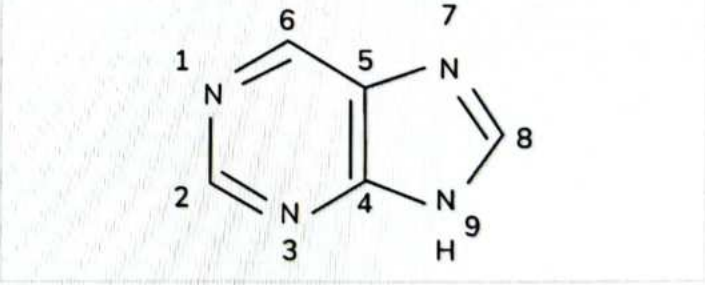
Heme Synthesis

- First step: Reaction takes place in mitochondria.
- Substrates used: Glycine.
- Glycine and amino acid react with succinyl CoA (intermediate of citric acid cycle in the presence of delta ALA synthase to form delta ALA
- It is a decarboxylation reaction.
- Coenzyme: Pyridoxal phosphate.
- Prerequisite: Glycine should enter into mitochondria.
- Transporter: SLC25A38

Purine Ring Formation

Purine	Pyrimidine
• Bigger structure: 2 rings and 9 atoms	• Small structure: 1 ring and 6 atoms

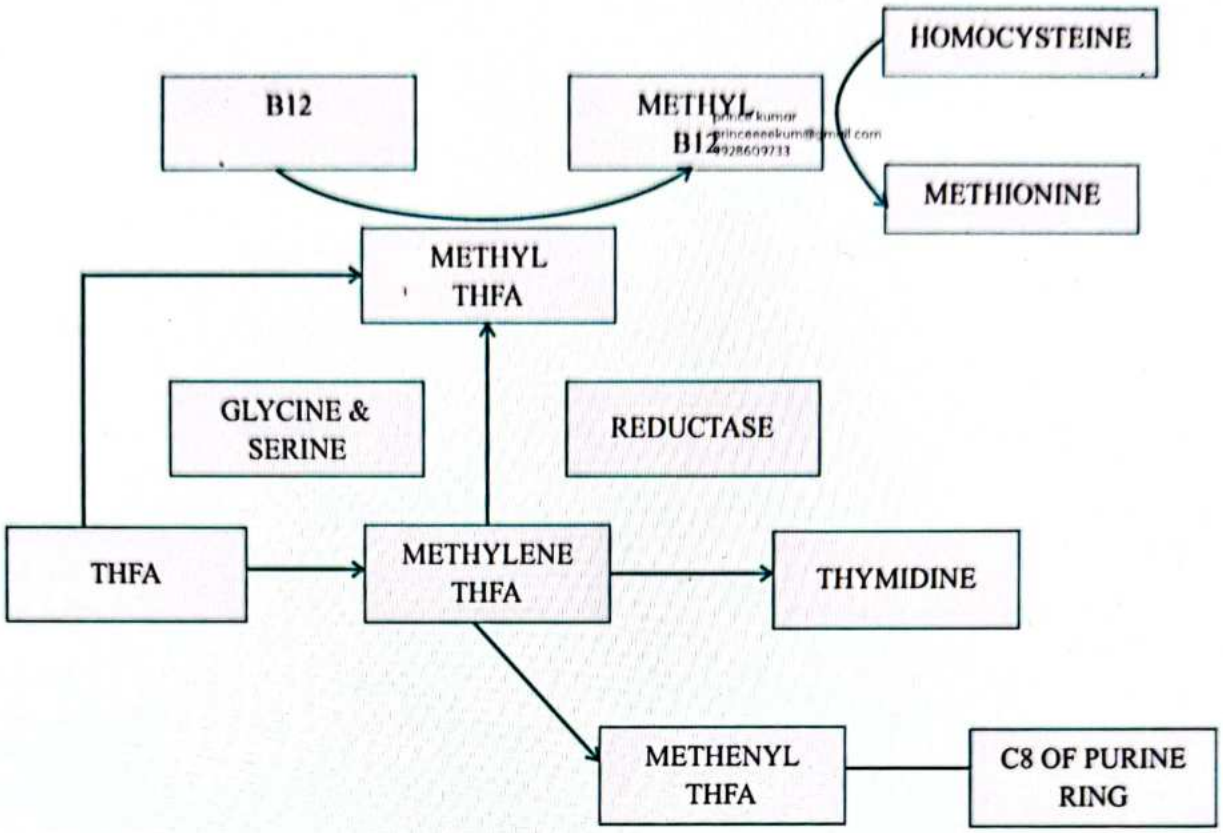
Sources of Purine Ring



- N1: Aspartate
- C2 and C8: Donated by tetrahydrofolate derivatives.
  - C2: N10 formyl tetrahydrofolate
  - C8: N5 N10 methenyl tetrahydrofolate
  - N3 and N9: Glutamine
  - C4, C5 and N7: Glycine
  - C6: CO_2

One Carbon Pool

- Pool of one carbon derivatives of THFA, their sources and their biological products



Reaction	Source and Enzymes
• THFA gives N5 N10 methylene THFA. • It is necessary for the conversion of uridine to thymidine	• Glycine Cleavage System (source) • Serine Hydroxy Methyl Transferase
• N5 N10 methylene THFA (undergoes irreversible chemical reaction) gives methyl tetrahydrofolate	• **Enzyme:** Reductase

Methyl tetrahydrofolate has very less group transfer potential where it transfers methyl group only to B12, converting to methyl B12.

↓

This methyl B12 has one coenzyme rule (act as a coenzyme for methionine synthase)

↓

Methionine is converted to homocysteine in presence of methionine synthase.

↓

This methionine is converted to S adenosyl methionine (used for transmethylation reactions)

↓

Transmethylation products: Creatinine, epinephrine, choline

Methyl folate Trap in B12 Deficiency

- When there is B12 deficiency there is no methyl B12.
- Inactivation of methionine synthase
- In B12 deficiency: Creatinine, choline, and epinephrine (decreased).
- Tetrahydrofolate will be trapped as methyl tetrahydrofolate.
- That causes functional deficiency of tetrahydrofolate occurs (because of low activity of methionine synthase)
- B12 deficiency indirectly causes folate deficiency.
- It is known as methyl folate trap.

Folate Deficiency with Macrocytic Anemia

When there is no folate, there is no tetrahydrofolate, no methylene tetrahydrofolate and no methenyl tetrahydrofolate

↓

Thymidine is not synthesized (without methylene tetrahydrofolate)

↓

Purine ring is not synthesized (without methenyl tetrahydrofolate and formyl tetra hydro folate)

↓

Optimum concentration of all nucleotides is required for replication process.

↓

In folate deficiency replication is not possible due to the absence of purines and pyrimidines

↓

Hence, cell division is inhibited.

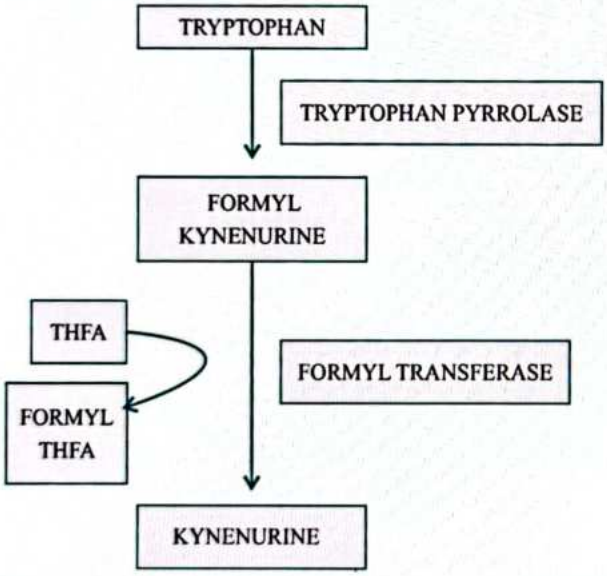
↓

In folate deficiency cells can grow but cell division is not possible resulting in macrocytic anemia.

↓

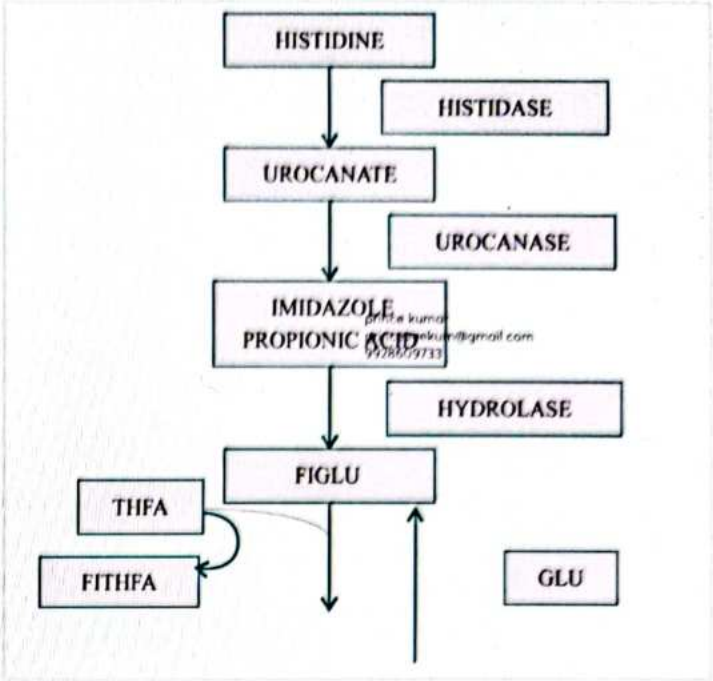
B12 deficiency indirectly causes folate deficiency.

Tryptophan in One Carbon Pool



Reactions	Enzymes
• Tryptophan gives formyl kynurenine	• Tryptophan pyrrolase
• Formyl kynurenine to kynurenine	• Formyl transferase
• The removed formal group is attached to tetrahydrofolate forming N10 formyl tetrahydrofolate (helps in the formation of C2 of purine ring)	

Histidine in One Carbon Pool



Reactions	Enzymes
• Histidine gives Urocanate	• Histidase
• Urocanate to imidazole propionic acid	• Urocanase
• imidazole propionic acid give FIGLU	• Hydrolase

- If there is tetrahydrofolate in a person, the FIG is transferred to THFA which is further converted to FITHFA.
- Therefore, FIGLU will become glutamate which is further converted to Alpha-Ketoglutarate
- It enters the citric acid cycle that comes out as carbon dioxide.

- If there is any folate deficiency, FIGLU is unable to donate its FI group.
- Hence, there will be accumulation of FIGLU.
- In urine, FIGLU levels will be increased (after histidine load)

THFA Derivatives, Sources and Significance

THFA Derivatives	Sources	Significance
N5 N10 methylene THF	Glycine, Serine	Conversion of dUMP to dTMP(uridine to thymidine)
N10 formyl THF	Tryptophan	C2 of purine ring
N5, N10 methanyl THFA	N5, N10 Methylene THFA	C8 of purine ring

One Liners

- The amino acid necessary for heme synthesis is **Glycine**
- The most common inhibitory neurotransmitter of brain is **GABA**
- The most common inhibitory neurotransmitter of spinal cord is **Glycine**
- N5 N10 methylene THFA is used for the formation of **Thymidine**
- C2 of purine ring is formed from **N10 formyl THFA**
- C8 of purine ring is formed from **N5, N10 methenyl THFA**

Multiple Choice Questions

Q. All of the following are aminoacids required for creatine synthesis except

A. Glycine
B. Alanine
C. Arginine
D. Methionine

Q. All of the following act as sources of atoms of purine ring except:

A. Glycine
B. THFA
C. Arginine
D. Aspartate

Q. All of the following are specialized products of Glycine except:

A. Collagen
B. Creatine
C. Glutathione
D. Pyrimidine

Q. Tryptophan acts as a source of:

A. N10 formyl THFA
B. N5. N10 methylene THFA
C. N5, N10 Metheny! THFA
D. N5 formyl THFA

Q. FIGLU test is done after a load of:

A. Tyrosine
B. Tryptophan
C. Histidine
D. Glycine

Q. Glycine acts as a source of:

A. N5, N10 methylene THFA
B. N5, N10 methenyl THFA
C. N10 formyl THFA
D. Formimino THFA

Q. All the following amino acids take part in one carbon pool except

A. Glycine
B. Tryptophan
C. Histidine
D. Proline

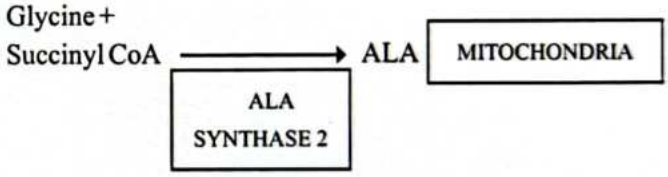
Case Based MCQs

Q. An 8-month-old boy was admitted because of paleness. Laboratory studies disclosed microcytic and hypochromic anemia. Serum iron and ferritin were high. Examination of bone marrow revealed prominent erythroid hyperplasia; 18% of the erythroblasts were distinct ringed sideroblasts. Electron microscopic studies found intramitochondrial iron deposits in the erythroblasts. Sideroblastic anemia was diagnosed. A point mutation in SLC25A38 (mitochondrial transporter) was found in this patient. What is the mode of inheritance of this condition?

A. X linked recessive
B. X linked dominant
C. Autosomal recessive
D. Autosomal dominant

Explanation

- Glycine reacts with succinyl CoA in the presence of ALA synthase to form ALA in mitochondria.



- When there is defect in ALA synthase2, protoporphyrin ring will not be formed.
- No protoporphyrin ring, iron cannot be chelated.
- So that iron accumulates in the mitochondria surrounding the nucleus (looks like sideroblast)
- Hence it is called sideroblastic anemia.
- If ALA2 synthase defect the inheritance is X linked recessive sideroblastic anemia.
- Sideroblastic anemia can also occur due to the defect of a transporter SLC 25A38. Therefore, glycine not able to enter the mitochondria because of the mutation in the transporter. The gene is present in Autosomal chromosome or Autosomal recessive inheritance.

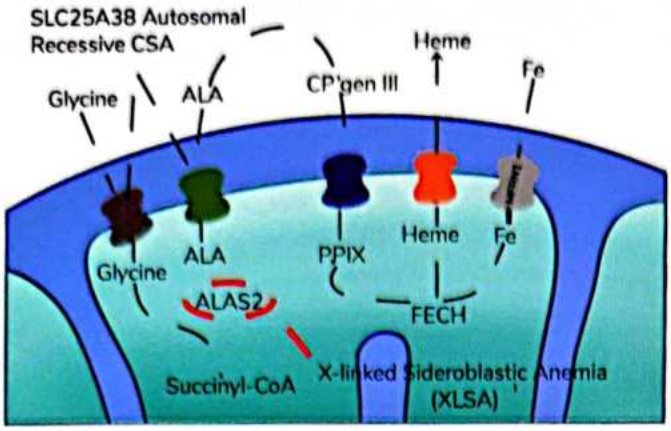
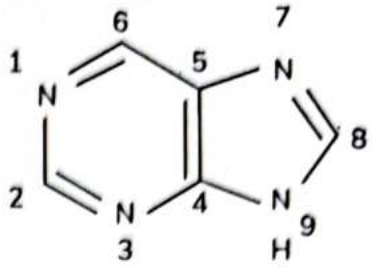


Image Based MCQs

Q. The source of C8 in the given ring is?



A. N5, N10 methylene THFA
B. N5, N10 Methenyl THFA
C. N10 Formyl THFA
D. N10 methenyl THFA

28 PHENYLALANINE AND TYROSINE METABOLISM

Metabolism of Phenylaniline

Major Fate

- Phenylalanine is converted to tyrosine (hydroxylated phenylalanine) catalyzed by phenylalanine hydroxylase.

Hydroxylases

- All hydroxylases are monooxygenases.
- Every hydroxylase will allow an oxygen molecule to react with the substrate.
- When an oxygen molecule reacts with Phenylaniline, one 'O' of the oxygen molecule will be incorporated into the substrate.
- Finally, the substrate is converted to the hydroxylated form.
- The other O will become water (hydrogen atoms are obtained from the co-enzyme tetrahydrobiopterin (BH2) that is converted to dihydrobiopterin).

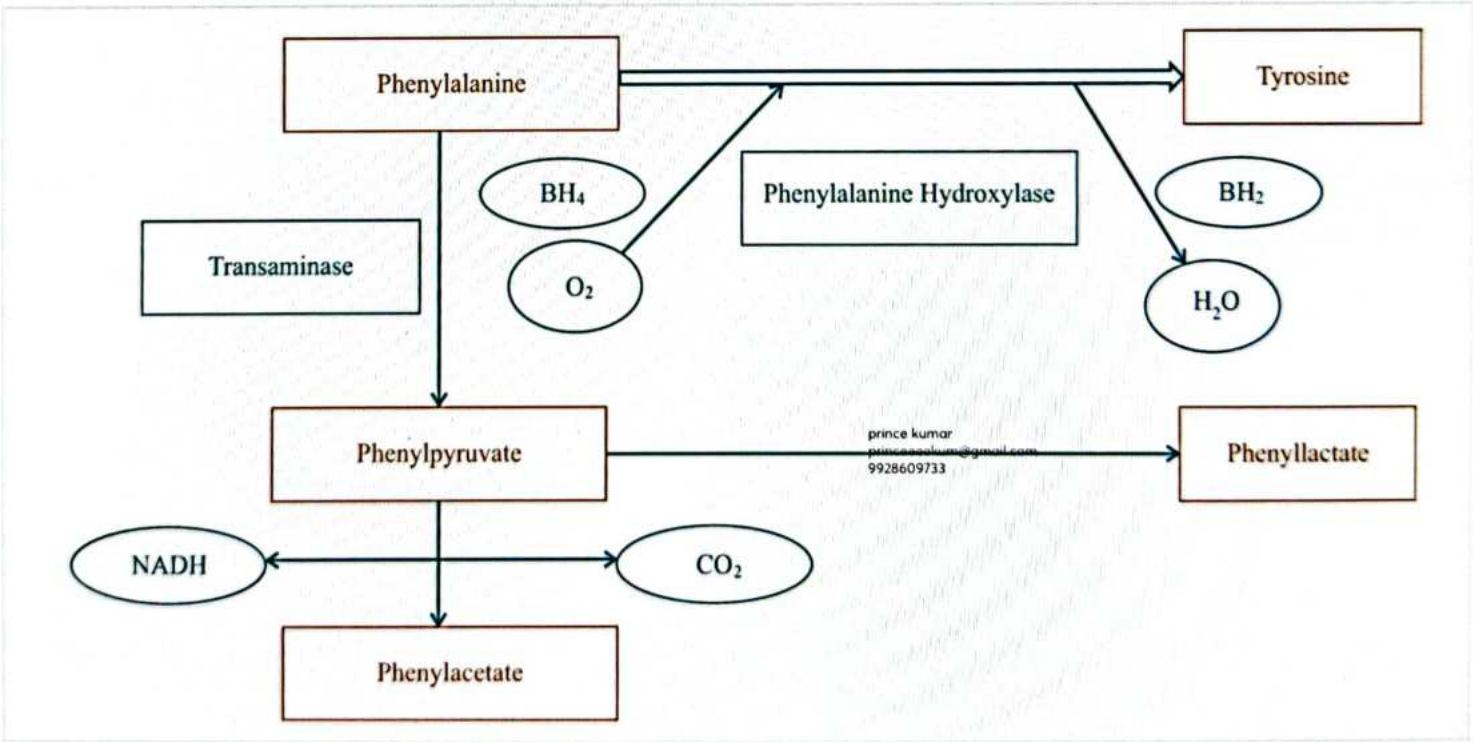
Minor Fate

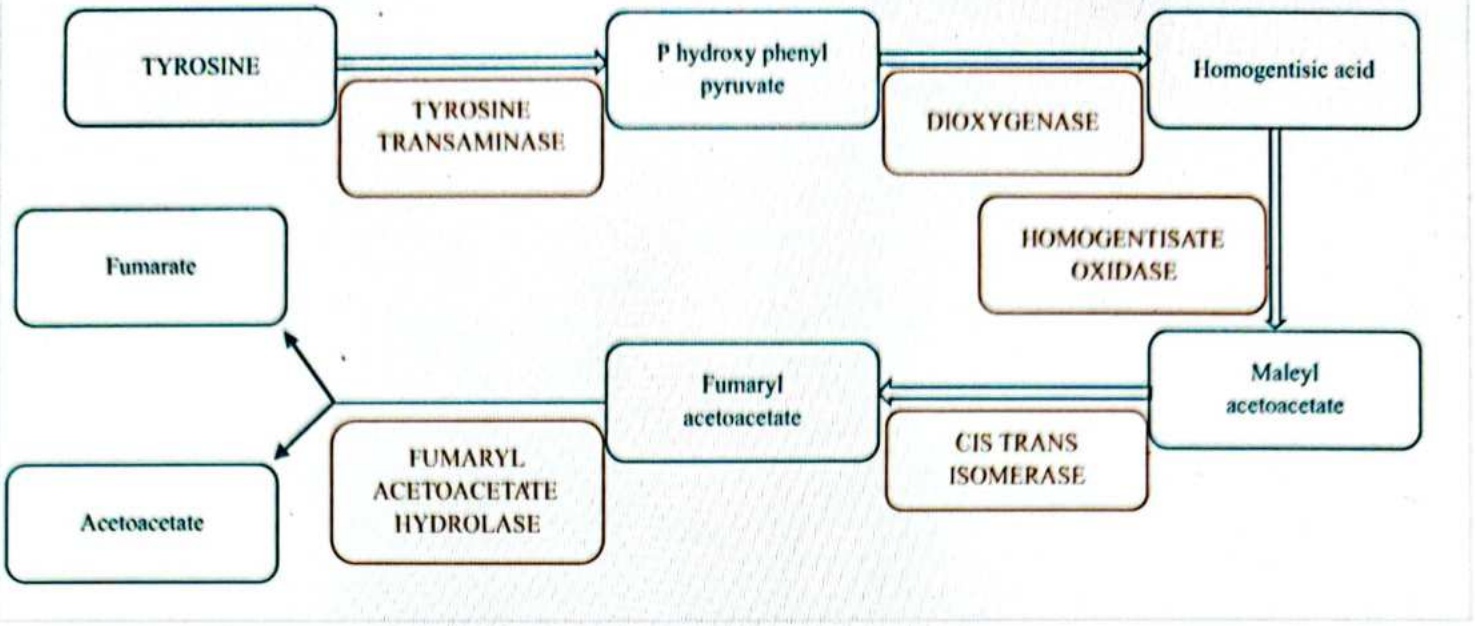
- Phenylalanine on Transamination forms phenylpyruvate
- Mnemonic: LP Lumbar puncture (Alanine on transamination forms pyruvate)
- Phenylpyruvate on reduction becomes phenyl lactate.
- On reduction, it is converted to phenylacetate.
- Phenylpyruvate (phenyl ketone) on oxidative decarboxylation forms phenylacetate
- It reaches the liver and undergoes conjugation with glutamine (phenylacetylglutamine) excreted through urine.

Tyrosine Metabolism

Reaction	Enzyme
Tyrosine (hydroxy phenylalanine) converted to P hydroxy phenylpyruvate	Tyrosine transaminase.
P hydroxy phenylpyruvate to homogentisic acid.	P hydroxy phenylpyruvate dioxygenase
Homogentisic acid is converted to maleylacetoacetate	Homogentisate oxidase
Maleylacetoacetate to fumarylacetoacetate.	Cis trans isomerase
Fumarylacetoacetate is converted to fumarate and acetoacetate	Fumarylacetoacetate hydrolase

- Amino acids, which are both ketogenic and glucogenic are phenylalanine, tyrosine, and tryptophan (aromatic amino acids).





Disorders of Tyrosine Metabolism

Disorder	Explanation
Type-I tyrosinemia (hepato renal syndrome)	• Deficiency enzyme: Fumarylacetoacetate hydrolase. • Fumarylacetoacetate (precursor) accumulate which is toxic to both liver and kidney parenchymal cells (Fanconi syndrome progress to renal failure) • It results in jaundice, hepatomegaly, hypoglycemia, HCC and liver cirrhosis. • Fanconi syndrome. • Renal failure. • Additionally, Fumarylacetoacetate is converted to succinyl acetone that inhibits the delta ALA dehydratase (heme synthetic enzyme). • Elevation of succinyl acetone is considered as a pathognomonic feature. • Many times, this disease is misdiagnosed as porphyria (due to neuropsychiatric manifestations).
Type-II tyrosinemia	• Other names: Occulo cutaneous syndrome, Richner-Hanhart syndrome • Deficiency enzyme: Tyrosine transaminase • Ocular: Painful corneal erosions • Cutaneous: Palmar hyperkeratosis
Type-III tyrosinemia	• Deficiency enzyme: P hydroxy phenylpyruvate dioxygenase • Indications: Recurrent seizures, intermittent ataxia • Gain of function mutation of P hydroxy phenylpyruvate dioxygenase causes hawkinsinuria (swimming pool odor of urine)
Alkaptonuria (Black urine disease)	• Deficiency: Homogentisate oxidase. • Homogentisic acid will be accumulated in the blood (undergoes oxidation to form benzoquinone acetate) • It polymerizes to form melanin-like fibers (pigmentation of the skin, mucus membrane) • Osler's sign: Black or brown pigmentation of sclera between the medial and lateral rectus (sign) • It accumulates in cartilage (cartilage destruction) • Finally, it causes ochronosis (a feature of Alkaptonuria) • Patients are normal till mid-age. • Indications: multiple joint involvements, multiple intervertebral disc bulges, knee pain • Symptom: Urine turns dark on standing • Treatment is done by inhibiting the oxidation (antioxidants: Vitamin C, Nitisinone)

Nitisinone

- Acts by inhibiting P hydroxy phenylpyruvate dioxygenase.
- Loss of gene mutations causes type-III tyrosinemia.
- Gain of function mutation causes hawkinsinuria.
- This drug was first used in the treatment of type-I tyrosinemia.
- Because when this enzyme is inhibited, the precursor fumarylacetoacetate is not formed.
- Hence, not toxic to liver and kidney parenchymal cells

Uses

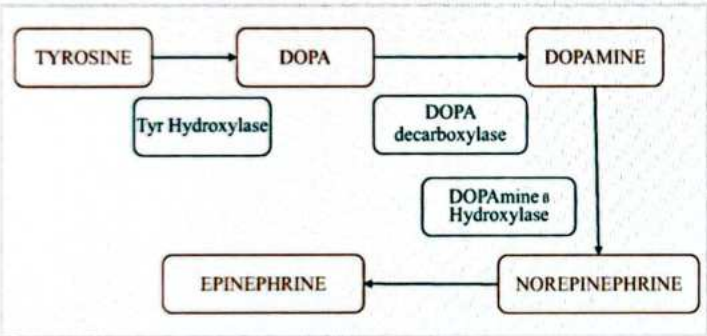
- To treat alkaptonuria (accumulation of homogentisic acid is inhibited)
- To treat hawkinsinuria

Contraindications: Type-II tyrosinemia

Specialized Products from Tyrosine

- Neurotransmitters (Dopamine, Norepinephrine, epinephrine) by-products of tyrosine
- Formation of melanin pigment
- Formation of thyroid hormone

Neurotransmitters: Dopamine, Norepinephrine, Epinephrine



Reaction	Enzyme
Tyrosine to dihydroxy phenylalanine (DOPA)	Tyrosine hydroxylase
DOPA to Dopamine	DOPA Decarboxylase (uses PLP as a coenzyme)
Dopamine gives Norepinephrine	Dopamine beta-hydroxylase (depends on Vitamin C)
Norepinephrine forms epinephrine	S adenosyl l methionine

- Norepinephrine: Released by postganglionic sympathetic nerve endings (fewer beta effects.
- Epinephrine: Adrenal medullary chromaffin cells.

Synthesis of Melanin Pigment

Reaction	Enzyme
Tyrosine to DOPA	Tyrosine hydroxylase/ Tyrosinase
DOPA to Melanin	Non-enzyme-catalyzed chemical reaction

- Tyrosinase enzyme defect causes albinism.

Synthesis of Thyroid Hormone

- Thyroid gland is organized in the form of follicles (have a central colloid)
- It is surrounded by a layer of follicular cells (active protein synthesizing machinery).
- Protein: Thyroglobulin (synthesized)
- It is a glycoprotein containing 5000 amino acids (around 123 are tyrosine residues)
- Tyrosine residues undergo iodination to form monoiodo and diiodo tyrosine.
- It undergoes coupling to form thyronine hormones.

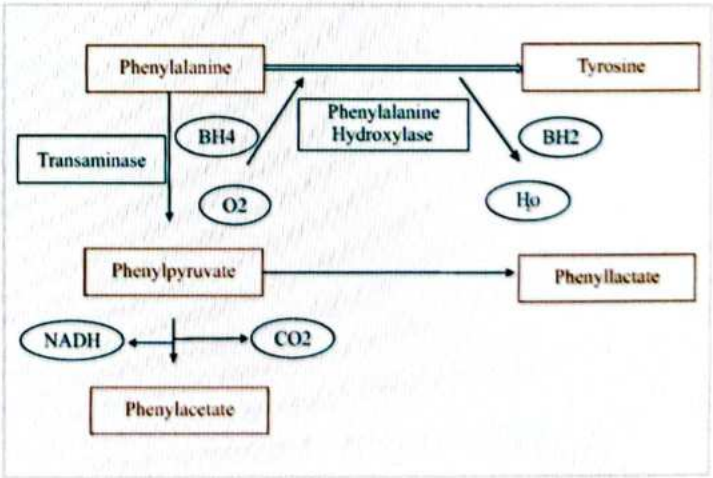
Example

- MIT + DIT - T3
- DIT + MIT - Reverse T3
- DIT + DIT - T4

During Coupling	
T4	Predominant
T3 (the active form of thyroid hormone)	Less
Reversal T3	Least

Phenylketonuria 00:29:10

- Defect: Phenylalanine metabolism (major fate- Phenylalanine hydroxylase)



- Accumulation of phenylalanine.
- Diverted to minor route.
- Phenyl ketones are synthesized.
- As the phenyl ketones are accumulated, it is called phenylketonuria.

Clinical and Biochemical Features of Phenylketonuria

Characterized with

- Mental retardation (decrease in tyrosine)
  - Another reason is increase in the accumulation of phenyl ketones.
  - These will compete with other neutral amino acids to cross BBB.
  - This results in decreased blood development.
  - Phenylalanine restricted diet along with tyrosine supplements (avoid mental retardation).
- Mousy odor (accumulation of phenylacetate)
- Hypopigmentation (decreased synthesis of melanin)

Screening Tests

- **Ferric chloride test:** Urine (non-specific finding)
  - Ferric chloride forms an adsorption complex with phenyl ketones (bluish or greenish precipitate)
- **Guthrie's test:** Blood
  - Organism: Bacillus subtilis needs phenyl ketones for its growth

Take a colony plate of Bacillus subtilis.
↓
Add drop of patient's blood
↓
After incubating, count the number of colonies.
↓
There is steady state increase of bacillus subtilis.
↓
It indicates the presence of phenyl ketones in the blood

Q. Which is required for the growth of bacillus subtilis?
Ans. Phenyl Ketones

- Both the above tests are false tests.
- Screening test: HPLC with tandem mass spectrometry using a heel prick blood sample (any inborn error of amino acids)
  - Blood sample is added to a adsorption pad (Whatman filter paper 1) and punched
  - Such that every analyte goes through HPLC and get quantified.
  - Depending upon the concentration, you can diagnose various disease with one sample (multiple diagnosis)

Urine Odor and Diagnosis

Odor	Disorders
Mousy	PKU
Fruity	DKA (diabetic ketoacidosis)
Cabbage	Tyrosinemia (type I)
Boiled cabbage	Hypermethioninemia
Oast house (urine and sweat smell as oasthouse) or beer baby syndrome	Methionine malabsorption
Swimming pool	Hawkinsinuria
Sweaty feet	Isovaleric acidemia
Tom cat Urine	Multiple carboxylase deficiency
Fish odor	Trimethyl amine urea

One liner

- Phenylketonuria is caused by the defect of **Phenylalanine Hydroxylase**
- Type I Tyrosinemia is caused by the defect of **FumarylAcetoacetate Hydrolase**
- Type II Tyrosinemia is caused by the defect of **Tyrosine Transaminase**
- Gain of function mutation of pHPP dioxygenase causes **Hawkinsinuria**
- Oast house odor is caused by **methionine malabsorption**
- Cabbage odor is a feature of **Type-1 Tyrosinemia**

Multiple Choice Questions

Q. Nitisinone treats all except?
A. Type I Tyrosinemia
B. Type II Tyrosinemia
C. Alkaptonuria
D. Hawkinsinuria

Q. Type II Tyrosinemia is characterized by all except:
A. Palmar hyperkeratosis
B. Corneal ulcer
C. Porphyria
D. Oculocutaneous syndrome

Q. All the following are specialized products obtained from tyrosine except:
A. Dopamine
B. Melanin

C. Epinephrine
D. Serotonin

Case Based MCQs

Q. A 6-year-old boy presents with periodic aggressive behavior. His urinary ALA is elevated. On examination, he is icteric. A mild hepatomegaly is observed. Blood examination revealed massive elevation of AFP. HPLC and TMS examination revealed an elevation of succinyl acetone. The probable diagnosis is.

A. Type II Tyrosinemia
B. ALA Dehydratase deficiency Porphyria
C. Type I Tyrosinemia
D. Type III Tyrosinemia

Q. A 6-year-old boy presented with spontaneous corneal erosion. On examination, his palms were found to be hyperkeratotic HPLC with TMS revealing a high Tyrosine peak and absence of Succinyl acetone peak. The most probable diagnosis is?

A. Type II Tyrosinemia
B. Alkaptonuria
C. Hawkinsinuria
D. Type I Tyrosinemia

Explanation

- Type I: Succinyl acetone will be elevated.
- Type II: No elevation of Succinyl acetone

Image Based MCQs

Q. 20-year-old boy with severe mental retardation, mousy odor in body fluids, and hypopigmentation. The patient has frequent episodes of seizures & aggressive behavior. What is your diagnosis?

A. Tyrosinemia
B. Albinism
C. Maple Syrup Urine Disease
D. Untreated PKU Phenylketonuria

Explanation

- **Albinism:** Hypopigmentation
- **Tyrosinemia:** Type-I - Hepatorenal syndrome and Type-II - Oculocutaneous syndrome
- **MSUD:** Burnt sugar odor of urine

29 AMINO ACIDS METABOLISM DISORDERS

Carbon Skeleton Catabolism

- In the urea cycle, at least every amino acid gives off its amino group to form a carbon skeleton which further undergoes catabolism.
- The amino group forms urea.

  Amino group → Ammonia → Urea
- The carbon skeleton is involved in catabolism.
- Depending upon the product obtained from the carbon skeleton catabolism, amino acids are classified into
  - Purely Ketogenic (On catabolism gives only acetyl CoA)
    - → Leucine
    - → Lysine
  - Purely Glucogenic (On catabolism gives only pyruvate)
    - → Alanine: On transamination yields pyruvate.
    - → Glycine: Simplest amino acid.
    - → 3-hydroxyl group-containing amino acids
      - Serine
      - Threonine
      - Hydroxyproline
    - → Sulfur-containing amino acid
      - Cysteine
  - Both Ketogenic and Glucogenic (Substance on catabolism gives one of the glycolytic intermediate - Pyruvate or TCA cycle intermediate other than Acetyl CoA)
    - → Aromatic amino acids
      - Phenylalanine
      - Tyrosine
      - Tryptophan
    - → Branched-chain amino acid
      - Isoleucine

Important Information

- **Ketogenic:** If Acetyl CoA Intermediate is produced.
- **Glycolytic intermediate:** Can reverse the glycolysis and reach glucose.
- **Citric acid cycle intermediate:** Can produce OAA.
- **OAA (gluconeogenesis intermediate):** Involved in gluconeogenesis.

Glucogenic AA to TCA Cycle Intermediates

Intermediates formed via TCA cycle

- **Mnemonics: Cats succeed Funny Oxes**
  - Cats - Alpha Ketoglutarate
  - Succeed - Succinyl CoA
  - Funny - Fumarate
  - Oxes - Oxaloacetate

On transamination the following amino acids form Alpha Ketoglutarate

- Glutamate
- Glutamine
- Histidine
- Proline
- Arginine

Amino acids which form Succinyl CoA are

- V - Valine
- I - Isoleucine
- M - Methionine

Amino acids which form Fumarate are

- Phenylalanine
- Tyrosine

On transamination, the amino acids which form Oxaloacetate are.

- Aspartate
- Asparagine

Tryptophan Metabolism

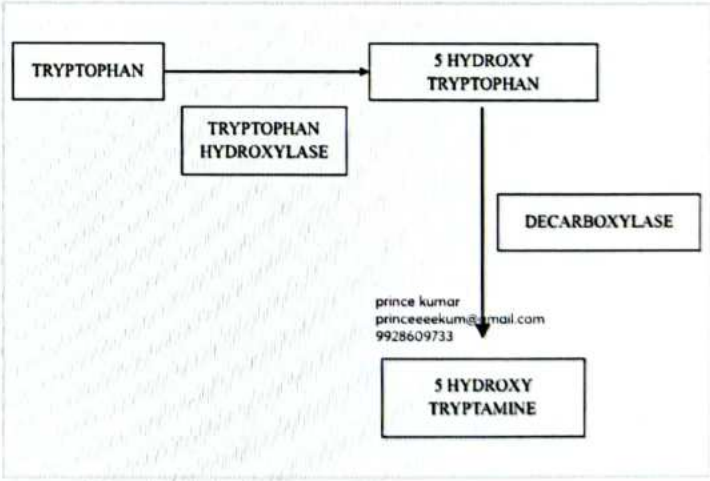
Tryptophan Metabolism - Specialized Products

- Serotonin - 5-hydroxytryptamine
- Niacin

Synthesis of Serotonin

Aim: To form coenzyme forms of niacin

- Niacin Mono Nucleotide.
- Niacin Adenine Dinucleotide.



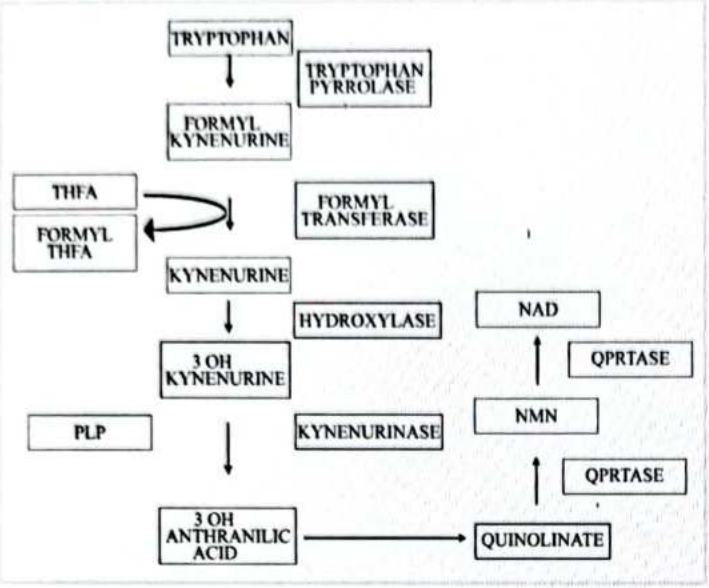
Step-01: Tryptophan → 5 Hydroxytryptophan

- Hydroxylation process.
- Catalyzed by Tryptophan Hydroxylase.

Step-02: 5 Hydroxytryptophan → 5 Hydroxytryptamine (Serotonin)

- Decarboxylation process.
- Catalyzed by Decarboxylase.

Synthesis of Niacin



Step-01: Tryptophan → FormylKynurenine

- Catalyzed by Tryptophan pyrrolase.

Step-02: FormylKynurenine → Kynurenine

- Catalyzed by Formyltransferase
  - Converts Tetrahydrofolate to formyl Tetrahydrofolate.
  - It enters One carbon pool and is used to form C2 of purine rings.

Step-03: Kynurenine → 3-OH Kynurenine

- Catalyzed by Hydroxylase.

Step-04: 3-OH Kynenurine → 3-OH Antitranilic acid

- It utilizes pyridoxal phosphate.
- Catalyzed by Kynenurinase and dependent on B6.
- B6 is necessary for
  - All transaminases.
  - Most of the Decarboxylases.
  - Kynenurinase.

Step-05: 3-OH Antitranilic acid → Quinolinate

- 3-OH Antitranilic acid undergoes a series of non-enzyme catalase reactions to form a ring (Quinolinate).

Step-06: Quinolinate → Niacin Mono Nucleotide

- Rings get attached to Ribose 5 Phosphate.
- Quinolinate phosphoribosyl transferase (QPRTASE) is involved in the reaction.
  - Role: It converts the ring form (In step 6 - Quinolinate) to nucleotide by adding ribose 5 phosphate.

Step-07: Niacin Mono Nucleotide → Niacin Adenine Dinucleotide

- Catalyzed by QPRTASE
- Another phosphoribosyl transferase that acts on NAD to form adds Ribose 5 Phosphate.

Step-08: NAD → NADP

- Kinase acts on the NAD to convert it into NADP.

Pellagra

- Deficiency of Niacin causes pellagra.
- Characterized by 3 D's
  - Diarrhea
  - Dementia
  - Dermatitis

Causes of Pellagra

Diet with

- Niacin deficiency.
- Tryptophan (60 mg Tryptophan= 1 mg Niacin) deficiency in the maize-based diet.
- Tryptophan malabsorption - Hartnup's disease.

Hartnup's Disease (Blue Diaper Syndrome)

- Tryptophan malabsorption syndrome.
- The defect of the neutral amino acid transporter specific for tryptophan causes it.
- It is responsible for both
  - **Absorption along the intestine**, if not
    - → Hartnup's disease.
    - → Niacin deficiency.
  - **Reabsorption from the renal tubule**
    - → It causes aminoaciduria.
    - → As the tryptophan is present in urine and exposed to air, the indole ring of tryptophan will oxidize to form ketol compounds and produce a blue color.

Important Information

- **Example:** If there is a family with an autosomal recessive pattern of inheritance of pellagra, accompanied by aminoaciduria in most family members and bluish urine discoloration, then
- Remember that Hartnup's Disease is another cause of pellagra.

In Carcinoid Syndrome

- Tryptophan is used to form Serotonin.
- Thus, tryptophan is not available for niacin synthesis.
- Niacin deficiency.

Vitamin B6 deficiency

- It inactivates Kynenurinase.
- The precursor kynurenine gets converted into Xanthurenic acid.
- After tryptophan load, estimate the xanthurenic acid in urine.

Leucine pellagra

- Inhibits Quinolinate phosphoribosyl transferase (QPRTASE).
- In a leucine-rich diet, for example, a sorghum diet.
  - In general, the farmers sell the costlier crops like rice and wheat and cultivate themselves grains like maize or sorghum as such.
  - Where
    - → **Maize:** Maize-rich diet causes niacin deficiency.
    - → **Sorghum:** Sorghum is rich in leucine, which may cause pellagra.

Homocysteine Metabolism

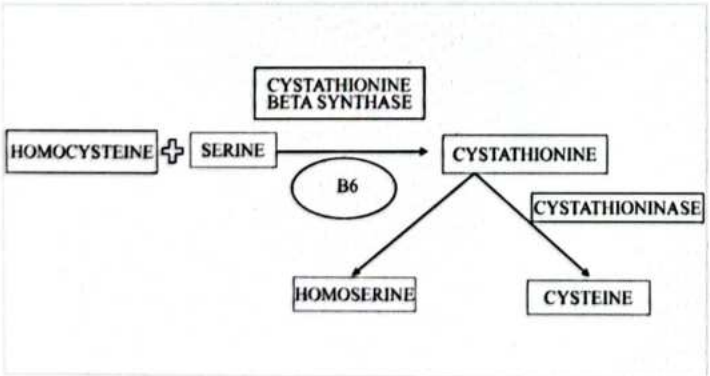
- Homocystinuria causes.
  - Accelerated atherosclerosis.
  - Accelerated thrombosis.
  - Skeletal deformities like
    - → Flat foot
    - → Charlie Chaplin gait
    - → Ectopia lentis.

Important Information

- **Example:** If a patient is presenting with Stroke or Myocardial Infarction, then Homocysteine levels need to be estimated.

- Homocysteine is a sulfur-based amino acid, which can get converted into one or another such sulfur-containing amino acids.
- Homocysteine may get converted into either Cysteine or Methionine.
- Major fate of Homocysteine is to form Cysteine.
- Minor fate of Homocysteine is to form Methionine.

Synthesis of Cysteine



Step-01: Homocysteine + Serine → Cystathionine

- It occurs in the presence of cystathionine beta-synthase, a B6-dependent enzyme.

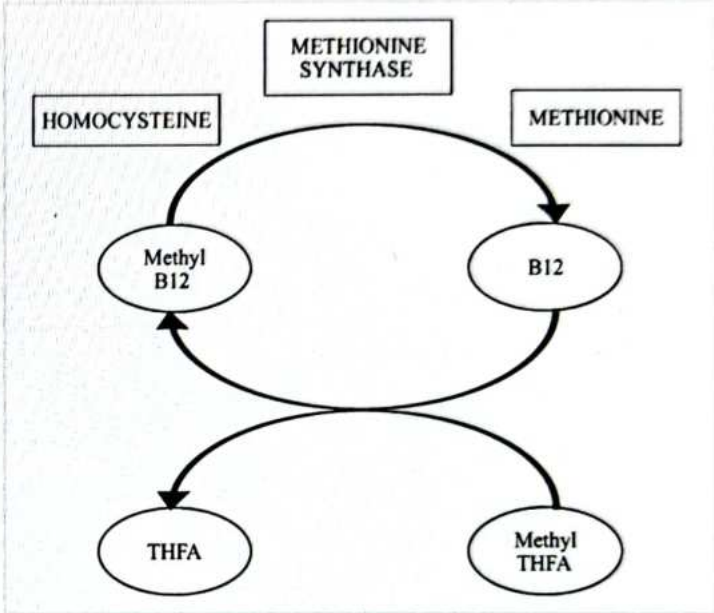
Quick Recap: Pyridoxal Phosphate is necessary for

- Transaminases
- Decarboxylases
- Kinenurinases (Converts Tryptophan → Niacin)
- Cystathionine beta-synthase (Converts Homocysteine → Cysteine).

Step-02: Cystathionine → Homoserine + Cysteine

- Catalyzed by Cystathioninase.

