Structured Notes According to

BIOCHEMISTRY

Revision friendly **Fully** Colored **Book/Structured Notes**

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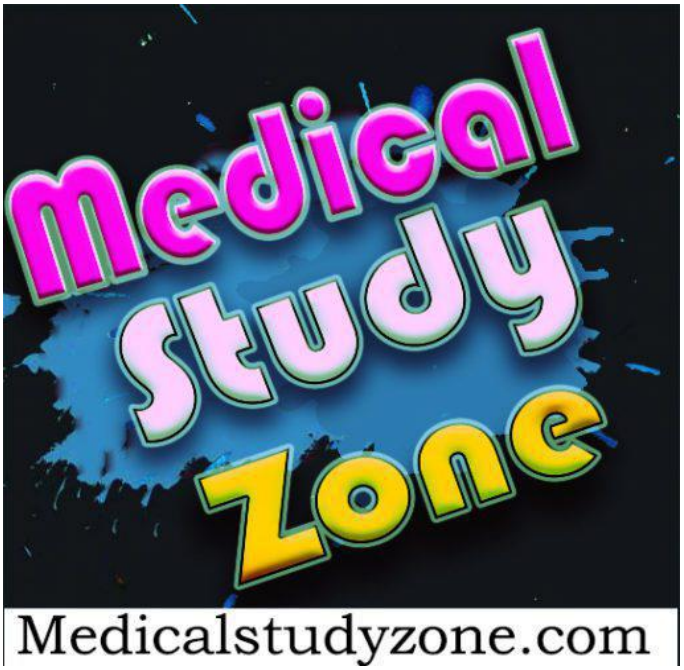


CONTENTS

BIOCHEMISTRY

LIST OF IMPORTANT TOPICS

MOST IMPORTANT: CYCLES

Multiple revisions must be given; to be studied with pediatrics inborn errors of metabolism; understand the step of defect of various disorders with absent enzyme and resultant substrate accumulation resulting in disease

METABOLISM

- Glycolysis
- Gluconeogenesis
- Krebs cycle
- ETC
- Glycogen Metabolism
- Fatty acid synthesis and Oxidation
- Purine and pyrimidine metabolism
- Lipoproteins
- Carbohydrate isomerism
- Energetics of all pathways
- GLUTS
- Ubiquitine proteasome pathway
- Polarity of amino acids
- 21st amino acid

GENETICS: To be done with Pathology and genetic disorders of Pediatrics

- Karyotyping
- PCR and types, esp. RT-PCR
- FISH
- Microarray, CGH
- Epigenetics
- Flow cytometry

VITAMINS AND ESSENTIAL FATTY ACIDS: Function, Deficiency

PROTEIN STRUCTURE, COLLAGEN STRUCTURE

DNA REPLICATION, TRANSCRIPTION, TRANSLATION

ENZYMES: Classification, Kinetics, Isozymes

LEARNING OBJECTIVES

UNIT I: CONCEPTS

- The first unit deals with explaining the basic concepts in biochemistry. Chapter 1 "Four formulas" contain unique "Smile formulas" designed to understand and solve questions related to various metabolic pathways, their categorization as catabolic and anabolic pathways, their hormonal regulation and rules to know their site of occurrence in the cell. The 2nd chapter "How to use formulas" gives you specific examples on use of these formulas. A short 3rd chapter give you information about secondary messengers cAMP & cGMP their functions. The next three chapters discuss about the molecules serving as fuel sources during fed, fasting and starvation condition of the body and the processes occurring in the body to maintain blood glucose which is primary energy source for vital organs of the body. A short chapter is included on molecule of central importance acetyl CoA that provide a link between carbohydrate, lipid and protein metabolism. A frequently asked metabolic disease Diabetes is discussed as a separate chapter. "Respiratory quotient" chapter discusses the RQ values of various biomolecules and how the body metabolic state affects these values.

- "Cell organelles" chapter gives a brief overview of structure and function of various cellular organelles and method to study them in the lab and biomarkers for their identification. The chapter "Basics of Carbohydrates and Enzymes" has important points on conventions used to denote oxidation-reduction reactions. It also provides a brief enzyme classification, and characteristics of important metabolic enzyme categories such as dehydrogenases, kinases, phosphorylases, phosphatases, carboxylases and decarboxylases. In the last chapter function of cellular energy storage and transformation molecules such as ATP, GTP, NADH etc. are discussed.