Synthesis of Methionine



Step-01: Homocysteine → Methionine

- Mediated by Methionine Synthase or Homocysteine methyltransferase, dependent on methyl B12 as its coenzyme.
- Methyl Tetrahydrofolate donates its methyl group to B12.

Type-I Homocystinuria	Type-II Homocystinuria
Block in the major fate.	Block in the minor fate.
Enzyme Defect: Cystathionine beta-synthase.	**Enzyme Defect:** Methionine synthase.
Biochemical Changes • Low levels of cysteine. • High levels of methionine.	**Biochemical Changes** • High levels of cysteine. • Low levels of methionine.
Treatment would differ for both types.	
It responds to the B6 administration.	It responds to B12 and folate administration.

Cystinuria

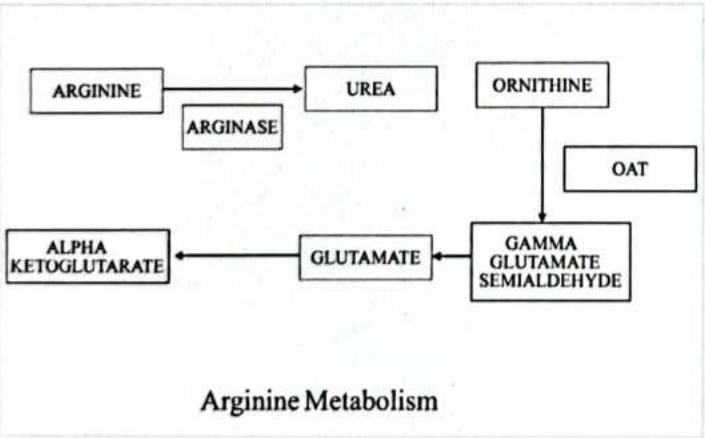
- Defective neutral amino acids transporter which is responsible for reabsorbing 4 amino acid along the PCT.
  - C - Cystine
  - O - Ornithine
  - L - Lysine
  - A - Arginine
- Cystine is formed as a result of dimerization because of the supersaturation of urine with cysteine.
- Cystine is insoluble and deposits to form recurrent cystine stones (Hexagonal in shape).

Cystinosis

- Lysosomal storage disorder.
- Defective efflux of Cysteine from lysosomes as a result there is chronic accumulation of cysteine in lysosomes of various organ results in lysosomal degranulation.
- The acidic pH is released into tissues, causing tissue degradation.
- **If occurs in PCT**
  - Fanconi syndrome (Manifestation of Cystinosis).
    - → Glycosuria
    - → Aminoaciduria
    - → Phosphaturia (Can cause short stature in children)
    - → Bicarbonaturia
    - → Polyuria
    - → Polydipsia
- **Local tissue damage** can also occurs in iris
  - Causes iris depigmentation.
  - Photophobia.
- On slit lamp examination, characteristic cystine crystals are seen (Sequin-like crystals in the cornea).

Gyrate Atrophy of the Retina

- It is related to arginine metabolism.



Arginine Metabolism

The flow of Reactions

- Arginine → Urea + Ornithine → Gamma glutamate semialdehyde → Glutamate → Alpha-ketoglutarate (Enters TCA cycle and comes out as carbon dioxide)

Enzymes involved

- Arginase mediates the conversion of arginine.
- Ornithine aminotransferase catalyzes ornithine to form gamma glutamate semialdehyde.
- It is caused by the defect in Ornithine aminotransferase.
- Ornithine is toxic to the retina and causes gyrate atrophy of the retina.
- Ornithine accumulation should be prevented.

Treatment involves

- Arginine restricted diet.
- B6 supplements.

One Liners

1. Kynenurinase depends on **Vitamin B6**.
2. Type I Homocystinuria is caused by the defect of **Cystathionine beta-synthase**.
3. Type II Homocystinuria is caused by the defect of **Methionine Synthase**
4. Gyrate Atrophy of the retina is caused by the defect of **Catabolism of Arginine**.

MCQs

Q. Type-I Homocystinuria responds to the administrator of?
A. B6
B. B12
C. Folate
D. Thiamine

Q. Cystinuria is characterized by?
A. Fanconi's syndrome
B. Photophobia
C. Porphyra
D. Renal stones (Answer)

Q. In gyrate atrophy of the retina, which amino acids should be restricted in the diet?
A. Phenylalanine
B. Tyrosine
C. Arginine
D. Lysine

Case Based MCQs

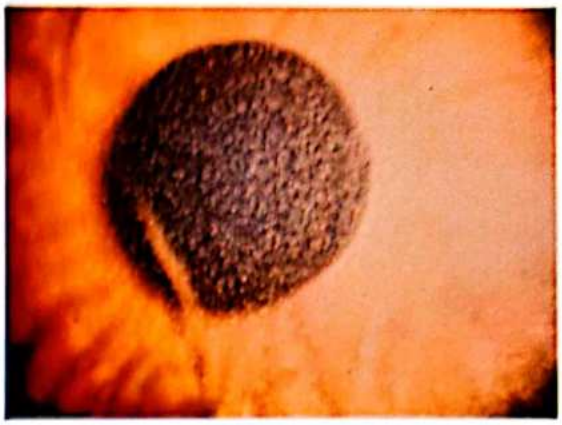
Q. A 4-year-old child presents with mental retardation, Charlie Chaplin gait, and ectopia lentis. Plasma homocysteine and methionine levels are raised, and plasma cysteine is markedly reduced. There is increased excretion of homocysteine and methionine in urine. The diagnosis is
A. Homocystinuria type I
B. Homocystinuria type II
C. Homocystinuria type III
D. Hypermethioninemia

Q. A 8-year-old child presents with short stature, polyuria, polydipsia, and photophobia. On slit examination, the cornea shows characteristics of crystals in the eye; the probable diagnosis is

A. Cystinuria
B. Cystinosis
C. Homocystinuria
D. Cystathioninuria

Image Based MCQs

Q. A 12 years old boy presented with photophobia, short stature, polyuria, and polydipsia; slit lamp examination of the cornea is shown in the image. The probable diagnosis is



A. Cystinuria
B. Cystinosis
C. Homocystinuria
D. Hartnup's disease

30 NUCLEOTIDES DNA CHROMOSOMES

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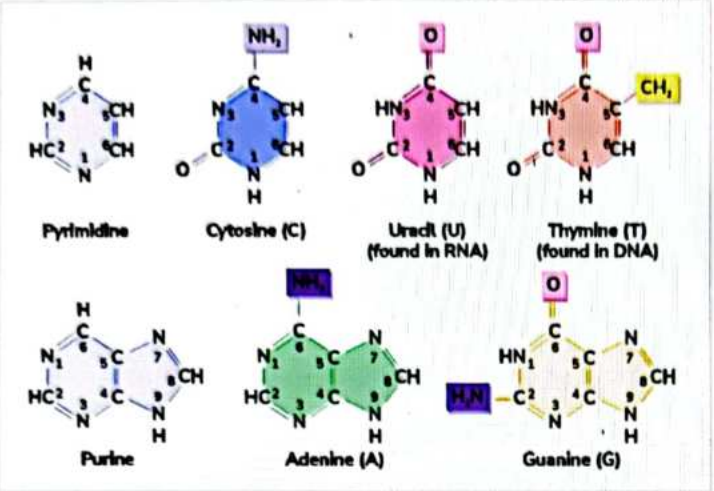
Types of Nitrogenous Bases

2 Types

- Purines - 2 rings and 9 atoms
- Pyrimidines - single ring and 6 atoms



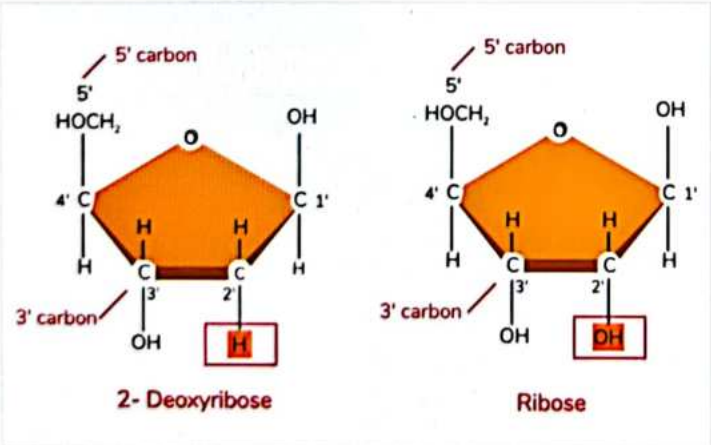
- **Pyrimidines bases.**
  - Cytosine, Uracil, Thymine - found in RNA
- **Purine bases –**
  - Major purine bases: Adenine and Guanine
    → Present in the polynucleotide chain
  - Minor purine bases:
    → Hypoxanthine
    → Xanthine
    → Uric acid

Xanthine Derivatives

- Caffeine - 1,3,7 trimethyl xanthine
- Theophylline - 1,3 dimethyl xanthine
- Theobromine - 3,7 dimethyl xanthine

Nucleoside

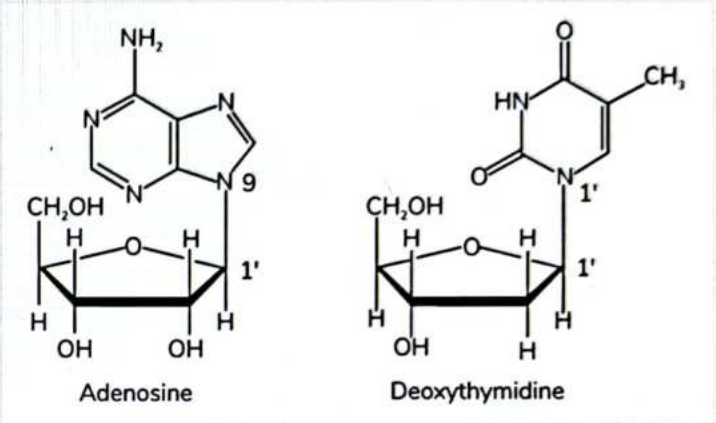
- Base + Ribose or Deoxyribose sugar → Nucleoside
  - Ribose is a pentose that has five carbon atoms.



- Deoxyribose means it has lost its oxygen.
- Purine or pyrimidine base gets attached to a ribose or deoxyribose sugar to form a nucleoside.

Structure of Nucleoside

- The first hydroxyl group of ribose or deoxyribose sugar gets attached to n-9 of purine or n-1 of pyrimidine to get nucleoside.
- N-Glycosidic linkage - formed with carbohydrate with the nitrogen atom.
- The link between base and sugar - Beta-N-Glycosidic linkage.



- Adenine - Adenosine
- Guanine - Guanosine
- Hypoxanthine - Inosine (nucleoside of Hypoxanthine)
- Cytidine
- Thymidine
- Uridine
- Deoxythymidine is a glycosidic linkage.

Significance of IMP

- IMP is the first nucleotide formed during purine synthesis de novo (the pathway that synthesizes nucleotides).
- To synthesize DNA and RNA, we need AMP and GMP. Inosine monophosphate on amination with aspartate will give AMP. The same Inosine on dehydrogenation by IMP dehydrogenase followed by amination with glutamine gene will provide GMP.
  - IMP dehydrogenase is inhibited by Mycophenolate mofetil which is an immunosuppressant.
  - Without GMP, replication is not possible.
  - Replication is the process by which you synthesize two double-stranded DNA from a single, double-stranded DNA.
  - To do this, the optimum concentration of all four nucleotides is needed.

- Without replication, cells cannot divide.
- All rapidly dividing labile cells will get affected. Lymphocytes get involved and cause immunosuppression.

- IMP is the third nucleotide of the anticodon of tRNA. This does not follow the Watson and Crick rule and is known as a Wobble phenomenon.
- This causes degeneracy of the codon.
- tRNA with Anticodon for glycine comes with CCI.

Important Information

Watson and crick base pairing rule:

- A with T: the purine adenine (A) always pairs with the pyrimidine thymine (T).
- C with G: the pyrimidine cytosine (C) always pairs with the purine guanine (G)

Degeneracy of Codon

- Choices: A, U, G, C
- Combination of 3 = 4 to the power 3 = 64
- Total 64 codons
- 3 stop codons.
- 64 - 3 = 61 codons code for 20 amino acids
- More than one codon codes for a single amino acid. This is degeneracy.

Hypothetical mRNA

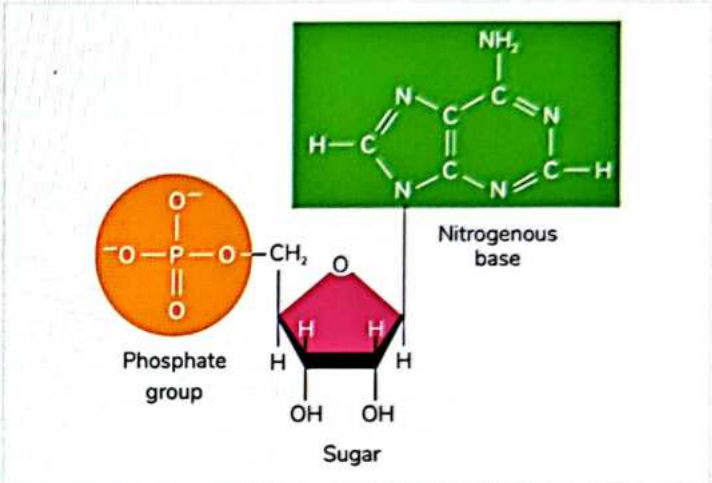
- 5'-GGG-GGC-GGA-GGU-3'
- tRNA, which carries glycine along with it, comes with anticodon CCI
- The third nucleotide of the anticodon of tRNA is inosine monophosphate.
- Inosine monophosphate does not follow Watson and Crick's rule.
- So tRNA is least bothered about the third nucleotide. It will check whether it follows the rule.
- Corresponding to CC, GG is everywhere, so tRNA will hybridize where glycine is the amino acid.
- The third nucleotide is pair nonspecific.
- Polarity in which mRNA takes part in translation - 5' to 3' polarity.
- tRNA polarity – 3' to 5'
- Inosine monophosphate might look like the 3rd nucleotide of the anticodon of tRNA. But the rule is any polynucleotide chain should be in the 5 '3' direction. So, Inosine monophosphate is the first nucleotide of the anticodon of tRNA and does not follow Watson and Crick rules. This is the Wobble phenomenon.
- **3 ways in which the wobble phenomenon can be stated.**
  - The third nucleotide between codon and anticodon is not specific.
  - The third nucleotide of the codon of mRNA is not specific.
  - The 1st nucleotide of the anticodon of tRNA is not specific.

Pseudouridine

- In uridine, the ribose sugar gets attached to n-1. Here the ribose sugar gets connected to c5. This abnormality is Pseudouridine.

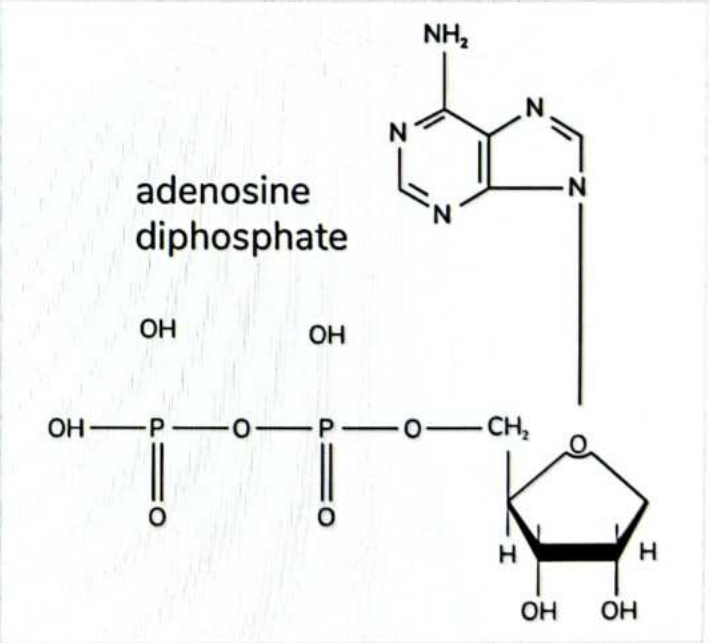
Converting nucleoside to nucleotide

- Attaching the phosphate group to the 5' hydroxyl group of nucleosides to get monophosphate nucleotide.
- Linkage - Beta N glycoside linkage
- Monophosphate nucleotide
- The linkage between the alcohol group and the acid group -> Ester
- So, the linkage is ester linkage (phospho ester linkage)

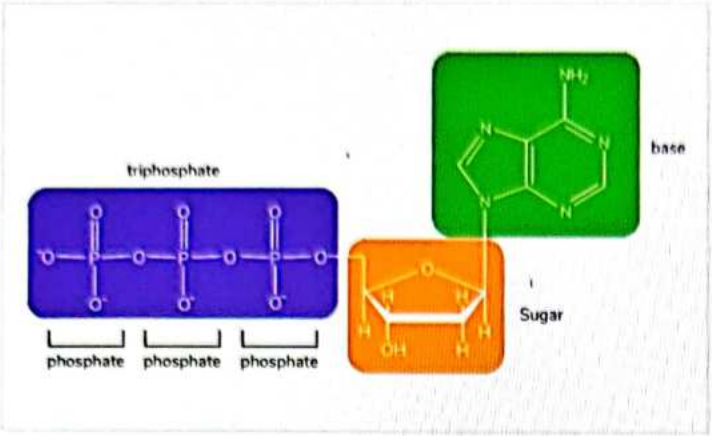


Monophosphate Nucleotide to Diphosphate Nucleotide

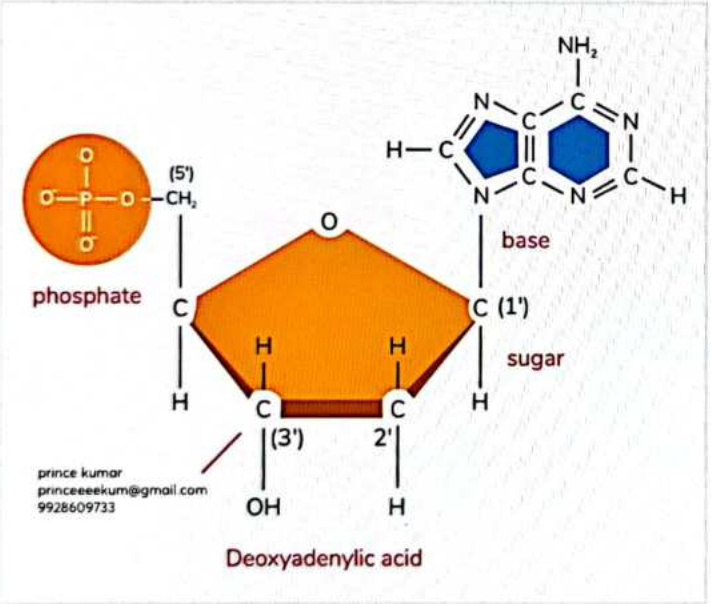
- Attach one more phosphate group to the existing phosphate group.



- Linkage -> two acid groups with the removal of water -> acid anhydride linkage
- Number of linkage in a triphosphate nucleotide- 2
- Number of linkage in a diphosphate nucleotide - 1
- Number of linkage in a monophosphate nucleotide – 0



- ATP is energy currency as it has 2 acid anhydride linkage.
- ATP gets converted to AMP + PPI
- Monophosphate nucleotide is present in the polynucleotide chain.
- For any substance to get linked in poly form, there must be a free hydroxyl group.
- 3' prime hydroxyl group, which is free in monophosphate. This links with the following 5" phosphate group of the next nucleotide.



Polynucleotide Formation

- For any substance to get linked in a poly-form, it has to be from the hydroxyl group.
- The first hydroxyl group in a monophosphate nucleotide is not free as it is involved in beta n glycosidic linkage formation with the base.
- We cannot rely on the first two and the last hydroxyl groups; only the 3' prime hydroxyl group is free in monophosphate.
- This links with the following 5" phosphate group of the next nucleotide.
- Now we can call this linkage as phospho- diester linkage.
- It starts from a 3' Prime carbon atom and links with the five Prime carbon atoms hence the name is 3' Prime 5' prime Phospho-diester linkage.

Refer image 30.1

MCQs

Q. All of the following are purine bases except
a. Adenine
b. Uric acid
c. Hypoxanthine
d. Uracil

Q. The linkage present in a nucleoside is
a. Alpha N glycosidic linkage
b. Beta N glycosidic linkage
c. Phosphoester linkage
d. Acid anhydride linkage

Q. The linkage present in a monophosphate nucleotide is
a. Alpha N glycosidic linkage
b. Beta N glycosidic linkage
c. Phosphoester linkage
d. Acid anhydride linkage

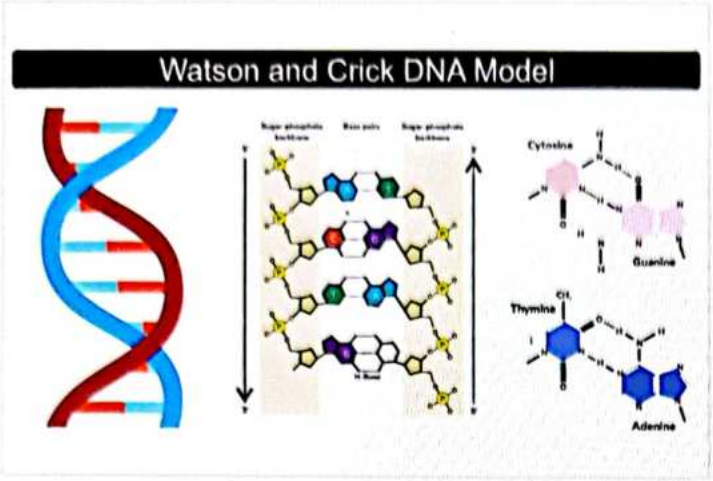
Q. The linkage present between phosphate groups in a triphosphate nucleotide is
a. Alpha N glycosidic linkage
b. Beta N glycosidic linkage
c. Phosphoester linkage
d. Acid anhydride linkage

Q. The linkage present between individual nucleotides in a polynucleotide chain is
a. 3'5' phosphodiester linkage
b. Beta N glycosidic linkage
c. Phosphoester linkage
d. 5'3' phosphodiester linkage

Q. Regarding DNA structure, which is true:
a. The double helical structure is stabilized by covalent bonds.
b. The individual strands are stabilized by 5'3' phosphodiester linkage.
c. The individual strands are stabilized by 3'5' phosphodiester linkage.
d. The term 5' end indicates that the 5' end is linked to the kinetochore.

DNA Structure

- DNA structure was given by Watson and Crick.
- Two strands are complementary (A-T, G-C) and anti-parallel (5'-3', 3'-5').



- Strands look like ladder like fashion made of phosphodiester linkages with deoxyribose sugar.
- Steps are made of nitrogenous bases linked by hydrogen bonds.
- There are 2 hydrogen bonds between A and T and 3 hydrogen bonds between G and C.

B Type of DNA

- Most common physiological form
- Right-handed helix
- 1 full turn has 10 bases.
- Full turn measure 34 A
- Width is 20 A
- Every full turn has a one major and one minor groove.

A Type of DNA

- It forms after dehydration.
- During DNA RNA hybrid, RNA RNA hybrid
- Right-handed helix
- Every complete turn has 11 bases.
- All grooves will be of the same dimension.

Z Type of DNA

- Seen in GC rich sequences.
- Left-handed helix
- Every complete turn has 12 bases.

Denaturation

- A double-stranded DNA unwinds to form two single strands by breaking hydrogen bonds at very high temperatures.
- Tm/ Melting temperature is at which 50% of a given double-stranded DNA undergoes unwinding to form two single strands.
- This melting temperature is directly proportional to GC content and inversely proportional to AT content. Melting temperature is directly proportional to salt concentration.
- Chemicals like formamide help denaturation.

DNA and Chromosome

- Condense chromosomes with the help of proteins using histones.
- Histones are essential proteins. They are positively charged and made of lysine and arginine.
- DNA is negatively charged. There is an electrostatic attraction between these, so a segment of double-stranded DNA bounds around and gives the first type of chromosomes.
- **Five types of histones:**
  - H1
  - H2A
  - H2B
  - H3
  - H4
- Except for H1, the remaining four forms are dimers. We call that octamer.
- Histone sits in the center and attracts double-stranded DNA that gets bound around.
- The string-on-bead appearance. The series is a double-stranded DNA and is octamer. This is known as a nucleosome.
- H1 Histone is not present in nucleosomes.
- A link of fragments connects nucleosomes.
- H1 histone is present in the link of the fragment.
- One such fibril is called a 10-nanometer fibril. This gets folded on itself to give a 30-nanometer fibril. This is the second level.
- Chromosome scafolds are tubular proteins with slits in the center.
- All chromosomes perform two functions, replication, and transcription.
- When the chromosomes are performing replication and transcription, there will be un condensation first and then unbinding.

MCQ

Q. The histone that is not present in a nucleosome is

a. H1
b. H2A
c. H2B
d. H3

Q. The most common form of DNA is

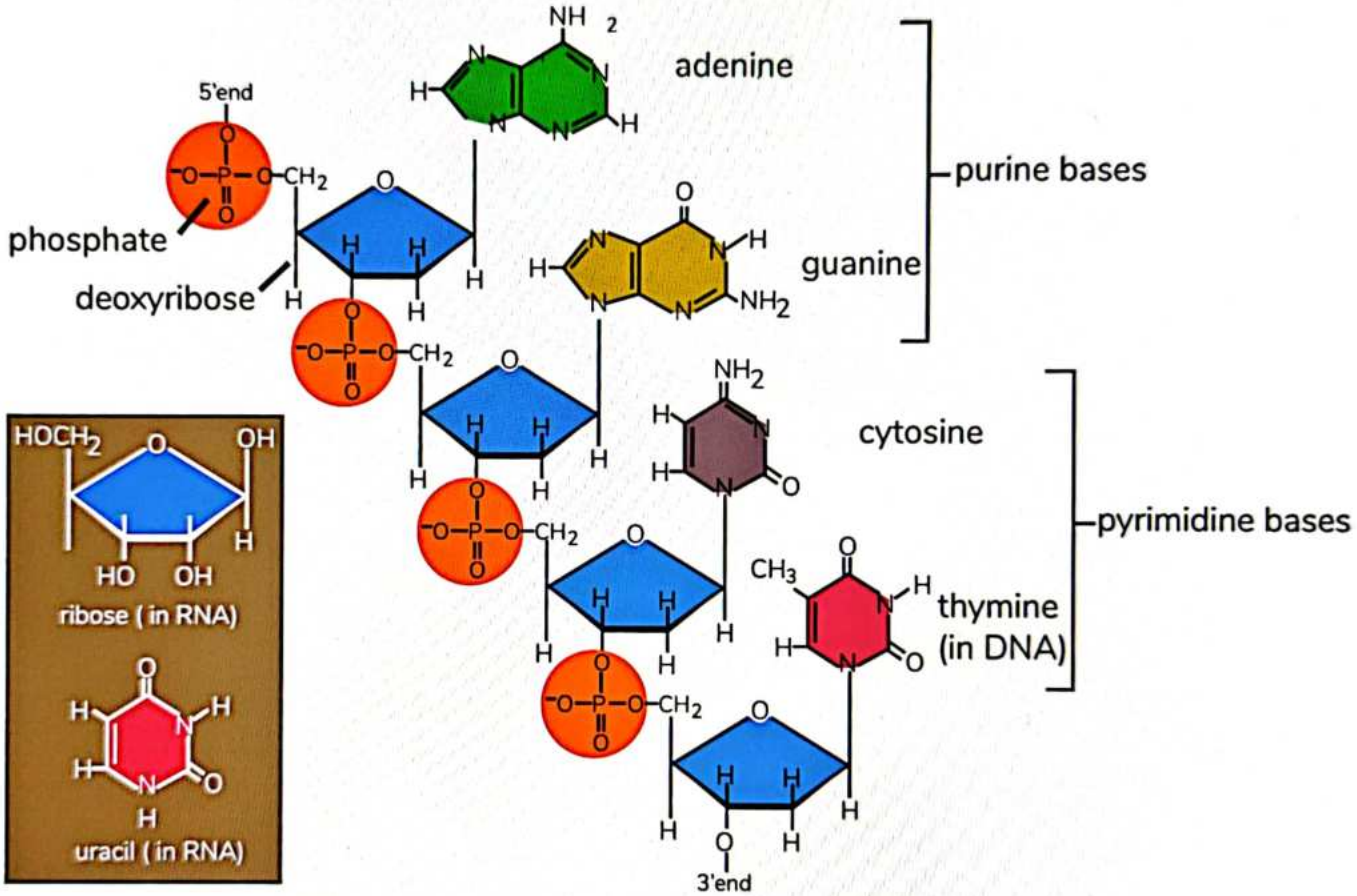
a. B DNA
b. Z DNA
c. A DNA
d. E DNA

Q. The features of B DNA include all except
a. It is a right-handed helix.
b. One full turn of DNA has 10 nucleotides and measures 34A width 20A.
c. The major groove is equal to the minor groove in terms of width.
d. It is the form that is present under physiological conditions.

Q. Denaturation of DNA is done by all except
a. Increasing the temperature
b. Increasing the salt concentration
c. Decreasing the salt concentrations
d. Formamide

Q. The left handed helix is seen in.
a. B DNA
b. Z DNA
c. A DNA
d. E DNA

Image 30.1



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32 MITOCHONDRIAL DNA AND REPLICATION

Mitochondrial DNA

00:01:05

- It is a closed circular double stranded DNA similar to plasmids of prokaryotes (which gained entry into eukaryotes).
- Though Mitochondria has its own DNA, not all proteins are coded by them.
- Mitochondrial DNA only codes 13 proteins of ETC.
- It codes only for 22tRNAs, 2rRNA.
- In Total, there are 37 genes, which means it is not sufficient on its own.
- Genetic code is different here.
- UGA Is a stop codon but here it codes for Tryptophan.
- Usually AGG & AGA codes for Arginine, but here they act as stop codons.
- Mutations are common. DNA polymerase Gamma which Synthesizes mitochondrial DNA does not have proofreading and repair mechanisms.
- Such Mutations are only maternally inherited because
- During zygote formation, all sperm contributes with pronucleus and the remaining part of sperm will be shut down and here we have mitochondria, so sperm mitochondrial DNA does not take part in Zygote formation and so the mutation of it will not be inherited.

Heteroplasmy:

It is the possibility of the presence of more than 1 type of genome in a single individual. This is possible in a mitochondrial DNA Mutation.

- Ex: Leyh Syndrome, NARP (middle) and Mental Retardation on the other end of spectrum. These 3 are caused by 1 mutation, which is ATP 6 gene present in mitochondrial DNA. And when there is Mutation of ATP 6 gene, there is a spectrum that can vary. On one hand, it can be mild mental retardation and on the other end it can have Leyh Syndrome and in between it can cause NARP.
- Not every sperm mitochondrial DNA is excluded from zygote, but a part of the middle of sperm manages to get into zygote and that has got mitochondria.
- The no. of sperm zygote gets is very less (1 or 2) as compared to the ones that ova gets (which gets lacs). However, sperm zygote does not have any effect in mitochondrial Sperm Mutation.
- But whenever there is a maternal DNA Mutation, depending upon the no. of sperm mitochondrial DNA entered in zygote, the presentation would differ. If it is 0 or 1, there is no dilution effect on the mother's mitochondrial DNA Mutation.
- When there is no inheritance of sperm mitochondrial DNA in zygote, the presentation of maternal mitochondrial DNA will be severe that presents Leyh Syndrome.

- When there are 5-10 copies of Mitochondrial DNA in zygote, the maternal mitochondrial DNA presentation will be mild because it's diluted and in this case it presents mild mental retardation.
- Between the two extremes it can present NARP, (Neuropathy ataxia retinitis pigmentosa).

Replication

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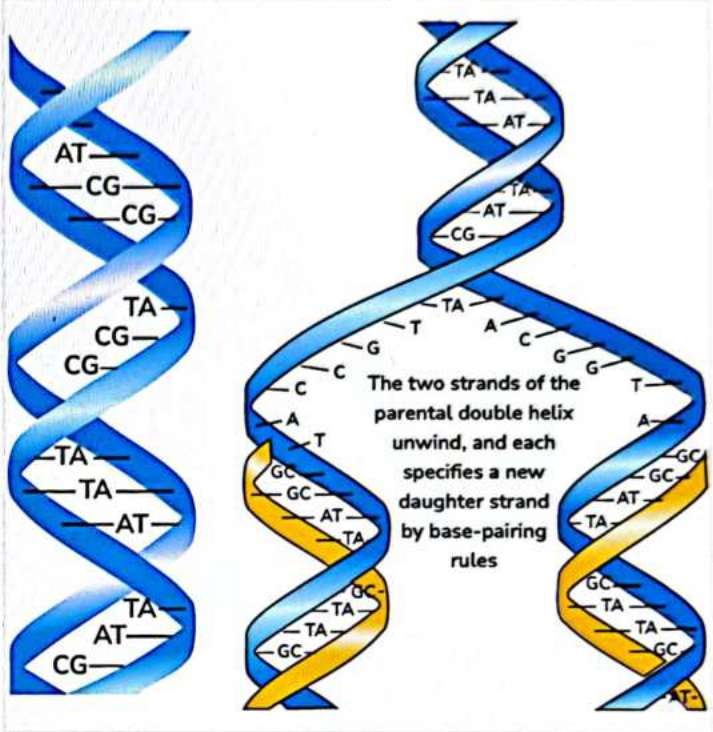
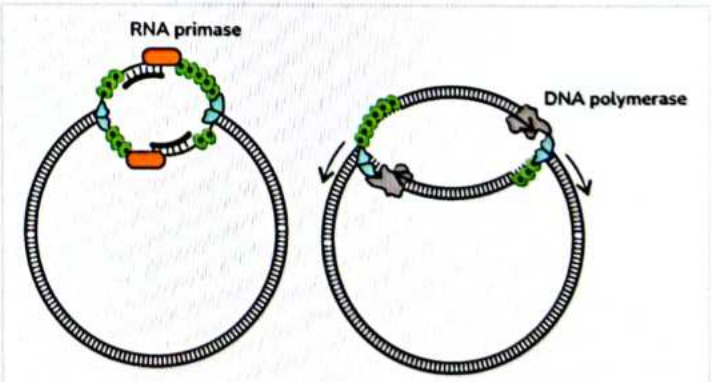


- Process by which 1 double stranded DNA undergoes replication to form 2 double stranded DNAs.
- For this to happen, the parent double stranded DNA should unbind. After unbinding, each of the two strands will act as a template based on which, we will be synthesizing 2 new strands.
- It is also called a semi conservative process because out of the two strands only 1 is new and the other one is conserved.



- It is a bidirectional process Because during Replication after the parent double strand DNA unbinds, all enzymes will start acting at the center.
- From the center, one set of enzymes acts in one direction & the other set acts in another direction.

DNA Polymerase

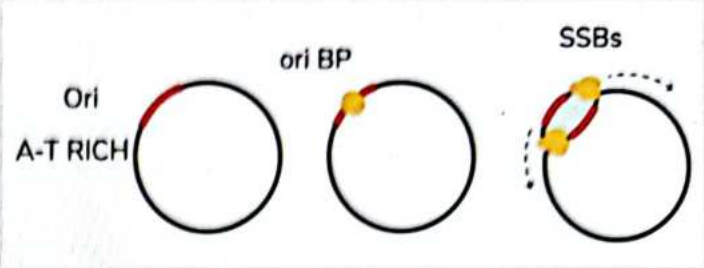
- All of them need a template strand to be already present. Only then can they synthesize new strands which are complementary and Antiparallel.
- All DNA polymerases can synthesize new strands only in 5 '3' direction because the linkage that is present is always 3'5' phosphodiester linkages, so no other go in the first nucleotide, the 5' Phosphate group will be free.
- The template strand has to be Antiparallel (3'5"Direction)

Requirement:

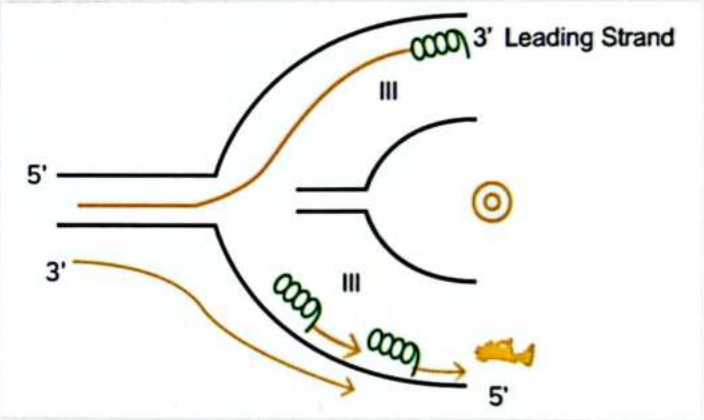
- Need a 3'5' strand to act as a template, only then they can synthesize new strand
- All of them need a primer to be already present, only then they will be able to elongate a new strand.
  - In Vitro replication, when replication is happening between yourselves, we use RNAs as primer where as in In vitro replication happens in the test tube which is PCR. In polymerase chain reaction, we use DNA as primer because handling RNAs is difficult.
- DNA polymerase needs all 4 Deoxynucleotide triphosphate.
  - All DNA polymerase reaches the temple strand from 3'5'. Suppose on the strand there is A and they can recruit complementary nucleotides which will be linked to the adjacent nucleotides. So, there should be a source of nucleotides.
  - The nucleotide present in a polynucleotide chain is monophosphate Nucleotide.
  - Triphosphate nucleotide is needed because when they recruit complementary monophosphate nucleotides, they are supposed to link adjacent ones by forming 3 '5' phosphodiester linkages. And for this, the enzyme will need energy which is coming from triphosphate nucleotides formed complementary.
- They always need magnesium, manganese or a divalent cation to act as a catalyst.
- Fifth requirement is a buffer.
- DNA polymerases have the property of proofreading and repair activity.
  - This means when they are synthesizing in 5'3' direction they might get some defect, they will start removing the defective strand from the other end.
  - For this they will need 3 '5' exonuclease activity.
    → DNA polymerase 1 alone has got both 3'5' and 5'3' exonuclease activity.
- All DNA polymerase can exhibit only 1 polymerase activity that is 5 '3' but they can exhibit 2 exonuclease activities which are 3' 5' & 5'3'.

Steps of Replication 00:19:10

- In the first step, the parent strand is supposed to unwind, which is initiated by identification of the origin of replication, shortly called as Ori



- Ori is a segment of chromosome which is rich in A-T sequences. This is chosen because they will have less no. of hydrogen bonds which makes unwinding easier.
- Once the origin of replication is identified, that will be found by the origin of replication binding proteins. These proteins bind to Ori and they initiate unwinding.
- Though they initiate unwinding, because the 2 strands are complementary bases, they try to stick together easily. So, single stranded binding proteins bind to both strands that prevents them from reannealing.
- What forms is known as the replication bubble. Once a Replication bubble is formed, all enzymes start acting from the center. From the center one set acts in one direction and the other set in another direction.



- From the center, the enzymes present try to identify the 3' 5' strands, which are on top (here). So they will acknowledge the top strand as a template strand.
- Once they identify, the first primase will Synthesize RNA primer and then DNA polymerase 3 will Synthesize DNA Strand.
- So, the 3' 5' strand against which DNA polymerase can synthesize new strands continuously will be called a leading strand.
- The other strand is in the 5 '3' direction from the center. Against this, no enzyme can synthesize. As they cannot synthesize from the center, they start synthesizing from the angle of separation.

- They start from an angle of separation because from there the direction in which there is a template strand is 3' 5'. And against these the enzyme can synthesize new strands.
- From the center to reach the angle of separation, first helicase and primase joined together to form Primosome. And they move along the strand to reach the angle of separation. Once they reach there, they will attach DNA polymerase 3 & single stranded binding protein and then they are known as the replication fork.
- So, the DNA polymerase 3 will continue the synthesis of DNA strands (that is why they are known as lagging strands & against these there are short RNA primer attached to DNA, known as Okazaki fragments (synthesized by DNA polymerase 3 & primase and joined by DNA ligases) until it comes across another RNA Primer in its view.
- The RNA primer would be synthesized by the previous replication fork (the smaller one having the same process).
- Now, once polymerase 3 seizes the RNA primer, it knows it can no longer continue the synthesis so it leaves the place after recruiting DNA polymerase 1.
- So, the new strands are synthesized in 3' 5' direction. So, polymerase 1 has arrived and has got 5' 3' exonuclease activity using which it removes the RNA Primer.
- Once the RNA primer is removed, there is a gap which is filled by polymerase 1 by extending the synthesis previous strand.
- Once the gap is filled, lipase will unite the ends.

Functions of Prokaryotic DNA Polymerases

00:36:30

S. No.	DNA polymerase	Functions
1	DNA polymerase 1	Removes RNA primer and filled the gap during lagging strand synthesis
2	DNA Polymerase 2	Proofreading and repair
3	DNA polymerase 3	helps leading strand synthesis and okazaki fragment synthesis

- All these DNA polymerase 1,2,3 are prokaryotic enzymes.

Difference between Prokaryotic & Eukaryotic Genome

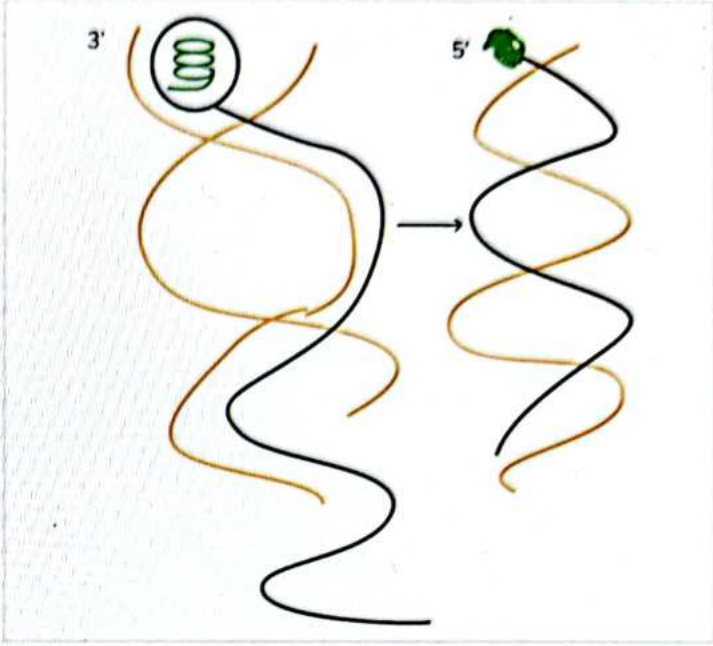
S. No.	Prokaryotic	Eukaryotic
1	Smaller	Larger
2.	Closed circular	Open Linear

- Because we have a larger genome if we try to replicate each and every one of our 46 chromosomes with only one origin of replication each, it is estimated that it will take 150 hours for all the 46 to be replicated.
  - But the actual time of Replication is only 4 hours. So, the duration is reduced from 150 to 4 hours due to the multiple sites rich in A-T

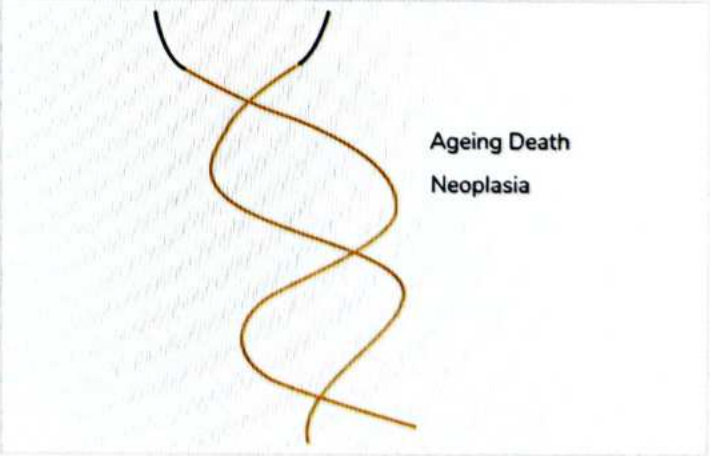
being chosen.

Telomeric end Shortening

00:40:07



- Suppose, this is one end of Chromosome and in this one strand is 3' 5' & other is 5' 3'.
- Between two strands, 3' 5' strands will be treated as the leading strand.
- Corresponding to this 3' strand, first RNA primase will Synthesize RNA primer and then DNA polymerase will Synthesize the DNA Strand.
- And the DNA polymerase will be proceeding towards the other end of Chromosome.
- As it's an open linear Chromosome, the DNA polymerase won't be able to come back and remove the RNA primer.
- So, the RNA primer goes unnoticed and left back.
- Following replication, when we get 2 dotted DNAs, only 1 of the two is new and in the new strand 1 of the two ends will be replaced by an RNA fragment which gets lost in due course.
- And that is the reason for Telomeric End shortening.



- When you are born, you are born with a particular telomeric length and as you push your cells into the cell cycle, there is progressive Decrease in the length of the Telomeric end.
- And there is the critical or minimal length of telomere that is necessary to maintain the stability of the Chromosome.
- So, you will allow yourselves to get into cell cycle/ replication, only till the day the critical length of telomere is reached.
- Once the critical length is reached, you will stop sending the cells into the cell cycle.
- That is associated with aging and death.
- A person is young only until the person is capable of replenishing the losses for which they need to push the cell into the cell cycle. But at one point the critical / minimal length of telomeric is reached and the person stops sending the cells into the cell cycle.
- And that is when aging starts and results in death.
- If the cell's telomere is reduced beyond the critical end, you should induce apoptosis of the cell because in that cell the Chromosome has lost its stability.
- Now, if there is an apoptotic gene Defect, the cell survives but its critical length of telomere is reduced beyond and stability is lost and that Chromosome accumulates Mutation. On accumulation of mutations that can cause neoplasia.

Telomerase 00:44:59

- It is a reverse Transcriptase.
  - Transcription is DNA to RNA and reverse Transcription is RNA to DNA.
- It is an RNA dependent DNA polymerase.