- **Major learning objectives**
  - To learn concepts in order to interconnect various processes on fuel metabolism
  - To learn about structure and function of various cell organelles
  - To know enzyme and metabolic terms to understand metabolism in later topics

1 FOUR FORMULAS

- Fed state (2 hrs after food intake) → Anabolism occurs

00:01:00

- Fasting state (12-18 hrs without food) } Catabolism occurs
- Starvation (1-3 days without food) }

SMILE FORMULA 1

00:04:04

- Tells about which pathway/enzyme is Anabolic and which pathway/enzyme is Catabolic?

Anabolic pathways

- Glycogenesis (Glycogen synthesis)
- HMP (Hexose Monophosphate Pathway)
  - Synthesis of NADPH and Ribose 5- P
- Fat Synthesis
  - Fatty Acid Synthesis
  - Triglyceride Synthesis
  - Cholesterol Synthesis
- Lipoprotein Lipase (LPL) enzyme

Catabolic pathways

- Glycolysis
  - Breakdown of glucose (6C) to 2 pyruvate (3C)
- Link Reaction (Pyruvate dehydrogenase reaction)
  - Pyruvate (3C) → acetyl CoA (2C)
- Glycogenolysis (glycogen breakdown)
- Oxidation of Fatty Acids
- Gluconeogenesis
  - Because it occurs in fasting state (catabolic state)
- Ketone Body Synthesis
  - Occur in starvation (catabolic state)
- Ketone Body utilization/breakdown
- Hormone Sensitive lipase (HSL) enzyme

Formula: This division is based on whether pathway occur in catabolic or anabolic state. Pathway occurring in fed state are anabolic while pathways occur in fasting/starvation are considered as catabolic.

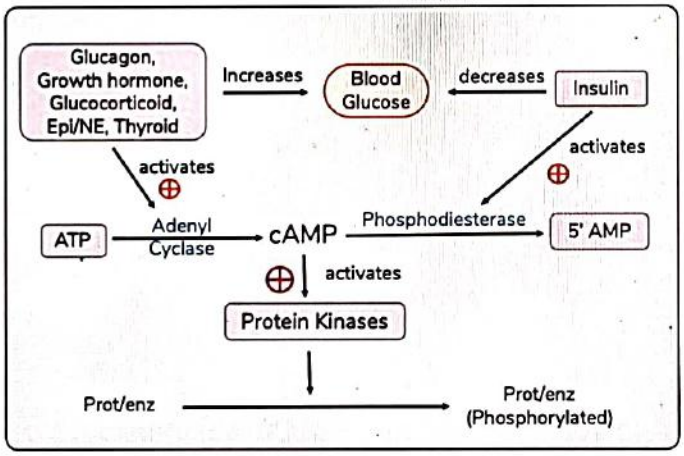
SMILE FORMULA 2

00:10:52

- Related to hormones Insulin and glucagon and their effects on pathways.
- Insulin is released in fed state to cause hypoglycemic action. So, consider insulin as anabolic hormone.
- **Formula:** Insulin activates all anabolic pathways/enzymes esp. regulatory enzymes of anabolic pathways.
  - Exception: Insulin also activates two catabolic pathway enzymes i.e. Glycolysis and Link Reaction.
- **Formula:** Glucagon activates all catabolic pathway/enzymes
  - Exception: glucagon does not activate two catabolic pathway enzymes i.e. Glycolysis and Link Reaction, as these are activated by Insulin.

Phosphorylation and dephosphorylation by cAMP

00:15:34



- cAMP causes phosphorylation

Important Information

- Why many hormones increase blood glucose while only one hormone decreases blood glucose?
  - Because hypoglycaemia is more dangerous than hyperglycaemia.

- Insulin activates phosphodiesterase so, ↓ cAMP and causes dephosphorylation
- Glucagon and epi/NE, GH, Glucocorticoid and thyroid

hormones activates adenyl cyclase so ↑ cAMP and cause phosphorylation

Previous Year's Questions

Q. Which enzyme is active during low insulin glucagon ratio? (NEET 2020)

A. Hexokinase
B. Glucokinase
C. Glucose 6 phosphatase
D. Phosphofructokinase.

Previous Year's Questions

Q. Gluconeogenesis is inhibited by? (FMGE Dec 2019)

A. Glucagon
B. Insulin
C. Cholecystokinin
D. 5 alpha hydroxylase synthase

SMILE FORMULA 3

00:21:08

- Related to Phosphorylation and Dephosphorylation states of enzymes

  Formula: Any Pathway or Enzyme which is activated by Insulin will be active in its Dephosphorylated state. It includes all anabolic pathways and glycolysis and Link rxn.