Function:

- Based on RNA fragments that are replaced in terminal ends of chromosomes, your telomerase can synthesize and substitute a DNA fragment. This is the function of Telomerase.
- The telomerase activity is present in germ cells and absent in somatic cells.
- If a somatic cell has increased telomerase activity, it will be able to replenish telomeric ends continuously and as long as the cell is able to replenish, the cell will always be in cell cycle. On repetitive cell cycles, it will result in Neoplasia.
- Increased telomerase activity is associated with malignancy.
- Only because the telomeric end becomes shortened, it is related to aging and death.
  - Dolly is the first sheep that was cloned. But cloning is not as new as Dolly's cloning.
  - Dolly is the first somatic cell cloned animal, whose breed life expectancy is 12-13 years but Dolly had a half-life (6) years only due to natural death (Pneumonia).
  - Dolly was having half-life expectancy because Dolly was cloned from a somatic cell that was taken from a sheep of age 6 years.
  - Germ cell cloning is like you trying to give clay a shape (not sure how the product will look) whereas somatic cell cloning is like you have dry clay (you cannot change the shape), all you can do is use the dry clay as mold and clay exacts replicas out of it.
  - If we take a somatic cell of a person, that somatic cell chromosome has got folded to give rise to that person. If we allow it will give rise to the same from which the cell has been taken.
  - Somatic cell products will have lesser life expectancy based on the period at which the somatic cell was taken from the person.

Functions of Eukaryotic DNA polymerases 00:49:22

S. No	DNA Polymerase	Functions
1	DNA Polymerase alpha	RNA Primase
2	DNA Polymerase beta	Proofreading and repair activity
3	DNA Polymerase epsilon (Have proofreading & repair activity)	Helps in initiating the leading strand synthesis (after initiation it is taken over by delta)
4	DNA Polymerase gamma	Helps in mitochondrial DNA Synthesised and does not have proofreading & repair activity.
5	DNA Polymerase delta	Completes the leading strand synthesis, fills gaps during lagging strands, it helps in okazaki fragment Synthesis, also helps in removing RNA,

One liners 00:52:23

Q. Mitochondrial DNA is synthesized by DNA:
Ans. Polymerase gamma

Q. Okazaki fragments in prokaryotes are synthesized by:
Ans. DNA polymerase III

Q. Okazaki fragments in eukaryotes are synthesized by:
Ans. DNA polymerase Delta

Q. The DNA polymerase with proofreading and repair activity in prokaryotes is:
Ans. DNA polymerase II

Q. The DNA polymerase with proofreading and repair activity in eukaryotes is :
Ans. DNA polymerase epsilon and beta

MCQ

Q. Which of the following is true about replication?
a. Conservative process
b. Bidirectional
c. Non conservation
d. Unidirectional

Q. DNA Polymerase requires all except
a. RNA primer
b. 3' to 5' strand to act as a template
c. dNTP
d. 5' to 3' strand as a template

Q. Replication fork includes all except?
a. Helicase
b. Primase
c. DNA polymerase III
d. DNA polymerase I

Q. Replication along lagging strand, true is:
a. Polymerase I synthesizes along 3' to 5' direction
b. Helicase and primase join to form primosome and moves along the lagging strand
c. Okazaki fragments are joined together by DNA helicase
d. DNA polymerase III removes RNA primer

Q. Function of DNA polymerase delta except:
a. Lagging strand synthesis
b. Leading strand synthesis
c. Okazaki fragment synthesis
d. RNA Primase

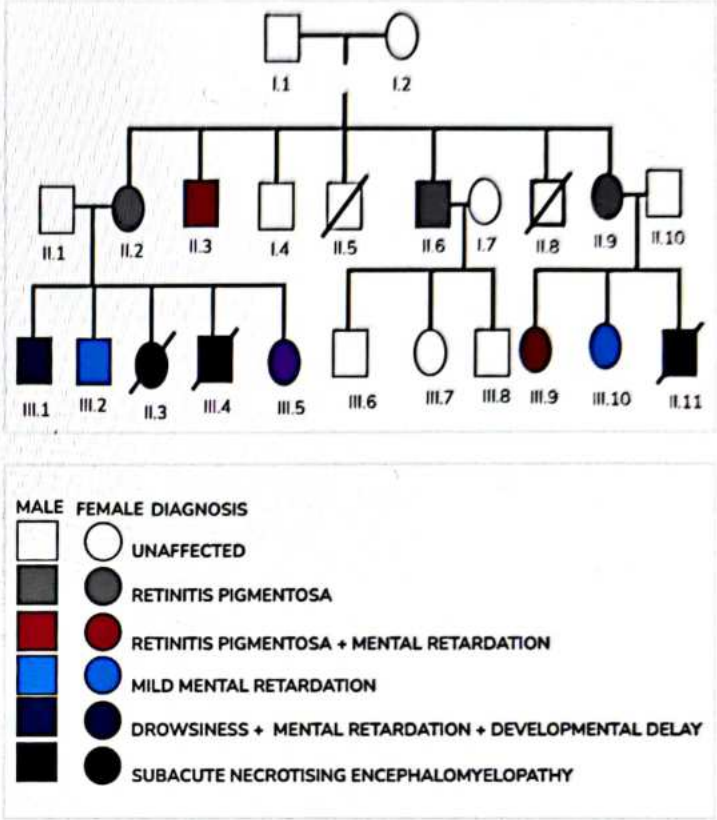
Q. DNA polymerase with repair mechanism is?
a. DNA polymerase 1
b. DNA polymerase II
c. DNA polymerase III
d. DNA polymerase a

Q. The True statement about telomerase are all except?
a. They are present at the ends of eukaryotic chromosome
b. Increased telomerase activity is associated with malignancy
c. DNA dependent RNA polymerase
d. DNA polymerase

Image based MCQ

00:57.00

Q. A family was reported with many cases of mental retardation, developmental delay. Some presented with retinitis pigmentosa. 3 neonatal deaths with lactic acidosis in the family forced them to consult a geneticist. A pedigree chart was drawn (attached). DNA was extracted from all affected members. PCR-RFLP detected a point mutation (transversion) at one specific site. The explanation for variable presentation caused by a single mutation site is:



a. Phenotypic heterogeneity
b. Pleiotropy
c. Allelic heterogeneity
d. **Heteroplasmy**

- Phenotypic Heterogeneity: Multiple site Mutations in a gene presenting with various presentations. Ex: Crigler Najjar Syndrome type 1, or 2 or sometimes mild rotor syndrome.
- Pleiotropy: It is one Mutation affecting multiple organs. Ex: Marfan's Syndrome caused by Fibrillin.
- Allelic Heterogeneity: Multiple sites of mutation causing one presentation. Ex: Cystic Fibrosis (m/c cause is DF 508)

33 DNA REPAIR MECHANISM

Four Repair Mechanisms

00:00:36

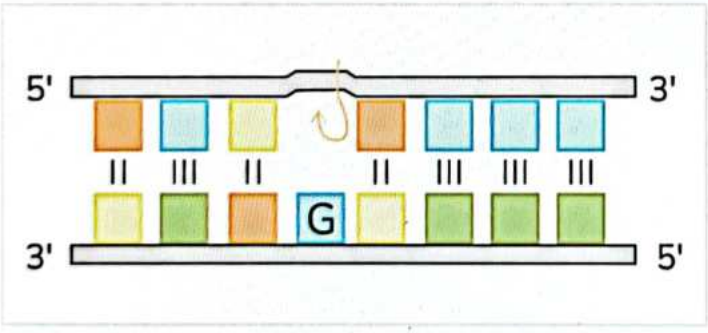
- Base excisiona repair (most common error)
- Mismatch repair
- Nucleotide excision repair
- Double stranded DNA Break Repair Mechanism

Question: Which is the most common error that can happen in a DNA?
Answer: Base excision error

- The backbone of the DNA is made up of 3' 5' phosphodiester linkages linking the ribose or the deoxyribose sugars.
- The nitrogenous base is attached to the ribose or deoxyribose sugars.
- The nitrogenous bases that can be found are A T G C.
- Steps are made up of nitrogenous bases.
- If A is present on one side, then T base will be present on the other side (A-T).
- This is how DNA looks. On the other side also, there will be a backbone.

What is the Base Excision Error?

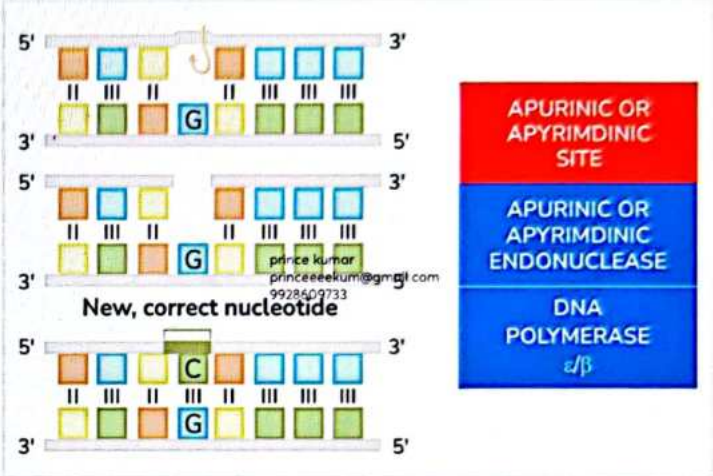
- In this case the backbone is maintained properly.
- The glycosidic linkage which links the base with the sugar is very thermolabile.
- Beta N-glycosidic linkage which links the base with sugar is very weak.
- Many times, this linkage gets broken, and the base will be lost.
- This error can happen most commonly in a DNA.



- If a purine is lost, we call it an Apurinic site and if a pyrimidine is lost, we call it an Apyrimidinic site.

Questions: Which enzyme do you think will repair this Base Excision error?
Answer: The enzyme is Apurinic or Apyrimidinic Endonuclease.

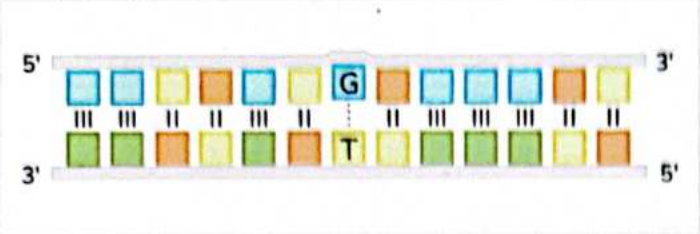
- Apurinic and Apyrimidinic Endonucleases make a nick close to the site, the base has been lost. If it makes a nick, the end gets exposed; that is what DNA polymerases need.
- **Eukaryotic DNA polymerases:** DNA polymerase epsilon and beta have proofreading and repair mechanisms.
- These polymerases have exonuclease activity.
- DNA polymerase epsilon or beta will start removing the defective strand, wherever the base has been lost, that place alone is removed.
- These same polymerases also have polymerase activity, which fills up with current nucleotides.
- DNA ligase will unite the end.



Mismatch Repair

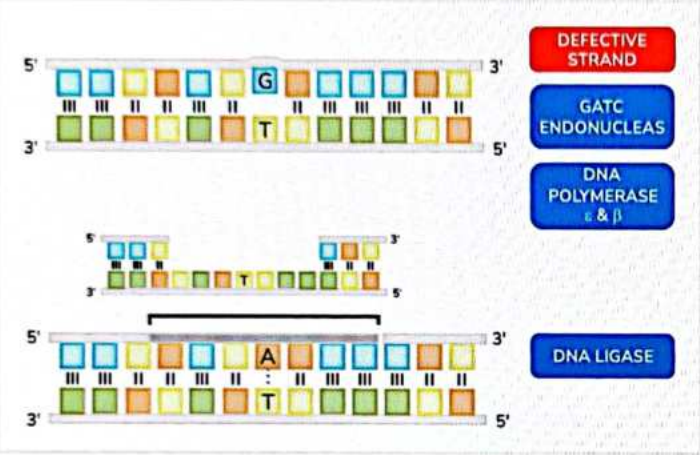
00:05:00

- Happens most commonly during replication.
- During replication, all that the DNA polymerases that does the template strand.
- Mostly, Template strand T the DNA polymerase is supposed to attach with A.
- If it gets attached with G or C, this leads to mismatch error.



Questions: How to identify the defective Strand?
Answer: This is done by methylases. Methylases methylate the normal strand. So, that another strand is considered defective

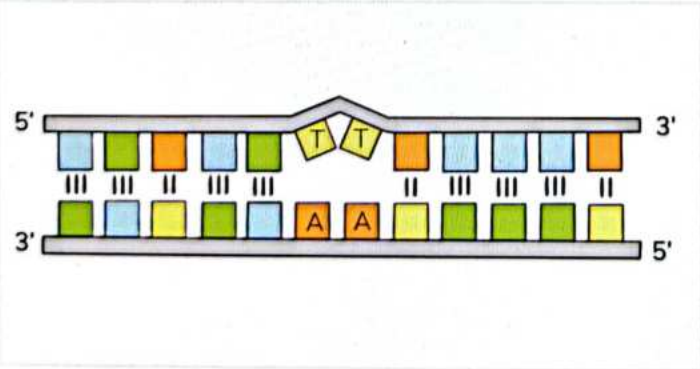
- **GATC Endonuclease,** it scans the defective strands on either side of the defect.
- It makes a nick with the nearest GATC sequence.
- Once the nick is done, the end is exposed. There comes DNA epsilon or beta to start removing the defective strands.
- DNA epsilon or beta will use their polymerase activity to substitute a new strand and ligase will unite the ends



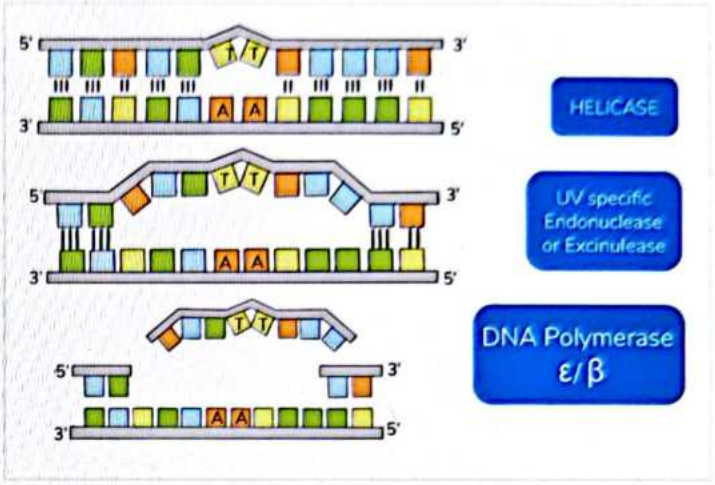
- Mismatch error defect causes hereditary nonpolyposis colon cancer also called as lynch syndrome.
- This syndrome is characterised by microsatellite Instability.
- Most common gene mutation causative of this lynch syndrome is human mutant s homology 2(hMSH2)

Nucleotide Excision Repair 00:10:09

- It is caused due to UV light mediator pyrimidine-pyrimidine dimerization.
- UV light causes adjacent pyrimidines to dimerise in DNA.



- Pyrimidine dimers create a kink, causing abnormality. It must be replaced with a normal strand.
- This is done by nucleotide excision repair.
- After pyrimidine-pyrimidine dimerisation, the 1st enzyme that is brought into action is helicase.
- Helicase breaks hydrogen bonds and unwinds the double stranded DNA to form single strand DNA.
- UV specific Endonuclease or Excinuclease that excises a nucleotide away.
- UV specific Endonuclease makes 2 Nicks.



- Defect of nucleotide excision repair causes Xeroderma Pigmentosum and Cockayne syndrome.
  - Xeroderma Pigmentosum is caused by replication coupled nucleotide excision repair.
  - Defect of transcription coupled nucleotide excision repair causes Cockayne syndrome.

DS DNA Break Mechanism

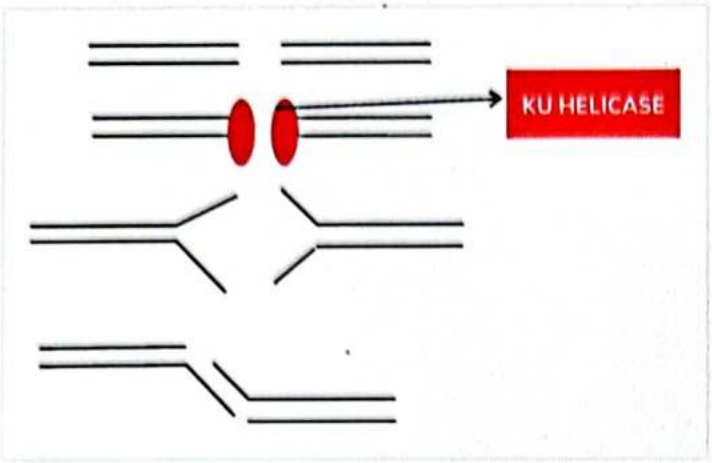
- The backbone has been broken, both the strands and the 3',5' phosphodiester linkage is broken.
- Ester linkages can be broken by adding water. This is called Hydrolytic cleavage.
- Only in the presence of Ionising radiation, does DSDNA break down.

The repair mechanism can be brought out in 2 ways:

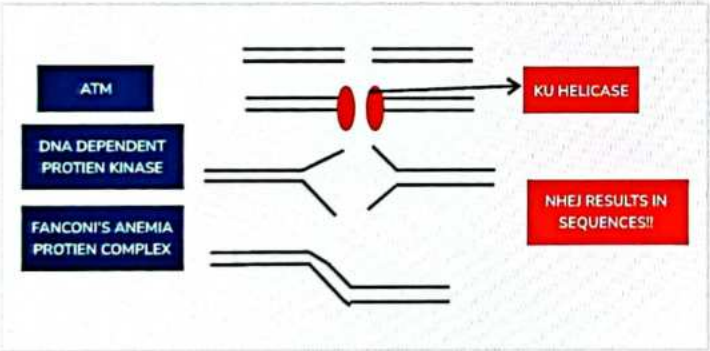
- Non homologous end joining (NHEJ)
- Homologous DNA repair (HDR)

Non homologous end joining (NHEJ)

- NHEJ is more common than HDR.
- NHEJ is mediated by Ku Helicase, a dimer.
- Helps in attachment of 2 broken units.
- 2 units on each side will cause unwinding of both the ends and approximation.
- Approximation continues until they find the base pairing and excess strand will be removed.
- Now DNA ligase will unite the ends.



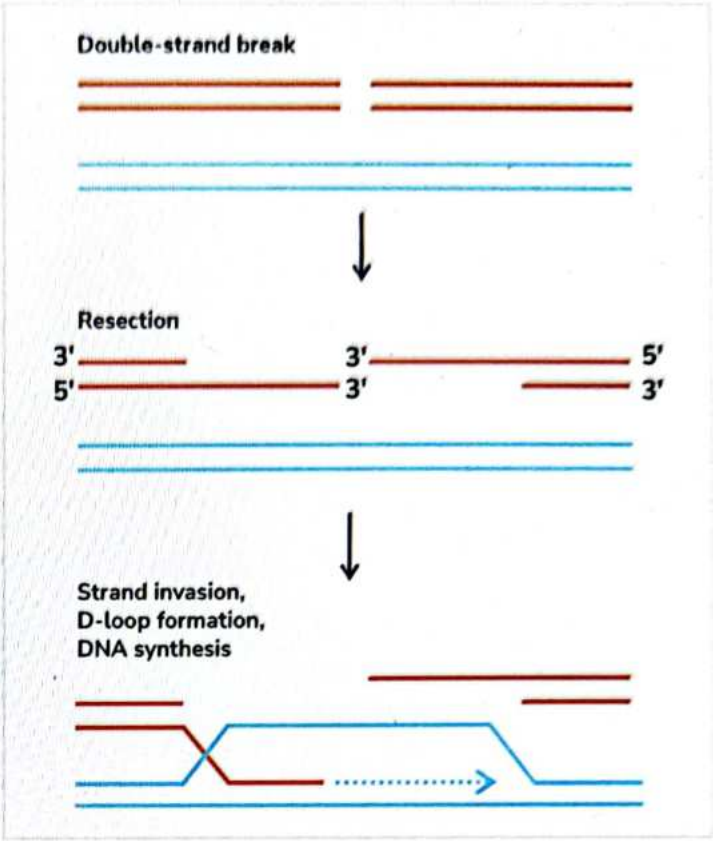
- There is loss of gene sequence in NHEJ.
- Ku Helicase is not active on its own. We need a kinase to make it active.
- ATM (ataxia telangiectasia protein) protein or DNA dependent protein kinase also called tumour suppressor protein.
- ATM protein phosphorylated and activate Ku Helicase.
- The actual action of ATM protein is to help in stopping the cell cycle at G1-S transition.
- The link between error detection in G1 phase and p53 activation is ATM protein.
- ATM protein phosphorylates P53 and also helps in a few repair mechanisms.
- Fanconi's Anaemia Protein Complex brings both the ATM protein and Ku Helicase in action.



Homologous DNA Repair

- DNA polymerase will try to create an Overhanging edge in the homologous DNA repair.
- DNA polymerase uses its exonuclease activity to remove a part of the strand to create overhanging.
- Strand invasion ability is caused due to overhanging edges.
- Whenever there is DS break due to Ionising radiation, another copy of the same chromosome will align close to the defect.
- After identification of Homologous DNA sequence, DNA polymerase will use its exonuclease activity to remove a part of the strand.
- Overhanging edge has got strand invasion ability. Ligase will unite the ends.

- Holliday junction, a good sign in Homologous DNA repair mechanism.



Defects of DSDNA Break Repair 00:28:26

- It causes many disorders.

Mnemonic: ABF

- Ataxia Telangiectasia
- Bloom syndrome
- Fanconi's anaemia
- HBOC (Human breast and ovarian cancer syndrome)
- Ataxia Telangiectasia and Bloom syndrome are caused by NHEJ
- Severe combined immunodeficiency- another disorder caused by NHEJ.
- Fanconi's anaemia, human breast and ovarian cancer syndrome are caused by defect of HDR

One Liners

01. The most common error in DNA is **Base Excision**
02. UV light damage to DNA causes **Pyrimidine dimer**
03. Ionisation radiation causes **DSDNA break**
04. The linkage that is broken in Base Excision is **beta N Glycosidic Linkage**
05. The linkage that is cleaved in double stranded DNA Break is **3'5 phosphodiester linkage**

MCQs

1. The most common error in a DNA is

A. Base Excision
B. Pyrimidine dimer
C. Mismatch
D. DS DNA Break

Ans: Base Excision

2. Base Excision Repair is done by

A. Apurinic endonuclease
B. DNA polymerase Gamma
C. GATC Endonuclease
D. Ku helicase

Ans: Apurinic endonuclease

3. Mismatch repair defect causes

A. HNPCC
B. Xeroderma pigmentosum
C. Fanconi's anaemia
D. Ataxia Telangiectasia

Ans: HNPCC

4. UV light damage to the DNA leads to

A. Purine dimers formed
B. DNA hydrolysis occurs
C. Specific endonuclease recognises the damage
D. Double stranded DNA Break occurs

Ans: Specific endonuclease recognises the damage

5. Double stranded DNA Break Repair defect causes all except

A. Hereditary Nonpolyposis colon cancer
B. Ataxia Telangiectasia
C. Bloom's syndrome
D. Fanconi's syndrome

Ans: Hereditary Nonpolyposis colon cancer

6. Cockayne syndrome is caused by the defect of

A. ds DNA Break Repair
B. Replication linked nucleotide excision repair.
C. Transcription linked nucleotide excision repair.
D. Mismatch repair

Ans: Transcription linked Nucleotide excision repair

7. Severe combined immunodeficiency is caused by the defect of

A. Non-Homologous End Joining
B. Homologous DNA Repair
C. Transcription linked Nucleotide Excision Repair
D. Mismatch repair

Ans: Non-Homologous End Joining

8. Homologous DNA repair defect causes

A. Ataxia Telangiectasia
B. Lynch syndrome
C. Bloom syndrome
D. Fanconi's anaemia

Ans: Fanconi's Anaemia

Case Based MCQs

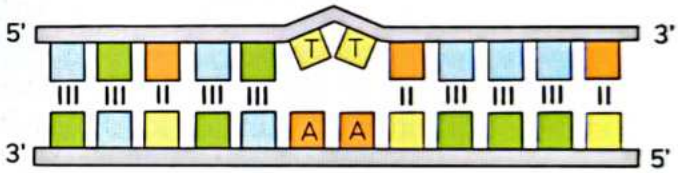
Q. 40-year-old male who was diagnosed with rectal cancer. Left colectomy was carried out. A histopathologic diagnosis of poorly differentiated adenocarcinoma. Family history revealed the five family members were diagnosed with colorectal cancer, and two successive generations affected. All malignancy were diagnosed before 45 years of age. Microsatellite instability was detected on genetic analysis of tissue of the proband. It is caused by a defect of

A. Base Excision repair
B. Mismatch repair
C. Nucleotide Excision repair
D. ds DNA Break repair

Ans: Mismatch repair

Image Based MCQs

Q. True about the DNA defect observed in the image are all except



A. Caused by UV light.
B. Exonuclease is involved in the repair.
C. Defect of the repair causes Xeroderma Pigmentosum
D. Defect of the repair causes Cockayne syndrome

Ans: Exonuclease is involved in repair

34 RNA TRANSCRIPTION AND POST TRANSCRIPTIONAL MODIFICATIONS

Differences between DNA and RNA

S.no	DNA	RNA
1.	Contains Deoxyribose sugars	• Contains Ribose sugars (More hydroxyl groups) • More alkali labile
2.	Have thymidine	• Have uridine. o During pyrimidine synthesis, uridine is first formed o On amination, it gives cytidine o On methylation, it gives thymidine
3.	Preferred to exist as double strand	• Preferred to exist as single strands. o Do not follow Chargaff's Rule of Base Pairing. o Watson and Crick Base pairing rule is followed here. → In any double-stranded structure, A should always hybridize with T, and G should always hybridize with C. → Chargaff's Rule of Base Pairing is A content = T content and G content = C content. → Chargaff's rule holds good for double-stranded DNA.
4	DNA polymerases have proofreading and repair activity.	• More prone to get mutated. o Because RNA polymerases do not have proofreading and repair activity.

Types of RNA

- rRNA - Ribosomal RNA
- mRNA - Messenger RNA
- tRNA - Transfer RNA
- sRNA - Small RNA

rRNA

- Associated with ribosomes.
- Eukaryotic ribosome is an 80S unit that dissociates into 40S and 60S ribosome subunits.
- **Four types of rRNA**
  - o 5srRNA - associated with 60s ribosomes.
  - o 5.8srRNA - associated with 60s ribosomes.
  - o 18srRNA - associated with 40s ribosomes.
  - o 28srRNA - associated with 60s ribosomes.
- All these four rRNAs are the product of one common gene, which on transcription gives rise to a primary transcript.
  - o The primary transcript is a very large 45srRNA.
  - o And during post-transcriptional modification, it will be broken to give rise to 5.8srRNA, 18srRNA, and 28srRNA.
  - o 5srRNA is transcribed separately from a different gene.
- 28srRNA is a ribozyme (RNA with enzymatic activity)
  - o Enzymatic activity that it exhibits is peptidyl transferase activity.
    - → It shifts a growing polypeptide chain from the P site to the A site.

Important Information

- Gene is a segment of a chromosome that can quote for either a protein of RNA, and this gene or transcription gives rise to a primary transcript.

mRNA

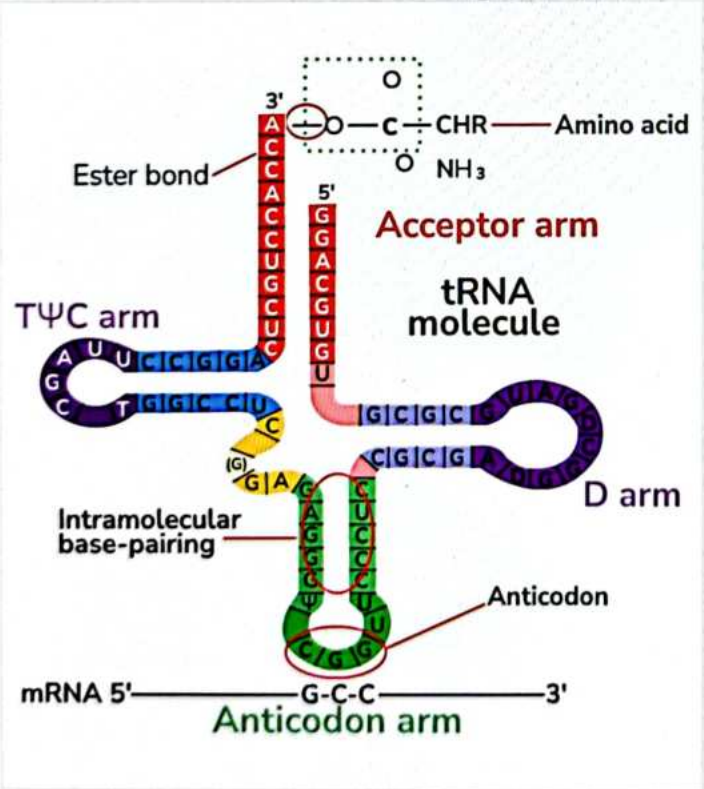
- Messenger RNA
  - o Carries message from the nucleus to the cytoplasm.
  - o Message for protein synthesis is in the form of genes which are in chromosomes which are in the nucleus.
  - o Protein is synthesized by the ribosome in the cytoplasm.
- **Synthesis**
  - o Genes do not only have coding sequences but also non-coding intervening sequences, otherwise called introns.
  - o The immediate product of transcription of this gene is not a functional mRNA but a primary transcript or heteronuclear RNA (formed within the nucleus).
  - o It should undergo at least three post-transcriptional modifications in the nucleolus.
    - → 7-Methyl guanosine cap gets added to 5 prime ends.
    - → Poly-A tail gets added to the 3 prime ends.
  - o Nucleus is rich in exonucleases (starts acting on a nucleotide chain from the exterior- either from 3 or 5 prime ends).

- To protect endogenous RNA from their attack, the head and tail are added.
- Removal of non-coding intervening sequence or Introns. Joining the coding sequence or exons in a process called splicing.
- After splicing, the result is a functional mRNA that comes out of the nucleus and reaches the cytoplasm.
- In the cytoplasm, the ribosome will start reading mRNA from the 5 prime ends to the 3 prime ends.
- Reads codon one by one.
- Depending on the codon while reading, the ribosome recruits a complimentary anti-codon containing tRNA.
- This tRNA brings along amino acid, which leads to the translation of the nucleotide sequence of mRNA as the amino acid sequences of a polypeptide chain.

- mRNA nucleotides are grouped in three to form a codon. Based on codon, the attachment of amino acids happens during translation

tRNA

- Called transfer RNAs.
  - Transfer amino acid from the amino acid pool to the ribosome.
  - To quote for 21 amino acids, there are 50-100 tRNA.
    → Each has a unique gene.
  - All are clover leaf shaped because they have four stable arms and one variable arm.
    → Anticodon arm
    → Acceptor arm
    → D arm
    → TΨC arm



- **Anticodon arm**
  - It has triplet nucleotide which is complementary to the codon of mRNA.
  - Third nucleotide pair formation is non-specific (more than one codon coding for a single amino acid) called the wobble phenomenon.
    → Wobble phenomenon - Reduced stringency when the third nucleotide pair is formed between codon and anti-codon.
  - When tRNA is written in 3 prime, 5 prime direction, the first nucleotide of the anticodon of tRNA is not specific.

Important Information

- Polarity of tRNA is 3 prime and 5 prime directions.

  - **Degeneracy** - More than one codon coding for a single amino acid because of the wobble phenomenon.
    → Wobble phenomenon also occurs when the third nucleotide of the codon of mRNA is not specific or the first nucleotide of the anti-codon of tRNA is not specific.
- **Acceptor arm**
  - Depending upon the anticodon present, this arm accepts the amino acids.
  - It has a triplet nucleotide.
    → CCA attached to the three prime ends - where adenosine amino acid gets attached.
    → This triplet is common to all the 50 - 100 tRNAs because it is not coded by the specific gene.

Important Information

- Initially, during their formation, all the tRNAs are truncated tRNAs.
- After they are synthesized in the nucleus and after they reach the cytoplasm, an enzyme by the name of nucleotidyl transferase which transfers the triplet CAA nucleotide uniformly to all 50 - 100 tRNA.

- **TΨC arm**
  - For ribosomal attachment during the translation process.
- **D arm**
  - Helps in the attachment of aminoacyl-tRNA synthetases.
- The moment any cell decides to get into the translation of protein synthesis, the first enzyme system to be activated will be the aminoacyl-tRNA synthetases.
  - After activation, aminoacyl-tRNA synthetases go to the amino acid pool and pick up a specific amino acid.

- Activates it using two high-energy phosphates and attaches it to themselves, forming an amino acyl-enzyme.
- This enzyme carrying amino acid goes to the tRNA pool.
  - → Attach to one of the arms of each tRNA - D arm.
  - → Check the anticodon at the arm.
  - → If they have their specific anticodon, they attach the amino acid to the acceptor arm.
  - → If not, they go to the next tRNA and go on till they get their specific anticodon.
- The moment the cell decides to get into the translation of protein, glycynl-tRNA synthetase gets activated and goes into the amino acid pool and picks up only glycine.
- Activates it using two high-energy phosphates.
- Attaches it to themselves, forming glycenlle enzymes.
- Attaches to the D arm and scans the anti-codon arm, and if it finds CCI here, it will allow glycine to get attached to the tRNA.
- Moves from tRNA to tRNA until it finds CCI.

Q. Fidelity of the gene is conferred by?
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Ans. Aminoacyl-tRNA synthetases.

Explanation

- Because they pick up a specific amino acid and attach it to tRNA.
- If this is not a choice, tRNA can be the right answer.
- Abnormalities of tRNA
  - Pseudouridine only found in tRNA.
  - Inosine found only in tRNA.
  - Some bases will be methylated in tRNA while some bases are alkylated.

Synthesis of tRNA

- tRNA has its own gene, which on transcription, gives rise to a primary transcript.
  - Simple straight chain shape
- It gets the cloverleaf shape due to the numerous intra-chain base pairing tendencies.
  - First post-transcriptional modification - intra-chain base pairing
- The primary transcript will only have uridine, which becomes pseudouridine
  - Glycosidic linkage is formed in uridine at N1 and in pseudouridine at C5
- Second post-transcriptional modification - rearrangement of glycosidic linkages giving rise to pseudouridine.
- Primary transcript has adenosine which converts to inosine.
  - Third post-transcriptional modification - Deamination creating inosine.
- Fourth post-transcriptional modification - randomly methylation happens.
- Fifth post-transcriptional modification - randomly alkylation happens.
- 1 to 5 post-transcriptional modifications happen in the nucleus.
- Then it leaves the nucleus and reaches the cytoplasm
  - Truncated tRNA - not attached to the CCA triplet nucleotide by a nucleotidyl transferase.
  - After attaching with the CCA triplet by nucleotidyl transferase (6th post transcription modification), it becomes complete tRNA and not functional.
    - → Not attached to the amino acid
- 7th post-transcriptional modification - Aminoacyl-tRNA synthetases attach the amino acid to it and make it a functional tRNA.

Small RNA

Types

- Small nuclear RNA (snRNA)
- Small interference RNA (siRNA)

snRNA

- Six types
  - U1 to U7 except for U3
- All except U7 help in splicing.
  - Splicing - Process wherein three prime and five prime phosphates diester linkages are broken at these Splice junctions.
    - → Introns will be removed, and exons one and two are joined by forming a three-prime and five-prime phosphates diester linkage.
  - snRNA acts as an enzyme in splicing
  - snRNA are examples of ribozymes.
- U7 helps in stem-loop structure attachment.

Important Information

- The three post-transcriptional modifications of mRNA during synthesis are not universal.
- Exception - Histone mRNA formation
  - Histone genes are of prokaryotic type (do not have introns)
  - No slicing happens.
  - No Poly (A) tail attachment in prokaryotes - acquired during evolution.
  - A stem lobe structure is attached in the place of Poly (A) tail at three prime ends to protect from exonucleases.
  - The stem lobe structure attachment is due to U7.

siRNA

- Full form - small interference RNA
- Interfere with gene expression.
  - Gene expression - Gene on transcription gives rise to mRNA, and mRNA on translation gives rise to protein.

- siRNA interferes with gene expression at the translation level.
- They interfere with their own sequences.
  - And if they are a complementary sequence on any of the fully formed mRNAs, they go and hybridize with them to form a double-stranded RNA structure.
  - The double-stranded formation is abnormal, and the ribosome starts reading from 5 prime ends to the 3 prime ends.
  - Ribosome recruits RNA transcription silencing complex at the double-stranded region
    → One of the proteins of this complex is an endonuclease - makes multiple mixes on the mRNA, fragmenting the mRNA, and thus the translation is inhibited.
  - This interference is one of the ways by which we regulate gene expression.
    → Eg. - Inflammatory focus is in a person - switching multiple cytokine genes of the person - all these are transcribed to give rise to cytokine mRNAs.
    - If they convert into protein - a hyperinflammatory state is induced.
    - The gene has introns that have siRNA gene.
    - These are to be transcribed, and they go and hybridize with mRNA and form a double-stranded RNA.
    - Then the mRNA gets fragmented, and the cytokine storm is stopped.
- Form of regulation of gene expression.

Interference Phenomenon

- Downregulates gene expression.
- Also called a mechanism for "gene knockdown"

Q. Where does siRNA help?
Ans: In the regulation of gene expression at the transcription level.

Q. Which brings down gene knockdown?
Ans. Interference

Important Information

- Gene knockout - removing a defective gene.
- Gene knockin - replacing a defective gene with a normal gene.
- This is done by mediated CRISPR gene editing.

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35 PROPERTIES OF GENETIC CODE MUTATION

Properties of genetic code mutations

- We often call the genetic code a degenerate codon.

Properties of Genetic Code

- Four choices of nucleotides (A, U, G, C)
- 3 combinations of nucleotides are used to form a codon (4C3 or 64 codons are possible)
- Stop codons: 3
- 61 codons code for 20 amino acids
- Selenocysteine is not included as it is coded by stop codon

Q. How many choices of nucleotides do we have to make a codon?

Ans: 4 choices of nucleotides (A, U, G, C)

1. Degeneracy

- More than one codon can code for a single amino acid.
- It is explained by the Wobble phenomenon.
- 2 amino acids which don't follow degeneracy: Methionine and tryptophan.
- **Wobble Phenomenon**
  - Basis: Inosine monophosphate is a nucleotide for hypoxanthine
  - According to it, the third nucleotide of the anticodon of t-RNA is inosine monophosphate.
  - IMP does not follow Watson and Crick Base pairing rules.
    → Watson and crick stated: A always hybridizes with T/U and G always hybridizes with C.
  - **Example:**
    → A hypothetical mRNA has four codons GGG, GGC, GGA, GGU.
    → These all will code for the same amino acid glycine.
    → Because tRNA which carries glycine has an anticodon CCI (I is the first nucleotide of the anticodon tRNA) that does not follow Watson and Crick Base pairing rules
    → Hence, this tRNA is least bothered about the third nucleotide in all the four codons.
    → So, it attaches glycine as an amino acid.
    → Polarity of mRNA 5' to 3'
    → Polarity of tRNA 3' to 5' (antiparallel).
    → So, the IMP might appear as a third nucleotide.
    → But, according to the genetics the polynucleotide chain should be in 5' to 3' polarity (so the IMP is considered as the first nucleotide of the anticodon tRNA).
- **Statements of Wobble Phenomenon**
  - Reduced stringency in the third nucleotide pair formation between the codon and anticodon.
  - 3rd nucleotide of the codon of mRNA is not specific.
  - 1st nucleotide of anticodon of tRNA is not specific.

Q. Which property of the genetic code explains the Wobble phenomenon?

Ans: Degeneracy

2. Unambiguous

- One amino acid will code for only one codon.

3. Non overlapping

- Codon should not be overlapped with each other.

4. Not punctuated

- There is no punctuation mark after Third nucleotide.
- Hypothetically, if there is a loss of one nucleotide, then the amino acid coded alone will be defective.
- Then the nucleotides will be combined with one another (the entire frame of reading will be shifted).
- This might lead to frameshift mutations.

5. Universal

- Unique genetic code for every cell with few exceptions.
- At one point of time genetics were very puzzling because they understood only G.
- The genetic composition of all the cells is identical but the expression of genes is different in every cell.
- UGA is a stop codon but it codes for tryptophan in mitochondrial DNA.
- Usually, AGG and AGA codes for arginine but in mitochondrial DNA, they act as a stop codon.

Q. What are the amino acids that do not follow degeneracy

Ans:

- Methionine
- Tryptophan

Q. All are the properties of genetic code except

Ans: Ambiguous

Types of Mutation

- Change in the sequence of amino acids.

These are classified at three levels.

- Effects on nucleotide sequence
- Effect on Amino acid sequence
- Effect of function of a protein

Effects on Nucleotide Sequence

Point Mutation	Frameshift Mutation
• One nucleotide is substituted by other	• When there is a loss or gain of nucleotides the entire frame of reading will be shifted
• Depending on the nucleotide substitution it is divided into. o Transition o Transversion	• It is two types. o Deletion o Insertion
Transition: • Purine is replaced with a purine. • Pyrimidine is replaced with pyrimidine	**Deletion:** • Loss of nucleotide
Transversion: • Purine is replaced with pyrimidine. • Pyrimidine is replaced with purine	**Insertion:** • Gain of nucleotide

Effect on Amino Acid Sequence

Type of mutations	Explanation
Silent mutation	• In spite of change in the sequence of nucleotides, if there is no change in the amino acid sequence. • It is based on degeneracy
Missense mutation	• There is a change in the nucleotide sequence which results in the change of amino acid sequence. • It is observed when a codon coding for one amino acid is replaced by another (that codes for a different amino acid)
Nonsense mutation	• Codon coding for an amino acid is replaced with the stop codon. • In this condition, premature termination of proteins synthesis (truncated proteins) is produced)

Effect of Function of a Protein

Types of Mutations	Explanation
Acceptable mutation	• There is a change in the nucleotide sequence. • That results in alternating the amino acid sequence. • But there is no change in the function of the proteins. **Example:** • All hemoglobin variants that are accidentally finding during electrophoresis or chromatographic separation • HbA1C is a glycated hemoglobin (to assess the long-term glycemic control of a person) • When chromatography or electrophoresis is done, you will observe the difference in the movement of hemoglobin.

Examples: Hb Milwaukee, Bristol and Sydney

- The 67th amino acid of beta globin chain is usually valine.
- If it is replaced with aspartic acid, it is called Bristol.
- Hb Milwaukee: Valine is replaced with glutamic acid.
- Sydney: Valine is replaced with alanine.
- But the oxygen carrying capacity is normal

Partially acceptable mutation	• There is a change in the nucleotide sequence. • That results in alternating the amino acid sequence. • Hence, the function of the proteins are altered • But it is not immediately life threatening.

Example: Sickle cell anemia-Hbs (Mutation it fits point mutation, transversion, missense mutation)

- Mutation of beta globin gene (6th codon is GAG that codes for polar glutamic acid)
- It is replaced with GTG (non-polar amino acid valine)
- Glutamic acid is replaced with valine.
- The 6th codon present in the inner region of hemoglobin, is released after dehydration and deoxygenation.
- If it is polar, it doesn't cause any damage.
- If it is nonpolar, it exhibits a sticky patch with the membrane (due to hydrophobic interaction).
- It results, in the accumulation of globin chains, it loses its deformability.
- Finally, it causes a sickling crisis as the membrane is pinched off.

Unacceptable mutation	• It is immediately life threatening

Example: Congenital methemoglobinemia- HbM

- Fe2+ is replaced with Fe3+ (present in the center of the tetrapyrrole ring)
- Fe2+ is covalently attached to one amino acid (histidine F8) of the globin chain.
- In this condition, histidine F8 is replaced with a tyrosine has a phenol (hydrophilic) group.
- It accepts the electrons from Fe2+
- As a result, it becomes Fe3+ (cannot carry oxygen)

One Liners

- HbS mutation is an example of **point mutation, Transversion, missense, partially acceptable** mutations
- Amino acids which do not follow degeneracy are **Methionine and Tryptophan**
- Hb Milwaukee is an example of **Acceptable mutation**
- In Congenital Methemoglobinemia Histidine F8 is replaced by **Tyrosine**

Multiple Choice Questions

Q. No of possible codons
A. 64
B. 61
C. 20
D. 31

Q. No.of codons which code for an amino acid
A. 64
B. 61
C. 20
D. 31

Q. Codon consists of:
A. 3 base pairs.
B. 2 base pairs
C. 5 base pairs
D. 3 nucleotides

Q. Stop codon:
A. UAG
B. UCA
C. UAC
D. AUG

Q. The amino acid which does not follow degeneracy of codon is,
A. Glycine
B. Glutamine
C. Tryptophan
D. Tyrosine

Q. Wobble phenomenon explains which of the following,
A. Degeneracy
B. Unambiguity
C. Ambiguity
D. Punctuation

Q. Transition mutation of GATCCT is
A. GGTCCT
B. GTTCCT
C. GAACCT
D. GATACT

Q. Transit what kind of mutation does the following exemplify
Normal: 51-AUG-GAU-GAU-GGU-3'
3HN-met-asp-asp-gly-COO-
Mutation: 51-AUG-GAC-GAU-GGU-3'
+H3N-met-asp-asp-gly-COO-
A. Frameshift
B. Silent
C. Missense
D. Point mutation with no appartent effect

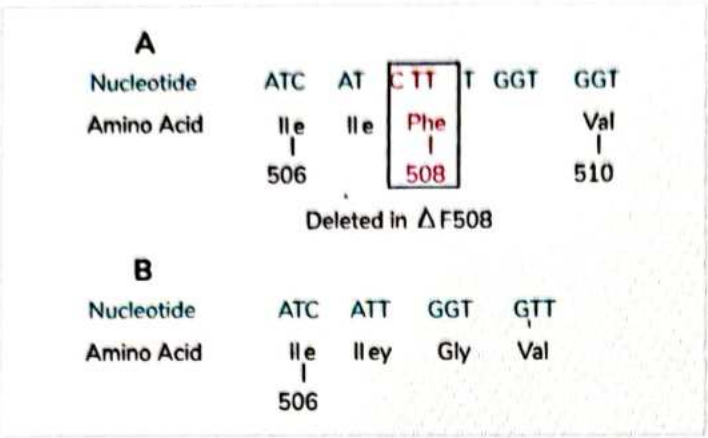
Q. There is no apparent change in the hemoglobin function if position 67 of globin chain which is occupied by valine is replaced by all except
A. Glutamate
B. Asparate
C. Alanine
D. Glycine

Case Based MCQs

Q. A 45-year-old male is detected to have fasting and postprandial hyperglycemia in the diagnostic range of diabetes. To understand his long term glycemic control, he is asked to estimate his HbA1C Chromatogram detects an abnormal Hemoglobin peak, corresponding to Hb Bristol. His oxygen carrying capacity is normal. Hb Bristol is an example of
A. Silent Mutation
B. Acceptable mutation
C. Partially acceptable mutation
D. Nonsense mutation

Image Based MCQs

Q. In the image A denotes the wild type allele. B is mutated. Identify the type of



A. Silent mutation
B. Point mutation.
C. Acceptable mutation
D. Deletion

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36 INHIBITORS OF TRANSLATION

Translation and inhibitors of translation

Translation

- **Translation happens in three phases:**
  - Initiation
  - Elongation
  - Termination

Initiation

- There are many factors involved and are given the prefix eIF.
- eIF stands for eukaryotic Initiation Factor.
- **eIF2C** - Helps ternary tRNA Methionine complex formation.
- **eIF3 & 1A** - Helps in stabilizing 40s Ribosome.
- **eIF 4G/4A** - Guides mRNA cap towards ribosome.
- **eIF4A/4B** - ATP dependent helicase.
- **eIF5** - Removes 3 & 1A.