  Formula: Any pathway or Enzyme which is activated by Glucagon (Catabolic enzymes) is active in Phosphorylated state.

  →Exception: ATP Citrate Lyase that is an anabolic enzyme (activated by insulin) but it is active in phosphorylated state

SMILE FORMULA 4

00:24:55

- Tells Which pathway occurs in which compartment of the cell?

  Formula: All Anabolic Pathways occur in Cytoplasm

  Formula: All Catabolic Pathways occur in Mitochondria

  o Exp: Two catabolic pathways also occur in cytoplasm.

  1. Glycolysis
  2. Glycogenolysis

- Pathways which occur in both Mitochondria and Cytoplasm

  1. Urea cycle
  2. Haem synthesis
  3. Gluconeogenesis

- Whenever a pathway occurs in both, it starts in mitochondria and finish in cytoplasm
- TCA, ETC→ vital pathways that occur in mitochondria and do not depend on fed or fasting state

Keep in mind that there are some other pathways such as urea cycle, nucleotide, DNA, RNA, Protein and AA synthesis which are neither regulated by hormones and do not depend on fed and fasting state. So, don't use formulas for these pathways.

ADENYL KINASE

00:30:27

- It's a phosphotransferase and perform conversion of ADP to AMP and ATP

$$2\,AMP \xleftrightarrow[\text{Kinase}]{\text{Adenyl}} AMP + ATP$$

- Used for interconversion of nucleotides ADP, ATP and AMP which are required for different functions in the cell.

LIPOPROTEIN LIPASE

00:32:28

- Present in the endothelium of blood vessels
- Breaks down fats (TG) travelling in the blood to FA and Glycerol
- FA and Glycerol are absorbed into adipose tissue and get stored as TG
- As LPL helps to store fats, hence considered as anabolic enzyme
- It is activated by anabolic hormone insulin and inhibited by glucagon

HORMONE SENSITIVE LIPASE (HSL)

- Present in the adipose tissues
- Breaks down fats (TG) stored in adipose tissue to FA and Glycerol during fasting and starvation
- Released FA and Glycerol is passed into blood and used for energy by liver and other organs
- As HSL helps to break fats, hence it is considered as catabolic enzyme
- It is activated by catabolic hormone glucagon and inhibited by insulin

Previous Year's Questions

Q. Insulin inhibits which of the following lipase enzymes? (INI CET July 2021)

A. Hormone sensitive lipase
B. Lipoprotein lipase
C. Acid lipase
D. Alkaline lipase

CLINICAL QUESTIONS

Q. A 28-year-old athlete who is training for a long race was advised by his coach to increases his carbohydrates intake after the workout for building his muscle glycogen storage. Muscle glycogen synthase is the main enzyme involved in this process the activity of which is increased by the action of which of the following:

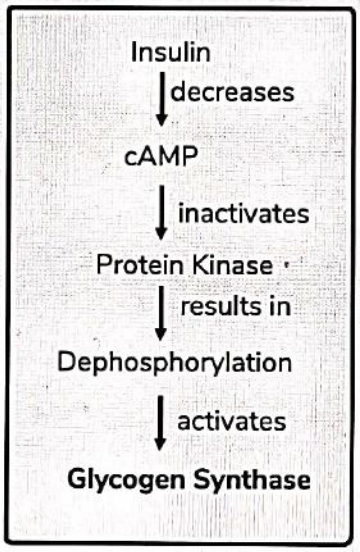
A. Epinephrine
B. Insulin
C. Glucagon
D. Phosphorylation

Answer: B

Solution

Glycogen synthesis (glycogenesis) in the resting muscle is an anabolic process. Insulin is an anabolic hormone, so increases the rate of the anabolic pathway.