Steps of Initiation

- Dissociation of 80s ribosomes into 60s and 40s subunits.
- Only the 40s subunit is involved, as the 60s subunit got a P site and A site which are not needed in the Initiation step.
- eIF3 & 1A is used to stabilize 40s ribosomal subunits.
  - **Reason:** To avoid reassociation of two subunits as they always have a tendency to do so.
- 43s PIC (Pre-Initiation Complex) is formed from 40s units by attaching three subunits.
  - **Three subunits are attached to the ternary complex are:**
    - → Methionine tRNA guided to the ribosome by eIF2C.
    - → eIF2C, a factor that helps ternary tRNA Methionine complex formation.
    - → GTP for activation.

Status of 43s PIC:

- 4G/4A guides mRNA cap towards ribosome.
- 4G/4A is attached to the cap through 4E.
- 4G/4A + 4E = 4F.
- m-RNA has hairpin loops; as long as it has, it cannot be involved in translation.
- To remove hairpin loops, hydrogen bonds should be broken by ATP-dependent helicase eIF4A/4B making mRNA single-stranded.
- Methionine tRNA should scan mRNA.
- It is to find the Initiation AUG codon present in the Shine-Dalgarno sequence in prokaryotes or Kozak consensus sequence [-3 and +4 should be purines - AUG (+1, +2, +3)] in eukaryotes.
- Once identified, methionine tRNA is attached to AUG at P-site.
- The 40s won't get reassociated with 60s units until it is stabilized by 3 & 1A.
- eIF5 is used to remove 3 & 1A.
- 40s is reassociated with 60s.
  - In the 60s, mRNA aligns itself in such a way that on P-site, AUG is attached along with the methionine tRNA.
  - The A-site is left free.

Facts about Elongation

- **Status of ribosome entering elongation phase:**
  - The 40s is reassociated with the 60s.
  - In the 60s, mRNA was present.
  - mRNA has come with AUG aligned in such a way that the AUG codon is on the P-site.
  - Methionine tRNA is attached to AUG.
- Corresponding to mRNA, a second codon is present for which a complementary anticodon should be recruited containing second aminoacyl tRNA.
- Any aminoacyl tRNA is synthesized by aminoacyl tRNA synthetases.
- Aminoacyl tRNA synthetases function to attach the specific AA to specific tRNA.
- It uses two high-energy phosphates to activate the amino acid.
- **Example:**
  - Glycinyl tRNA is formed by Glycinyl tRNA synthetase.
  - Tyrosinyl tRNA is synthesised by tyrosinyl tRNA synthetase.
- In such a way, the tRNA is selected and guided to the ribosome.
- eEF stands for Eukaryotic Elongation Factors
- eEF1A, with the help of GTP (3rd high energy phosphate), guides aminoacyl tRNA to attach to the A-site.
- **Peptidyl transferase** - Shifts the growing polypeptide chain from P-site to A-site.
  - A ribozyme (RNA with enzymatic activity) is not a protein.
  - It is a 28srRNA in eukaryotes.
  - It is a 23srRNA in prokaryotes.
- Between methionine and the second amino acid, the peptidyl transferase forms a peptide linkage.
  - This step doesn't use energy because amino acid is already activated by aminoacyl tRNA synthetase.
- After methionine gets attached to the second amino acid, tRNA has no role to play and leaves its site.
- Translocation is done by eF2 with the help of GTP (4th high energy phosphate).
- In translocation, the ribosome is translocated ahead.

- **The same happens for the third codon.**
  - The A-site is free, which corresponds with the third codon for which the complementary anticodon containing third aminoacyl tRNA is recruited.
  - eEF1A, with the help of GTP (3rd high energy phosphate), guides aminoacyl tRNA to attach to the A-site.
  - Peptidyl transferase shifts the growing polypeptide chain from P-site (2nd amino acid) to A-site (3rd amino acid) and forms a peptide linkage.
  - After methionine gets attached to the third amino acid, tRNA has no role to play and leaves its site.
  - Translocation is done by eF2 with the help of GTP (4th high energy phosphate).

Important Information

- Total 4 high-energy phosphates are needed to attach the amino acids to the growing polypeptide chain.
  - 2 - Activation of amino acids.
  - 1 - Activation of eEF1A.
  - 1 - Activation of eE2.
- Ribosome is translocated ahead.

The growing polypeptide is on P-site, and A-site is free.

- **The same goes with the fourth codon until**
  - The A-site is free, which corresponds with the fourth codon for which the complementary anticodon containing fourth aminoacyl tRNA is recruited.
  - The ribosome is moved along with the length of mRNA by eF2 with the help of GTP.
  - The ribosome continues moving until a stop codon arrives at A-site.
  - No complementary anticodon containing aminoacyl tRNA, so the RF1 factor is brought to action.
  - RF1 factor uses GTP to release the polypeptide chain, which is still attached to the tRNA on the P-site. This Is Termination
  - Even termination we need a high energy phosphate.

Energetics of Translocation for AA

S. No	Step	Number of ATPs
1.	Activating amino acids by aminoacyl tRNA synthetase	2
2.	Activating of eEF1A	1
3.	Activating of eEF2	1
Total	**4**	

Differences Between imet tRNA & Other tRNAs

S.No	imet tRNA	Other tRNAs
1.	Guided by eIF2C.	Guided by eEF1A.
2.	Gets attached to P-Site of 60s ribosome.	Gets attached to the A-Site of 60s ribosomes.
3.	3 high energy phosphates are required for attachment.	4 high energy phosphates are required for attachment.

Translation Energetics

- $3 + (n - 1) \times 4 + 1 = 4n$
- Where
  - 3 are the high energy phosphates required by imet tRNA.
  - (n - 1) are the other amino acids.
  - (n - 1) × 4 are the number of high-energy phosphates required other than methionine.
  - 4n is the total number of high-energy phosphates required by the n number of amino acids.

Translational Inhibitors

S.No	Inhibitors	Mechanism of Action
1.	**Aminoglycosides**	• Protein synthesis inhibitors with cidal action. • Inhibits every step of translation like- o Freeze initiation o Inhibits polysome formation.
2.	**Tetracyclines**	Inhibits EF1A (Prokaryotic elongation factor 1A). • It inhibits the attachment of aminoacyl tRNA to the A-site.
3.	**Chloramphenicol**	• Prokaryotic Peptidyl transferase inhibitors - 23srRNA inhibitors.
4.	**Cycloheximide and Ricin**	• Eukaryotic Peptidyl transferase inhibitors - 25srRNA inhibitors.
5.	**Macrolide and clindamycin**	• Prokaryotic translocation step inhibitors.
6.	**Diphtheriae and pseudomonas toxins**	• Eukaryotic translocation step inhibitors. Inhibits EF2.
7.	**Puromycin**	• Amino acyl tRNA analogue causes premature termination of protein synthesis

Puromycin Action

- Aminoacyl tRNA analogue.
- Puromycin attaches to the A-site instead of Aminoacyl tRNA.
- Peptidyl transferase tries to shift the growing polypeptide chain from P-site and A-site.
- Peptide linkage cannot be formed between methionine and puromycin.
- Elongation stops.
- The peptide chain will become free, causing premature termination of protein synthesis.

Inhibitors List-Mnemonic

- **Buy AT 30 and CCEL at 50.**
- Acts at 30s
  - **A**-Aminoglycosides
  - **T**-Tetracyclines
- Acts at 50s
  - **C**-Chloramphenicol
  - **C**-Clindamycin
  - **E**-Erythromycin (Macrolides)
  - **L**-Linezolid

One Liners

- Peptide linkage is formed by **Peptidyl Transferase.**
- Number of high-energy phosphates required for attaching an amino acid to a growing polypeptide chain is **4.**
- Initiation methionine tRNA binds to the **P** site of ribosomes.
- Aminoacyl methionine tRNAs bind to **A** site of 60s ribosomes.

MCQs

Q. P and A sites are components of
a. 80s
b. 40s
c. 60s (Answer)
d. 30s

Q. The function of the Shine Dalgarno sequence is to
a. Identify the termination signal
b. Help in guiding mRNA to ribosome
c. Identify initiation codon (Answer)
d. Dissociate ribosome

Q. The translation factor which guides aatRNA to A site is
a. eIF1A
b. eEF4A
c. eEF1A (Answer)
d. eIF3

Q. The total number of ATPs required for attaching every amino acid to a growing polypeptide chain is

a. 1
b. 2
c. 3
d. 4 (Answer)

Q. The total number of ATPs required for forming a polypeptide chain with n amino acids is
a. 4n-1
b. 4n+1
c. 3n
d. 4n (Answer)

Q. mettRNA is attached to
a. P site of 40s ribosome
b. A site of 40s ribosome
c. P site of 60s ribosome (Answer)
d. A site of 60s ribosome

Q. The step which does not require high energy phosphate is
a. met tRNA synthesis
b. Aminoacyl tRNA synthesis
c. Peptide bond formation (Answer)
d. Termination

Q. For Peptidyl transferase, all are true, except
a. It is a protein (Answer)
b. It is an RNA
c. It does not require high-energy phosphate
d. It is inhibited by chloramphenicol

Q. Tetracycline inhibits
a. IF1A
b. EF1A (Answer)
c. IF2C
d. EF2C

Q. Ricin inhibits
a. aatRNA synthetase
b. Peptidyl transferase (Answer)
c. Termination
d. Initiation

Q. Diphtheria toxin inhibits
a. IF1A
b. EF1A
c. IF2C
d. EF2 (Answer)

Q. Macrolide inhibits

a. Peptide bond formation
b. Termination
c. Translocation (Answer)
d. Initiation

Case Based MCQs

Q. A 25-year-old male presents with a skin infection with yellowish crusts on the face. Impetigo was diagnosed, and mupirocin ointment was prescribed. Mupirocin acts by

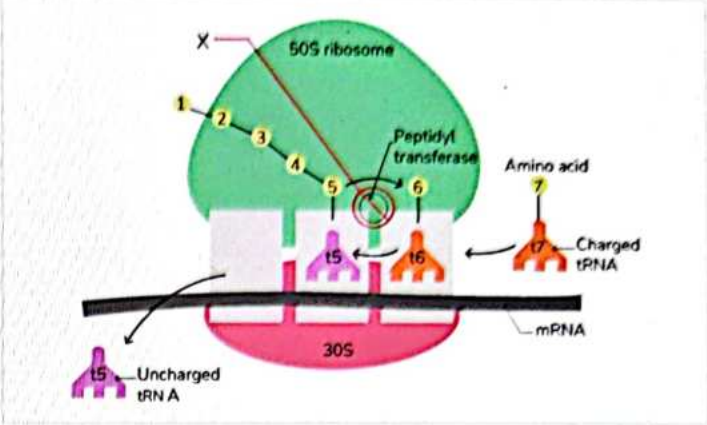
a. Inhibiting the binding of RNA polymerase to +1 site
b. Causing premature termination of mRNA synthesis
c. Causing premature termination of protein synthesis (Answer)
d. Inhibiting translocation of ribosomes

Explanation:

- Puromycin is the medicament in mupirocin ointment.
- Aminoacyl tRNA analogue.
- Premature termination of protein synthesis.

Image-Based MCQs

Q. Name X shown in the blue box, given the clue that it is an antibiotic, which inhibits the blue circle shown in the image.



a. Puromycin
b. Macrolide
c. Clindamycin
d. Chloramphenicol (Answer)

Explanation:

- Peptidyl transferase is inhibited by chloramphenicol.

37 PCR

Objectives

- Steps involved in PCR (Polymerase chain reaction)
- Steps to follow when- Mutation site is known or unknown.
- Significance of real-time PCR
- Difference between real-time PCR & conventional PCR.
- Steps that are followed from molecular diagnosis of SARS-COV-2.
- Sanger sequencing

Cloning

- It is a production of identical copies.
- It replicates DNA multiple times.

Applications of DNA

- **Genetic engineering application**: If we try to produce insulin on a large scale, the first raw material required will be the insulin gene.
- We will be copying the insulin gene multiple times; only then will we get the multiple copies of insulin mRNAs then we can expect multiple copies of insulin protein.
- **Forensic application**: From the crime site, we can expect a blood stain or a hair follicle.
- From this, we can extract one or two copies of DNA.

Applications of Replication

- Replicating DNA multiple times.
- The enzyme which replicates: DNA polymerases

Requirements of replication

- DNA polymerases
- Primer
- All four dNTPs
- Magnesium or manganese acts as a catalyst
- Buffer - to maintain the pH.

Types of Cloning

In-vivo cloning/Cell-based cloning

- Wherein all the requirements will be provided by a whole living cell like E. coli (recombinant DNA technology)
- **Recombinant DNA Technology**: It's a cell-based cloning wherein a cell equally provides all the requirements of replication, it provides all the requirements of cloning, and cloning happens in vivo within a cell.

In-vitro cloning or enzyme-based cloning

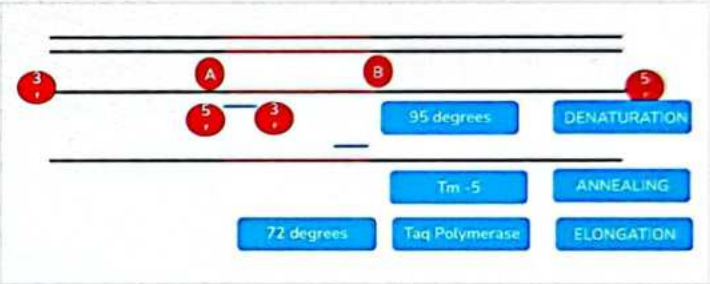
- It is a polymerase chain reaction.

Facts about PCR

- It is an in-vitro amplification of a desired fragment of DNA.
- We can specify what we want by constructing primers.
- We choose primers in such a way that they complement to flanking sequences of the desired fragment (terminal sequences).

- Suppose this represents the entire chromosome, and the red line represents what we want.
- So, we will get what we want by constructing primers.
- We will construct the primers in such a way that they are complementary to the flanking sequences of that desired fragment so that we will successfully get what we want.

Steps of PCR



Step 1

- **Denaturation**: We unwind the two strands of double-stranded DNA to form 2 single strands.
- It is carried out at 94/95 degrees (at this temperature, the hydrogen bonds will be broken).

Step 2

- **Annealing:** Adding primer which will anneal to the flanking sequences of the desired fragments (the temperature should neither be too high nor too low- it should be optimal)
- It should be 5 degrees < than the melting temperature of the primers.
- Two primers are required which are complementary to 3' flanking sequences of both the strands.

Step 3

- **Elongation**: It should be done by the DNA polymerase, which is thermostable. The enzyme that is used in elongation is Taq (Thermus aquaticus) DNA polymerase.
- DNA polymerase elongates new strand in 5' 3' direction.
- Optimal temperature required for elongation is 72 degrees.

Q. Why should DNA polymerase used be thermostable?

Ans. It is because PCR it's one step addition; when you take a PCR tube then add all the requirements of PCR (Primer, all four dNTPs, Magnesium or manganese- they act as a catalyst and buffer - to maintain the pH), then we place it in a machine known to be a thermocycler.

Q. Why is it called a thermocycler?

Ans. It is because it cycles between temperatures.

- First, it raises the temperature to 94 or 95 degrees; then, it reduces the temperature to an annealing temperature (i.e. 5 degrees less than the melting temperature of the primers).
- For elongation, it increases to 72 degrees (this is one cycle, and it continues).
- **Thermus aquaticus**: The T. aquaticus organism enables the PCR (polymerase chain reaction). It can withstand high temperatures, specifically those above 70% C, making it a "thermophile."
- If we allow PCR to go through N number of cycles, then we can expect the following:
  - 2^n products
  - Suppose we set PCR at 30 cycles; at the end of 30 cycles, we can expect the product to be 2^{30}

Disadvantages of Using PCR

- It is mainly because it uses sophisticated equipment- a thermocycler that is costlier and needs expertise.
- Isothermal amplification: Lamp assay/loop mediator amplification assays
- We use DNA extracted from an organism called Bacillus stearothermophilus (BST) or Bacillus smithii.

Advantages of Using BST

- They have a very high strand invasion ability. It invades strands and elongates new strands. It can be carried out at just 60 degrees.
- It is an isothermal amplification technique that doesn't need a thermocycler because all steps of PCR will be carried out at 60 degrees.
- An incubator is required to keep the desired temperature.

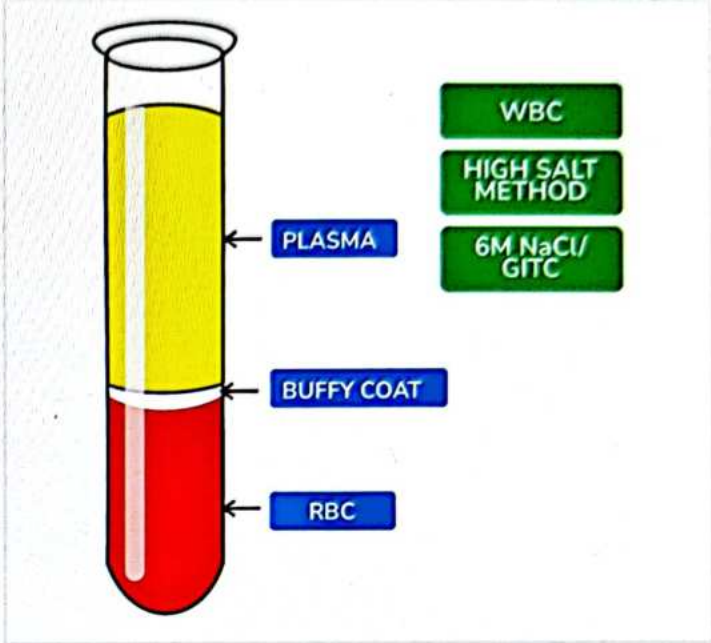
Steps of molecular diagnostics of the known mutation site

- Example: sickle cell anemia the mutation in 6th codon of Beta globin gene.

Step 1: Blood sample collection

Step 2: DNA extraction (by centrifuge)

- **Centrifugation of blood sample**
  - The blood sample will be differentiated into three zones.
  - 1st layer of plasma in the blood sample
  - 2nd layer is a buffy coat.
  - And the 3rd layer is RBC.
- Out of these three layers, the 2nd layer, which is a buffy coat layer, is used for the extraction of DNA.
- In the buffy coat layer, the WBCs are present. WBC has a nucleus that is rich in DNA.
- Then there is the cell lysis of DNA by high salt method - 6M NaCl/GITC - osmotic lysis of the cell, as well as the nucleus and then the DNA, comes out.



Step 3: 46 chromosomes with all 25 thousand genes (we want only the beta globulin gene)

Step 4: PCR (PCR does not help in direct diagnosis. It helps with fragmenting what we want)

Step 5: RFLP or sequencing (Restriction Fragment Length Polymorphism) or SSCP/ DGGE

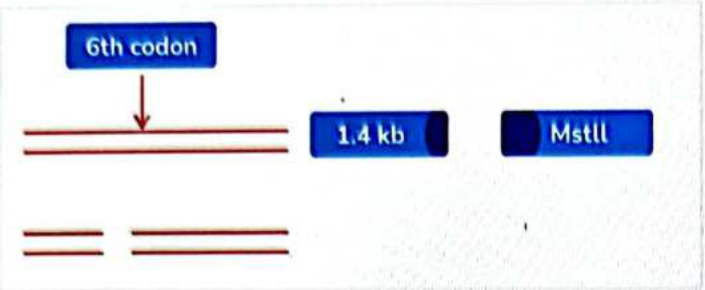
RFLP: Restriction Fragment Length Polymorphism

- Restriction enzyme
- Specific sites
- Cut or uncut

Example

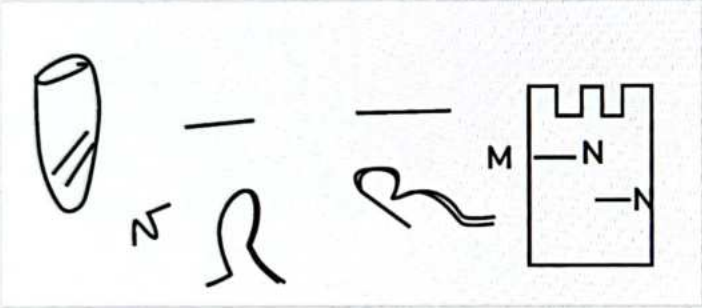
- Suppose you have extracted a beta globulin gene from a person. You have obtained a 1.4 kb fragment of the beta globulin gene fragment.
- This is why you have the 6th codon, and the 6th codon is the endonuclease restriction site by the name MstII.
- This enzyme cuts if this is normal, so if it is normal, 1.4 kb is cut into 0.2 and 1.2; if it is abnormal, it will not be cut.
- In the PCR tube, you have the beta globulin gene fragment. Now you will add the restriction enzyme MstII.

- We will go for electrophoresis. By running it into electrophoresis, we can see if it is cut or uncut because shorter fragments migrate faster, and longer fragments will not be able to move.
- If it is not moving, that means it is not cut, and if it is not cut, it is mutated. And if it moves, it is cut.



Q. Can you use RFLP for molecular diagnosis of any disorder?
Ans. No, only when a non-mutation site causes the disease.

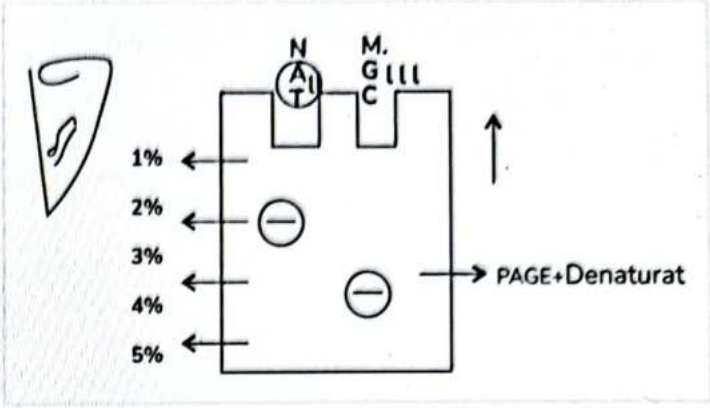
Steps of molecular diagnostics of known mutation site- SSCP (Single-strand conformation polymorphism)



Example

- Suppose we have the PCR tube, and we have the beta globulin gene fragment in it.
- Now we will denature this then we will get two single strands. Once they become two single strands depending upon the sequence present in these two single strands, they will form a happen loop.
- The single strand's secondary structure will differ depending on the sequences.
- This will be confirmation of the normal allele and the mutated allele.
- When we run electrophoresis, their migration would differ depending on the confirmation differences.
- One will move faster, and the other one will move slowly. Depending upon whether it is slow or fast, we will know whether it is normal or mutated.
- **PCR is common for**
  - Sequencing
  - RFLP
  - SSCP
  - DGGE

Steps of molecular diagnostics of known mutation site- DGGE (Denaturing gradient gel electrophoresis)



- Here, we will not denature it within the tube; we will denature it within the gel.
- So, after electrophoresis, we will run the fragment through electrophoresis directly on a gel, but this gel will have an increasing denaturing concentration.
- This is not a usual polyacrylamide gel alone.
- We have added the gel to the denaturant in an increasing concentration.

For example

- In the topmost portion, there will be 2% of denaturant, and as you go down, it becomes 3%,4% and 5% and so on.
- Suppose this is a normal allele, and this is a mutated allele.
- Suppose the normal allele has AT and the mutated allele has GT.
- At will have two hydrogen bonds, and GC will have three hydrogen bonds.
- When we run it through the electrophoretic gel, with an increasing concentration of denaturing gel, then each of them undergoes denaturation.
- The one which has got 80 has got less number of hydrogen bonds, and it undergoes denaturation quickly.
- Even at 3%, it will go denaturation; once it undergoes denaturation, it stops here.
- The one with GC has more hydrogen bonds, with which it will not undergo denaturation; it stops here.
- So based on the migration panel, we will know whether it is normal or abnormal.

RT PCR (Reverse Transcription PCR)

- We take RNA and convert it into DNA. Real-time PCR is always represented by qPCR.

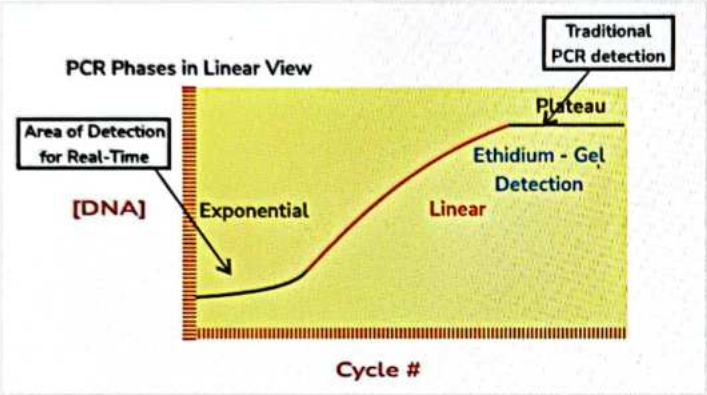
Steps involved in molecular diagnostics of SARS-COV2 infection.

- **Step 1**- Swab sample collection
- **Step 2**- Viral transport medium - It consists of Hanks' balanced salt solution, which has calcium and magnesium

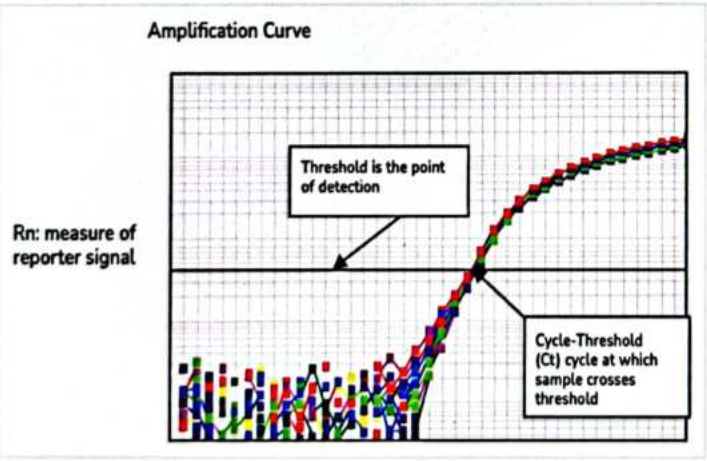
and heat inactivated bovine serum albumin, which will keep the microorganism intact.

- **Step 3-** Add gentamicin and Amphotericin B (which is going to kill bacteria and it will kill the fungus, and only the virus will be left)
- **Step 4-** Cold chain maintenance - we have to keep the virus intact; it should be kept at 2-8 degree celsius to maintain the viability of the virus.
- **Step 5-** RNA extraction
- **Step 6-** RT PCR
- **Step 7-** Real-time PCR (it is not used for amplification, it is used for quantification)
- **Step 8-** Molecular transport medium

Q. Why can't we use the conventional PCR of quantification?
Ans. It is because of the kinetics of PCR.

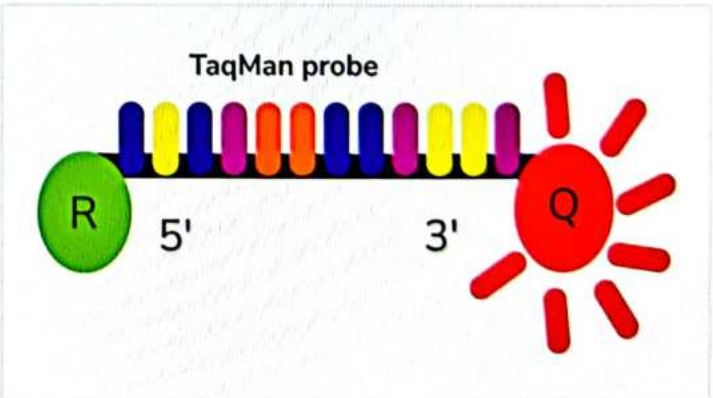


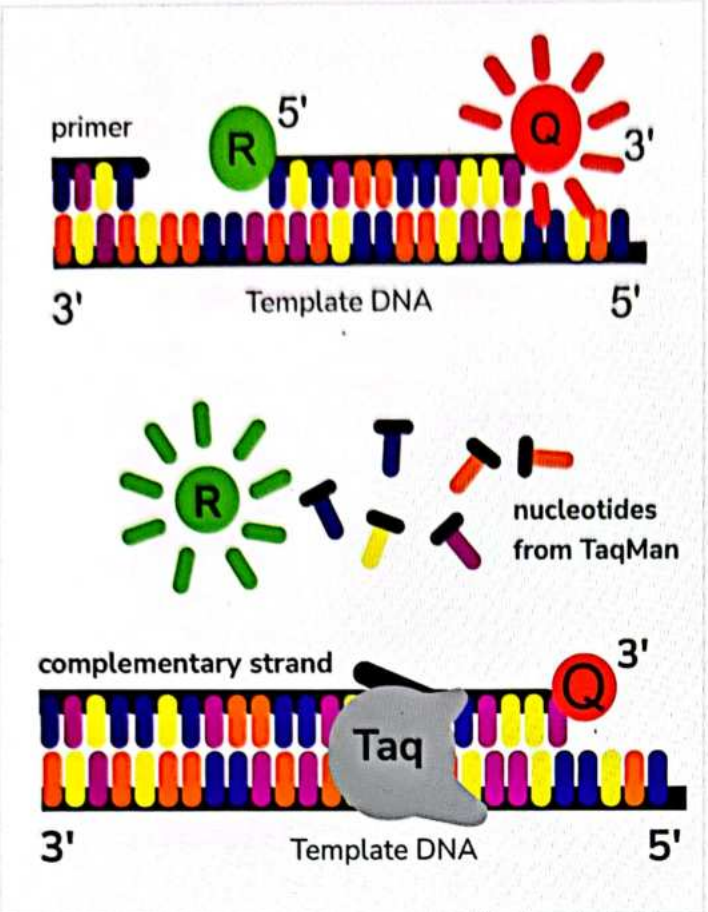
- So, this is a graph, and it's drawn along with the number of cycles on the X-axis and the number of products on the Y-axis.
- Any PCR goes through three phases.
- **Phase 1:** Initial lag phase or an exponential phase
- **Phase 2:** Rapid linear phase
- **Phase 3:** Plateau phase



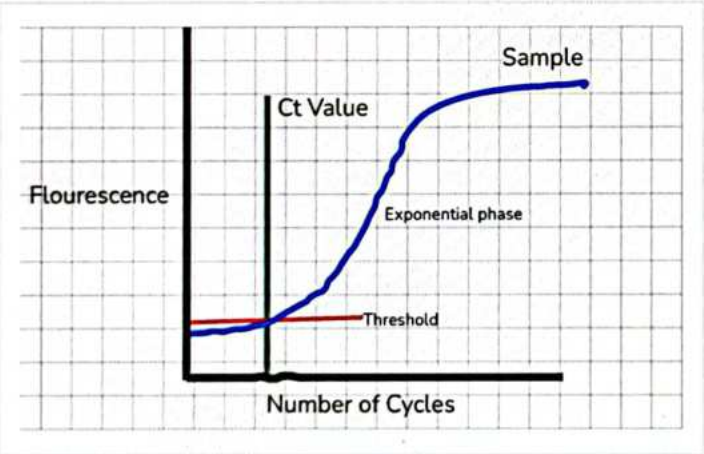
- The plateau phase is not the right time for quantification. The initial lag phase is the right time.
- Real-time PCR is so-called because it quantifies real-time.

- For this quantification, we use SYBR green (it is outdated) as a dye, or we use a TaqMan probe.
  - → Taq man probe will have oligonucleotide sequence, which is attached to two fluorescence dyes: reporter dye and quencher dye.
  - → Oligonucleotide sequence is very specific - this sequence should be complimentary to the mid sequence of the gene of interest.
  - → For example, in SARSCov-2 the oligonucleotide sequence can be S gene, N gene, E gene and RdRp gene.
- Test tube contains taqman probe, RNA from the swab and primers.
  - → Primers will be complementary to flanking sequences of the desired gene.
- Suppose the sample contains the template DNA
  - → Suppose the swab contains RNA of SARSCov2 reverse transcriptase enzyme converts RNA into DNA. That will act as the template DNA.
  - → Now primer attaches to the flanking sequence and probe attaches to the mid sequence of the gene of interest.
  - → Once the primer gets attached the tag DNA polymerase will start elongating the strand.
  - → Tag DNA polymerase have exonuclease activity therefore it remove the oligonucleotides.
  - → Once the oligonucleotides are fragmented now the reporter dye is detached from the quencher dye.
  - → Reporter dye detaches from the Quencher dye and now the reporter dye starts fluorescence.
  - → We measure the fluorescence; the fluorescence intensity is directly proportional to number of reporter dye getting detached from quencher dye that is directly proportional to tag polymerase activity.
  - → The tag polymerase activity is directly proportional to number of template DNA available.





- Now graph is drawn, number of cycles along X axis and fluorescence intensity along Y axis
- We have a threshold fluorescent intensity.



Q. What is CT value?

- The CT value is the minimum number of cycles at which the fluorescence intensity crosses the threshold intensity.
- A high CT value indicates that crossing the threshold intensity takes a long time.
- A higher CT value means less template DNA is available, so a higher CT value means a lower bacterial or viral load.
- There is a cut-off of 18-35 for CT values.
- If the CT value exceeds 35, the sample doesn't have an adequate load.
- The result can be considered negative if the CT value is beyond 35.
- CT= 1/bacterial load or viral load (inversely proportional)

One-Liners and MCQ's

- The instrument used for PCR is **Thermocycler**
- The enzyme used in conventional PCR is Taq **DNA polymerase**
- The enzyme used in LAMP PCR is **Bst or Bsm DNA polymerase**
- Real-time PCR quantifies in the **Exponential or lag phase**

Q. The temperature of the denaturation step in PCR is

A. 60 degree
B. 72 degree
C. 55 degree
D. 95 degree

Q. Annealing temperature in PCR is

A. Variable
B. 72 degrees
C. 55 degrees
D. 95 degrees

Q. All the following are the requirements of PCR except?

A. DNA Polymerase
B. dNTP
C. Restriction enzyme
D. Magnesium

Q. All the following are the requirements of PCR except?

A. DNA Polymerase
B. ddNTP
C. Two primers
D. Magnesium

Sanger's Sequencing

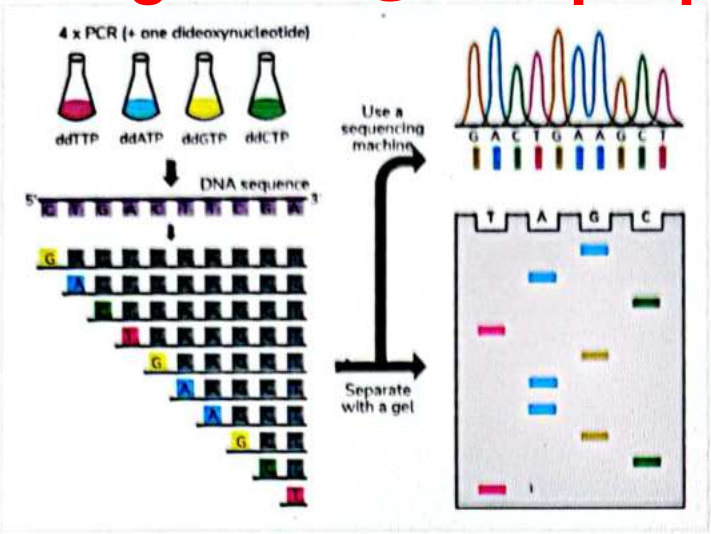
- Sanger sequences protein and DNA first.

Q. What is the method Sanger uses for DNA sequencing?

- It is Sanger's ddNTP(Dideoxynucleotide triphosphates) method or sangers termination method.

Q. What is the principle that is used?

- Whenever ddNTP gets inserted into a chain, the chain terminates them.



- Suppose this purple color structure is the DNA sequence we are trying to identify.
- Subjecting it to PCR. After subjecting it, we get multiple copies of the fragment we are trying to sequence, and we add an equal number of those copies into four test tubes. In this, the tubes differ for which the ddNTP has been added.
  - In the 1st test tube, we have added ddTTP
  - In the 2nd test tube, we have added ddATP
  - In the 3rd test tube, we have added ddGTP
  - In the 4th test tube, we have added ddCTP

Q. What happens when we add ddGTP?

- It starts sequencing and elongating this, corresponding to A; the enzyme will happily attach T, Corresponding to G, and the enzyme will attach C, corresponding to C, it is supposed to attach G. It has confusion about whether to attach dGTP or ddGPT because this test-tube has both.
- Out of the hundred copies of this fragment to 50 copies, it will attach dGTP like always; if it attaches dGTP, the elongation continues.
- To the remaining 50 by chance, it must have added ddGTP, but if it adds ddGTP, the elongation stops there.
  - If it stops, the length of the oligonucleotide will be 3.
  - To the remaining 50, when it has attached ddGTP, the elongation continues to T; it will attach A to T, in the same manner it attaches it to D, corresponding to C.
  - Out of 50, it attaches 25 to dGTP, and the elongation continues; to the remaining 25, it attaches to ddGTP, and the elongation stops. When it stops, the length will be 6
  - In these test- tubes ddGTP, there are three oligonucleotides of lengths-3,6 & 10 that we will see when we run it through electrophoresis.

Q. Which of the following is true about the molecular diagnosis of SARS-COV-2 infection?

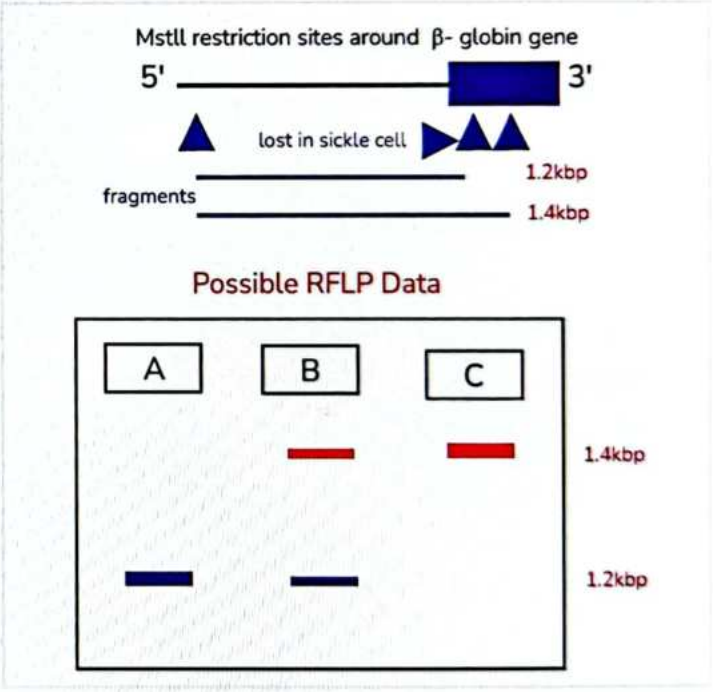
A. A higher CT value implies a higher viral load.
B. Always Involved in the RNA extraction step
C. Necessitates the use of thermocycler.
D. Involves reverse transcription.

Q. A 21-year-old man wants a molecular diagnosis of Sickle cell anaemia, as 3 of his maternal cousins have been diagnosed with sickle cell anaemia. The intern knows a few steps involved in molecular diagnostics in a jumbled way. Help him choose appropriate steps and arrange them in the right sequence.

1. RT PCR
2. Sample collection
3. FISH
4. RFLP
5. Cytogenetics
6. Conventional PCR
7. DNA extraction

A. 2,7,1,3
B. 2,7,6,4
C. 7,2,1,3
D. 2,6,7,3

Q. Three individuals, A, B and C, of a family of sickle cell anaemia want to get their molecular diagnosis done. With the provided RFLP data, choose the genotype of persons A, B and C.



A. Carrier, normal and disease
B. Disease, normal and carrier
C. Normal, disease and carrier
D. Normal, carrier and disease

38 RECOMBINANT DNA TECHNOLOGY

Recombinant dna technology

Cloning

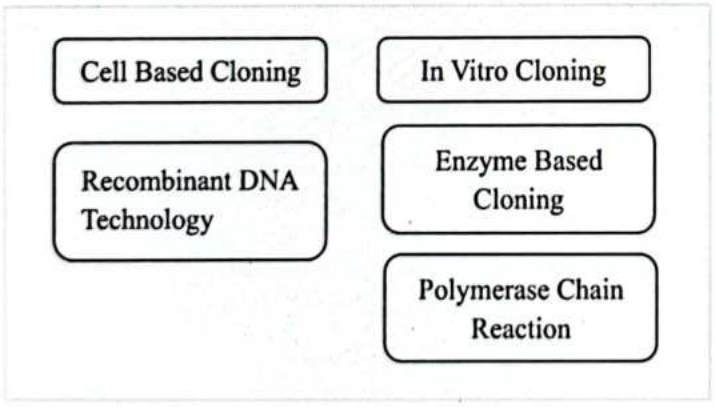
- Production of multiple identical copies of DNA
- Involves genetic engineering.
- Has forensic applications
- Refers to the replication of DNA multiple times.

Requirements of Replication

- DNA polymerase
- Primer
- All 4 deoxy nucleoside diphosphate (dNTPs)
- Magnesium or manganese - acts as catalyst
- Buffer

Types of Cloning

Cell Based Cloning	Enzyme Based Cloning
• Whole cell like E-coli provides all the requirements. • Introduce desired DNA fragments in E-coli. • E-coli provides all the requirements of Cloning. • In vivo cloning. • Includes recombinant DNA technology (rDNA).	• A test tube is taken, and all the requirements are added to it. • Cloning occurs in the test tube. • In vitro cloning. • Includes polymerase chain reaction (PCR).



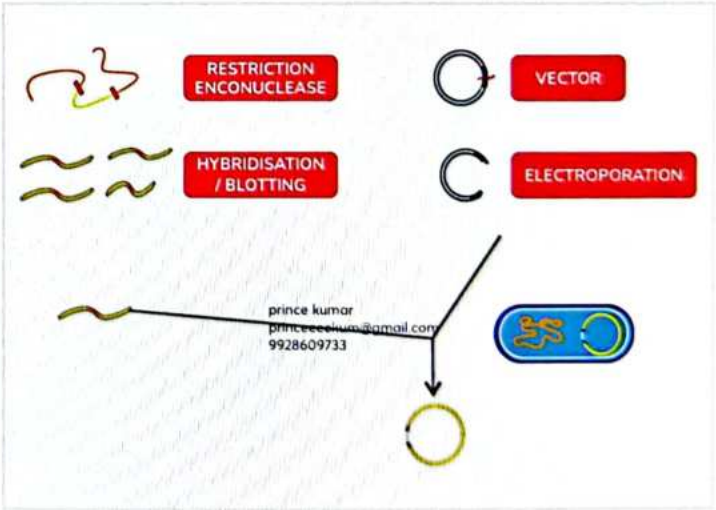
Steps of Recombinant DNA Technology

(Assume Production of insulin on a large scale).

- First requirement is the human chromosome from which the insulin gene is separated.

Take the human chromosome.
↓
Cut it into small fragments using restriction endonucleases.
↓
Hybridization or blotting technique should be employed (to identify the gene of interest)
↓
Get a vector.
↓
Cut the vector with restriction endonucleases.
↓
Attach the gene of interest with vector (important for insertion into E-coli)
↓
Forms recombinant vector (a combination of prokaryotic vector and eukaryotic gene)
↓
Treat E-coli with the technique electroporation (formation of pores due to electric current)
↓
Pores are formed on the cell wall of E. coli.
↓
Introduce the recombinant vector into E-coli through the pores.
↓
When vector replicates, or cell divides multiple copies are formed

Rate Limiting Step: Introduction of the recombinant host into E-coli.



Restriction Endonucleases

- Cut the DNA at specific sites called palindromic sequences.

Important Information

Palindrome: Something which has the same pronunciation when read from both sides. Ex: ROTOR

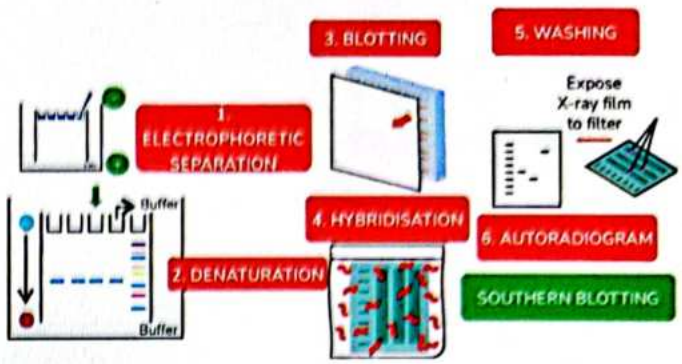
- Palindromic sequence: Double-stranded sequence is the 5' to 3' sequence read in the reverse direction.
- **Ex:** 3'G-G-A-T-C-C 5'
  5'C-C-T-A-G-G 3'
- The sequence from 3' to 5' on the first strand is the same as the sequence on the second strand when read from 5' to 3'.
- **Two types of restriction endonucleases:**
  - Sticky end RE
  - Blunt end RE

Sticky end Restriction Endonucleases	Blunt end Restriction Endonucleases
• Cut the DNA strands at different sites on the two strands.	• Cut both strands at the same site.
Ex: 3' G-G-A-T-C-C 5' - cuts the sequence between G-G. • 5' C-C-T-A-G-G 3' - cuts the sequence between G-G. • Forms two complexes with overhanging ends. • The gene of interest having a sticky end combines with a similar vector having a sticky end.	**Ex:** 3' G-G-A-T-C-C 5' 5' C-C-T-A-G-G 3' • Cuts the strands between A-T • From two complexes with blunt ends. • When genes of interest and vectors are brought together, they don't stick easily. • Difficult to form a recombinant vector.

- Today, mostly sticky end restriction endonucleases are used.
- The only blunt end restriction endonuclease used is Hpal.

Hybridization or Blotting Technique

- **Steps involved:**
  - Electrophoretic separation
  - Denaturation of separated fragments
  - Blotting
  - Hybridization
  - Washing
  - Autoradiogram



Electrophoresis

Electrophoretic tank contains gel, and Wells is taken.

↓

All DNA fragments are placed in Wells.

↓

Top side is negatively charged, and the bottom is positively charged.

↓

Negatively charged DNA fragments move towards positive electrode.

↓

Short fragments move faster than the long fragments.

Denaturation

The DNA fragments are then denatured.

↓

The DNA fragments are double stranded.

↓

To make them available to the probe they are covered to single-stranded by denaturation.

↓

Blotting

Gel is blotted on a nitrocellulose paper.

↓

Hybridization

The blotting paper is then dipped into radiolabelled probe taken in a petri dish.

↓

The radiolabelled probe attaches with the complementary sequences.

↓

Hybridization takes place.

↓

Washing

Take away the hybridised blotting paper.

↓

Wash out the excess unbound probe.

↓

Autoradiogram

Expose the hybridised blotting paper to X-ray.

↓

The gene of interest with probe creates a band.

↓

Fragments with a gene of interest is identified.

Southern Blotting	Northern Blotting	Western Blotting
Used to identify an unknown DNA fragment.	Used to identify an unknown RNA sample.	Used to identify unknown proteins.
• Denaturation is required to convert dsDNA to ssDNA. • Labelled DNA is used as a probe.	• RNA is single stranded. Mild denaturation is required to remove the hairpin loops. • Labelled DNA is used as a probe.	• Antibody probe is used. • Confirmatory test for HIV.

Western Blot

Take the HIV antigens.

↓

Separated by electrophoresis.

↓

Collect the patient's serum which may or may not have antibodies.

↓

Add the serum to separated HIV antigens.

↓

If antibodies are present in the patient's serum, they hybridise with the antigen.

↓

Wash the unbound proteins.

↓

Add a labelled probe to Ag-Ab complex which is directed against the human antibody.

↓

If the labelled probe gives a signal, it indicates the presence of antibody in the patient's serum

Qualities of Vector

- Should be capable of self-replication.
- Should have one or more origins of replication.
- Should have a particular or desired restriction site.
- Should have one or more marker genes to identify E-coli colonies with recombinant vectors.
- If natural vectors are used like plasmids or phage DNA, choose them if they have:
  - Ampicillin resistance gene
  - Tetracycline resistance gene
- Most commonly used marker genes are BAC or YAC.
- Artificial marker genes used are Beta galactosidase.

Choices of Vector

Vector	Insert Size
Plasmid	6 to 10 KB
Phage DNA	10 to 20 KB
Cosmids	20 to 30 KB
BAC	>50 KB
YAC	>500 KB

- Plasmids are small, circular and extrachromosomal DNA present in the Prokaryotes.
  - The advantage of using plasmids is that, after forming a recombinant vector introducing it to the E. coli will not be difficult and Electroporation step will not be required.
  - The disadvantage is, plasmid cannot be used as a vector if the length of the genome to be altered is more than 10KB.
- The bacteriophage is a virus that can infect a bacteria.
  - The advantage of using a phage DNA as vector is that, it has got an inherent ability to inject its gene into E. coli and doesn't require electroporation.

One Liners

1. Unknown DNA fragment is identified by **Southern Blot**
2. Unknown protein is identified by **Western blot**
3. The probe used in the western blot is **Antibody**
4. The most commonly used plasmid vector is **pBR322**
5. The most commonly used marker gene in the artificial vector is **Beta Galactosidase**

MCQs

Q. Which of the following is a cell-based cloning technique?

A. Polymerase Chain Reaction

B. Restriction Fragment Length Polymorphism

C. Microarray

D. Recombinant DNA technology

Q. All the following are the tools required for recombinant DNA technology except.

A. Restriction endonucleases

B. Blotting

C. Thermocycler

D. Vector

Q. Which is true about recombinant DNA technology?
A. It requires Taq DNA polymerase.
B. It is an in vitro cloning process.
C. It requires a vector.
D. The equipment required is a thermocycler.

Q. Which is not true about sticky end-producing restriction endonucleases?
A. They cut both strands
B. They cut at palindromic sequences
C. HpaI is an example
D. It favours the formation of recombinant vector

Q. The rate-limiting step of recombinant DNA technology is
A. Formation of recombinant vector
B. Introduction of the recombinant vector into a cell
C. Identification of the fragment with a gene of interest
D. Cloning of recombinant vector

Q. Which of the following is not true about Northern Blot? -
A. cDNA is the probe
B. Does not involve denaturation step
C. Separates fragments based on length
D. The probe can be labelled with a radioactive substance

Q. The most commonly used plasmid vector is:
A. pBR322
B. pUC19
C. pCEV
D. pUC18

Q. To produce Insulin on a large scale, what is introduced into E.Coli?
A. Insulin gene
B. Insulin mRNA
C. Insulin cDNA
D. Insulin tRNA

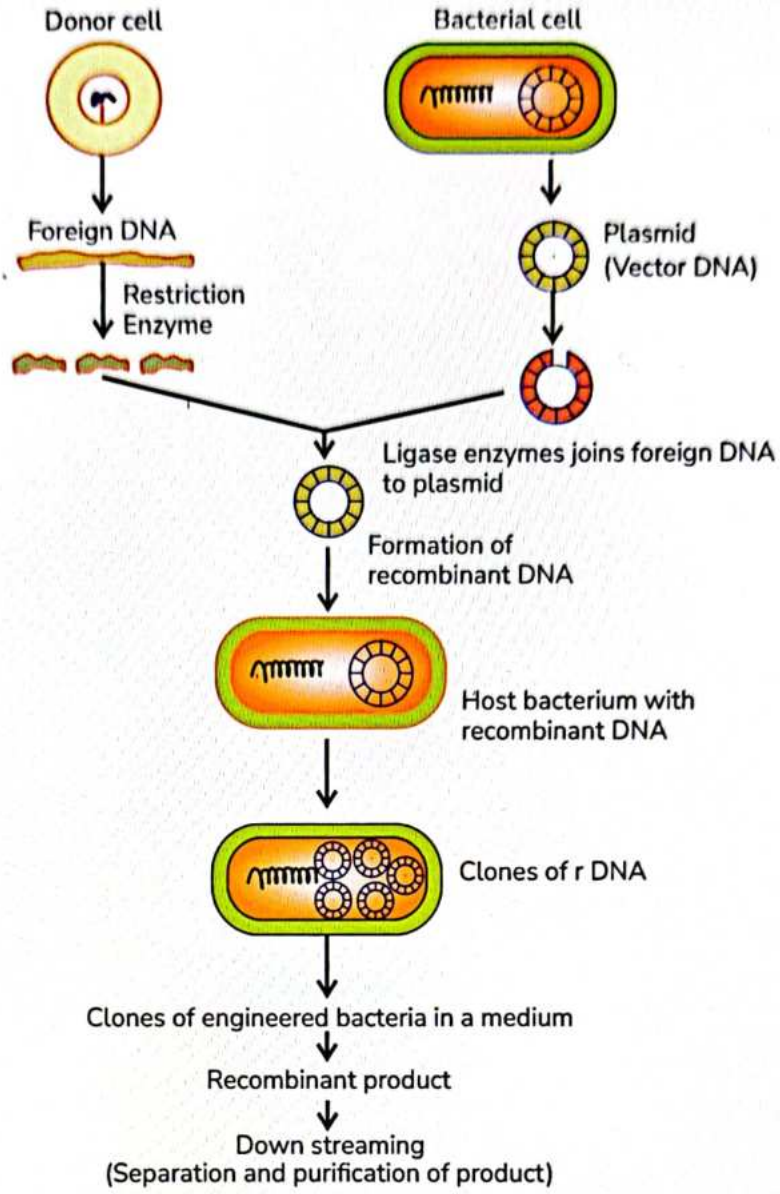
Explanation - Significance of cDNA

- Human genes can be cloned but can't be expressed in E.coli.
- Human genes have non coding intervening sequences called introns.
- When human genes are introduced in to E.coli, they can be cloned but cannot be expressed.
- The reason is the prokaryotes do not have introns and in turn do not have splicing machinery.
- When genes are getting expressed by the E.coli, it will be able to produce something analogous to the primary transcript.
- Since prokaryotes do not have splicing machinery, E.coli will not a be able to synthesize something analogous to the functional mRNA.
- So, insulin protein cannot be synthesized.
- To overcome this, we have to use in vivo splicing machinery.
- Here, in vivo pancreatic beta cells culture is maintained.
- Beta cells have an inherent ability to splice out the insulin genes and produce functional mRNAs of insulin.
- So, spliced mRNAs of insulin is extracted from the pancreatic beta cells and reverse transcriptase is used.
- Here, mRNA is used as a template and synthesize a single strand of DNA.
- Using this single stranded DNA, another strand of DNA is synthesized.
- This double stranded DNA is called cDNA (It is a double stranded DNA complimentary to a fully spliced mRNA).
- This cDNA does not have introns and it is introduced into the E.coli.

Case Based MCQs

Q. A 25-year-old man was scheduled for an emergency appendectomy. As a part of the preoperative screening, HIV testing was done, and his serum was found to be repeatedly reactive in enzyme Immunoassay. To confirm the HIV diagnosis, a method in which individual proteins of an HIV-1 lysate were separated according to size by polyacrylamide gel electrophoresis. The viral proteins were then transferred onto nitrocellulose paper and reacted with the patient's serum. An antihuman immunoglobulin G (IgG) antibody conjugated with an enzyme was used as a label. Name the method used and mention the analyte that is detected by this method.
A. Southern Blot, HIV DNA
B. Northern Blot, HIV RNA
C. Western Blot, HIV Antibody
D. Southern Blot, HIV RNA

Q. A and B in the image, respectively are



A. Recombinant Vector and Vector
B. Vector and Recombinant Vector
C. Restriction enzyme and Vector
D. Vector and Restriction enzyme

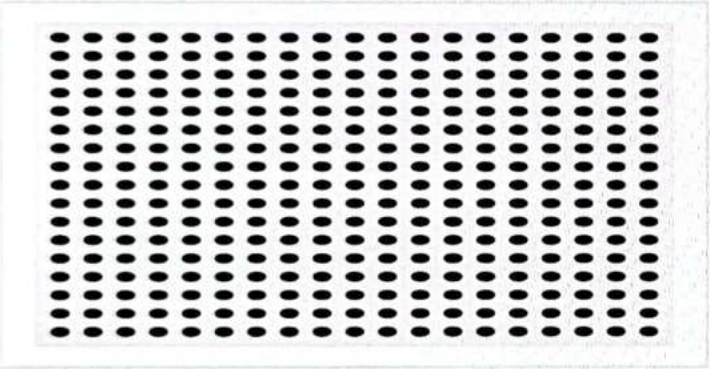
39 MICROARRAY, CRISPR, LAC OPERON

Comparative Genomic Hybridization 00:01:20

- A cytogenetic version of microarray.
- Cytogenetics is at the chromosome level.
- Chromosomal aberration level - there are genes where you have mutations.
- Molecular diagnostic is a mutation at the micro level.

Mirco Array 00:02:10

- Chip technology
- Not diagnostic but pathogenesis of a disorder.
- Differences in the level of gene expression.
- Example: breast cancer patient
- Remove cancer tissue
- Extract mRNAs from that tissue
- mRNA converts into cDNA through reverse transcriptase
- Then label cDNA with a fluorescent dye
- References cDNA from normal tissue - from a cDNA library
- Label it with a different fluorescent dye
- Both the cDNA that is differently labelled are mixed and laid out in a microarray chip



- Multiple spots
- There is an oligonucleotide that is complementary to the mRNA of interest in that particular disease
- It is a particular chip of a particular disease
- Oncore type 21 SA-Chip will have 21 spots-5 are housekeeping genes and 16 oligonucleotides complementary to mRNA of breast cancer
- Spread both cDNAs on the microchip, cancer tissue cDNA, and Normal cDNA.
- They will compete with each other to bind to oligonucleotides.
- If there is an equal expression of a particular gene, you can have personal preferences of labelling.
- Extract from cancer tissue is labelled in red fluorescent dye, and extract from normal tissue is labelled in green fluorescent dye.
- Spread on the microarray chip.
- If a particular mRNA is equally expressed in both tissues, the dyes mix and form a yellow color.
- Read it with a fluorescence reader.
- Red and green are mixed equally, which means it gives. Yellow color
- Red spots - over-expressed in disease tissue.
- Green spots- no representation in cancer tissue means. Under expressed.
- Based on this score will be given
  - Bad score - chemotherapy is suggestive
  - Good score- no need for chemotherapy
- Not used for diagnosis but decision making

Comparative Genomic Hybridization 00:07:52

- Cytogenetic version of microarray.
- Chromosomal aberration like microdeletion or amplification.
- A child with Syndromic manifestation with different syndrome.
- Chromosome painting - at the end; paint it with red, green and yellow
-

Steps involved are

- Two children of a family are suffering from neurodevelopmental delay.
- Cause unknown.
- Extract DNA - all 46 chromosomes.
- These are broken into fragments known as restriction digestion.
- Small fragments are denatured.
- And label it with a fluorescent dye (red).
- Labelled with red dye - DNA from neurodevelopmental delay.
- Normal chromosomal fragments library-these will also be denatured and that will be labelled with fluorescent dye (green).
- Both the single strands are mixed and spread it on microarray chip.
- If some spots are green dye - normal with no representation of that segment from the diseased person.
- It means that the chromosome has undergone deletion on the diseased person.
- It means microdeletion corresponding to that spot.
- CGH chip
- Yellow colour means that means normal.
- Green colour means micro deletion.
- Red colour on chip means micro amplification.

Mental Algorithm for Chromosomal Aberration

Chromosomal aberration can be:

- Micro deletion
- Amplification
- Copy number variations
- Structural Alterations - BCR and ABL translocation

Example: A family has two children. One with angel man syndrome. There is a microdeletion in the first kid. Find out if the second sibling has the same disorder.

Known	Unknown
FISH - fluorescent in-situ hybridization	Can not use FISH CGH will be used
Mutation is already known and specific	Mutation is unknown - shoot in dark

If you are looking for BCR and ABL translocation

- BCR is probed with fluorescent green dye.
- ABL is probed with fluorescent red dye.
- Fusion fragments - detrimental for a person.

Copy number variations	Structural alteration		
Known	Unknown	Known	Unknown
FISH	CGH	FISH	Spectral Karyotyping

Q. The technique used to identify unknown chromosomal deletions or duplication is?

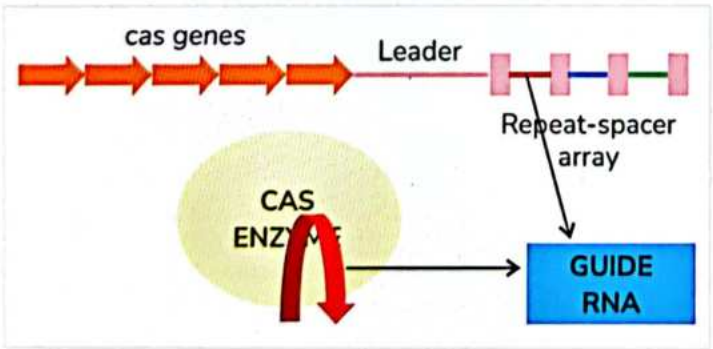
A. FISH
B. Microarray
C. Comparative Genomic Hybridization
D. Recombinant DNA technology

CRISPR - CAS System

00:17:45

- Helps in gene editing.
- CAS enzyme system:
  - Cas enzyme-Endonuclease: Makes a nick at a specific site, complementary to the guide RNA
  - Guide RNA-Complementary to the space sequences found in CRISPR
- Clustered Regularly Interspersed Short Palindromic Repeats - CRISPR

Structure of Crispr Sequence



- Linear array of multiple cas genes.
- On translation and transcription will give rise to cas enzyme.
- Results in leader sequence.
- Following the leader then having 50-100 palindromic repeats.
- Between palindromic is spacer sequence.
- Spacer sequence will transform guide RNA.
- Present in Bacteria and archaea from bacteriophages.
- Immunological memory

CRISPR Sequences

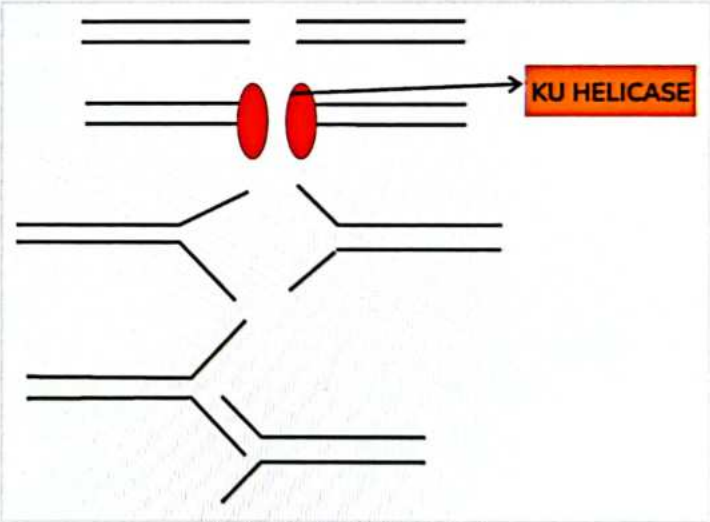
- Discovered by a Japanese scientist Yoshizumi Ishino in 1987
- Effectively repurposed for gene editing by Emmanuelle Charpentier in 2020 by engineering cas 9 to use a single guide RNA instead of crRNA and TranscrRNA
-

Gene Knockout and Gene Knock In

- Gene knock out/down - the defective gene is removed – sequence remains same and down regulate the gene expression and interfere with gene level at translation level
  - Nothing to do with CRISPR cas enzyme
- Gene knock in - the defective gene is replaced with a normal gene – better one

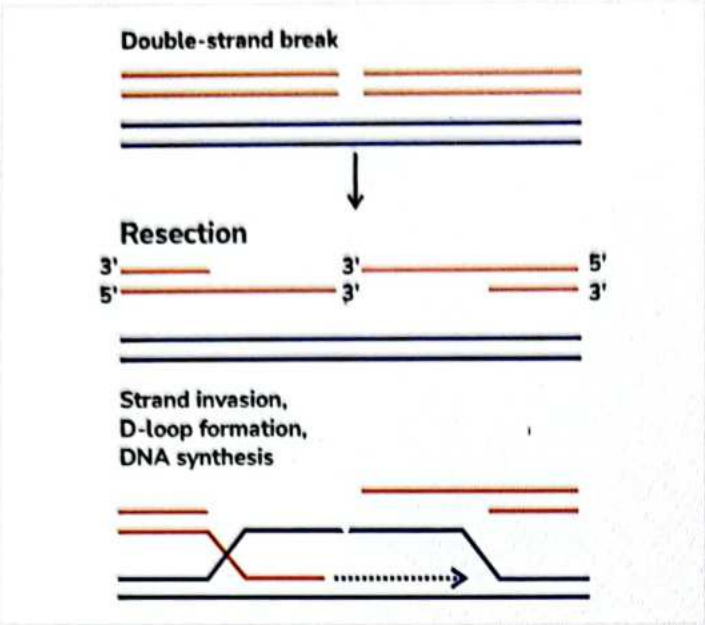
What does CRISPR CAS system do?

- Double-stranded DNA break
- Following, in DNA repair mechanism by two mechanisms:
  - NHEJ (nonhomologous end joining) - most common repair mechanism - Ku helicase
  - HDR (homologous DNA repair).

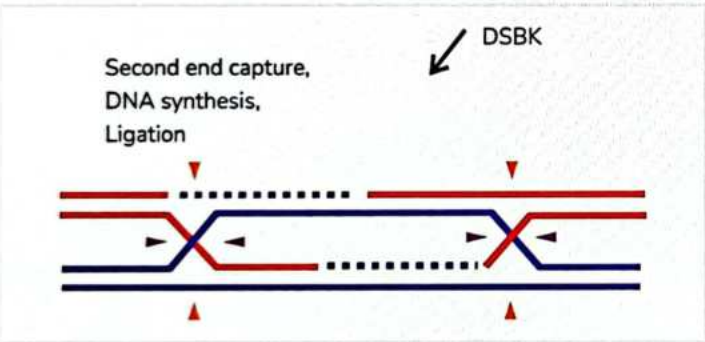


- Each of the units have gotten attached to both the ends
- Unwinding of both the ends
- Approximation of both the ends
- This will continue until they find base pairing
- Strands will be removed
- Ligase unites the ends - a part is removed of gene sequence
- That is removal of defective gene - gene knockout
- NHEJ results in gene knockout

Double stranded DNA



- If body is lucky enough, it will get to see homologous DNA sequence
- If you have chromosome 13 (1) copy, other copy will help in DNA repair
- Polymerase removed a pat of the top strand - exonuclease activity
- Overhanging strand will have a invasion ability
- Template dictation will be done by the overhanging strands - it will result in gene knock in



Q. Which is true about CRISPR cas system?
A. Bacteriophages exhibit extensive CRISPR cas system
B. The repeat sequence code for the guide RNA
C. Emmanuel Charpentier received a nobel prize to identify the first CRISPR system
D. PAM sequence helps in spacer acquisition

Q. Following CRISPR mediated gene nicks, which of the following can result in gene knock in?
A. Non homologous end joining
B. Homologous DNA repair
C. Interference
D. Ku helicase mediated repair

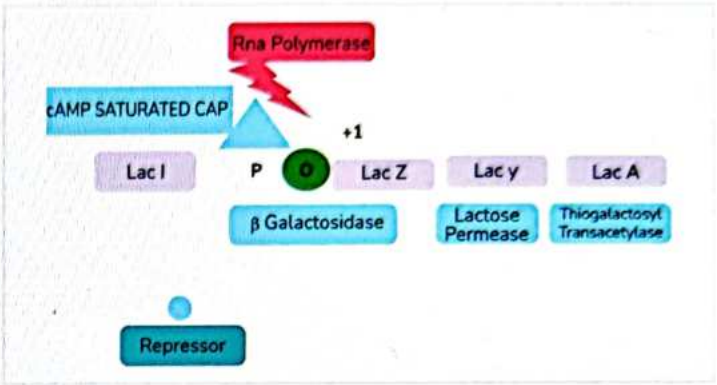
LAC Operon

00:32:54

- Positive regulator of lac operon: Cyclic AMP saturated CAP
- LAC-A codes for Thiogalactyl transacetylase

Introduction

- Not present in human genome
- It is found in E.coli
- Related to lactose utilisation E.coli
- Needs three enzymes:
  - Beta galactosidase
  - Lactose permease
  - Thiogalactoside transacetylase

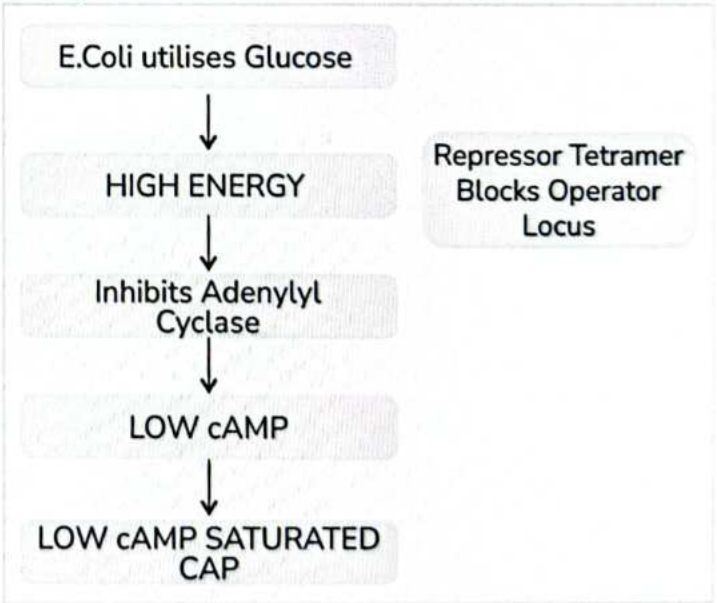


- These three genes share a common promoter and operator.
- **OPERON:** a linear array of genes involved in a metabolic pathway with a common promoter and common operator.
- Not regulated effectively.

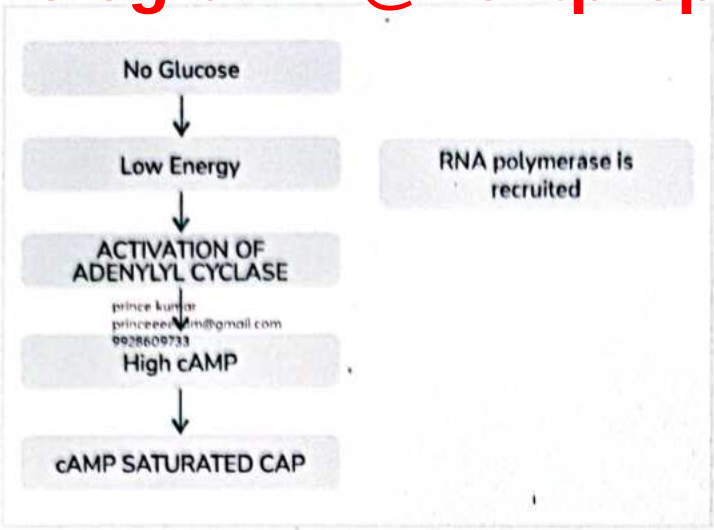
Preferred fuel for E. coli

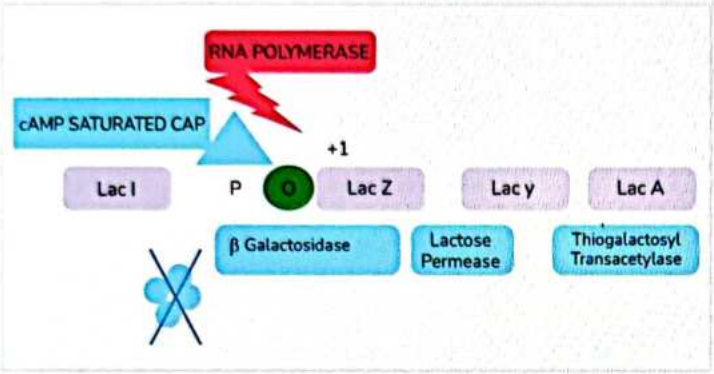
- Glucose
- Lactose

In the presence of Glucose



In the presence of Glucose & Lactose
In the absence of Glucose – Providing Lactose





One Liners

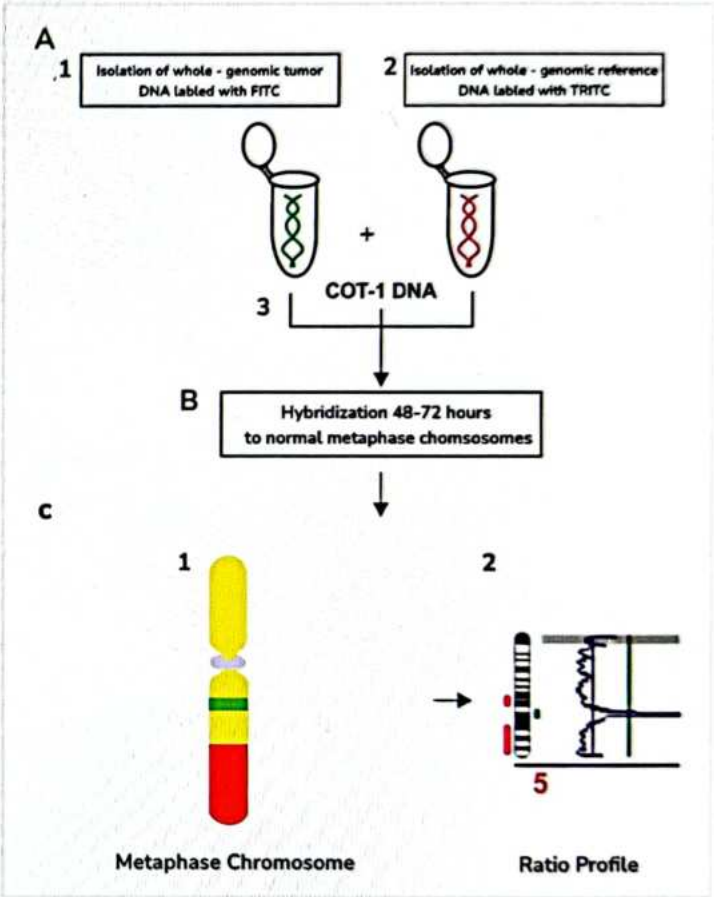
- Unknown DNA fragment is identified by Southern blot.
- Unknown protein is identified by Western blot.
- The probe used in western blot is antibody.
- The positive regulator of lac operon is cyclic AMP saturated CAP.
- Non homologous end joining causes gene knock out.
- Homologous DNA repair causes gene knock in.

Q. LAC A codes for?
A. Beta galactosidase
B. Lactose permease
C. Thiogalactose transacetylase
D. Isopropyl thiogalactosyl pyranoside

Q. The positive regulator of LAC operon is?
A. CAP
B. cAMP saturated CAP
C. Lac I product
D. Lac A product

Q. A child presented with unexplained mental retardation. He also showed two other congenital anomalies - microcephaly and cleft palate. There were no cardiac or other systemic defects.
A microdeletion syndrome was suspected. Which of the following investigations can be used?
A. Conventional cytogenetic analysis
B. PCR
C. Recombinant DNA technology
D. CGH

Q. The metaphase chromosome in comparative genomic hybridisation painted green?
A. Balance DNA content
B. Amplification in tumour DNA
C. Under expression in tumour DNA
D. Over representation of the whole chromosome in tumour DNA

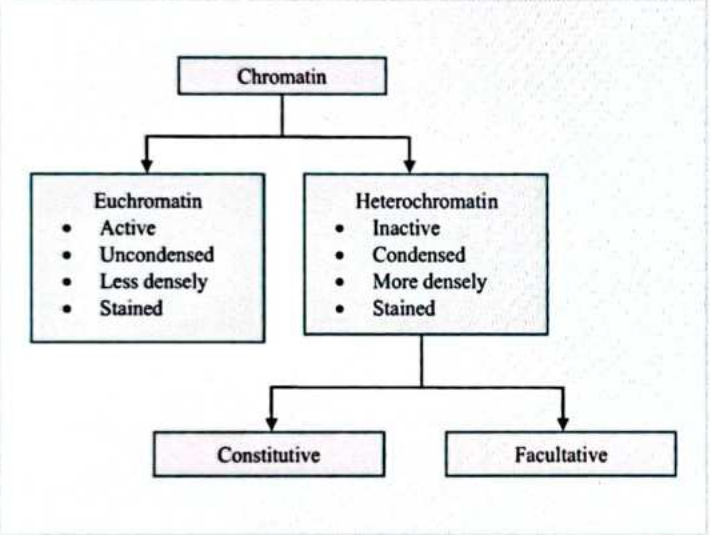


31 CHROMOSOME AND CELL CYCLE

Chromosomes 00:01:01

- Chromosomes: one long double-stranded DNA which condensed with help of histones - positively charged.
- Histone acetylation: histone will loose positive charges and loose interaction with DNA leads to DNA uncondensed.
  - When chromosome need not perform replication and transcription, to enable the function DNA will get condensed.
  - For both replication and transcription, unbinding has to be done first.
  - For unbinding to be mediated, prior to that DNA has to be in uncondensation stage.
  - Retinoblastoma protein is called tumor suppressor protein this is because Rb histone deacetylase retains the condensed form of chromosome.

Types of Chromatin 00:04:12



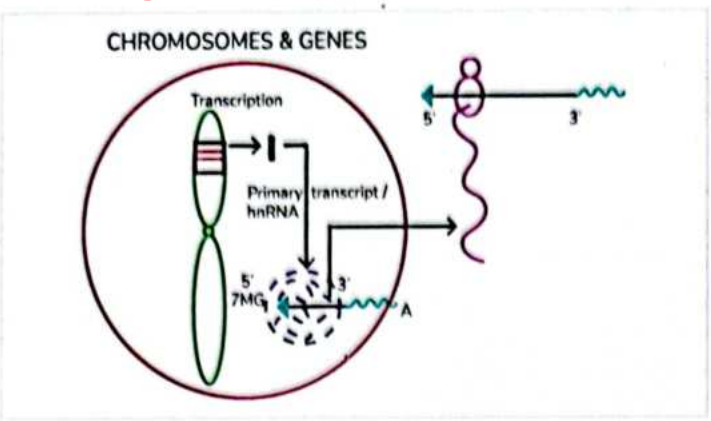
- Constitutive heterochromatin will found in centromeric region and in telomeric ends
- In cell cycle, M phase - mitiotic spindle is formed and gets attached to the centromere and in anophase the mitotic spindle contracts and centromere will breaks longitudinally.
  - Centromere will subjected to repeated breakage and has the tendency for one daughter chromosome to gain some sequences and also have tendency for other daughter chromosome to miss some sequences
    → Following every cell division, when we get two daughter cells, one cell will undergo duplication and other cell will undergo deletion of gene sequences.
    → Centromere: transcriptionally inactive
  - Constitutive heterochromatin in telomeric ends: Following every cell division there is telomeric end shortening, if coding present in ends there will be deletion or duplication. To avoid this, constitutive heterochromatin is present in telomeric ends.
- Facultative heterochromatin: eg: female 'X' chromosomes.

Epigenetics 00:09:35

- Inheritable stable changes in the DNA not caused by sequence alterations
- Altered chromatin remodelling
- Mechanism of chromatin remodelling
  - Histone deacetylation
  - Methylation of cytidine residues of CG islands
    → Both these occur to result in a part of chromosome to become Heterochromatin.

Chromosomes and Genes 00:12:13

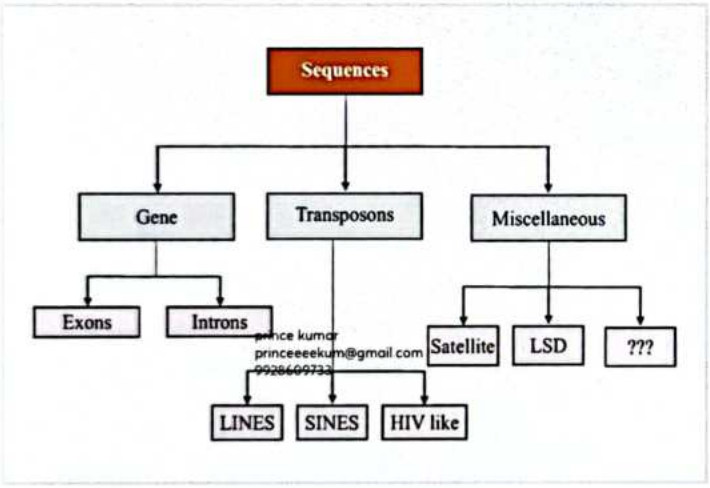
- Chromosomes: Long double-stranded DNA which condensed with help of histones
- Gene: Every gene is a part of a chromosome which code for either protein or for RNA.
  - In gene, there is also a non-coding intervening sequences called interons.
  - During transcription, look for template strand sequences and attach complimentary sequences. First product formed will have both coding and non-coding sequences called as Primary transcript.
  - Primary transcript: first transcript noted based on the gene sequences.
  - Primary transcript also known as Heteronuclear RNA (transcription happens within the nucleus). This undergoes atleast 3 post-transcriptional modifications in nucleolus.
  - 3 Post-transcriptional modifications:
    → 7-methyl guanosine cap added to the 5' end
    → Poly A tail added to 3'end
    → Splicing
  - After Post-transcriptional modifications, these mRNA will come out of nucleus and reach cytoplasm. After reaching cytoplasm, this is called as Functional cytoplasmic mRNA.
  - In Functional cytoplasmic mRNA, ribosomes will read mRNA from 5' end to 3'end and polypeptide chain will start growing is known as Translation.



One liners

- Number of basepairs per human haploid genome = 3.3×10^9
- Number of genes identified so far: 20000 to 25000
- Average size of a gene: 27000 bp
- Average size of a functional mRNA: 2000 nucleotides
- Largest gene: RBFOX1 (RNA binding protein - fox1-homolog1)
- The gene with maximum number of exons and largest exon: Titin
- Genes without introns: Histone and cytochrome b

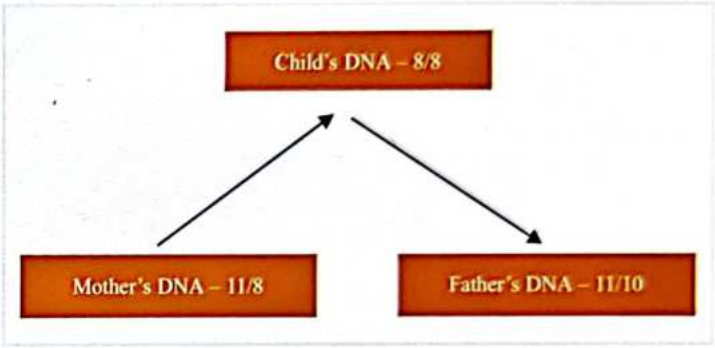
Types of Human Genomic Sequences 00:20:46



- Class I - 30% - Gene Sequences
  - 1.5% are coding exons
  - 28.5% are non coding intervening sequences or introns
- Class II - 45% - Transposons or Jumping Genes or Moderately Repetitive Sequences (repeated between 10^3 to 10^6
  - LINES: Long interspersed sequences (6 to 8 kb in length)
  - SINES: Short interspersed sequences (100 to 200 kb in length)
    - → Majority of SINES comes under Alu family
    - → Alu is a restriction endonuclei that is extracted from an organism by name Anthrobacter luteus
    - → Mutation of one of the restriction site is one of the causes of Neurofibromatosis.
- Class III - 25%- Miscellaneous Sequences
  - 3% is called as Satellite DNA.
  - Satellite sequences is also called Highly repititive sequences (HRS) or Simple segment repeats (SSR)
  - HRS is named because it repeats >10^6 times
  - Based on length, satellite DNA is divided into Microsatellite repeats and minisatellite repeats
    - → Minisatellite repeats is otherwise known as Variable number tandem repeats (VNTR)

Applications of Satellite DNA 00:28:17

- Forensic application
- To clear off paternal dispute cases
  - Steps involved in paternal dispute cases
    - → Blood sample from mother, child and the suspected father
    - → DNA is extracted
    - → Atleast analyse 5MSR in 5 loci each



    - → Not the biological father
- Microsatellite instability disorders
  - CAG: Huntington's chorea and spinocerebellar ataxia
  - CTG repeat: Myotonic dystrophy
  - CGG: Fragile X syndrome
  - GAA: Friedrich's ataxia
    - → All these are characterised by Anticipation
    - → Magnitude of disease ∝ No. of repeats (number of repeats increasing following every meiosis)

MCQS

Q. Barr body is an example of
A. Euchromatin
B. Constitutive heterochromatin
C. Facultative heterochromatin
D. Hypersensitive heterochromatin

Q. DNA basepairs per haploid genome in human
A. 2.3×10^9 base pairs
B. 3.3×10^9 base pairs
C. 2.3×10^{10} base pairs
D. 1.3×10^6 base pairs

Q. The largest gene in human genome is
A. Dystrophin

B. Titin
C. RBFOX1
D. Ferritin

Q. The largest protein is
A. Dystrophin
B. Titin
C. Transferrin
D. Ferritin

Q. The gene with maximum number of exons is
A. Dystrophin
B. Titin
C. Transferrin
D. Ferritin

Q. The gene with the largest exons is
A. Dystrophin
B. Titin
C. Transferrin
D. Ferritin

Q. Alu family belongs to
A. Unique non repetitive sequence
B. Moderately repetitive LINE sequence
C. Moderately repetitive SINE sequence
D. Highly repetitive sequence

Cell Cycle

00:36:49

Refer Image 31.1

- G0 phase: Complete resting phase
- G1 phase: Proofreading and repair
- S phase: Replication
- G2 phase: Again proofreading and repair
  - G1-S-G2 phase: Interphase (interplaced between complete resting phase and active cell division phase)
  - Condensed form of chromosome is done with Histone deacetylase(HDA)
  - When cell decides to get in cell cycle, cyclin level raises: → Cyclin D activates CDK-4

One liners

- The most common form of DNA is B type
- Number of basepairs per full turn in Z type of DNA is 12
- Replication occurs in S phase of cell cycle
- Condensed form of chromosome is seen in G0 and M phases of cell cycle
- Myotonic dystrophy is associated with CTG instability

MCQS

Q. Replication occurs in
A. G0 phase
B. G1 phase
C. S phase
D. M phase

Q. The phase in which chromosome are uncondensed is
A. G0 phase
B. G1 phase
C. Metaphase
D. M phase

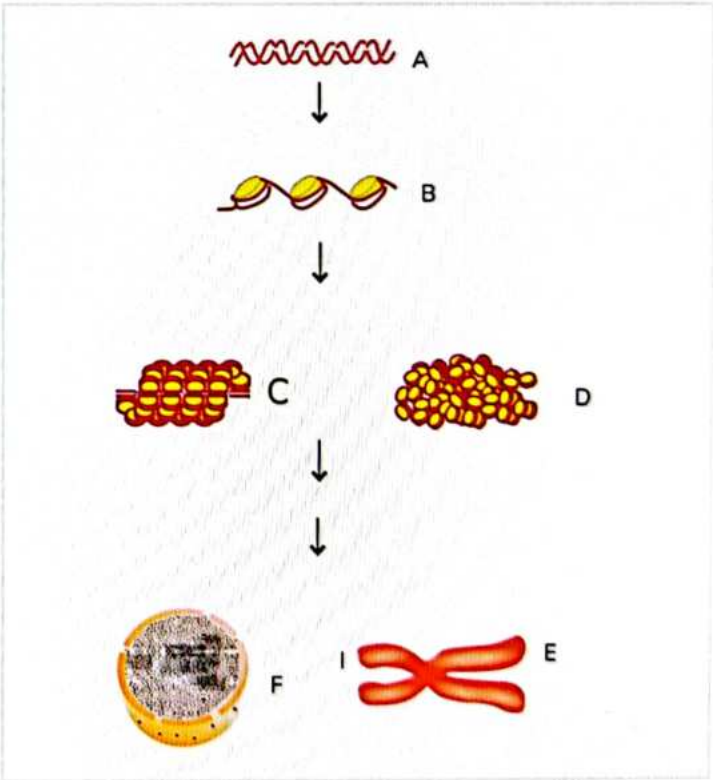
Q. A paternal dispute case was filed by a mother. The forensic centre checked 5 VNTR systems given below. Which of the following repeats should be observed in the putative father's DNA for him to be considered as the biological father of the child?

Individual	VNTR1	VNTR2	VNTR3	VNTR4	VNTR5
Child	9/10	8/11	10/12	9/8	6/7
Mother	7/9	8/9	11/10	9/11	6/9

A. 7/8, 9/11, 11/12, 8/10, 6/8
B. 7/8, 9/11, 10/12, 8/10, 6/8
C. 10/11, 11/10, 12/11, 8/7, 7/9
D. 10/11, 11/10, 11/10, 11/9, 9/10

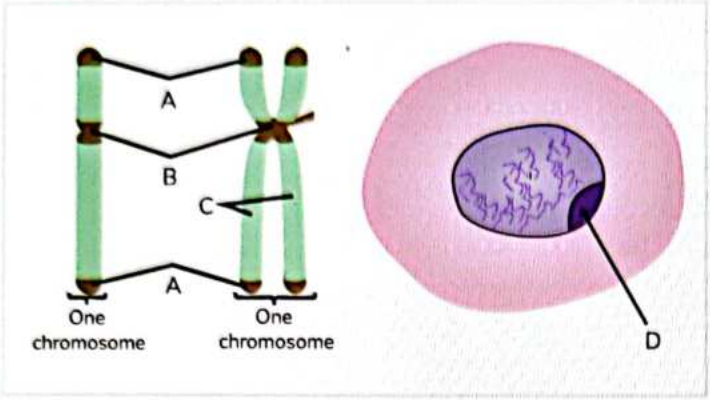
Image Based MCQ's

Q. Identify the structure B



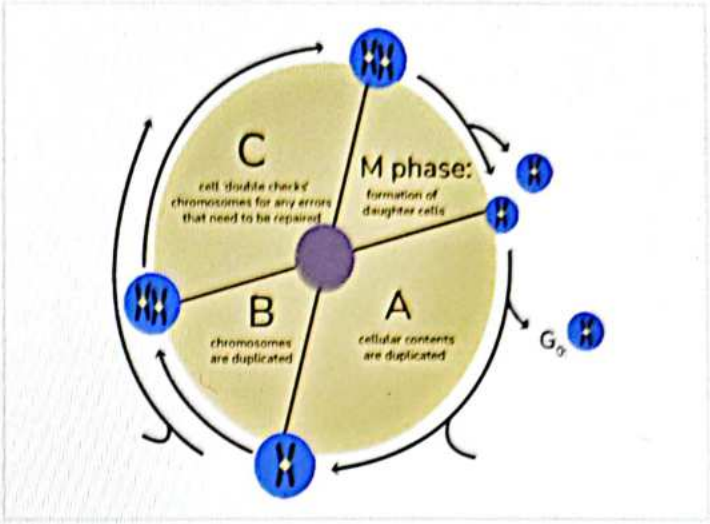
A. dsDNA
B. Nucleosome
C. 10 nm fibril
D. 30 nm fibril

Q. Based on the image provided, which of the following is not a heterochromatin



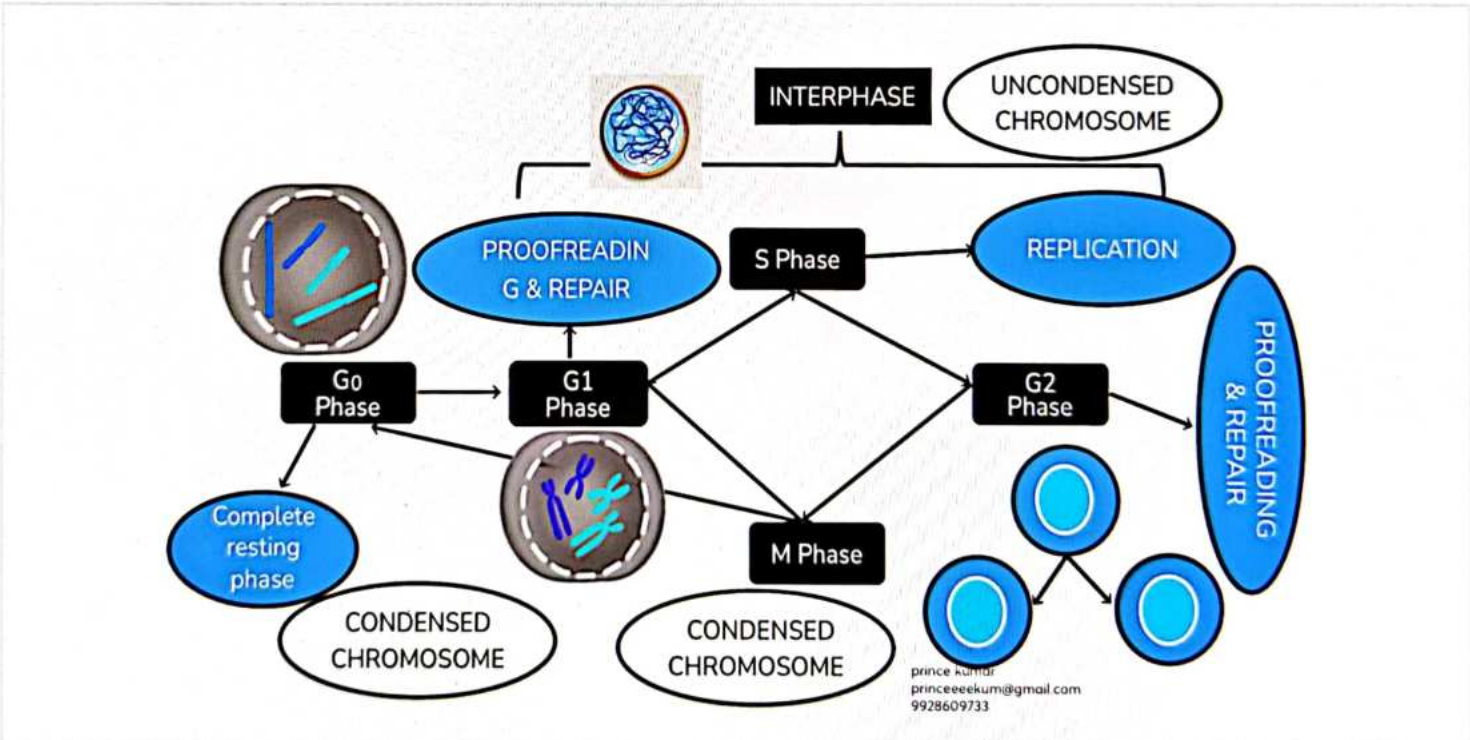
A. A
B. B
C. C
D. D

Q. Which of the following is a true statement?



A. Replication happens in 'B' phase. In B phase, the chromosome is highly condensed
B. Replication happens in 'C' phase. In C phase, the chromosome is highly condensed
C. Replication happens in 'B' phase. In B phase, the chromosomes in uncondensed
D. Replication happens in 'C' phase. In C phase, the chromosome in uncondensed.