Insulin increases the activity of muscle glycogen synthase after the intake of carbohydrates & increases glycogen reserves in liver & muscles. It acts by cAMP mediated dephosphorylation of glycogen synthase (See fig below)



Simultaneously, it also decreases the activity of glycogen phosphorylase thereby decreasing glycogenolysis.
(For details refer to chapter Glycogen regulation)

Reference: Harper's 30th/e pg. 150

2 HOW TO USE FORMULAS

HOW TO USE FORMULAS

Q1. Which of the following is active in dephosphorylated state? [PGI type] 00:00:30

A. Glycogen Synthase → Synthesis → Anabolic
B. Pyruvate Carboxylase → Gluconeogenesis → Catabolic
C. Glycogen phosphorylase → Break down → Catabolic
D. Acetyl CoA Carboxylase → FA Synthesis → Anabolic
E. Pyruvate dehydrogenase → Link reaction → Catabolic

Ans. A, D, E

- Apply Smile formula 3: Pathways or Enzymes which are activated by Insulin (Anabolic Enzyme) are always active in Dephosphorylated state with link reaction as an exception.

Q2. Which of the following does not occur in mitochondria? (AIIMS Nov 2016) 00:02:46

A. Beta oxidation
B. Fatty acid synthesis
C. DNA synthesis
D. Protein synthesis

Ans. B

Pathway	Nature	Compartment
Beta oxidation	Catabolic	Mitochondria
Fatty acid synthesis	Anabolic	Cytoplasm
DNA synthesis	Mitochondrial DNA and protein synthesis	Mitochondria
Protein synthesis		

Q3. Insulin promotes lipogenesis by all except 00:04:07

A. Decreasing cAMP
B. Increasing Glucose uptake
C. Inhibiting Pyruvate Dehydrogenase
D. Increasing Acetyl CoA

Ans. C (Apply Smile formula 3)

Q4. Mitochondria are involved in all of the following except 00:05:18

A. ATP Production
B. Apoptosis
C. Tri carboxylic Acid Cycle
D. Cholesterol Synthesis

Ans. D (Refer to Smile formula 4)

Q5. Hormone Sensitive lipase is NOT activated by 00:05:52

A. Insulin → Lipoprotein Lipase → Anabolic
B. Glucagon } → Hormone Sensitive lipase
C. Catecholamines } → catabolic
D. Thyroid

Ans: A (Apply Smile formula 1 and 2)

Q6. Which of the following is not seen in low insulin–Glucagon Ratio? (AIIMS 2019) 00:06:40

A. Gluconeogenesis → catabolic
B. Glycogen Breakdown → catabolic
C. Ketogenesis → catabolic
D. Glycogen Storage → Anabolic

Ans. D (Apply Smile formula 1 and 2) Low Insulin means catabolic state

Q7. Which of the following is active in dephosphorylated State? 00:07:50

A. Glycogen Synthase → Anabolic
B. Pyruvate carboxylase → catabolic
C. Glycogen Phosphorylase → catabolic
D. PEPCK → catabolic

Ans. A (Apply Smile formula 3)

Q8. All occur in mitochondria except: [PGMEE 2015] 00:08:38

A. Glycolysis
B. TCA Cycle
C. ETC
D. Ketogenesis

Ans. A (Apply Smile formula 4)

Q9. The biosynthesis of the enzymes Pyruvate Carboxylase is repressed by: (PGMEE 2012-13)

00:09:18

A. Insulin
B. Cortisol
C. Glucagon
D. Ketogenesis

Ans. A (Apply Smile formula 2)

Q10. Which of the following is active in phosphorylated state?

00:09:51

A. Glycogen Synthase
B. Glycogen Phosphorylase
C. Acetyl Co A Carboxylase
D. G6PD Enzyme

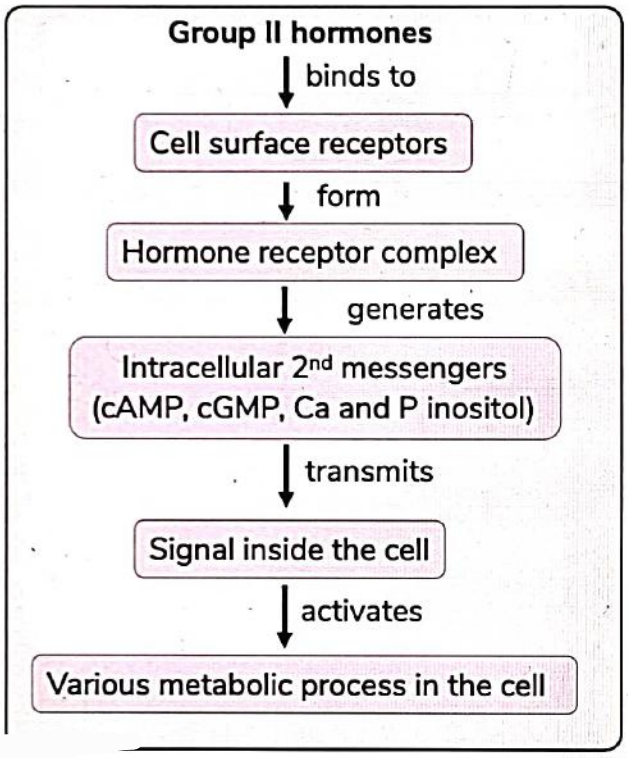
Ans. B (Apply Smile formula 3)