Image 31.1



40 ENZYMES

Objectives

- What are enzymes?
- Types of enzymes
- Difference between prosthetic group, coenzyme and cofactors
- Enzyme kinetics
- Enzyme Inhibition

Q. All of the following are proteins except:

A. Aminoacyl tRNA synthetase

B. Peptidyl transferase

C. RNA polymerase

D. siRNA dicer

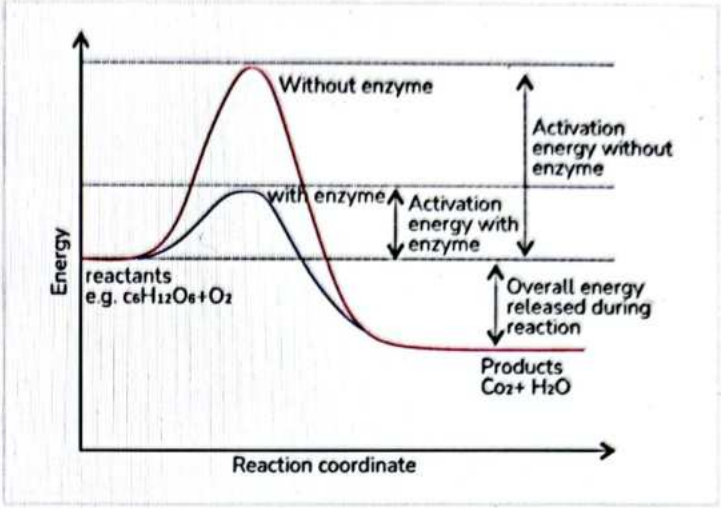
- All enzymes are proteins except a few exceptions that are RNAs
- RNA with enzymatic activity - Ribozyme
- Examples:
  - Peptidyl transferase
    - → 28 srRNA - eukaryotes
    - → 23 srRNA - prokaryotes
  - snRNA - small nuclear RNA
    - → Help in splicing - Breaking and reforming covalent linkages
    - → 6 types - U1 to U7 except U3

Enzymes

00:03:10

- Enzymes are Biocatalysts and most of them are proteins.
- Few exceptions are RNAs
- RNA with enzymatic activity - Ribozyme
  - 28 srRNA
  - snRNA
- Enzymes are called catalysts because they increase the rate of chemical reaction without getting utilized during the process
- Needed only in minute quantities
- Act by reducing the activation energy
- No effect on equilibrium constant of a chemical reaction
- They cannot shift the equilibrium either to the right or to the left
- Do not change the enthalpy of chemical reaction

Activation Energy



- Reaction coordinate
- X axis - progress of chemical reaction
- Y axis - energy changes
- Reactants of higher energy forming products of lower energy.
- They should spontaneously break down to form products.
- But this is not how it happens.
- Reactants are first converted into a transition state.
- The energy required to convert reactants to a transition state is called activation energy.
- If activation energy is higher, longer will be the time required to form the transition state.
- The Enzymes from an alternative transition state with lesser activation energy.
- So, the time required to reach the transition state is less.
- In the presence of enzymes:
  - Lesser the activation energy.
  - Shorter will be the time required to form the transition state.
  - Faster will be the chemical reaction.

Q. All are ribozymes except

A. Peptidyl Transferase

B. U1

C. U6

D. tRNA

- tRNa - has no enzymatic activity
- It translates amino acids to RNA.

Q. Glycogen phosphorylase belongs to
A. Class 6
B. Class 4
C. Class 3
D. Class 2

- Glycogen phosphorylase is a Transferase enzyme - class 2

Classification of Enzymes 00:08:35

- There are 7 classes of enzymes:
  1. Oxidoreductases
  2. Transferases
  3. Hydrolases (hydrolytic cleavage)
  4. Lyases
  5. Isomerases
  6. Ligases
  7. Translocases

Oxidoreductases

- Either oxidizes or reduces the primary substrate
- Name ends with oxidase, reductase, oxygenase or dehydrogenase

Instances when Oxidoreductases are confused:

Catalase

- Allows two molecules of hydrogen peroxide to react with each other.
- One hydrogen peroxide molecule gets oxidized and forms oxygen.
- The other molecule gets reduced to form two molecules of water.
- $H2O2 + H2O2 \rightarrow O2 + H4O2$
- Catalase is oxidoreductase.

Hydroxylases

- Monooxygenases.
- If Substrate – RH.
- It reacts with oxygen, only one atom of oxygen gets incorporated to form R-OH.
- The other oxygen atoms form water molecules.
- $R-H+O2 \rightarrow R-OH+H2O$
- The enzyme oxidizes primary substrate - so it is oxidoreductase

Transferase 00:12:15

- Transfer a certain group from one substrate to another.
- $AX + B \rightarrow A + BX$
- Name has trans in it:
  - Transketolase.
  - Transaldolase.
  - Acyltransferase.

Instances when Transferase are confused:

Kinase

- Hexokinase.
- Glucose + ATP → G6P + ADP
- Allows glucose to react with ATP
- Form Glucose 6- phosphate and ADP
- They are phosphotransferase
- They are transferase

Glycogen synthase

- Allow UDP glucose to react with glycogenin.
- Glycogenin - Central protein where all glucose molecule gets attached
- UDP glucose + glycogenin → glycogen primer
- Glycogen synthase is a transferase that transfers one glucose molecule from UDP - glucose to glycogenin.

Glycogen phosphorylase

- Mediates Phosphorolysis.
- Adds inorganic phosphate and cleaves glycogen.
- $(glycogen)n + iP \rightarrow (glycogen)n-1 + glucose-6P$
- Acts on glycogen with n number of molecules and cleaves it.
- Glucose molecule is transferred from glycogen to phosphate.

Hydrolase 00:15:55

- Mediates hydrolytic cleavage.
- Reaction where you add water and split a compound
- We are able to break a linkage by adding water - when it is ester or ether linkage
- Ester linkage - between alcohol and an acid
- $R-OH+HO-(C=O)-R \rightarrow R-O-(C=O)-R+H2O$
- When you add water to ester
- H - added to R-OH - to form R-OH
- OH, gets added to R-C=O → R-COOH

Lyases

- Breaks a linkage without adding water

	Hydrolases	Lyases
Break linkage by	Adding water	Without using water
Bonds	Break ester and ether linkage	C-N and C-C linkages
Linkages examples	**Ester linkages:** Esther's Phosphate Amide Peptide linkage **Ether linkages:** O - glycosidic linkages - carbohydrates N- glycosidic- nucleosides and nucleotides	

Examples of enzymes	Names end with	Lyases
	Hydrolase	Synthases
	Esterase	Decarboxylase
	Phosphatase	Hydratase - add water
	Phospholipases	Dehydratase - remove water
	Amidases	
	Peptidase	
	Glucosidases	
	Nucleosidases	
	Nucleotidases	

Important Information

- Synthase - lyases - Class 4
- Synthetase - ligases - Class 6
- Hydroxylase forms hydroxyl group - monooxygenase - oxidoreductase
- Hydrolase breaks a linkage by adding water
- Hydroxylase - Class 1
- Hydrolase - Class 3
- Hydratase and dehydratase- Class 4

Isomerases

- Converts one isomer into another.
- End with isomerase.
  - Phosphohexose isomerase
  - Phosphotriose isomerase
- Racemases- interconverts D and L from.
- Epimerase - glucose to galactose.
- Mutase mediates positional isomerism.
  - Phosphoglycerate mutase
  - 3- phosphoglycerate to 2-phosphoglycerate

Ligases

- Form a covalent linkage by adding ATP as a source of energy.
- Ligases
- Synthetases

Important Information

- ALA synthase- Class 4
- Carbamoyl phosphate synthetase- Class 6

Lyases	Ligases
Do not use ATP AS a source of energy	Use ATP as A source of energy
Synthases	Synthetase
Decarboxylases	Carboxylases

Important Information

Carboxylases - BBA

- Bicarbonate
- Biotin as coenzyme

ATP source of energy

Translocase 00:27:30

- Protein which translocates substrates from one sub-compartment to another sub-compartment
- Tricarboxylate transporter
  - Translocates Citrate from mitochondria to cytoplasm
  - Citrate then takes part in fatty acid synthesis

Q. Glycogen synthase is a
A. Ligase
B. Lyase
C. Transferase
D. Hydrolase

- Though the name ends with synthase, they do not belong to lyase category.
- It is a Glucosyl transferase.

Q. ALA synthase is an example of
A. Lyase
B. Ligase
C. Transferase
D. Hydrolase

- All synthase - lyases

Q. PLP is a
A. Prosthetic group
B. Cofactor
C. Apoenzyme
D. Holoenzyme

- All B complex vitamins - prosthetic group

Parts of an Enzyme 00:31:40

- All enzymes have a protein part and a non-protein part.
- Protein part → apoenzyme
- Non - protein part:
  - Prosthetic group
  - Cofactor
  - Coenzyme

Prosthetic Group

- If the non-protein part is in a tight, stable incorporation with the protein part.

- Examples:
  - All B complex vitamins except tetrahydrofolate
  - PLP - Pyridoxal phosphate
    - → Needed for transaminases.
    - → Decarboxylases.
    - → Kynureninase - convert tryptophan to niacin.
    - → Glycogen phosphorylase.
  - B12
    - → Methyl B12 - methionine synthase.
    - → Adenosyl B12 and methyl malonyl co-A mutase.
  - B1 in transketolase.
  - Most of the minerals
    - → Magnesium in kinases.
    - → Copper in tyrosinases.
    - → Copper in Superoxide dismutase.
    - → Zinc in carbonic anhydrase.

Important Information

- Tetrahydrofolate - group transfer agent - coenzyme
- Transfers one carbon from one system to another

Cofactor

- Non-protein is in a transient dissociated incorporation with the protein part.
- Examples:
  - Calcium dependent kinases.
  - Kinases are generally active.
  - When calcium is there it gets activated more.

Coenzymes

- Act as recyclable shuttle or group transfer agents.
- Transfer group from one system to another system.
- Examples: Mnemonic MAO
  - Methyl group- transferred by Tetrahydrofolate.
  - Acyl group transfer - Coenzyme A.
  - Oligosaccharides – Dolichol.

Q. Folate derivatives act as
A. Prosthetic group
B. Cofactor
C. Apoenzyme
D. Coenzymes

- B9 - tetrahydrofolate - act as group transfer agents - coenzyme

Q. Cobalamin in methionine synthase act as
A. Prosthetic group
B. Cofactor
C. Apoenzyme
D. Coenzymes

- Cobalamin in methionine synthase Is in a tight stable corporation - prosthetic group

Q. Dolichol act as
A. Prosthetic group
B. Cofactor
C. Apoenzyme
D. Coenzymes

- Dolichol act as Group transfer agent - coenzyme

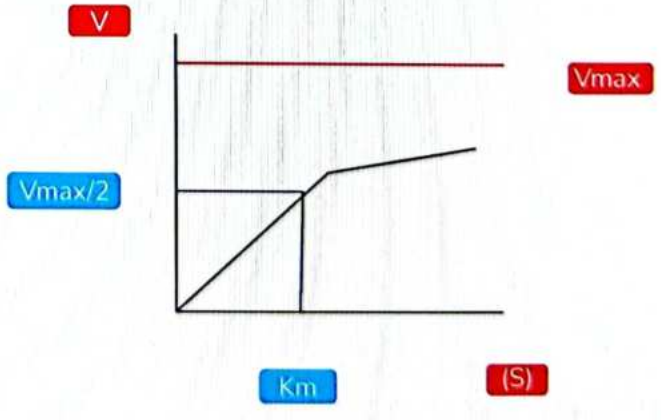
Enzyme Kinetics 00:37:30

Q. Michaelis Menten equation is used for enzymes following
A. Linear kinetics
B. Saturation kinetics
C. Sigmoid kinetics
D. Hyperbolic kinetics

- Monomeric enzymes - having only one subunit - follow saturation kinetics.
- Polymeric subunit – follow sigmoidal kinetics.
  - Myoglobin - follow saturation kinetics.
  - Hemoglobin - 4 subunits - follow sigmoidal kinetics.
- Michaelis Menten equation is used for enzymes following saturation kinetics.

Saturation Kinetics

- If you draw a graph:
  - X axis - subtraction concentration
  - Y axis - velocity of enzyme-catalyzed chemical reaction
- When you increase substrate concentration.
- Initially there is an increase in velocity.
- Beyond a point even when you increase the substrate concentration.
- The velocity cannot increase further.
- At this point all the available substrate binding sites of the enzymes are saturated with substrates.
- At this point the velocity reaches a plateau.
- This maximum velocity achievable is called Vmax.



- Vmax is never achievable
- Both in in-vitro experiments and in in-vivo experiments, never have we been able to achieve Vmax
- ½ Vmax - at ½ Vmax - draw horizontal line.
- Allow it to touch the curve.
- From there, drop a vertical line - touch the x axis at Km.
- Km - Michaelis constant
  - Substrate concentration at half maximal velocity.
- Example:
  - If Km is 100 umol.
  - Means if the substrate concentration is 100 umol - enzyme can achieve ½ maximal velocity.
  - If Km is 1000 umol.
  - The substrate concentration has to be 1000 umol for the enzyme to achieve the same ½ Vmax.
- If Km is high more substrate has to be supplied to achieve half maximal velocity.
- If Km is high, the affinity of enzymes for the substrate is low.
- Km and affinity are inversely proportional.

Michaelis Menten Equation 00:42:35

$$V = Vmax [S] / Km + [S]$$

- V - Velocity of enzyme catalyzed chemical reaction for a given substrate concentration.
- Vmax - The maximum velocity achieved by an enzyme when all the substrate binding sites are saturated with substrates.
  - Not measurable
- Km substrate concentration at half maximal velocity.

Uses:

- To calculate the velocity of an enzyme catalyzed chemical reaction for a given substrate concentration.
- We cannot use the equation as such as Vmax is never measurable.

Significance of Km

Substitute V= ½ Vmax in michaelis menten equation:

- V = Vmax [S] / Km + [S]
- ½ Vmax = Vmax [S] / Km + [S]
- ½ = [S] / Km + [S]
- Km + [S] = 2[S]
- Km = [S]
- Means at ½ Vmax; Km = substrate concentration
- Km is the substrate concentration at half maximal velocity

Q. Equation used to study the kinetics of enzymes following sigmoidal kinetics is:

A. Lineweaver Burke equation
B. Michaelis menten equation.
C. Hill's equation
D. Dixon equation

Important Information

- Oligomeric enzyme
  - Creatine kinase
  - LDH

Double reciprocal plot 00:46:33

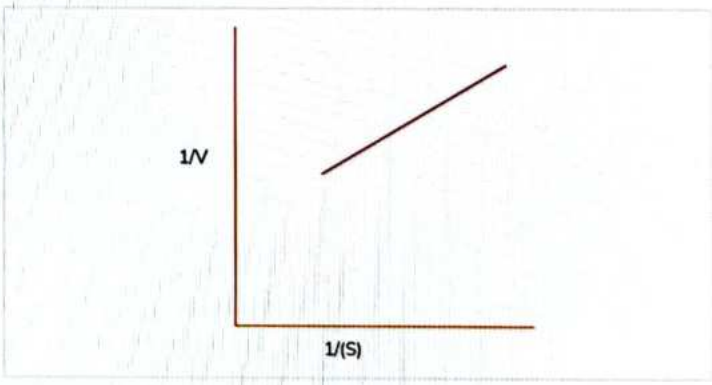
Q. TRUE about double reciprocal plot is

A. 1/v is plotted along x axis
B. 1/[S] is plotted along y axis
C. 1/ Vmax is the x intercept
D. -1/ Km is the x intercept

- Along y axis – 1/v
- Along x - 1/[S]
- 1/ Vmax is the y intercept
- -1/ Km is the x intercept

Lineweaver Burke plot / Double reciprocal plot

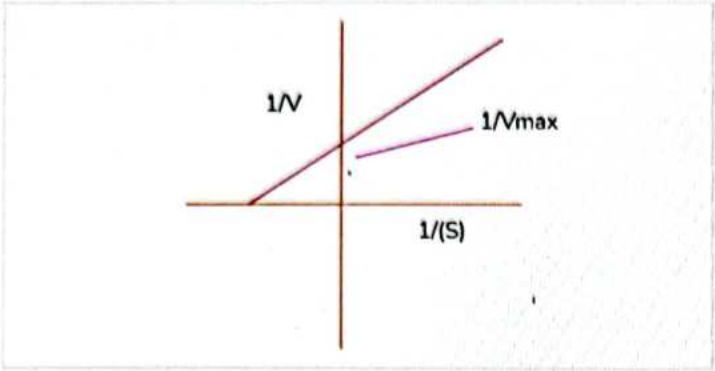
- V = Vmax [S] / Km + [S]
- Taking Reciprocals on both sides
- I/V = Km + [S] / Vmax [S]
- I/V = Km/Vmax [S] + [S] / Vmax [S]
- I/V = Km/Vmax [S] + 1/Vmax
- This equation is in the form of y = ax + b
  - Equation of a straight line
  - y = 1/V
  - x = 1/[S]
  - This gives a straight line



Uses:

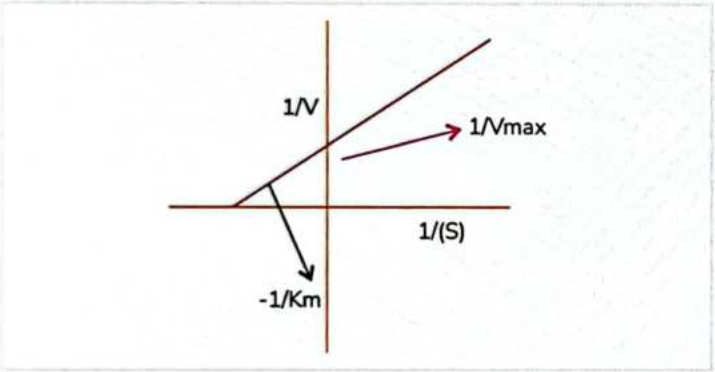
- If we have a new enzyme.
- Subject the enzyme to activity at various substrate concentrations.
- The velocity of the product concentration is calculated.
- The reciprocal of substrate concentration is plotted along the x axis.
- The reciprocal of velocity is plotted at y axis.
- This helps to find out Vmax and Km.
- If we extrapolate the line it cuts x axis at one point and y axis at another point called x and y intercept

- Y-intercept:
  - x=0
  - 1/V=Km/Vmax[S]+1/Vmax
  - 1/V=0+1/Vmax
- Y-intercept=1/Vmax



- X intercept:
  - y=0
  - 1/V=Km/Vmax[S]+1/Vmax
  - 0=Km/Vmax[S]+1/Vmax
  - −1/Vmax=Km/Vmax × 1/[S]
  - 1/[S]=−1/Km

- X-intercept -1/Km



Q. True regarding competitive inhibition of an enzyme

A. Km is increased

B. Km is unaltered

C. Km is decreased

D. Vmax is decreased

- In presence of competitive inhibition,
- Inhibition can be overcome by increasing the substrate concentration
- Vmax is unaltered
- Km will be high

Enzyme Inhibition

00:55:55

Types of enzyme inhibition

- Reversible
- Irreversible
- Pharmacological agents - reversible inhibitors

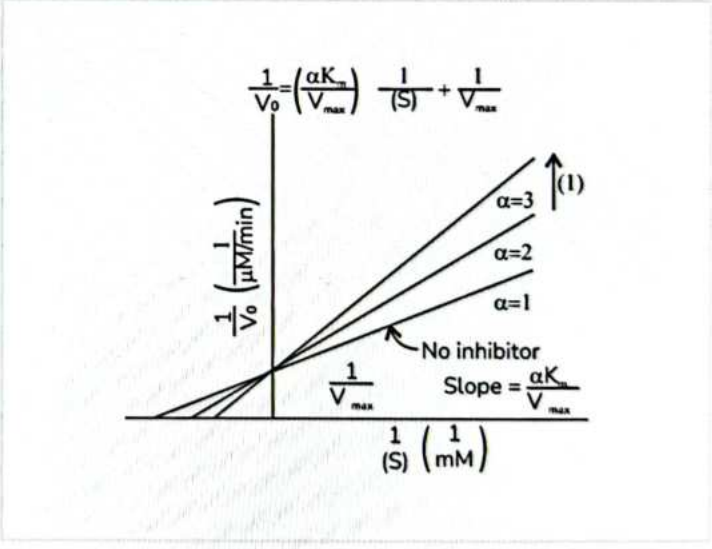
Reversible Inhibition

- Competitive
- Uncompetitive
- Mixed
  - Noncompetitive - a special type of mixed inhibition

Competitive inhibition

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

- Enzyme reacts with substrate to form enzyme substrate complex
- It may reverse to form enzyme and substrate or can form enzyme and products
- Competitive inhibitor is a substrate analog
- Compete with substrate to go and bind with the active site of an enzyme
- Form enzyme inhibitor complex
- Does not allow the products to form
- It reduces the probability that the enzyme binds with substrate
- Reduces the probability of formation of enzyme substrate complex
- It reduces probability of product formation
- So, when substrate concentration is increased, substrate wins the competition and the product is formed
- So, Vmax is unaltered as the inhibition can be overcome
- But to reach the Vmax the substrate concentration has to be increased
- Km is the substrate concentration at half maximal velocity
- This is increased in competitive inhibitor
- Km is increased



- Vmax is unaltered
- Km is increased or 1/Km is decreased
- This shows the inhibitor is competitive inhibitor
- Effects of competitive inhibitor
  - Vmax is unaltered
  - Km is increased

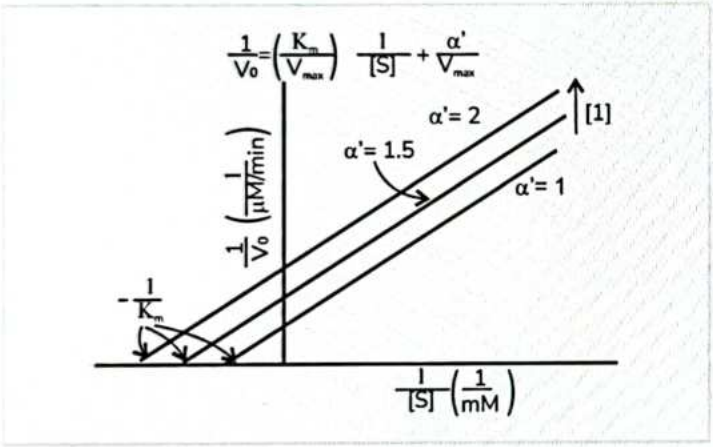
Uncompetitive inhibition

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

- Goes and binds to the enzyme substrate complex.
- From enzyme substrate inhibitor complex.
- Does not allow the product formation.
- Increasing the Substrate concentration will not reverse the inhibition
- More substrates → more enzyme- substrate complex → more enzyme substrate inhibitor complex
- Vmax is decreased
- Km is also decreased
- In the presence of uncompetitive inhibitor:

$$E + S \leftrightarrow ES$$

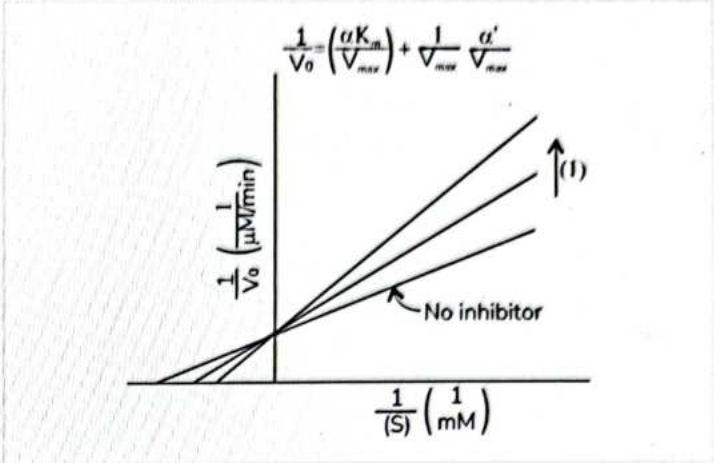
  - Enzyme substrate is diverted to form something else (enzyme substrate inhibitor complex).
  - Right side of equilibrium is always kept low - the enzyme substrate complex gets used up.
  - The equilibrium shifts to that side.
  - More and more enzymes and substrates will react to form enzyme substrate complexes.
  - The affinity of enzymes to substrate increases.
  - Affinity and Km are inversely proportional so Km decreases.



- Uncompetitive inhibition - everything is parallel.
- 1/Vmax and 1/Km is high.
- Parallelly shifted line.

Mixed inhibition 01:08:25

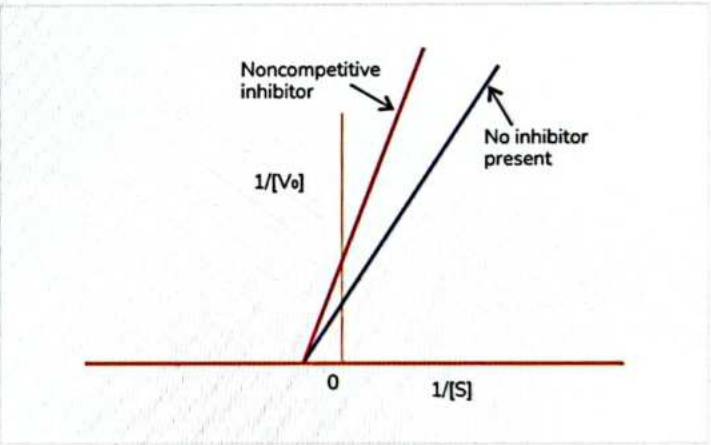
- Binds to both free enzymes and enzyme substrate complexes.
- Forms enzyme substrate complex and enzyme substrate inhibitor complex.
- The product is not formed.
- Increasing the substrate concentration will not overcome inhibition.
- So, Vmax is low.
- Km is high.
- Competes with substrates to bind with enzymes.
- Affinity for the enzyme to the substrate is reduced.
- Km is increased.
- The factors are altered in wrong directions.
- Low Vmax and high Km.



- 1/Vmax is increased
- 1/Km is reduced

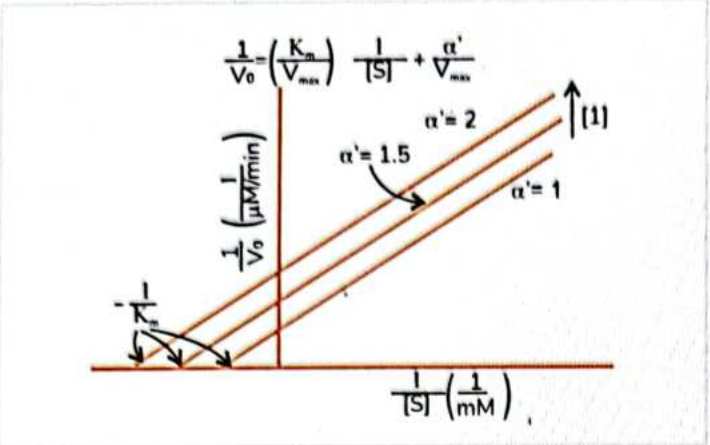
Non-competitive inhibitor 01:11:45

- Special type of mixed inhibitor.
- Binds to both free enzymes and enzyme substrate complexes with same affinity.
- Vmax is low.
- Km is normal.
- Non-competitive- normal Km



Type of inhibition	Vmax	Km
Competitive inhibition	N	↑
Uncompetitive inhibition	↓	↓
Mixed inhibition	↓	↑
Non-competitive inhibition	↓	N

Q. What kind of inhibition is this?



A. Competitive inhibition
B. Uncompetitive inhibition
C. Non-competitive inhibition
D. Mixed inhibition

Q. An enzyme was mixed with 4 mM substrate. The initial rate of product formation was 25% of Vmax. the Km of the enzyme is

A. 2 mM
B. 4 mM
C. 9 mM
D. 12 mM

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Explanation:

- [S] = 4 mM
- V = 25% Vmax
- V = Vmax / 4
- Km = substrate concentration at ½ Vmax
- V = Vmax [S] / Km + [S]
- Vmax / 4 = Vmax × 4 / Km + 4
- 1/4 = 4/Km + 4
- Km + 4 = 16
- **Km = 16-4 = 12**

Q. An enzyme - catalyzed reaction was carried out with the initial substrate concentration 1000 times greater than Km for that substrate. After 9 minutes, 1%of the substrate had been converted to the product, and the amount of product was 12 mmol. If one-third as much enzyme and twice as much of the substrate is combined, how long would it take for the same amount (12 mmol) of product to be formed?

A. 13.5 mins
B. 27 min
C. 3.8 mins
D. 4.9 mins

Explanation:

When,

- [S] = 1000 Km
- V = 12 mmol / 9 min

Now if,

- [S] = 2000 Km
- Enzyme concentration = ⅓ of initial
- V = Vmax [S] / Km + [S]

- **If [S] = Km**
- V = Vmax Km / Km + Km
- V = Vmax Km / 2 Km
- V = Vmax / 2
- Or when substrate concentration is Km, velocity is 1/2 Vmax

- **If [S] = 10 Km**
- V = Vmax [10 Km] / Km + 10 Km
- V = 10/11 Vmax
- V = 0.9 Vmax

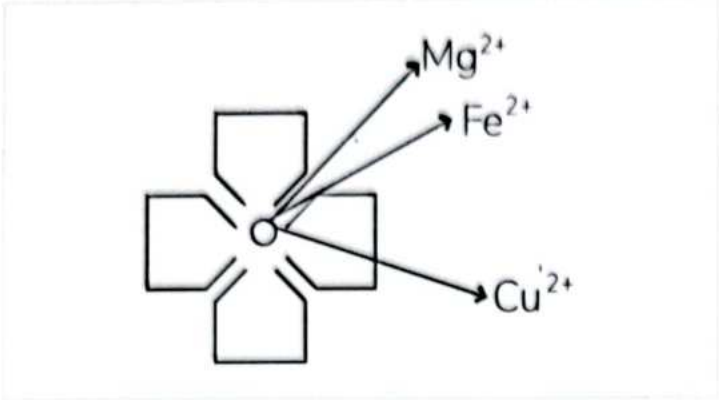
- **If [S] = 100 Km**
- V = Vmax 100 Km / Km + 100 Km
- V = 100/101 Vmax
- V = 0.99 Vmax

- So, if substrate concentration is increased from 10 times to 100 times, there is hardly any increase in velocity.
- If substrate concentration is increased to 1000 times, velocity becomes - 0.999 Vmax.
- When substrate concentration is increased beyond 10 times Km, there is no change in velocity.
- So here, the change in substrate concentration will not change the velocity.
- It is the enzyme concentration which is the determinant of velocity.
- It linearly affects the velocity.
- Enzyme concentration decreases by ⅓.
- Time taken will increase by 3 times.
- 3 times 12 minutes = 27 minutes.

41 HEME-SYNTHESIS AND PORPHYRIA'S

Heme

60 61 66



- Heme means a metal-containing protoporphyrin ring.
- There are four pyrrole rings.
- In the middle of this ring is the metal which can be magnesium in the case of chlorophyll, and it can be iron as observed in haemoglobin, and myoglobin or cytochrome protein.
- The copper containing heme is cytochrome A and A3, which complex 4 of ETC.

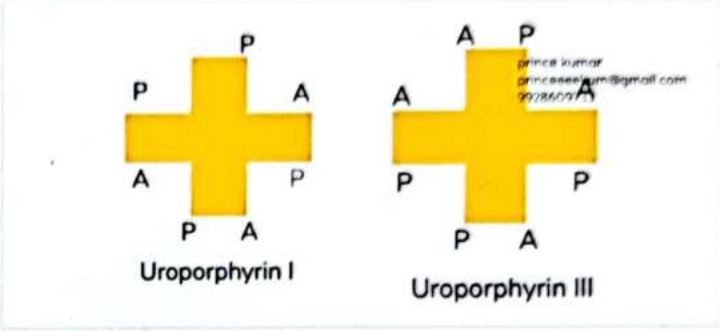
Two types of ring are possible

- **Porphyrinogen Ring:** If all four pyrrole rings are connected by methylene bridges with no double bond that is called a porphyrinogen ring.
- **Porphyrin Ring:** If all four pyrrole rings are connected by methine bridges with a double bond that is called a porphyrin ring. These double bonds are capable of absorbing light at 400 nm and emitting light at 600 nm. This causes three effects.
- When the urine of a patient will be visually examined through a spectroscope, who is suspected to be suffering from porphyrias. So, if the urine sample has porphyrins, the light source will give rise to white light this light will pass through the test tube containing the urine. If the urine has porphyrins with methanol bridges, it will start absorbing the light at 400 nm.
- **Soret Band:** The light which is emitted from the test tube will not have 400 nm and the spectroscope placed on the other side will see a dark line corresponding to the 400nm which is called the Soret band.
- **Erythrodontia:** The urine sample will emit light at 600nm, it will correspond to the red wavelength. This is the reason for the red fluorescence of porphyrins, accumulation of porphyrin precursors induces a deep red-brown or yellow-brown discolouration (erythrodontia).

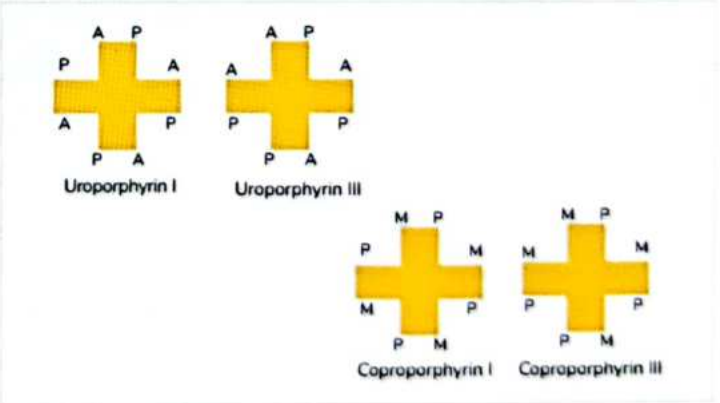
- Porphyrins absorb light of shorter wavelengths, which means they have higher energy. The energy difference will be liberated as free energy, This excites the oxygen molecules which are present in the medium, and the superoxide radicals cause photo-oxidative damage. This is the reason for photosensitivity in porphyrias.

Structure of Heme

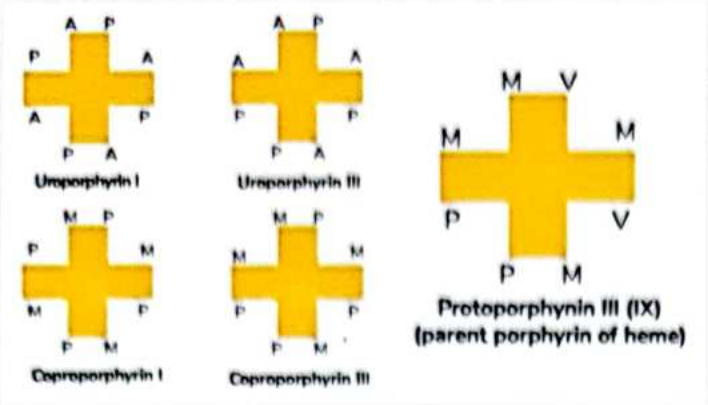
- There are 8 positions in the four pyrrole rings which can be substituted into one group or another. Depending on what a substituted in these 8 positions one can identify



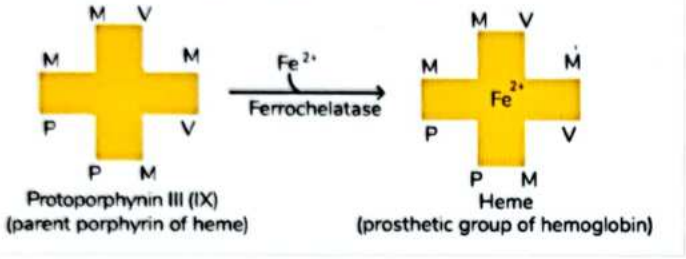
- **Uroporphyrinogen:** And all these 8 positions if there are alternating acetyl and propionyl groups that are called Uroporphyrinogen.
  - Uroporphyrinogen I is when there is an symmetry in all the rings.
  - Uroporphyrinogen III is when there is an asymmetry in the last ring, heme belongs to this series.
  - CH_3COO is the acetyl group and CH_3 is the methyl group. The conversion from the acetyl group to the methyl group is done by decarboxylating.
- Uroporphyrinogen decarboxylase enzyme converts uroporphyrinogen to coproporphyrinogen.



- **Coproporphyrinogen:** In this case, there are methyl propionyl groups. The conversion of uroporphyrinogen to coproporphyrinogen is decarboxylating.



- **Protoporphyrinogen**: In this case, only in rings 1 and 2 there is a methyl propionyl and vinyl group. Coproporphyrinogen to protoporphyrinogen conversion is done by oxidation. Protoporphyrinogen can be converted into protoporphyrin by oxidation as well as or removal of hydrogen and the enzyme protoporphyrinogen oxidase.



- Conversion of Protoporphyrinogen to Protoporphyrin by oxidation and the enzyme is Protoporphyrinogen oxidase.
- The difference between protoporphyrin and heme is chelating iron. The enzyme is Ferrochelatase.

Heme Synthesis Steps

00:15:04

- Heme is synthesised partly in mitochondria and cytoplasm. There is a total of 8 steps of heme synthesis, which are equally divided into both locations. The steps are as follows:
  - The first step happens in the mitochondria. Glycine and amino acids react with succinyl CoA (an intermediate of the citric acid cycle) in the presence of delta ALA synthase to form delta ALA. This is a decarboxylation reaction and the enzyme used is vitamin B6. Since it is a decarboxylation presence of a coenzyme such as a pyridoxal phosphate is necessary.
  - The next step occurs in the cytoplasm. ALA comes out of mitochondria and reaches the cytoplasm. And cytoplasm to molecules of delta ALA condenses with each other in the presence of delta ALA dehydratase. Two molecules of water get removed here and form porphobilinogen. Delta ALA dehydratase enzyme depends on zinc. The porphobilinogen is one pyrrole ring that is attached to an amino group. Heme is a tetrapyrrole group.
  - This step requires four porphobilinogen molecules to react with each other for the renewable of 4 ammonia. The enzyme is called porphobilinogen de aminase. When this enzyme acts it produces linear tetrapyrrole first which is otherwise called Hydroxymethylbilane. Porphobilinogen de aminase is also known as Hydroxymethylbilane synthase and known as Uroporphyrinogen I synthase.
  - First porphyrin ring formed here is Uroporphyrinogen I
  - In order to convert Hydroxymethylbilane into Uroporphyrinogen 3 the enzyme used is Uroporphyrinogen 3 synthase.
  - In order to convert uroporphyrinogen into coproporphyrinogen requires the help of a decarboxylase. The enzyme used is uroporphyrinogen decarboxylase which forms coproporphyrinogen I and 3.
  - In order convert Coproporphyrinogen into protoporphyrinogen is done with the help of an oxidase. Oxidation happens in the mitochondria which means the enzyme Coproporphyrinogen oxidase is present in the mitochondria. Only CP 3 enters into the mitochondria as CP1 gets excreted out of the body.
  - In the mitochondria, the coproporphyrinogen oxidase will convert protoporphyrinogen 3 to protoporphyrinogen. The protoporphyrinogen to protoporphyrin by oxidising. And the enzyme used for this conversion is protoporphyrinogen oxidase.
  - Lastly to convert protoporphyrin to heme, for that iron needs to be chelated. The last enzyme used is ferro chelatase.

Regulation of Heme Synthesis

00:24:36

- The rate-limiting enzyme of Heme synthesis is delta ALA synthase.

There are two isoforms of delta ALA synthase

- **ALA Synthase I:** It is present in the liver where it is related to cytochrome P450 enzyme synthesis. Delta ALA Synthase I get suppressed and repressed by heme. In the presence of any cytochrome p450 inducer when heme gets induced, heme gets used up. When is heme unavailable there is feedback stimulation of delta ALA synthase 1 and more intermediates go through heme synthesis which causes aggravation of porphyria. Delta ALA synthesis is also stimulated by fasting or starvation.
- **ALA Synthase II:** It is present in the RBC precursors where it is related to haemoglobin synthesis. This gets regulated based on the availability of the heme and based on the rate of erythropoiesis.
- Additionally, the heme synthetic pathway also gets regulated at the hydroxymethylbilane synthase step. HMB synthesis is also inhibited by coproporphyrinogen and protoporphyrinogen.

Heme Synthesis Disorder

- Only complete defect delta ALA synthase II. In this case, the protoporphyrin ring will not be synthesised. Which will future prevent the chelating of iron, which will accumulate in the mitochondria. Such iron-accumulated mitochondria surround the nucleus of RBC precursors making it look like a ring which is why it is called sideroblastic anaemia. It is an X-Linked sideroblastic anaemia because the ALA synthase II gene is present in the X chromosome.
- In the case of an autosomal chromosome, the SLC 25 A 38 transporter will be defective, which means it is unable to take the glycine into the mitochondria. This will also cause sideroblastic anaemia. But it is autosomal recessive sideroblastic anaemia.
- Other than ALA synthase II every other defect is partial. Initially, the Heme synthesis rate will decrease. This will cause the feedback simulation of the ALA synthase; this stimulation will lead to the pushing of all the substrates into the heme synthetic pathway. There is an increase in the flux of intermediate in the pathway. Whenever there is excess intermediate wherever the block is it will be overcome which will lead to the heme synthesis. Anaemia is not a feature. Precursor accumulation causes porphyria. If ALA and PBG accumulate both are toxic to a neuron which will cause Neuropsychiatric manifestation.
- Initially these patients present with aberrant pain like recurrent acute abdominal pain. Imaging studies and exploratory laparotomy findings are normal. Therefore, it is k/as tic tac toe disease.
- Porphyrins accumulation causes photosensitivity.

Sr. No	Enzyme defect	Name of the disorder
1	ALA synthase II	X linked sideroblastic anaemia
2	ALA Dehydratase	ALA dehydratase deficient porphyria
3	Porphobilinogen Deaminase (Hydroxymethylbilane Synthase)	Acute intermittent porphyria
4	Uroporphyrinogen III synthase	Congenital erythropoietic porphyria (most severe)
5	UP Decarboxylase defect	Porphyria Cutanea Tarda (PCT)
6	Coproporphyrinogen oxidase	Hereditary Coproporphyria
7	Protoporphyrinogen oxidase	Variegate porphyria
8	Ferro chelatase	Erythropoietic protoporphyria (EPP)

Refer Image 41.1

Classification of Porphyriyas

Porphyrias are classified into three categories

- **Neuropsychiatric Manifestations**
  - ALA dehydratase deficient porphyria - accumulation of only ALA
  - Acute intermittent porphyria - accumulation of only ALA and PBG
- **Photosensitivity**
  - Congenital erythropoietic porphyria - accumulation of Uroporphyrin I and Coproporphyrin I.
  - Porphyria Cutanea Tarda (PCT): It is the most common porphyria.
  - Erythropoietic protoporphyria (EPP)
- **Both**
  - Hereditary Coproporphyria
  - Variegate porphyria.

Lead Poisoning

- Lead can cause acquired porphyria.
- **Lead inhibits 3 enzymes of heme synthesis.**
  - Delta ALA dehydratase
  - Coproporphyrinogen oxidase
  - Ferro chelatase
- **Biochemical features of lead poisoning**
  - Delta ALA dehydratase – ALA elevation
  - Coproporphyrinogen oxidase – elevated coproporphyrin
  - Ferro chelatase – Elevated Zinc protoporphyrin levels – most sensitive marker

Revision Questions

One Liners

- Porphyrins absorb light at 400 nm
- Porphyrins emit light at 600 nm
- The rate limiting enzyme of heme synthesis is ALA Synthase
- Acute Intermittent porphyria is caused by the defect of HMB Synthase
- The most common porphyria is PCT

MCQ

Q. All of the following are associated with photosensitivity except?

A. HEP

B. EPP
C. PCT
D. AIP

Q. Which of the following porphyria's is autosomal recessive
A. PCT
B. ALA synthase defect
C. CEP
D. AIP

Q. Lead inhibits all except
A. ALA dehydratase
B. ALA synthase
C. Ferrochelatase
D. Coproporphyrinogen oxidase

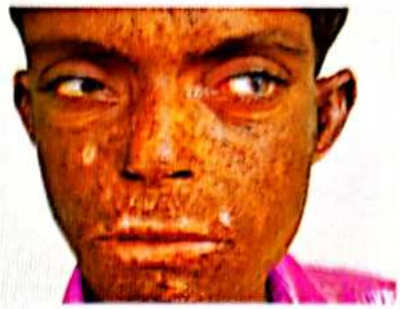
Q. The Coenzyme required for ALA synthase is
A. Vitamin B1
B. Vitamin B6
C. Zinc
D. Copper

Q. The Coenzyme required for ALA dehydratase is
A. Vitamin B1
B. Vitamin B6
C. Zinc
D. Copper

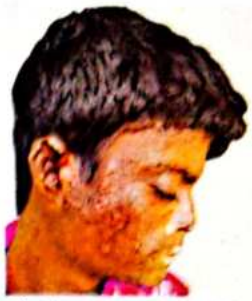
Q. A 35-year-old man has a history of intermittent abdominal pain and episodes of confusion and psychiatric problems. High amounts of -aminolevulinate and porphobilinogen is also detected in his urine analysis. The patient also has a mutation in the gene for uroporphyrinogen I synthase (porphobilinogen deaminase). The probable diagnosis of the patient is:
A. X-linked sideroblastic anemia
B. Acute intermittent porphyria
C. Congenital erythropoietic porphyria
D. Porphyria cutanea tarda

Q. A person working in a paint industry presents with sudden onset of photo sensitivity. The physician suspects lead poisoning. What is the most sensitive parameter that can be prescribed by the physician for an early identification of lead poisoning?
A. Elevated ALA
B. Elevated coproporphyrin levels
C. Elevated zinc protoporphyrin levels
D. High PBG levels

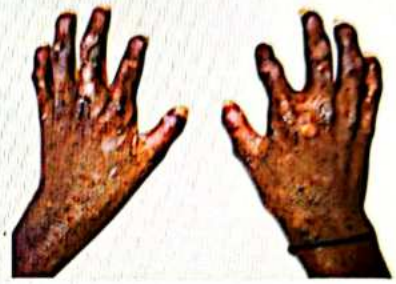
Image-based MCQS



Vesicles, scarring, pigmentation, hypertrichosis on the face and scleromalacia of the left eye



Vesicles, scarring, pigmentation, hypertrichosis of the face and mutliation of the ear lobe

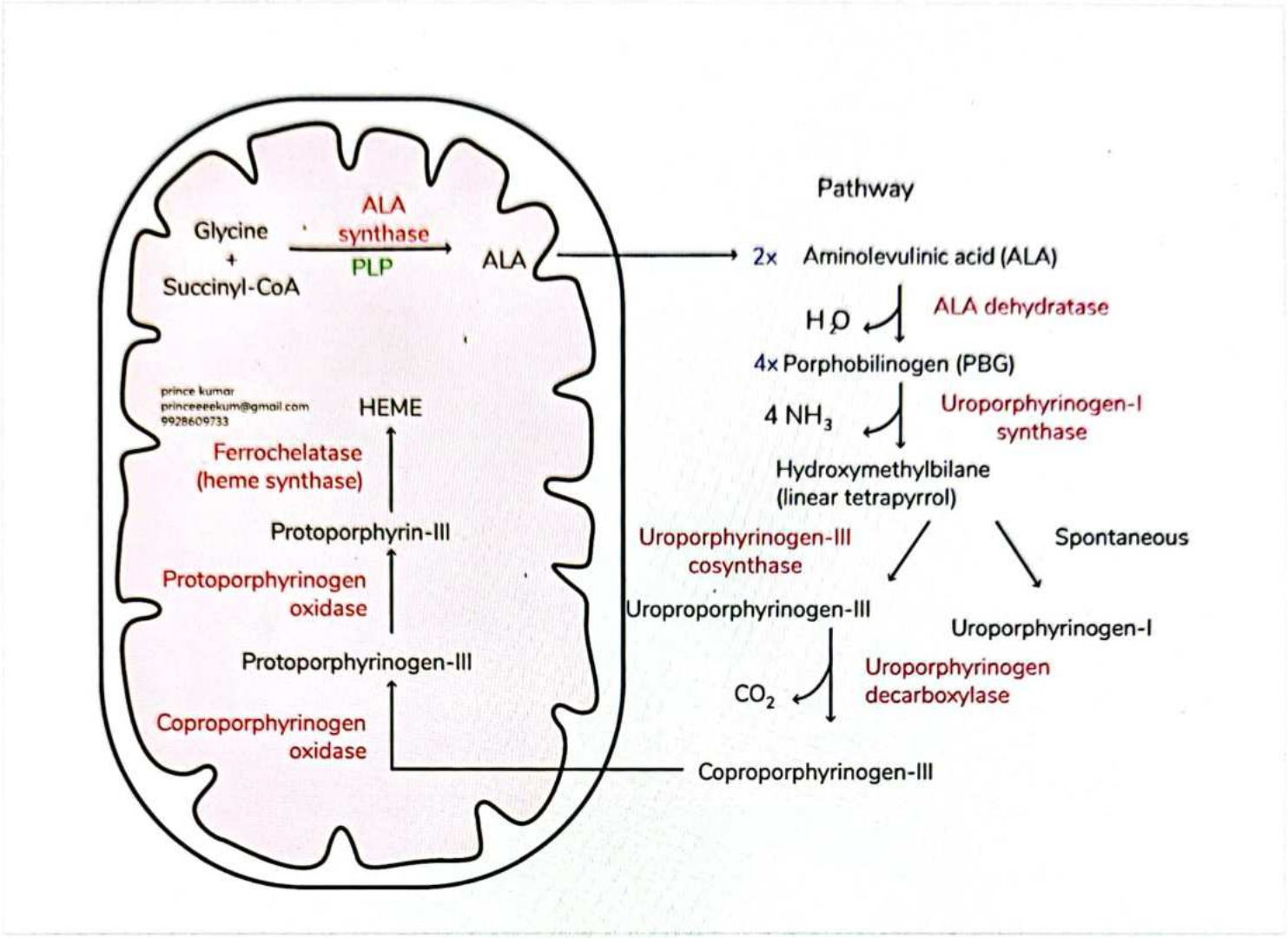


Vesicles, scarring of the hands, multilating deformities of the fingers and dystrophic nails

Urine turning reddish pink on standing

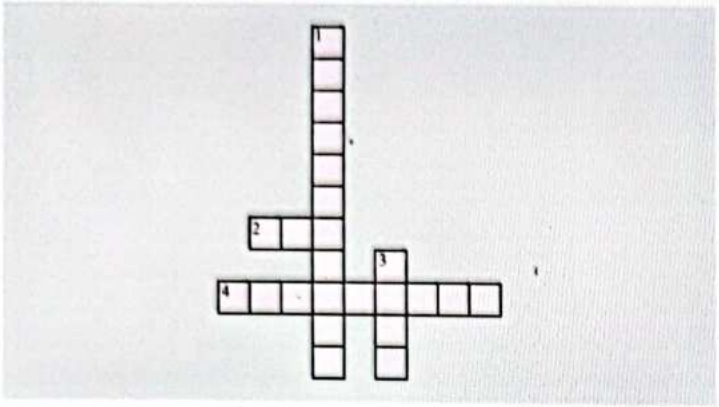
Q. A 15-year-old boy presented with severe photosensitivity, atrophic scarring, mutilated fingers and port wine urine. There was no neurological involvement. The most probable enzyme defect is
A. Uroporphyrinogen III synthase
B. ALA dehydratase
C. Coproporphyrinogen oxidase
D. Protoporphyrinogen oxidase

Image 41.1



CROSS WORD PUZZLES

Crossword Puzzle 1



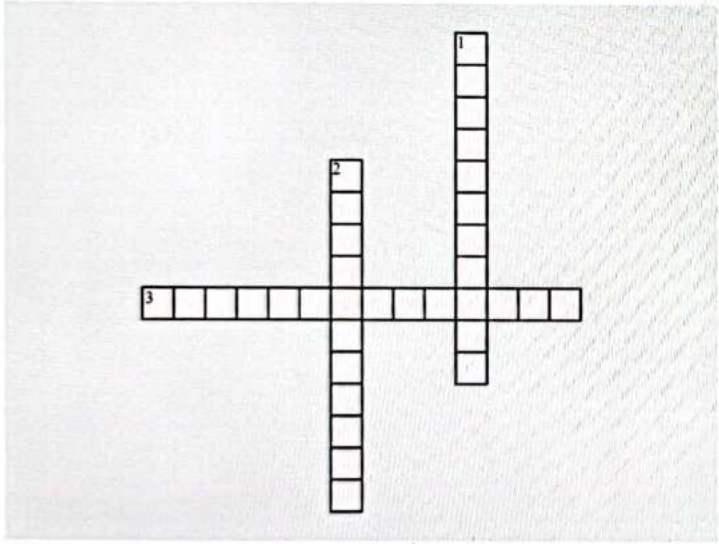
Across

2. The most common porphyria is _____.
4. The coenzyme required for ALA synthase is_____.

Down

1. Lead inhibits all expect _____.
3. The coenzyme required for ALA dehydratase is_____.

Crossword puzzle 2



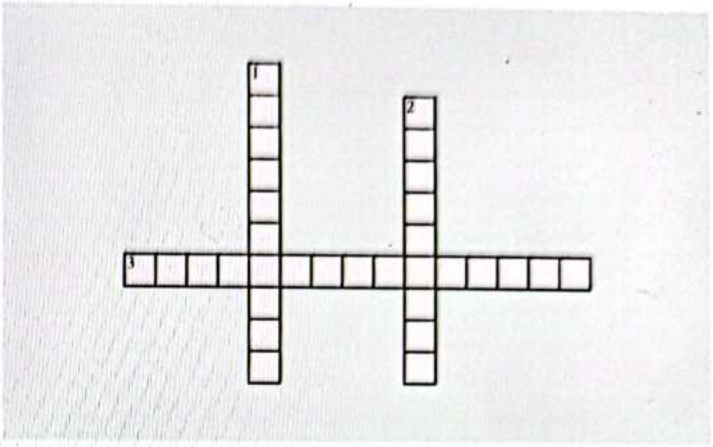
Across

3. Heme means a metal-containing _____ ring.

Down

1. In the middle of this ring is the metal which can be magnesium in the case of _____.
2. In the middle of this ring is the metal which can be iron in the case of _____.

Crossword puzzle 3



Across

3. The conversion from the acetyl group to the methyl group is done by _____.

Down

1. _____ absorb light of shorter wavelengths, which means they have higher energy.
2. _____ bridges with no double bond that is called a porphyrinogen ring.

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42 FAT SOLUBLE VITAMINS PART-1

Part 1 of fat-soluble vitamins

- Fat malabsorption causes the deficiency of fat-soluble vitamins indirectly.
- All these fat-soluble vitamins can be stored in the body.
- Deficiency of these fat-soluble vitamins are comparatively rare compared to water soluble vitamins.
- As they are stored, the probability of the toxicity is high.
- Fat soluble vitamins are prone to develop toxicity manifestation.

Vitamin A

Forms of Vitamin A

- **Retinol:** Alcohol form
- **Retinal:** Aldehyde form
- **Retinoic acid:** Acid form

Significance of Retinol

- This is the form in which vitamin A is absorbed.
- It is transported and stored.
- It is stored as Retinyl palmitate or Retinyl esters

Significance of Retinal

- In the word Retinal, Retina is hidden. This is a clue which says this is present in the eye.
- It is present in Rods and Cones in the form of Rhodopsin and Conopsin.
- As long as there is no light, Rhodopsin is 11 cis Retinal in the center attached to Opsin.
- Retinal is 11 cis Retinal + Opsin

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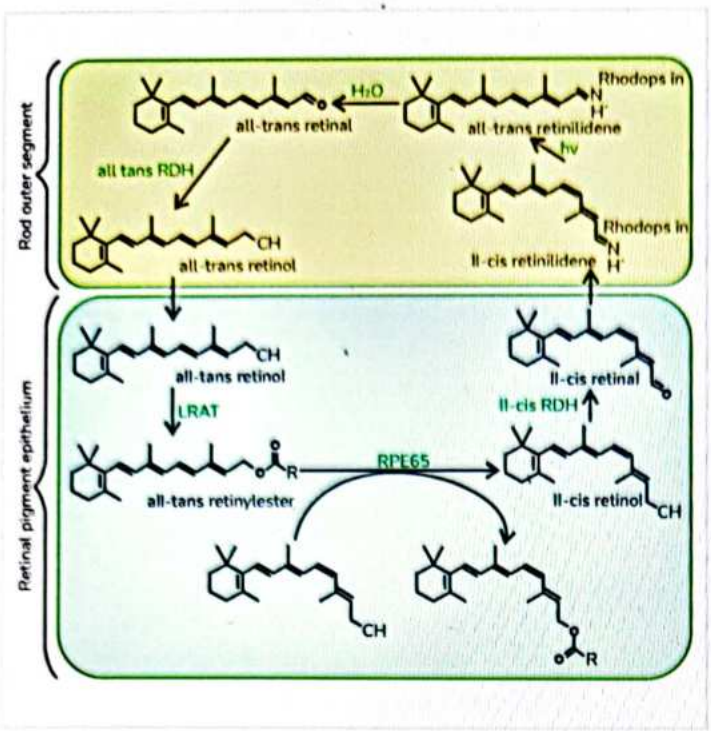
Significance of Retinoic Acid:

- There are 2 forms of Retinoic Acid
  - All Trans Retinoic Acid
  - 13-Cis Retinoic Acid
- All Trans Retinoic Acid helps in growth and differentiation of epithelium.
- 13-Cis retinoic acid suppresses keratinisation of epithelium.
- As it suppresses keratinisation of epithelium it leads to Phrynoderma.
- Phrynoderma is a manifestation of Vitamin A deficiency.
- Phrynoderma is nothing but follicular hyperkeratosis.
- Small papillary lesions in the extended surfaces of the limb with the keratin plug in the tip is known as follicular hyperkeratosis.
- In Vitamin A deficiency without suppression of keratinisation of epithelium, conjunctiva will undergo keratinisation.
- This causes conjunctival xerosis.
- Keratin will accumulate as Bitot's spots.
- Bitot's spots are nothing but triangular foamy white or yellowish spots.
- These are present on lateral side of the eye (Bilateral)
- These are keratin accumulations.
- These spots are evidence that there is an increase in keratinisation of epithelium that happens in vitamin A deficiency.
- 13- Cis Retinoic Acid causes apoptosis of sebaceous glands.
- It stimulates NGAL production.
- It is for Resistant acne.
- It is used in grave conditions like Harlequin ichthyosis.
- Harlequin ichthyosis has armor like skin scars in children.
- In this condition, children can't breathe.
- So, we need to suppress keratinization and 13-Cis Retinoic Acid is prescribed.
- Pharmacological actions of 13-Cis retinoic acid are to treat
  - Resistant acne
  - Harlequin ichthyosis

Wald's Visual Cycle

- It is all about what happens when light falls on the retina.
- It leads to photobleaching of the rhodopsin.
- It helps to regenerate rhodopsin after the photobleaching.
- It mainly happens in two cells.
  - Retinal pigment epithelium
  - Rods and cones
- Vitamin A is transported in the form All trans retinol.
- The form of the vitamin A in rhodopsin is 11 cis retinal + opsin.
- In the retinal pigment epithelium, the all trans retinol is converted into the 11 cis retinal
- To achieve the above process the retinal epithelium has three enzymes.
  - Lecithin retinol acyltransferase (LRAT)
    - → This enzyme transfers acyl group of the fatty acids from lecithin to retinol
    - → This leads to the conversion of retinol to retinyl ester.
  - RPE 65 (Isomerohydrolase)
  - Retinol dehydrogenase



- Wald's visual cycle will happen in two cells retinal pigment epithelium and in rods, cones.
- From the circulation retinal epithelium manages to get retinol (All trans retinol)
- It is converted into 11 cis retinal in order to enter rhodopsin.
- LRAT is lecithin Retinol acyltransferase.
- It transfers the fatty acid to L trans retinol.
  - Obtained from the circulation.
- Converting all trans retinol into all trans retinyl esters.
- RTE65 is present in Retinal pigment.
  - Also called isomero hydrolase.
  - Simultaneously hydrolyses the ester and isomerizes it.
- On hydrolysis, all trans Retinyl ester will become all trans retinol (Alcohol form).
- Isomerase converts all trans retinol into 11 cis retinol (Alcohol form).
- Aldehyde form is inserted into Rhodopsin.
- Another enzyme, 11 cis retinol dehydrogenase converts 11 cis retinol into 11 cis Retinal.
- 11 cis retinal gets incorporated into Opsin.
  - A G protein coupled receptor.
- 11 cis retinal gets incorporated and forms Rhodopsin.
- When light falls on the retina, 11 cis retinal undergoes photoisomerization.
  - Gets converted to all trans Retinal.
- All trans Retinal comes out.
- This is called photobleaching.
- To regenerate Rhodopsin, 11 cis retinal is needed.
- In the presence of adequate Vitamin A, all the sequence of events would have already happened.
- The person will have a ready-made 11 cis Retinal.
  - Gets incorporated into Opsin becoming Rhodopsin.
- Person can absorb another photon.
- Dark adaptation time means a light that causes photobleaching.
  - To adapt darkness, you should be able to regenerate Rhodopsin.
  - It requires adequate Vitamin A
  - Then the sequence of events would have already happened.
  - 11 cis Retinal gets incorporated into Opsin and becomes active.
- In a person with lack of Vitamin A, all trans Retinal detached from Opsin should be converted and recycled.
- This is done by alcohol dehydrogenase.
- Rods and cones have Retinal dehydrogenase.

Important Information

- One way of regenerating Rhodopsin is having a ready-made 11 cis Retinal which gets incorporated into Opsin.
- Another way is you have to regenerate in the rods and cones or in the retinal pigment epithelium.
- This takes a longer time.

RDA Of Vitamin A

- Adult men: 1000 ug/day
- Adult women: 850 ug/day
- Pregnancy: 900 ug/day
- Lactation: 950 ug/day
- Children: 400-650 ug/day

Sources of Vitamin A

- **Animal sources:** (Dairy products)
  - Milk, butter, cream, cheese
  - Egg yolk and liver, fish liver oils.
- Liver is the major source of Vitamin A (as it gets stored in Vitamin A)

Important Information

- Consumption of polar bear liver causes Vitamin A toxicity.
- Polar bears have a large quantity of Vitamin A.

- **Plant sources:** (Yellow in color)
  - Carrot, papaya, mango
  - Pumpkins and green leafy vegetables
- Plant sources are in the form of beta carotene.
- To convert beta carotene into Vitamin A, an enzyme beta carotene dioxygenase is required.
- Dioxygenase is dependent on thyroxine.

- **In hypothyroidism,** dioxygenase will be inactive.
  - And is not able to convert beta carotene into Vitamin A.
  - So, carotene accumulates in the skin.
  - This is called carotenemia.
  - This is yellow discoloration of the skin.

Vitamin A Deficiency

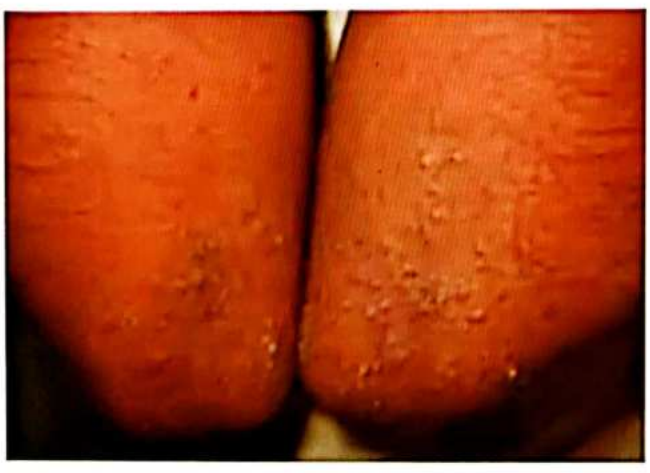
- **Causes**
  - **Primary cause** - Dietary deficiency
  - **Secondary cause** - Due to fat malabsorption, loss of retinol binding protein.
- To transport Vitamin A, retinol binding protein is required in the circulation.
- Due to the lack of retinol binding protein in the circulation, vitamin A cannot be transported properly.
- Causes Vitamin A deficiency.

Secondary causes of Vitamin A deficiency:

- **Example of fat malabsorption**
  - Obstructive jaundice
- **Example of loss of retinol binding protein**
  - Liver cirrhosis
  - Nephrotic syndrome
  - Chronic alcoholism

Vitamin A- Deficiency Manifestations

- **Ocular manifestations**
  - **XN:** Night blindness or nyctalopia (XN)
  - **X1A:** Conjunctival xerosis
  - **X1B:** Bitot's spots
  - **X2:** Corneal xerosis.
  - **X3A:** Corneal ulcer involving 1/3rd of cornea.
  - **X3B:** Corneal ulcer involving more than 1/3rd of cornea.
  - **XS:** Corneal Scar
  - **XF:** Xerophthalmic fundus
- **Skin manifestations**
  - Phrynoderma: Follicular hyperkeratosis



  - Xerophthalmia
  - Bitot's spots
  - Keratomalacia
  - Corneal opacity

Vitamin A Toxicity

- Consumption of more quantities of liver results in toxicity.
- More common in Eskimos.
- Eskimos feed on the livers of polar bears.
- This causes Vitamin A toxicity.

Manifestations of Vitamin A toxicity

- They mainly occur due to the increased intracranial tension and they include
  - Anorexia
  - Nausea, vomiting.
  - Irritability, headache
  - Hypertension and bradycardia
- Based on the above manifestations you might assume that as a tumor
- Nothing will be detected when the imaging studies are done.
- Hence this is called as cause of pseudotumor cerebri.
- **It also causes**
  - Bony exostosis
  - Hepatomegaly

Important Information

Pseudotumor cerebri:

- No tumor but there will be increase in the intracranial tension with hypertension, bradycardia, vomiting, and headache

Mechanism of Vitamin A toxicity

Accumulation of Vitamin A in lysosomes
↓
It releases lysosomal enzymes.
↓
Finally, it causes the cell death.

Vitamin E

- Other Name: Tocopherol
- It is an antioxidant.
- It is a chain breaking antioxidant.

Chain Breaking Antioxidant

- It has an extra pair of electrons.
- It donates its electron and convert the intermediates of lipid peroxidation into stable intermediates.
- Therefore, these are known as the Chain breaking antioxidants.

- The chain propagation occurs due to lipid peroxidation intermediates.
- These are of two types.
  - **Lipid phase chain breaking antioxidant.**
    - → **Fat soluble vitamin:** Tocopherol or alpha tocopherol
    - → This alpha tocopherol will accept or donate the electrons from lipid peroxidation intermediates to form tocopheroxyl radical.
    - → The intermediates will become stable intermediates.
  - **Aqueous phase chain breaking antioxidant.**
    - → The most popular one is Vitamin C (Water soluble vitamin)
    - → This will help in regenerating the tocopherol, polyphenol flavinols like epitachin gallate.
    - → It is also known as the indirect chain breaking antioxidant.
    - → Green tea contains polyphenol flavonoids like epigallocatechin gallate.

Other Facts of Vitamin E

- Vitamin E RDA will depend on the lipid intake of a person as the lipid intake stimulates the oxidative stress.
- If a person take the selenium the Vitamin E requirements will be reduced
- **RDA of Vitamin E**
  - **Male:** 10 mg/day
  - **Female:** 8mg/day
  - **Pregnancy:** 10 mg/day
  - **Lactation:** 12mg/day
- Vitamin E is the part of wheat germ oil or vegetable oil.
- Vitamin E deficiency is quite uncommon.
  - If there is any deficiency, there will no antioxidant.
  - RBC will act as a major source for oxidative stress.
  - So RBCs are susceptible to lysis following exposure to oxidative stress
- Deficiency presents as the hemolytic anemia which is uncommon.

Toxicity of Vitamin E

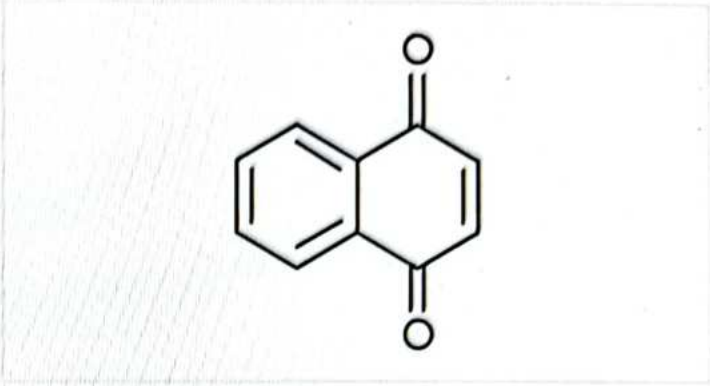
- It is more common as it is a fat-soluble vitamin which is stored in the liver
- Vitamin E toxicity will present with hemorrhagic tendency

Vitamin E toxicity	Vitamin K toxicity
We will find the features resembling with Vitamin K deficiency (Bleeding)	We will find the features resembling with Vitamin E deficiency (Hemolytic anemia)
It causes Hemorrhagic tendency	It will cause hemolytic anemia

Vitamin K

Structure Vitamin K

- It has a naphthoquinone ring.



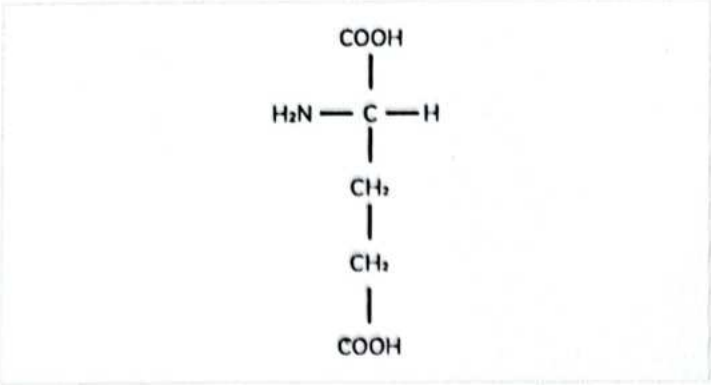
- The side chain has the poly isoprenoid side chain.
- This side chain is responsible for lipid solubility.
- It is called as the fat-soluble vitamin because of its side chain
- If the isoprenoid side chain has
  - **20 carbons:** K1
  - **30 carbons:** K2
  - If there are no carbon atoms in the side chain: Menadione (Only H)
    - → Menadione is not fat soluble because it doesn't have isoprenoid side chain.
    - → So, Menadione is water soluble.
- Vitamin K deficiency is more common in the patients with fat malabsorption.
- Suppose there is an individual with cystic fibrosis then pancreatic insufficiency is expected.
- Pancreatic enzymes are essential for fat absorption.
- When there is pancreatic insufficiency, fat malabsorption occurs along with vitamin K.
- So, the patient will present with bleeding.
- To treat this type of individuals, Menadione is recommended because absorption is not dependent on the pancreatic enzymes.

Important Information

- If there is Vitamin K malabsorption due to fat insufficiency the prescribed medication is Menadione.

Uses of Vitamin K

- It acts as a coenzyme or prosthetic group for gamma carboxylase.
  - This gamma carboxylase will add a additional carboxyl group to the glutamate residues present in few proteins (Calcium dependent)
- The glutamic acid has C-H group in the center that attached to carboxyl and amino groups as the functional groups



- The R group in the glutamic acid is CH2 - CH2 - COOH
- H is the alpha carbon atom
- Below the alpha, beta carbon atom is present.
- Below the beta, gamma carbon is present.
- Gamma carboxylase will attach one more carboxylic group to the gamma carbon atom.
- As there are two negative charges all the calcium.
- Calcium will bind with the two negative atoms.
- So that all the calcium dependent proteins will be activated.
- The calcium dependent proteins are clotting factors II, VII, IX, and X
- Anticoagulant factors like protein C and S
- Osteocalcin is involved in the mineralization of the bone by depositing calcium.
- For depositing calcium, it needs two negative charges, so gamma carboxylase is required.

Important Information

Forms of Vitamin K

- Vitamin K exist in two forms.
  - Epoxide form
  - Hydroquinone form

- Hydroquinone is necessary as a coenzyme for gamma carboxylase.
- It will change into epoxide form while converting glutamic acid to gamma glutamic acid.
- Epoxide reductase is used for regenerating the hydroquinone.
- This epoxide reductase is inhibited by the warfarin (Anticoagulant)
- Without this active form of vitamin K, vitamin K dependent coagulation factors will not be activated.
- Hence warfarin functions as an anticoagulant.

One Liners

1. The form in which Vitamin A is stored is **Retinol.**
2. The RDA of Vitamin A in adult male is **1000ug/day.**
3. **Vitamin E** is a lipid phase chain breaking antioxidant.
4. The RDA of vitamin E in males is **10 mg/day.**
5. Vitamin E deficiency causes. **Hemolytic anemia**
6. Hypervitaminosis E causes **Hemorrhagic tendency.**
7. Vitamin K is required as a coenzyme for **Gamma carboxylase.**

Multiple Choice Questions

Q. Rhodopsin until stimulated by light is.
A. 11 cis Retinol
B. 11 cis retinal
C. All trans Retinal
D. All trans retinol

Explanation: It is surrounded by the opsin

Q. The form in which vitamin A is transported is?
A. 11 cis retinol
B. All trans Retinoic acid
C. All trans retinal
D. All trans retinol

Explanation: It is transported in alcohol form (Trans retinol)

Q. Retinoic acid plays a role in all except?
A. Growth
B. Differentiation of epithelium
C. Reproduction
D. Stimulates production of NGAL

Explanation

- All trans Retinoic acid will help in growth, differentiation.
- 13C retinoic acid suppresses the keratinization and helps in causing the Apoptosis of sebaceous glands
- It also stimulate NGAL production

Q. True statement regarding Wald's Visual Cycle is?
A. Rhodopsin is all trans retinal + Opsin
B. Retinal pigment epithelium converts 11 cis retinal to all trans retinal
C. Light converts 11 cis retinol to all trans retinol
D. RPE65 helps in the formation of 11 cis retinol

Explanation

- It occur in the retinal pigment epithelium and rods
- It will convert all trans retinol into 11 cis retinal
- Then it will get incorporated into opsin
- Other name of RPE65 is isomerohydrolase
- It helps to convert retinyl esters (All trans retinyl ester) to 11 cis retinol
- It is further converted into 11 cis retinal by retinol dehydrogenase
- Circulatory form is all trans retinol
- The one expected in rhodopsin is 11 cis retinal

- All trans retinol is converted to 11 cis retinal ultimately.
- It is done by LRAT, RPE 65 or isomerohydrolase and 11 cis retinol dehydrogenase

Q. Chain breaking antioxidants are all except?
A. Tocopherol
B. Ascorbic acid
C. Polyphenolic flavonoids
D. Superoxide dismutase

Explanation
- Lipid phase chain breaking antioxidant is Vitamin E tocopherol
- Aqueous phase chain breaking antioxidant is Vitamin C Ascorbic acid
- We also have polyphenolic flavonols and epithelien galate
- Superoxide dismutase inhibits the chain initiation
- Chain initiation is done by the superoxide radical becoming hydrogen peroxide that further becomes peroxide radical.
- Hence chain propagation will start

Q. Warfarin causes increased concentration of which of the following?
A. Menadione
B. Hydroquinone
C. Epoxide form
D. Factor II

Explanation
- Warfarin inhibits epoxide reductase that leads to the increase in the epoxide form
- It decreases the hydroquinone form

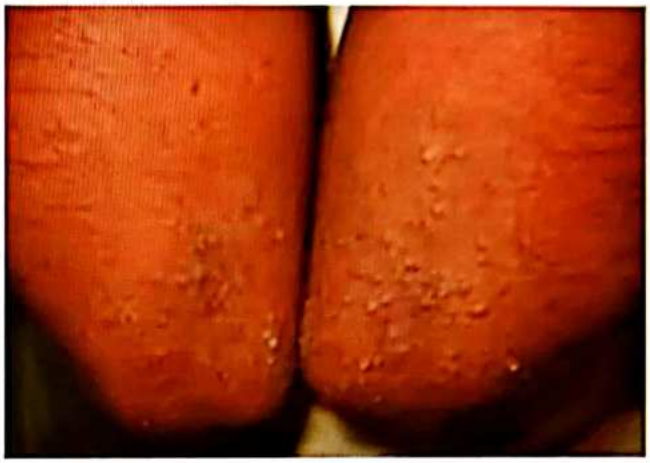
Case Based MCQs

Q. A 27-year-old sailor presenting with chief complaints of flushing, headache, nausea, and joint pain. He had consumed 800 g of grilled ocean perch liver the day before and had experienced numbness shortly after. Two days later he presented with exfoliation of skin, persisting, headache and vomiting. On examination his BP was high and heart rate was low. Imaging studies did not detect any SOL in the brain. The most probable cause is.
A. Seasickness
B. Stroke
C. Vitamin A toxicity
D. Vitamin D toxicity

Explanation
- All these are the manifestations due to increased intracranial pressure.
- Hence it is called as pseudotumor cerebri because imaging studies did not detect any SOL in the brain

Image Based MCQs

Q. A 7-year-old girl presents with bilaterally symmetrical discrete papules with central keratinous plug localized to elbows as shown in the image. The probable cause is deficiency of.



A. Retinol
B. Retinal
C. Retinoic acid
D. Vitamin D

Explanation
- It is phrynoderma or follicular hyperkeratosis.
- It is caused by the deficiency of vitamin A (13 cis retinoic acid)

43 FAT SOLUBLE VITAMINS PART-2

Vitamin D

Bioactivation of Vitamin D

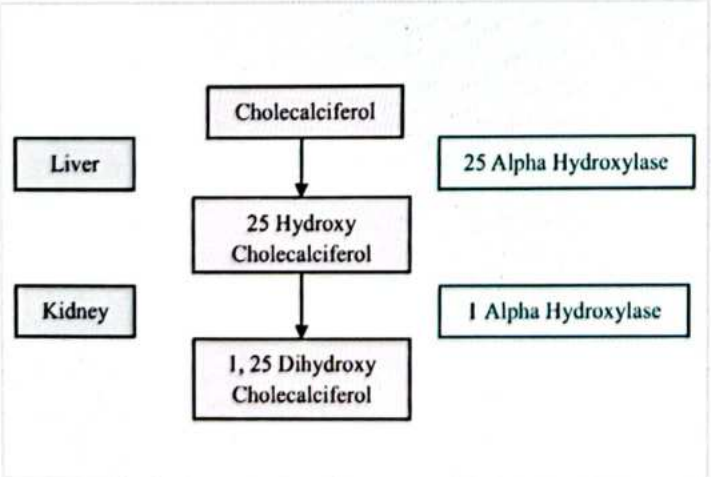
- Vitamin D is endogenously produced and synthesized by the action of UV light.

UV rays fall on skin.
↓
7 dehydrocholesterol is present in the epidermis of skin.
↓
UV rays act on the 7 dehydrocholesterol
↓
Open a ring of 7DHC.
↓
Converts 7DHC to cholecalciferol

- The endogenous Vitamin D is inactive.
- The active form of Vitamin D is cholecalciferol.
  - Not a true steroid.
  - It is a secosteroid - because one ring is opened.
    → Steroids have all the 4 rings intact.

Cholecalciferol enters the circulation.
↓
Enters the liver - contains 25 alpha hydroxylases.
↓
Converts to 25 hydroxy cholecalciferaol
↓
This 25 Hydroxy cholecalciferaol enters the circulation again.
↓
Enters the kidney - contains 1 alpha hydroxylase.
↓
Converts to 1,25 dihydroxycalciferol

Synthesis of Active form of Vitamin D



Actions of Vitamin D

Vitamin D play its role in 3 tissues:

- Intestine
- Bone
- Kidney

Intestine

- Calcium is absorbed by enterocytes of the intestine by active transport.
- Vitamin D increases calbindin expression. Therefore, increasing calcium uptake.

Bone

- Acts directly on osteoblasts and activates it.
- Helps in mineralization of the Bone.
- Activates osteoclasts indirectly.
  - Increases serum calcium and phosphate levels.

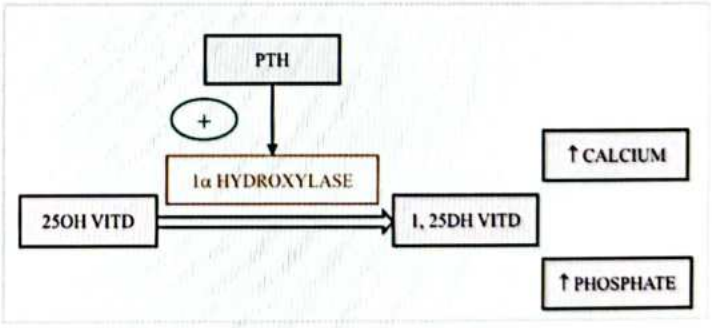
Important Information

- When osteoblasts are activated the osteoclasts also get activated.
- This is due to rank ligand activation.

Kidney

- Increases calcium and phosphate reabsorption.
- Opposite action of parathyroid hormone.
  - Increase calcium reabsorption and inhibits phosphate reabsorption.
- Net effect of Vitamin D: Increases serum calcium and serum phosphate.

Regulation of Vitamin D Synthesis



- **Rate Limiting step.**
  - Conversion of 25 cholecalciferol to 1,25 cholecalciferol.
  - Catalyzed by 1 alpha hydroxylase enzyme.
  - Activated by parathyroid hormone.
- The vitamin D Synthesis is regulated depending on the amount of calcium and phosphorus.

- When there is Hypercalcemia and hyperphosphatemia vitamin D should not be synthesized.

Important Information

- If more calcium and phosphorus is present in blood and more vitamin D is synthesized.
  - Leads to hypercalcemia and hyperphosphatemia.
  - Hypercalcemia leads to:
    - → Depression
    - → Kidney stones
    - → More gastrin release - gastric ulcers
    - → Constipation

- Hyperphosphatemia inhibits the 1 alpha hydroxylase.
- Hypercalcemia does not inhibit 1 alpha hydroxylase directly.
  - Inhibits parathyroid hormone.

Calcium sensing receptors are present in parathyroid follicles.

↓

Senses the serum calcium levels.

↓

If Hypercalcemia is present

↓

Suppresses parathyroid hormone

↓

1 alpha hydroxylase is not stimulated

↓

1, 25 dihydroxy cholecalciferol can't be synthesized

- 1,25 dihydroxy cholecalciferol inhibits 1 alpha hydroxylase as a feedback mechanism.
- Therefore, regulatory factors are:
  - Hypercalcemia
  - Hyperphosphatemia

Biochemical Features of Vitamin D Deficiency

- **Vitamin deficiency causes:**
  - Hypocalcemia
  - Hypophosphatemia

Less vitamin D

↓

Causes Hypocalcemia and hypophosphatemia.

↓

Hypocalcemia activates PTH - Secondary hyperparathyroidism.

↓

Leads to increase in calcium reabsorption and phosphate secretion.

↓

Causes calcium levels to become normal.

↓

Phosphate secretion further decreases the phosphate levels

- Normal calcium
- Hypophosphatemia
- High PTH
- High ALP
  - Because PTH stimulates osteoclasts and osteoblasts.

Important Information

- **Primary hyperparathyroidism** - Caused due to direct damage to the gland.
  - Due to adenoma.
- **Secondary hyperparathyroidism** - Caused due to other factors.
  - Gland is normal but releases more PTH.
  - Due to:
    - → Hypocalcemia
    - → Hyperphosphatemia
- **Tertiary hyperparathyroidism** – long standing secondary hyperparathyroidism leads to tertiary hyperparathyroidism.
  - PTH is released autonomously.
  - No improvement even if underlying causes are treated.

Normal Level of Vitamin D:

- Normal Level of Vitamin D: 30 to 80 ng/ml.
- Estimated parameter - Total 25 hydroxy vitamin D.
  - Sum of 25 hydroxyvitamin D2 and D3.
    - → Functionally both are the same.

Vitamin D2	Vitamin D3
• Ergocalciferol • Artificially synthesized. • Double bond is present in the side chain.	• Cholecalciferol • Naturally formed.

- **1,25 dihydroxy cholecalciferol** - Has a short half-life.
  - Have short term fluctuations.
  - So not used as an estimating parameter.
- **25 hydroxy vitamin D** has a longer half-life.
  - Not subjected to short term fluctuations.

Significance of 1,25 Dihydroxy Vitamin D

Used in the estimation of

- **Chronic Kidney Disease:**
  - Leads to hyperphosphatemia.
  - Also, as 1 alpha hydroxylase is present in kidneys it is also inhibited.
  - This indicates - low levels of 1,25 dihydroxy cholecalciferol.
  - Hence, measuring its levels is helpful rather than 25 hydroxy vitamin D. Leading to:
    - → Hypocalcemia - activates PTH.

→ Hyperphosphatemia
→ Secondary hyperparathyroidism

- **2 causes of hyperparathyroidism**
  → Vitamin D deficiency
  → Chronic kidney disease
- **The secondary hyperparathyroidism** causes - Renal osteodystrophy.
  → There may be pathological fractures.
  → Measure ALP levels to identify Secondary hyperparathyroidism,
  – If High, the result is positive.
  – Should be treated soon.
- **Treatment includes:**
  → Dialysis
  → Calci mimicking drugs - Cinacalcet and etelcalcetide.
  – Mimics calcium
  – Binds to calcium sensing receptors.
  – Inhibits PTH.

Important Information

- Care should be taken with Calci mimicking drugs.
- May lead to more PTH inhibition - Adynamic Bone disease.
  - Cause pathological fractures.
- Doses should be titrated by estimating ALP levels.
  - **High ALP** - Increase the dose - Signifies secondary hyperparathyroidism.
  - **Low ALP** - Decrease the dose - May signify adynamic bone disease.

- **Hypoparathyroidism** - Less 1,25 dihydroxy vitamin D.
- **Sarcoidosis** (Granulomatous disease) - High 1,25 dihydroxy vitamin D.

One Liners

1. Active form of Vitamin D is **1,25 dihydroxy cholecalciferol.**
2. Rate Limiting enzyme of vitamin D synthesis **1 alpha hydroxylase.**
3. 1 alpha hydroxylase is stimulated by **PTH.**
4. Normal serum vitamin D is **30 to 80 ng/ml.**

MCQs

Q. True about vitamin D action is.
A. It decreases calcium absorption along Intestine.
B. It stimulates osteoblasts indirectly.
C. It stimulates osteoclasts directly.
D. It increases serum calcium and phosphate.

Q. True about vitamin D Synthesis and regulations all except
A. 1 alpha hydroxylase is the rate Limiting enzyme.
B. 1 alpha hydroxylase is stimulated by PTH.
C. 1 alpha hydroxylase is directly inhibited by High phosphate.
D. 1 alpha hydroxylase is directly inhibited by High calcium.

Q. 1,25 Dihydroxy Vitamin D is increased in
A. Chronic kidney disease
B. Hypoparathyroidism
C. Pseudohypoparathyroidism
D. Sarcoidosis

Q. True about vitamin D deficiency is.
A. High calcium
B. High phosphorus
C. High PTH
D. Low ALT

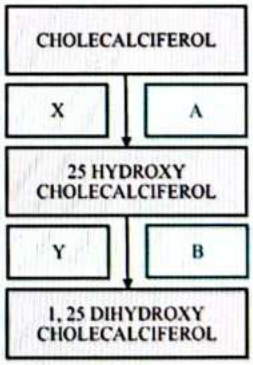
Explanation

- Vitamin D deficiency:
  - Normal calcium
  - Hypophosphatemia
  - High PTH
  - High ALP

Q. A known patient with chronic kidney disease presents with low serum calcium and high serum phosphorus. Which of the following is true?
A. His 1,25 DHCC is expected to be High.
B. His PTH is expected to be low.
C. Cinacalcet is recommended.
D. His ALP is expected to be low.

Image Based MCQ

Q. X, Y, A and B respectively are



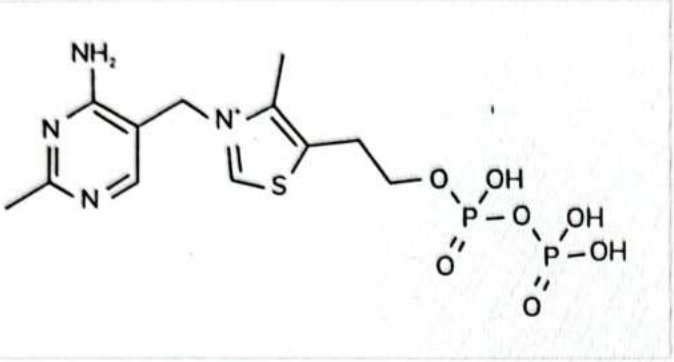
A. 25 alpha hydroxylase, 1 alpha hydroxylase, liver, and kidney
B. 25 beta hydroxylase, 1 beta hydroxylase, liver and kidney
C. 25 beta hydroxylase, 1 beta hydroxylase, kidney and liver
D. 25 beta hydroxylase, 1 beta hydroxylase, kidney and liver

44 WATER SOLUBLE VITAMINS

Water Soluble Vitamins

- Vitamin B Complex are involved in the core metabolic pathways.

Thiamine - Vitamin B1



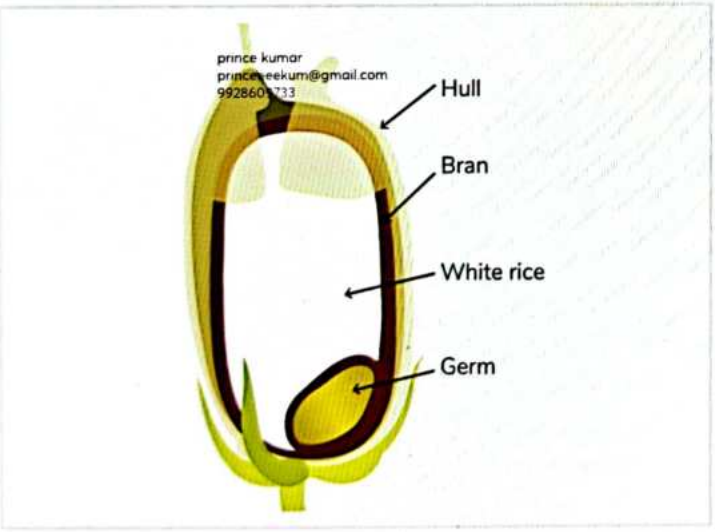
- It is a sulphur containing vitamin.