3 cAMP and cGMP

cAMP AND cGMP

- They are 2nd messengers
- Used by group II hormones for their signal transduction

00:00:52



Differences between cAMP and cGMP

00:02:06

cAMP	cGMP
• More common	• Less common
• Less sustained response	• Long term and sustained response
ATP —Adenylate Cyclase→ cAMP —Phospho-diesterase→ 5' AMP	GTP —Guanylate Cyclase→ cGMP —Phospho-diesterase→ 5' GMP
	• Guanylate cyclase exist in 2 forms: 1. membrane bound form 2. cytoplasmic form
• Activate protein kinase A	• Activate protein kinase G
• The hormones which use cAMP as second messenger are called as group IIa hormones such as: o Glucagon o LH o FSH o hCG o Calcitonin o Catecholamines	• The hormones which use cGMP as second messenger are called as group IIb hormones • It has role in o visual cycle o vasodilatory effect by NO (nitric oxide) o activation of peptide hormones like ANP and BNP (atrial and brain natriuretic peptides) where membrane bound guanylate cyclase is used.

Previous Year's Questions

Q Which of the following has cyclic GMP mediated action? (JIPMER Nov 2018)
A. Photochemical reactions of visual cycle
B. Steroidogenesis
C. Thyroid hormone action
D. Recruitment of glucose transporters to cell membrane

Previous Year's Questions

Q. Nitric oxide acts by increasing? (NEET May 2018)
A. BRCA I
B. BRCA 2
C. Interleukin
D. cGMP

4 SOURCES OF BLOOD GLUCOSE

SOURCES OF BLOOD GLUCOSE

Q. Sources of Blood Glucose? 00:00:11

1. Food (till 2 hours of diet)
2. Liver Glycogen (provide glucose for 12-18Hrs)
3. Gluconeogenesis (Requires High Energy)

Q. Main/Preferred fuel for the body? 00:02:50

Ans: Fat

- In fed state body will use carbohydrates preferably
- In fasting and starvation, body will first use mainly fats and then shift to using proteins

Important Information

- Exercise done in morning when body glycogen stores are depleted helps body to consume a lot of energy in gluconeogenesis. hence, is much more beneficial for reducing weight than exercise done at other times.

Previous Year's Questions

Q. A person had a meal at 8 pm and records blood glucose at 7 am on the next day which was 180mg/dl. What's the source of glucose in this patient? (NEET PG Sep 2021)

A. Dietary Glucose
B. Hepatic Gluconeogenesis
C. Hepatic Glycogenolysis
D. Muscle glycogenolysis

CLINICAL QUESTIONS

Q. A 28 years old man suffering from intractable vomiting is presented to a hospital and was unable to eat or drink for the past 3 days. His blood analysis still shows normal glucose level. Which of the following process is mainly responsible for the blood glucose maintenance in this patient?

A. Liver glycogenolysis
B. Muscle glycogenolysis
C. Liver gluconeogenesis
D. Dietary Glucose

Answer: C

Solution

The patient is in starvation state and the main source of glucose in starvation is gluconeogenesis which occurs mainly in the liver. So, the answer is liver.

Regarding other options:

Glycogen breakdown in the liver can maintain blood glucose for 12-18 hours. After that period, liver gluconeogenesis is the main source of blood glucose during fasting/starvation.

The glycogenolysis of muscle gives glucose 6-phosphate as end-product and not glucose due to the absence of enzyme glucose 6-phosphatase in muscles. Hence, muscle glycogen cannot contribute to blood glucose directly.