Important Information

- There are three sulphur containing vitamins are present;
  - Thiamine
  - Biotin
  - Lipoamide

- It is present in the aleurone layer of the grain.

Aleurone Layer



- Polishing these grains can cause the removal of aleurone layers and so does the thiamine.
- Parboiling of grains, attaches the aleurone layer to the grain, making it intact during polishing.

Biochemical Role

It is important in two biochemical reactions.

- **Oxidative decarboxylation**
  - PDH Complex
    - → Significance of PDH Complex: It converts pyruvate (From Glucose) into acetyl CoA, which in turn is involved in the CAA cycle and releases it as carbon dioxide.
    - → It is necessary for oxidative utilisation of glucose.
    - → If thiamine deficiency - Lactic acidosis.
      - The Pyruvates has two fates, either it gets converted into acetyl CoA or lactate upon action by PDH or Lactate dehydrogenase.
      - In thiamine deficiency, PDH is blocked.
      - Thus, pyruvate gets converted into lactic acid.
      - Lactic acid accumulation causes lactic acidosis.
  - **Alpha KGDH**
  - **Branched chain ketoacid dehydrogenase.**
    - → It is involved in branch chained amino acid metabolism.
    - → Thiamine Deficiency - MSUD.

Important Information

- Mitral valve prolapse has late systolic murmur
- Chronic mitral regurgitation has Pan systolic murmur
- Acute mitral regurgitation has early systolic murmur

  - **Transketolase**
    - → They're involved in HMP Shunt, which utilises glucose.
    - → Thiamine Deficiency - Estimate RBC transketolase activity.

RDA

- It is dependent on calorie or carbohydrates intake.
- 0.5 mg/1000 calories.

Beri-Beri

- Dry Beri-Beri - CNS involvement.
  - Neurons use glucose for energy derivation.
  - Neurons suffer due to the thiamine deficiency.
- Wet Beri-Beri - CVS involvement.

Alcoholism and Thiamine

- Alcohol is metabolised by Alcohol Dehydrogenase and forms one aldehyde.
- Aldehyde is converted into an acid via Aldehyde Dehydrogenase.
- Both the enzymes remove one H-Atom from their substrate and add it to NAD (Coenzyme) to form NADH.
- It signifies, alcohol can be demonstrated as ↑NADH/NAD ratio and ↑ATP/ADP ratio.
- This causes energy status to become higher, which decreases the appetite of the person.
- This causes that person to miss the mixed balanced diet.
- It can cause essential micronutrients deficiencies.
- Thus, alcoholism is the source of "Empty Calorie."

Important Information

- **Zero calorie** - No calories.
- **Empty calorie** - Excess calories are generated, but these calories are not fortified with vitamins.
  - **No alcohol** like vodka, gin are not fortified with vitamins.

- Thiamine deficiency is the first seen micronutrient deficiency in chronic alcoholism, as alcohol interferes with thiamine absorption.
  - **Reasons**
    - → High energy status.
    - → Missed mixed balance diet.
    - → Essential micronutrient deficiencies.
- Even thiamine supplements are taken without a stop on alcohol consumption there will be no change in the symptoms as the alcohol interferes with the thiamine absorption.
- **Magnesium malabsorption** - Hypomagnesemia.
  - Any thiamine in the system can't be transformed into thiamine pyrophosphate (Coenzyme Form of Thiamine).

Thiamine manifestations in chronic alcoholism

- **Wenicke's encephalopathy** - Acute thiamine deficiency.
  - **Manifestations:**
    - → **G** - Global Confusion.
    - → **O** - Opthalmoplegia.
    - → **A** - Ataxia.

Important Information

- In thiamine deficiency, the most affected parts of the brain are:
  - **Mammillary body.**
    - → It is a part of papez circuit.
    - → It is related to processing information.
    - → If this gets affected, then the person may present with global confusion.
  - **3, 4, 6 cranial nerves.**
    - → If it gets affected, it causes ophthalmoplegia.
  - **Cerebellar neurons.**
    - → If it gets affected, it presents ataxia.

- **Korsakoff syndrome** - Chronic thiamine deficiency.
  - **Manifestations:**
    - → Amnesia
      - Retrograde amnesia
      - Anterograde amnesia.
      - Confabulation.
        - Fabrication of stories to fill up the memory gaps.
      - Sensory agnosia.

Case

- A woman who is a chronic alcoholic with Korsakoff syndrome shows her daughter in the photo and asks her who this is, the woman might say that she is her sister's daughter.
- It is because the woman has forgotten the fact that she gave birth to her daughter, this is confabulation.

Important Information

- Mammillary body and cerebellum undergo atrophy (Irreversible Damage).
  - Mammillary body.
    - →If damaged - Poor processing of information and memory.

Summary

- It is sulphur containing vitamins.
- It is present in the aleurone layer of grains, so parboiling is important before polishing.
- Thiamine deficiency causes lactic acidosis.
- If neurons get affected or are called dry Beri-Beri.
- If CNS is involved, it is called wet Beri-Beri.
- Alcoholism with thiamine deficiency, it can cause:
  - Wernicke's encephalopathy - Acute thiamine deficiency.
    - → Manifestations:
      - G - Global Confusion.
      - O - Ophthalmoplegia.
      - A - Ataxia.
  - Korsakoff syndrome - Chronic thiamine deficiency.
- **RDA**
  - It is dependent on calorie or carbohydrates intake.
  - 0.5 mg/1000 calories.
- If suspect thiamine deficiency, estimation of RBC transketolase activity.

Riboflavin - Vitamin B2

- It has a carbohydrate, ribitol in its structure.

Coenzyme Role

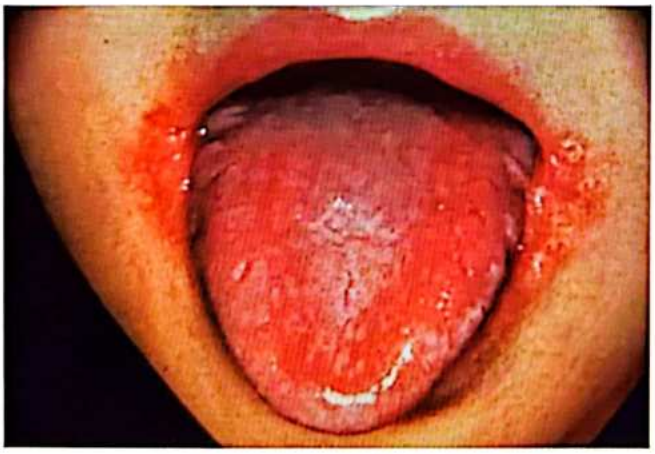
- Coenzyme role of riboflavin is in the form of:
  - FMN
  - FAD

Biochemical Role

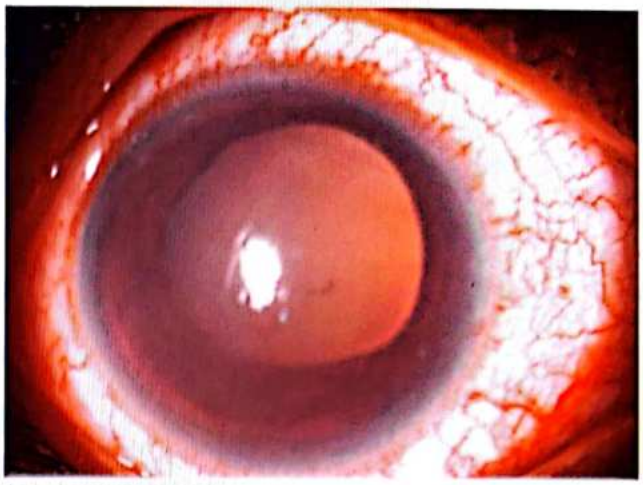
- **ETC**
  - Both FMN and FAD are used.
- **Carbohydrate metabolism**
  - PDH uses FAD as its coenzyme.
    - → It is an enzyme complex and has 3 subunits and 5 coenzymes.
    - → 5 coenzymes:
      - TPP
      - Lipoamide
      - CoA
      - FAD (Involved in aerobic form of glucose)
      - NAD
- **Lipid metabolism**
  - Acyl CoA dehydrogenase - 1st enzyme for fatty acid oxidation.
    - → It uses FAD as its coenzyme.
- **Amino acid metabolism**
  - L-Amino acid oxidase - It uses FAD as its coenzyme.
- **Citric acid cycle**
  - Succinate Dehydrogenase - It uses the FAD.

Deficiency Manifestations

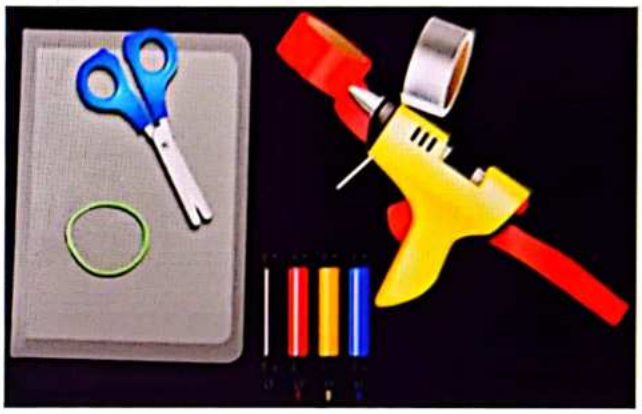
- Riboflavin is involved in many metabolic pathways and is necessary for rapidly dividing labile cells, thus epithelium gets affected.



- **Example:** Angular stomatitis.
- **1st sign of riboflavin deficiency** - Circumcorneal congestion.



- For Vitamin B2 deficiency - RBC or erythrocyte glutathione reductase activity should be checked.



- **Trick** - Ribbon and Glue go hand in hand in artwork.
  - Ribbon - RBC.
  - Glue - Glutathione reductase activity.

Niacin or B3

- It can be synthesised via metabolic pathways.
- 60 mg of tryptophan = 1 mg of niacin.

Synthesis of Niacin

Step-01: Tryptophan → FormylKynurenine

- Catalysed by Tryptophan pyrrolase.

Step-02: FormylKynurenine → Kynurenine

- Catalysed by Formyltransferase
  - Converts Tetrahydrofolate to formyl Tetrahydrofolate.
  - It enters One carbon pool and is used to form C2 of purine rings.

Step-03: Kynurenine → 3-OH Kynurenine

- Catalysed by Hydroxylase.

Step-04: 3-OH Kynenurine → 3-OH Anthranilic acid

- It utilises pyridoxal phosphate.
- Catalysed by Kynenurinase and dependent on B6 or Pyridoxal phosphate.
- B6 is necessary for
  - All transaminases.
  - Most of the Decarboxylases.
  - Kynenurinase.

Step-05: 3-OH Antitranilic acid → Quinolinate

- 3-OH Antitranilic acid undergoes a series of non-enzyme catalase reactions to form a ring (Quinolinate).

Step-06: Quinolinate → Niacin Mono Nucleotide

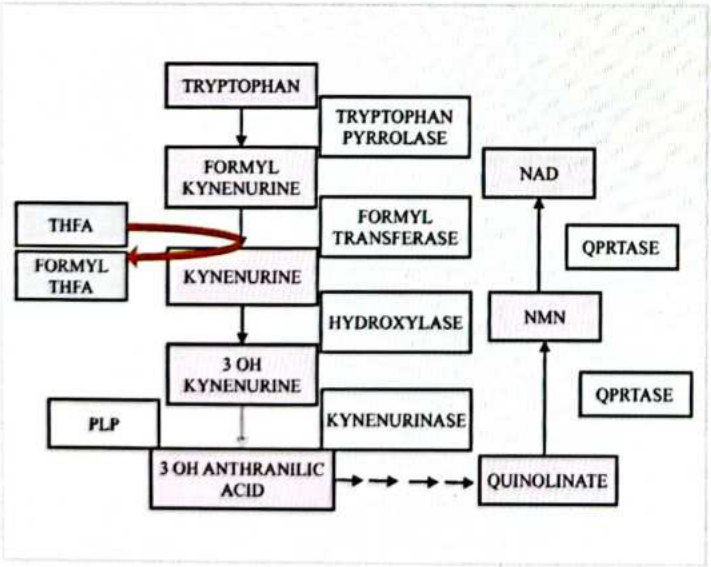
- Rings get attached to Ribose 5 Phosphate, and form nucleotides
  - Examples
    - → Adenine + Ribose 5 Phosphate = AMP.
    - → Guanine + Ribose 5 Phosphate = GMP.
- Quinolinate phosphoribosyl transferase (QPRTASE) is involved in the reaction.
  - **Role:** It converts the ring form (In step 6 - Quinolinate) to nucleotide by adding ribose 5 phosphate.

Step-07: Niacin Mono Nucleotide → Niacin Adenine Dinucleotide

- Another phosphoribosyl transferase that acts on NAD to form adds R5P.
- Niacin mono nucleotide is the first coenzyme form of niacin.

Step-08: NAD → NADP

- Kinase acts on the NAD to convert it into NADP.



Important Information

- The precursor of niacin is tryptophan.

Pellagra

- Deficiency of Niacin causes pellagra.
- Characterised by 3 D's;
  - Diarrhoea
  - Dementia
  - Dermatitis (Photosensitivity).
    - → Castles necklace.

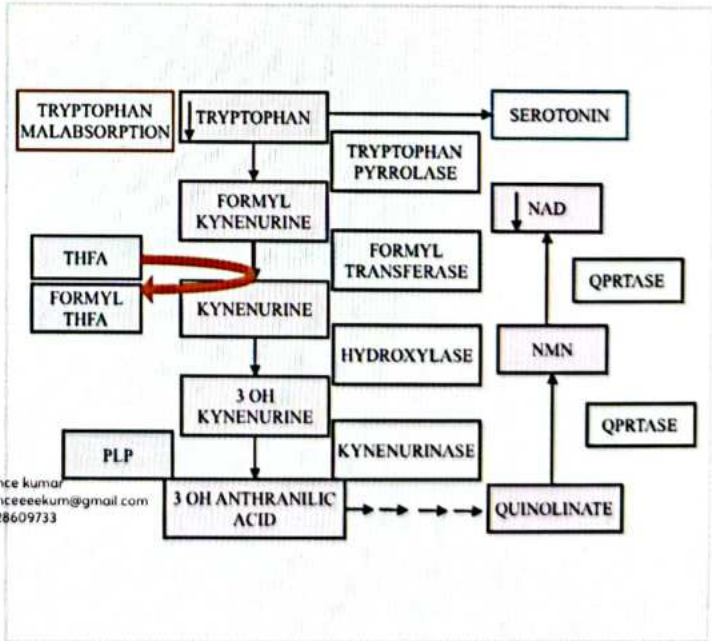
Causes of Pellagra

Diet with;

- Niacin deficiency.
- Tryptophan deficiency in the maize-based diet.
  - Farmers cultivate many crops like rice, wheat, and maize, but the economic value of rice and wheat is high.
  - They sell off the rice and wheat and consume only maize.
- Tryptophan malabsorption - Hartnup's disease.
  - It is caused by the malabsorption of tryptophan in the intestine.

Hartnup's Disease (Blue Diaper Syndrome)

- Tryptophan malabsorption syndrome.
- It is caused by the defect of neutral amino acid transporter specific for tryptophan.
- It is responsible for both.
  - Absorption along the intestine, if not:
    - → Hartnup's disease.
    - → Niacin deficiency.
  - Reabsorption from the renal tubule:
    - → It causes amino aciduria.
    - → As the tryptophan is present in urine and exposed to air, then the indole ring of tryptophan will get oxidised to form ketol compounds and produce blue colour.
    - → Thus, causes blue discoloration of urine - Blue Diaper Syndrome.
    - → produce blue colour.

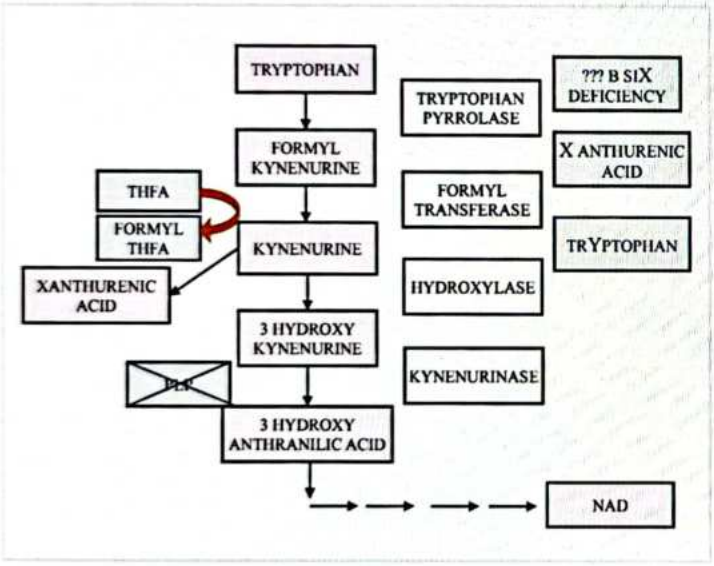


- **In carcinoid syndrome.**
  - Tryptophan is predominantly used to form Serotonin (5 hydroxytryptamine).
  - Thus, tryptophan is not available for niacin synthesis.
  - Niacin deficiency.
- **Vitamin B6 deficiency:**
  - It inactivates Kynenurinase.
  - Thus, the precursor tryptophan cannot be used in niacin synthesis.
- **Leucine pellagra:**
  - Leucine inhibits Quinolinate phosphoribosyltransferase (QPRTASE).
  - In a leucine rich diet, for example sorghum diet.

Causes of Pellagra

- Niacin deficiency in the diet.
- Tryptophan deficiency in the diet - Maize diet.
- Tryptophan malabsorption - Hartnup's disease.
- Carcinoid syndrome
- B6 deficiency
- Leucine pellagra - Sorghum based diet.

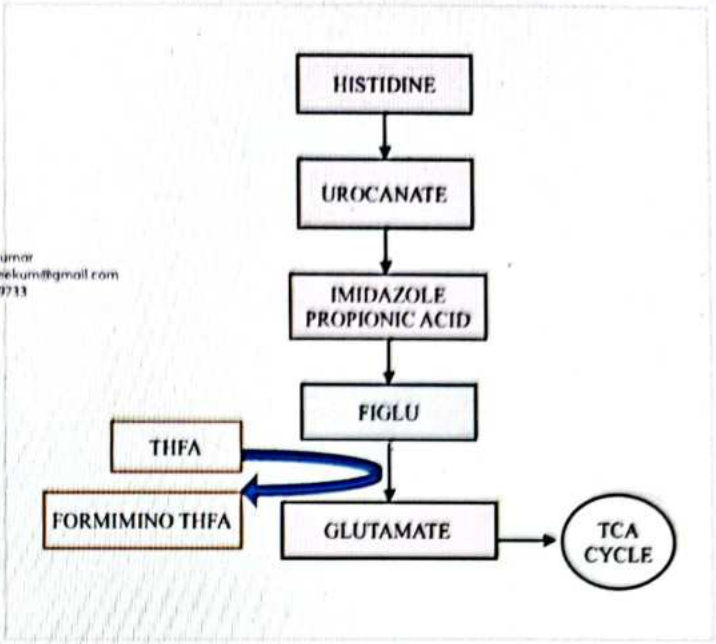
Fact - B6 Deficiency Detection



- In B6 deficiency, the kynenurinase enzyme becomes inactive.
- The precursor kynurenine gets converted into Xanthurenic acid.
- After tryptophan load, estimate the xanthurenic acid in urine.

Folate Deficiency Detection

- Histidine gets metabolised to form FIGLU (Formiminoglutamic acid).



- **In general:**
  - FIGLU will transfer its imino group to THFA, thus converting THFA into FITHFA.
  - FITHFA enters 1 carbon pool.
  - FIGLU becomes glutamate.
  - Glutamate converts into alpha ketoglutarate and enters the CAA cycle.

- **In folate deficiency:**
  - After histidine load, high FIGLU levels in urine is seen as it cannot transfer its imino group to THFA.

Vitamin B6

- Its coenzyme form is pyridoxal phosphate.

Vitamin B6 - Coenzyme Roles

- It is necessary for the activity of:
  - Glycogen phosphorylase
    → McArdle's disease - Type 5 GSD.
    → Muscle glycogen phosphorylase enzyme is defective.
    → It responds to B6 administration.
  - Transaminases
  - Decarboxylases
    → Examples:
      - Glutamate decarboxylases
  - Kynenurinase
    → It involves the conversion of tryptophan to niacin.
  - Cystathionine beta synthase
    → It helps in the conversion of homocysteine to cysteine.

Important Information

- Carboxylases need biotin.

Glutamate Decarboxylase

- It converts glutamate into GABA.
- The decarboxylation reaction is catalysed by glutamate decarboxylase which is dependent on PLP.
- Alpha carboxyl group is removed in the form of carbon dioxide.
- Thus, forming the gamma amino butyric acid, an inhibitory neurotransmitter.
- Structure:
  - Every amino acid has an alpha carbon atom which is attached to the amino group and carbohydrate group.
  - Propionic acid (With gamma and beta carbon atoms) gets attached to form the glutamate.
- In B6 deficiency, glutamate gets accumulated making the person GABA deficient.
- Low levels of excretory neurotransmitters and high levels of inhibitory neurotransmitters, thus causes seizures.
- Seizures respond to B6 administration.
- **Case** - If a neonate presents with seizures.
  - The most common causes:
    → Hypoglycemia
    → Hypocalcemia.
- To treat this condition calcium glutamate is given.
- If the seizures are not getting decreased, before any investigations on the causes of seizure, the seizure activity should be brought down.
- A B6 shot can be given in the IM route.
- Any glutamate present in the muscle gets converted into the GABA.
- Thus, reducing excitatory neurotransmitter levels and increasing inhibitory neurotransmitter levels.

ALA Synthase (heme synthesis)

- 1st step of heme synthesis - Decarboxylation reaction which needs pyridoxal phosphate.
- Glycine reacts with succinyl CoA in the presence of delta ALA synthase to form delta ALA.
- In pyridoxal phosphate deficiency;
  - Heme will not be synthesised, thus it causes anaemia.
  - Anaemia responds to B6 administration.

Important Information

Clinical conditions that respond to B6 administration:

- McArdle's disease
- Seizures
- Anaemia

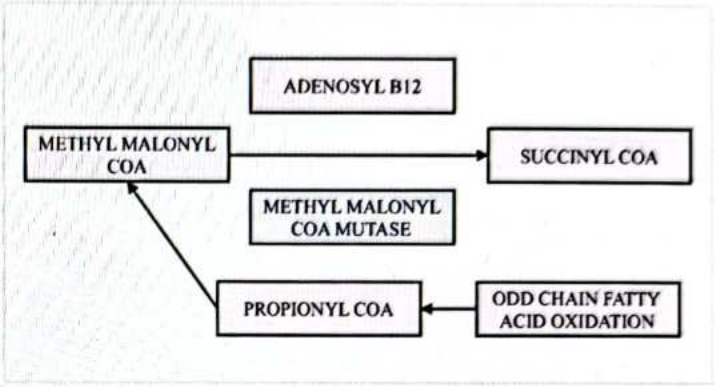
- Pellagra
  - Kynenurinase is dependent on B6.
- Homocystinuria
  - Cystathionine beta synthase, B6 dependent, converting homocysteine into cysteine.

Vitamin B12

- It has got 2 coenzyme roles:
  - Methyl B12
  - Adenosyl B12

Adenosyl B12

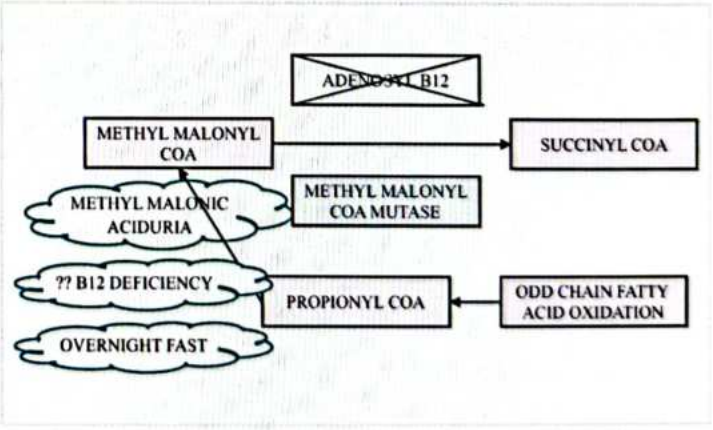
- It acts as a coenzyme for methyl malonyl CoA mutase.



In odd chain fatty acid oxidation:

- Propionyl CoA → Methyl malonyl CoA.
- Methyl malonyl CoA → Succinyl CoA.
  - It is catalysed by methyl malonyl CoA mutase, dependent on adenosyl B12.
- The succinyl CoA is involved in the CAA cycle and gets converted into carbon dioxide.
  If any defect on the metabolism can cause the manifestations of accumulation of the respective substrates.

In B12 deficiency:



- Adenosyl B12 will not be available.
- Methyl malonyl CoA mutase becomes inactive.
- It causes methyl malonic aciduria.

Investigation of B12 deficiency

- To rule out B12 deficiency, urine levels of methyl malonic acid are estimated after overnight fasting.
- **Reason:** Peripheral lipolysis occurs which pulls the odd chain fatty acids to the liver, which ultimately metabolises into the methyl malonyl CoA.
- The methyl malonic acid gets incorporated into myelin, which causes formation of abnormal myelin, in due course neurons undergo demyelination.
- Initially patients present with paraesthesia (Tingling sensation) in the periphery.
- In severe cases, it forms the SACD (Subacute combined degeneration).
  - **Presents with:**
    - → Sensory neuropathy
    - → Motor neuropathy
      - Cranial nerve involvement.

Suspected Vitamin Deficiency	Investigations	LOAD
Vitamin B6	Xanthurenic acid in urine.	After tryptophan load.
Folate	FIGLU levels in urine.	After histidine load
Vitamin B12	Methyl malonic acid in urine.	After overnight fast.

Methyl B12

- It is a coenzyme for methionine synthase or homocysteine methyltransferase.
- It helps in the conversion of Homocysteine to Methionine.
- **Homocysteine has two fates:**
  - It can be converted into cysteine upon action by cystathionine beta synthase.
  - It can be converted into methionine, upon action by methionine synthase.
- Methyl THFA donates its methyl group to be methyl B12.
- The THFA form is recycled for other THFA purposes.

In B12 deficiency:

- Methyl THFA cannot donate its methyl group and is trapped as such.
- This causes the functional deficiency of methyl THFA and is called Methyl Folate Trap.
- This condition occurs due to the low activity of methionine synthase or homocysteine methyltransferase.
- Indirectly B12 deficiency causes the folate deficiency, which in turn leads to macrocytic anaemia.
- The decreased activity of methionine synthase, homocysteine gets accumulated, homocystinuria.

Features of B12 Deficiency

- **Adenosyl B12 deficient.**
  - Methyl malonyl CoA mutase becomes inactive;
    - → Demyelination - Neurological
- It causes paresthesia and in severe terms SACD.
  - Methyl malonic aciduria
    - → Urine levels of methyl malonic acid are estimated after overnight fasting.
- **Methyl B12 deficient:**
  - Low functional THFA.
  - Methyl Folate Trap, it causes Macrocytic anaemia.
  - Homocystinuria.

Factor Affecting Vitamin B12 absorption

- **Non vegetarian sources:** All the vitamin B12 sources are non-vegetarian.
- **Pancreatic enzymes (Important):** Vitamin B12 is attached to a protein (Blocks B12 absorption), which can be separated upon action by pancreatic enzymes.
- **Cobalophilin receptors in terminal ileum:** Once vitamin B12 is attached to the intrinsic factor, the cobalophilin receptors accept the vitamin B12.
- **Intrinsic Factor bound Vitamin B12 (Important):** IF is released by gastric mucosa.
- **Intestinal Microorganism:** Excessive microorganisms in the colon utilise vitamin B12.
- **Terminal ileum (Important):** It should be healthy for good vitamin B12 absorption, if not it won't get absorbed.

Causes of Vitamin B12 Malabsorption

They're;

- Pancreatic insufficiency.
- IF deficiency / Atrophic gastritis / Autoimmune gastritis / Type A gastritis.
- Blind loop syndrome - Excess colonic microorganisms.
- Terminal ileum - Crohn's disease.
  - Regional ileitis.

Schilling's Test

Two purposes:

- Detects vitamin B12 malabsorption.
- Identify vitamin B12 malabsorption.

Important Information

Schilling's test doesn't diagnose the vitamin B12 deficiency.

Steps Followed in Schilling's Test

Step 01: To treat Vitamin B12 and folate deficiency.

- Both vitamins are necessary for the health of rapidly dividing labile cells in the intestinal mucosa.

- If intestinal cells are not healthy it itself results in the vitamin B12 malabsorption.
- In this case malabsorption is the effect, not the cause.

Step 02: Oral radiolabelled Vitamin B12 is provided.
- They are in measured quantities.
- Once given orally, vitamin B12 has two fates.
  - It binds with the B12 receptors in the liver.
  - If overflow of receptors in the liver occurs, then the excess is released out of the body via urine.
  - In Schilling's Test, vitamin B12 levels in the urine are estimated.
    → Before the test, all the receptors for B12 binding should get saturated.

Step 03: IM unlabelled vitamin B12 is administered to saturate all vitamin B12 receptors present in the liver.

Step 04: 24 hours urine is collected and labelled vitamin B12 is estimated.
- **Inference:** If less than 10% of orally administered Vitamin B12 is found in urine - malabsorption is confirmed.
- **Normal -** >10% of orally administered vitamin B12 should be excreted in urine, if there is no malabsorption.

Step 05: Test is repeated after oral IF.
- **Inference:** If less than 10% of orally administered Vitamin B12 is found in urine- Autoimmune gastritis is excluded.

Step 06: Test is repeated after 3 weeks of antibiotics.
- **Inference:** If less than 10% of orally administered Vitamin B12 is found in urine- Blind loop syndrome is excluded.

Step 07: Test is repeated after 2 days of pancreatic enzymes.
- **Inference 01:** If less than 10% of orally administered Vitamin B12 is found in urine- pancreatic insufficiency is excluded.
- **Inference 02:** Probably, Crohn's disease or celiac sprue.

One Liners

1. Schilling's test is used to diagnose **Vitamin B12 malabsorption**.
2. Vitamin K form used to treat Vitamin K deficiency due to fat malabsorption is **Menadione**.

Explanation
- Discussed in vitamin A, D, E, and K.
- In menadione, the polyisoprenoid side chain is absent, thus it is water soluble and not dependent on factors which are necessary for fat absorption.

3. Vitamin E RDA is dependent on **Fatty acid** intake.

Explanation
- Vitamin E is an antioxidant.
- Major source of oxidative stress is fat intake.

4. RDA of Vitamin B1 is dependent on **carbohydrate intake.**

Explanation: Thiamine is predominantly involved in aerobic utilisation of glucose.

5. Tryptophan load test is done to detect **B6 deficiency.**

6. Histidine load test is done to detect **folate deficiency.**

MCQs

Q. Thiamine deficiency is diagnosed by measuring which of the following?
A. RBC Glutathione reductase activity
B. RBC Transketolase activity
C. RBC G6PD activity
D. RBC Glutathione peroxidase activity

Important Information
- Thiamine is B1, 1 means one meal a day, a part of keto diet.
- Thus, RBC transketolase activity is estimated.

Q. Riboflavin deficiency is diagnosed by measuring which of the following?
A. RBC Glutathione reductase activity
B. RBC Transketolase activity
C. RBC G6PD activity
D. RBC Glutathione peroxidase activity

Important Information
Trick - Ribbon and Glue go hand in hand in artwork.
- Ribbon - RBC.
- Glue - Glutathione reductase activity.

Q. Methylfolate trap in Vitamin B12 deficiency is because of the inhibition of?
A. Methyl Malonyl CoA mutase
B. Methylmalonyl CoA isomerase
C. Methionine synthase
D. Cystathionine Beta synthase
Explanation: Methyl B12 acts as a coenzyme for methionine synthase or homocysteine methyltransferase.

Q. Is PLP necessary as a coenzyme for all except?
A. Transaminases
B. Glutamate decarboxylase

C. Methionine synthase
D. Cystathionine Beta synthase

Explanation

- PLP is necessary for.
  - Glycogen phosphorylase
  - Transaminases
  - Decarboxylases
    - → Examples:
      - Glutamate decarboxylases
      - ALA synthase
  - Kynenurinase
  - Cystathionine beta synthase.
- Methionine synthase is dependent on vitamin B12 and folate.

Q. All the following are PLP dependent states except?
A. Anaemia
B. Seizures
C. Pellagra
D. Albinism

Explanation

- Clinical conditions that respond to B6 administration.
  - McArdle's disease
  - Seizures
  - Anaemia
  - Pellagra
  - Homocystinuria
- Albinism - It is caused by the defect of tyrosinase which is dependent on copper.

Q. A chronic alcoholic present with an episode of hypoglycaemia. He is treated with dextrose infusion and thiamine injection. Before discharge, he is advised about effects of chronic alcoholism, and he wants to check his thiamine availability status. What is the investigation you would prescribe?
A. RBC transketolase activity
B. RBC Glutathione reductase activity
C. Urine methyl malonic acid level
D. Urine Xanthurenic acid level

Explanation

- RBC Glutathione reductase activity - For riboflavin deficiency.
- Urine methyl malonic acid level - B12.
- Urine Xanthurenic acid level - B6.

Q. A known patient of Tuberculosis is on INH and he presents with microcytic hypochromic anaemia. His serum ferritin and transferrin saturation are normal. A vitamin deficiency is suspected. Which vitamin deficiency can cause microcytic hypochromic anaemia and what is the investigation of choice for diagnosing this condition?
A. B12, methylmalonic acid in urine
B. Folate, FIGLU level in urine
C. B2, Glutathione reductase activity in RBCs
D. PLP, Xanthurenic acid in urine

Explanation

- In a patient with microcytic hypochromic anaemia, the first suspect should be iron deficiency.
- If serum ferritin and transferrin are normal, then suspect for vitamin deficiency.
- In heme synthesis, delta ALA synthase is dependent on B6.
- If B6 deficiency is suspected.
  - Xanthurenic acid levels in urine are estimated.
- **INH causes B6 deficiency**, because the active form of vitamin B6 (Pyridoxal phosphate) cannot be formed.
  - Reason: Both B6 and isoniazid is an aldehyde.
  - B6 PLP in the presence of PLK.
  - INH is a **competitive inhibitor of PLK**.
  - Thus the active form of vitamin B6 has not formed.

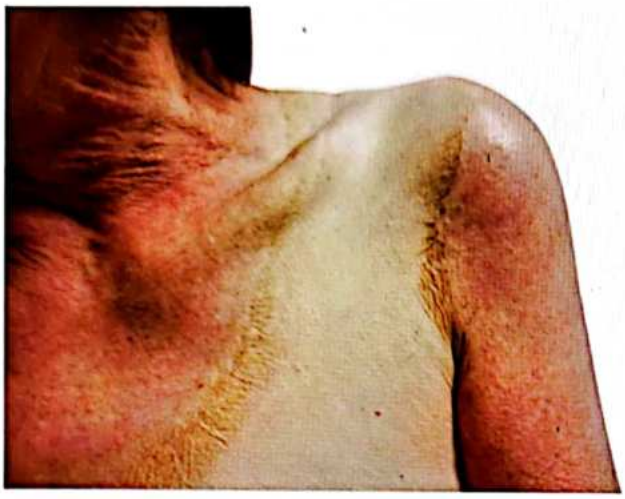
Q. A person, who is on a mixed balanced diet, presents with tiredness, weakness, macrocytic anaemia and subacute combined degeneration. He was given radiolabelled B12 orally and then intramuscular unlabelled B12 was injected. 24 hours urinary radiolabelled 812 was less than 10% of oral administered dose. After oral IF, 24 hours urinary labelled B12 was more than 10% oral administered dose. Which of the following is the probable cause?
A. Dietary B12 deficiency
B. Methyl Folate trap
C. Pancreatic insufficiency
D. Type A gastritis

Explanation

- If given SACD, think of vitamin B12 deficiency.
- Schilling's test is being done.
- Vitamin B12 deficiency is most commonly caused by nutritional causes.
  - In case, if a person is vegan or vegetarian.
- In this case, the person is on a mixed balanced diet still presenting vitamin B12 deficiency, probably B12 malabsorption is the suspicion.
- If it is confirmed, supplement IF.
- The IF administration has treated the condition in this case.
- Thus, the diagnosis is Type A gastritis or autoimmune gastritis, where the parietal cells get damaged.

Image Based MCQs

Q. A 65-year-old man with alcohol use disorder, presented with insomnia, irritability and confusion. His son attributed the confusion to a probable dehydration caused by diarrhoea. On examination, there were erythematous non itchy skin lesions in the sun exposed areas as shown in the image. The probable cause of confusion is



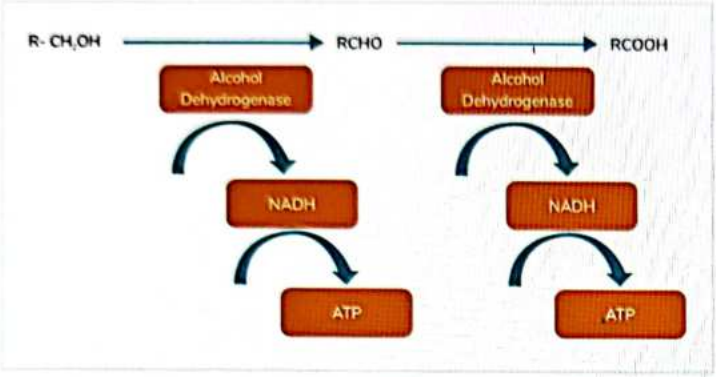
A. Wernicke's encephalopathy
B. Delirium tremens
C. Korsakoff syndrome
D. Pellagra

Explanation

- There is dermatitis, dementia, and diarrhoea.
- Thus 3D's - Pellagra.
- Wernicke's encephalopathy is also associated with confusion and also.
  - **G** - Global Confusion.
  - **O** - Ophthalmoplegia.
  - **A** - Ataxia.
- Alcoholism can present with multiple micronutrients deficiencies, as the person is missing a mixed balanced diet.
- Alcohol interferes with vitamin absorption. Like thiamine and many others.
- Delirium tremens is a manifestation of alcohol withdrawal, wherein presents with economic hyperactivity, that means:
  - High blood pressure,
  - High heart rate,
  - Tremors,
  - High body temperature.
- Korsakoff syndrome - Chronic thiamine deficiency.
  - It presents with confabulation.
  - Predominantly, psychological manifestation (Psychosomatic).

45 ALCOHOL METABOLISM

- **Alcohol:** RCH2OH
- Alcohol gets converted to aldehyde in the body - by Alcohol dehydrogenase.
- Aldehyde gets converted to acid - by Aldehyde dehydrogenase.
- Both enzymes remove hydrogen from Alcohol and aldehyde.
- Adds the removed hydrogen to its coenzyme (NAD).
- NAD + H gives NADH.



Important Information

- Metabolic status of chronic alcoholic: High NADH and NAD ratio.

Other Features of Chronic Alcoholism 00:02:00

- High energy - suppresses hunger.
- Person misses a mixed balanced diet.
- Essential micronutrient deficiency.

Important Information

- Alcohol is a source of empty calories.

- More prone for thiamine deficiency due to:
  - Alcohol interferes with thiamine absorption.
  - Interferes with Magnesium absorption.

Important Information

Relation between Hypomagnesemia and Thiamine deficiency

- Thiamine works in its active form called thiamine pyrophosphate (TPP)
- To convert to TPP it requires thiamine kinase and this enzyme needs Magnesium.
- If Magnesium is less thiamine doesn't convert to TPP.
- Leading to thiamine deficiency.

Thiamine Deficiency and Neurons 00:04:31

- Nerves use glucose aerobically.

Important Information

Glucose
↓
converts to pyruvate.
↓
Uses PDH and converts to Acetyl CoA
↓
Enters citric acid cycle.
↓
Releases carbon dioxide

- PDH requires thiamine to work.
- Therefore, thiamine deficiency affects Neurons more.
- Thiamine deficiency presents as Wernicke's encephalopathy in acute thiamine deficiency.
- **Nerves more susceptible to thiamine deficiency**
  - Mammillary body - causes global confusion.
  - 3,4,6 cranial nerves - causes ophthalmoplegia.
  - Cerebellar neurons - causes ataxia.
  - Mnemonic: GOA.

Features of Chronic Thiamine Deficiency 00:06:52

- Presents as Korsakoff syndrome.
- **Characterized by**
  - Amnesia (retrograde and anterograde).
  - Confabulation.
  - Sensory agnosia.

Chronic Alcoholism and B12 Deficiency 00:08:12

- Tetrahydrofolate (THFA): Important in purine and pyrimidine synthesis.
- B12 and folate deficiency inhibits purine and pyrimidine synthesis.
- Inhibits replication.
- No cell division but cell growth occurs.
- Leading to macrocytic anemia.

Chronic Alcoholism and Hypoglycemia 00:09:04

- Hypoglycemia because of High NADH/NAD ratio.
- Conversion of more pyruvate to lactate - with the help of NADH.
- Conversion of more OAA to malate - with the help of NADH.
- Decreases pyruvate and OAA levels (first intermediates in gluconeogenesis).

- Leading to impaired gluconeogenesis.
- Finally, leads to Hypoglycemia.

Hypoglycemia and Wernicke's Encephalopathy 00:11:00

What is one of the causes of Wernicke's encephalopathy in chronic alcoholics?

- Treating the chronic alcoholic patient with hypoglycemia using Dextrose.

Important Information

To treat chronic alcoholic patient with hypoglycemia
↓
Dextrose is administered (contains D-glucose)
↓
Converts glucose to pyruvate.
↓
To utilize pyruvate PDH is needed.
↓
PDH needs thiamine to perform its function.
↓
Utilizes all the leftover thiamine in the body.
↓
Precipitated Wernicke's encephalopathy

How to avoid Wernicke's encephalopathy in chronic alcoholic patients with hypoglycemia?

- Before treating with direct Dextrose
  - Get the personal history of the patient.
  - If alcoholic - give thiamine injection.
  - Then start Dextrose infusion.

Chronic Alcoholism and Fatty Liver 00:12:58

- Fat accumulation in the liver i.e., accumulation of triacylglycerols and cholesterol esters in the liver.
- Anabolism leads to Fatty Liver - High NADH/NAD ratio and high ATP/ADP ratio.
- More production and deposition of triacylglycerols and cholesterol esters in the liver.

Delirium Tremens 00:14:15

- One of the features of Alcohol withdrawal.
- Presents between 2nd and 10th day of Alcohol withdrawal.

Manifestations of Delirium Tremens

- **Mental manifestations**
  - Global confusion
  - Hallucinations
  - Nightmares
  - Mnemonic: Government Hospital Nurse.
- **Physical manifestations**
  - Tremors
  - Hyperthermia
  - Tachycardia
  - Hypertension

Important Question 00:15:54

Q. What is the most common deficiency in chronic Alcoholism?
Ans. Thiamine

Q. What does acute thiamine deficiency cause?
Ans. Wernicke's encephalopathy

Q. What causes chronic thiamine deficiency?
Ans. Korsakoff syndrome

MCQs 00:16:2

Q. A chronic alcoholic's metabolic status is characterized by?
A. High NADH
B. High NAD
C. High ADP
D. Hypervitaminosis

Q. All the following are features of Chronic Alcoholism except
A. Thiamine deficiency
B. Hypoglycemia
C. Fatty Liver
D. Polycythemia

Q. A chronic alcoholic presents with hypoglycemia. The intern starts a Dextrose infusion and very soon finds that the person presents with global confusion and ophthalmoplegia. The diagnosis is.
A. **Wernicke's encephalopathy**
B. Korsakoff syndrome
C. Delirium Tremens
D. Diabetic ketoacidosis

Q. A 55 year old alcoholic was brought to the emergency department by his friends. During their usual hangout at the local bar, he had passed out and they were unable to revive him. On admission, his blood glucose was 55 mg/dL. Which of the following is the explanation for hypoglycemia in him?
A. Thiamine Deficiency
B. Low NAD
C. Low pyruvate
D. High oxaloacetate

Q. A 55 year old alcoholic was brought to the emergency department by his friends. During their usual hangout at the local bar, he had passed out and they were unable to revive him. On admission, his blood glucose was 55 mg/dL. Which of the following proteins would have no significant activity in this patient?

A. Glucokinase
B. GLUT1 transporter
C. Glycogen phosphorylase
D. Hexokinase

Q. Hooch Tragedy is related to which of the following alcohols?

A. Methanol
B. Ethanol
C. Ethylene glycol
D. Hypoglycin

Hooch Tragedy

00:21:54

- Spurious Alcohol Tragedy.
- Related to methanol.

Methanol
↓
Methanol Converts to formaldehyde by Alcohol dehydrogenase.
↓
Formaldehyde converts to formic acid by Aldehyde dehydrogenase (toxic to neurons e.g., optic nerve damage)

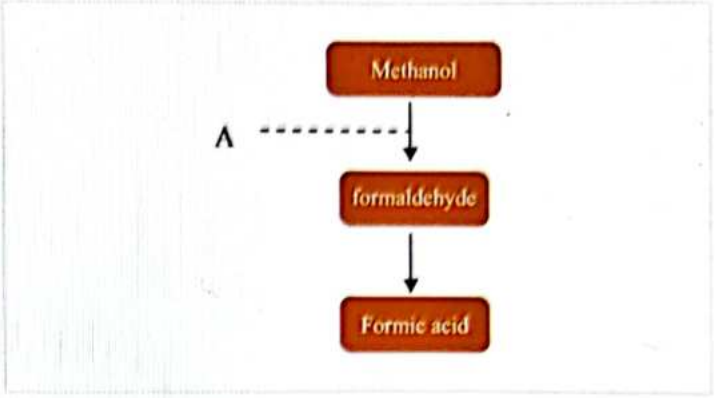
- **Formic acid leads to**
  - Inhibition of ETC. (particularly inhibits cytochrome C oxidase)
  - Leads to histotoxic hypoxia.
- **This methanol poisoning can be treated with**
  - Ethanol: Competitive inhibitor of methanol
  - Fomepizole: Inhibits Alcohol dehydrogenase

Image Based MCQs

00:25:13

Q. Identify the drug A which inhibits the step depicted in image.

00:25:14



A. Fomepizole
B. Salicylate
C. Disulfiram
D. Acetaldehyde