Reference: Harper's 30th/e pg. 178

5 FUEL IN FED, FASTING AND STARVATION

FUEL IN FED, FASTING AND STARVATION

Table: Substrates utilized for energy production

00:00:35

	Fed	Fasting	Starvation
Brain	Glucose	Glucose	KB
Heart	FA	FA	KB
Liver	Glucose	FA	Amino Acids
Muscle	Glucose	FA	FA and KB
Adipose tissue	Glucose	FA	FA
RBC	Glucose	Glucose	Glucose
Main fuel for body	Carbohydrate	Fat	Amino acids

In fasting

- RBCs cannot use fatty acid or ketone bodies (KB) as fuel even during fasting as they don't have mitochondria where beta-oxidation occurs.
- Brain cannot use FA as FAs cannot cross blood brain barrier.

In Starvation

- Vital organs Brain and heart are adapted to use KB during starvation so that if glucose is exhausted by utilization in other organs, death can be avoided as heart and brain can still function by using ketone bodies as an alternative fuel.
- Muscle cells avoid or delay using proteins/AA as fuel to avoid body muscle loss

Important Information

- Fetal heart and also in case of heart failure, main fuel is Glucose (GLUT-4 allows glucose transport in heart muscles during these conditions)
- Heart is adapted to use FA as main fuel as it works 24 hours and require continuous supply of energy by utilizing energy dense fats as compared to glucose.

Previous Year's Questions

Q. RBC uses which of the following substances during fasting/starvation? (INICET July 2021)

A. Glucose
B. Ketones
C. Amino acids
D. Fatty Acid

CLINICAL QUESTIONS

Q. A 32-year old on a trekking expedition was stranded in forest without any food supplies but was rescued after a week. On medical examination his plasma showed very high levels of ketone bodies. Which of the following cannot use ketone bodies for its energy supplies?

A. Brain
B. Adipose tissue
C. Muscle cells
D. Erythrocytes

Answer: D

Solution

- The body glycogen stores can supply glucose for 12-18 hours of fasting. After that liver perform gluconeogenesis to maintain glucose levels for vital organs.
- In prolonged fasting or starvation as is the case in the question, the body start to use fat deposit by performing beta-oxidation of fatty acid which results in the formation of lots of acetyl CoA in liver mitochondria.
- As oxaloacetate is in short supply in liver (due to its use in gluconeogenesis) to combine with this acetyl CoA to undergo TCA cycle, so, acetyl CoA is diverted to the formation of ketone bodies in the liver. The ketone bodies are released into plasma and travel to different tissues to provide energy.
- Now this process of ketone body utilization happens in mitochondria. As RBC lacks mitochondria, they cannot use these ketone bodies for their energy. So, they are always dependent on the glucose synthesized by gluconeogenesis.

Other tissue which also cannot use these ketone bodies is liver due to lack of enzyme thiophorase.

References: Harper's 30th ed/pg. 227

6 FASTING STATE

SCENE IN FASTING STATE

00:00:33

Refer Figure 6.1

Sequence of utilization of acetyl CoA

1. For use in TCA in liver cells to provide energy to liver cells for ketone bodies synthesis.
2. Once liver has generated enough energy for synthesis, acetyl CoA can now enter KB synthesis.
3. Once KB synthesis is up and running well and supplying the energy to vital organs, now gluconeogenesis will proceed, and acetyl-CoA can now be used for stimulation of 1st step of gluconeogenesis.

Important Information

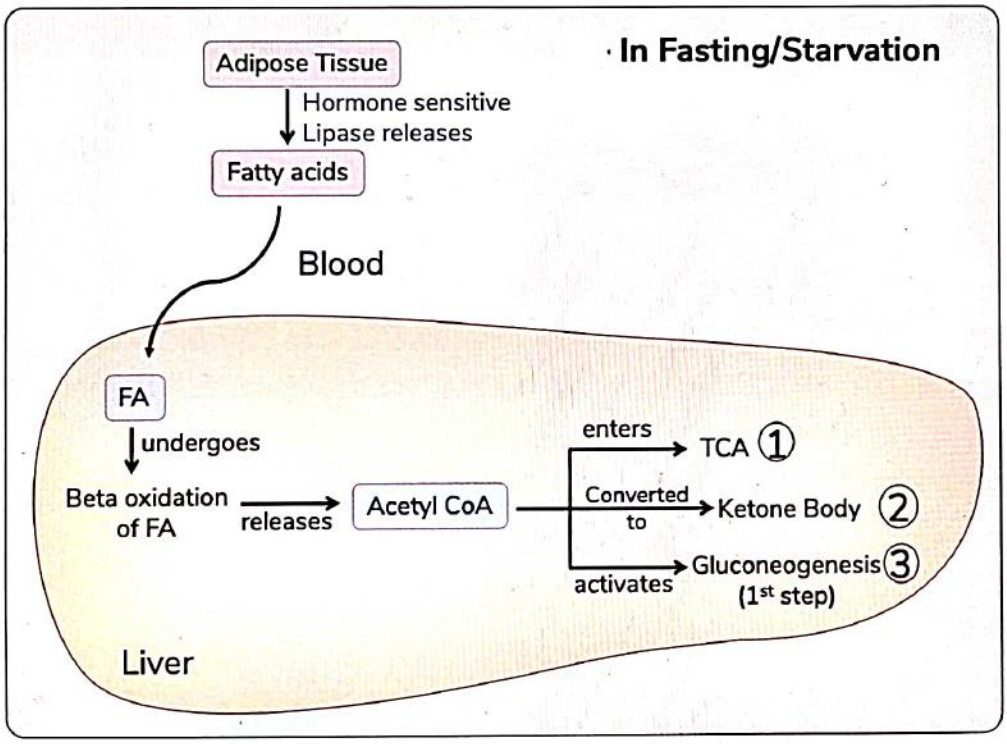
Any defect of beta-oxidation in liver will lead to non-ketotic hypoglycaemia due to non-formation of ketone bodies and no gluconeogenesis.

Previous Year's Questions

Q. Which of the following enzyme activity decreases in fasting? (AIIMS May 2018)

A. Hormone sensitive lipase
B. Glycogen phosphorylase
C. Acetyl CoA Carboxylase
D. Pyruvate carboxylase

Figure 6.1



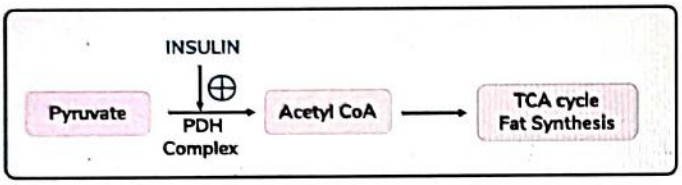
7 ACETYL CoA

ACETYL CoA

00:00:33

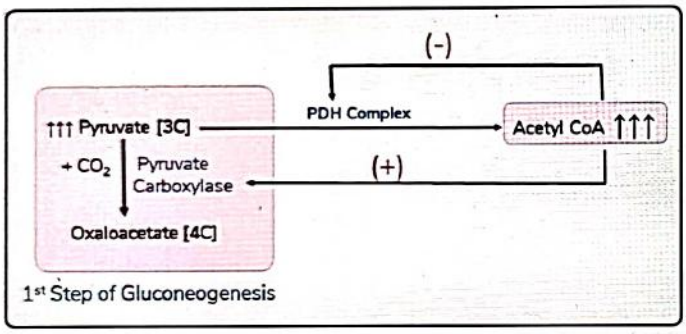
Fates of Acetyl CoA

1. In Fed state



2. In Fasting/Starvation 00:01:26

- **For first 2 fates** refer to chapter Fasting state
- **Third fate of acetyl CoA:** Activation of 1st step of gluconeogenesis



1st Step of Gluconeogenesis

Important Information

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

CLINICAL QUESTIONS

Q. A 30-year-old female was admitted to emergency with intractable vomiting, nausea and fever for past 48 hours. This condition severely affected her ability to eat or drink. Despite her prolonged fasting state, liver can maintain a blood glucose level of 72 mg/dl as shown in her blood glucose analysis by converting the various pyruvate forming substances into glucose. Which among the following is most important activator for first step for this gluconeogenic process?

A. Alanine
B. Citrate
C. Oxaloacetate
D. Acetyl CoA

Answer: D

Solution

During gluconeogenesis, pyruvate can be formed from gluconeogenic substrates such as lactate, glucogenic amino acids and glycerol. Pyruvate formed cannot give rise to phosphoenolpyruvate as this reaction is highly irreversible, so pyruvate is first converted to oxaloacetate by carboxylation reaction. This step is catalyzed by pyruvate carboxylase and acetyl CoA which is abundantly available from fatty acid oxidation is allosteric activator of this step. Acetyl CoA is also a negative regulator of PDH complex (see 2nd figure in text).

Oxaloacetate is then converted to malate in mitochondria which comes out into cytosol and converted back to oxaloacetate by malate dehydrogenase. Oxaloacetate is then converted to phosphoenolpyruvate by PEP carboxykinase.

Regarding other options:

Alanine is allosteric inhibitor of pyruvate kinase and hence inhibits glycolysis but has no effect on gluconeogenesis.

Citrate formed in first step of citric acid cycle is an allosteric activator of acetyl CoA carboxylase which is rate limiting enzyme of fatty acid synthesis. It is also a positive regulator of gluconeogenesis enzyme fructose 1,6-bisphosphatase. It also inhibits glycolysis by inhibiting PFK-1 and PFK-2.

References: Harper's 30th ed/pg. 164

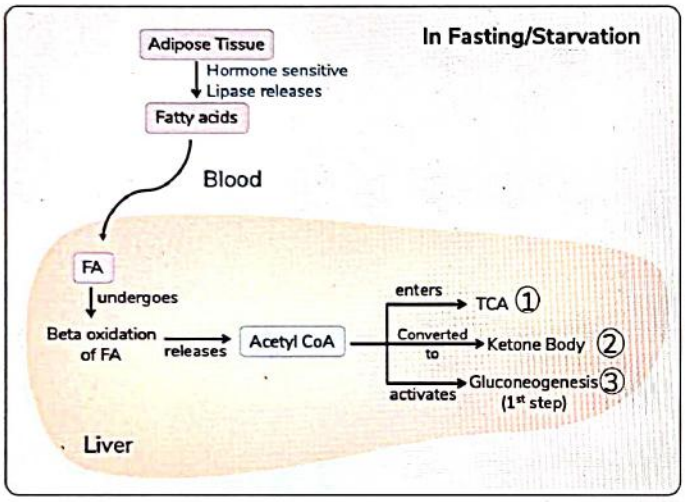
8 DIABETES

DIABETES

00:00:26

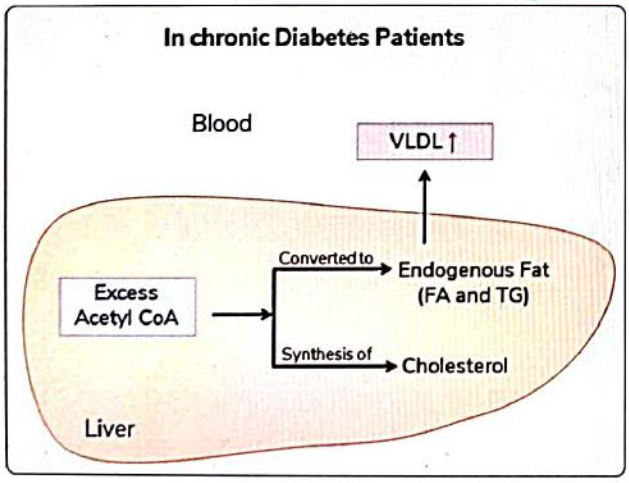
- Diabetes is fasting or starvation like state
  - As insulin is absent so, GLUT-4 is not active
  - Blood glucose cannot enter peripheral cells causing hyperglycaemia
  - Cells will feel as if they are in fasting/starvation

00:02:02



- It is catabolic state (all catabolic pathways will be activated)
- Generally, all anabolic pathways will be inhibited
- But only one anabolic process will occur in chronic Diabetes patients: Fat formation in Liver
  1. VLDL synthesis ↑
  2. Cholesterol synthesis ↑

00:03:24



Previous Year's Questions

Q. In type I diabetes, which of the following is true:- (NEET PG 2019)

A. Increased lipolysis
B. Decreased protein catabolism
C. Decreased hepatic glucose output
D. Increase glucose uptake

Previous Year's Questions

Q. Patient with Type I diabetes mellitus with complains of polyuria. Which of the following will occur normally in his body? (AIIMS Nov 2018)

A. Glycogenesis in muscle
B. Increased protein synthesis
C. Increased conversion of fatty acid to acetyl CoA
D. Decreased cholesterol synthesis

Previous Year's Questions

Q. Ketone bodies may be seen in: (JIPMER May 2019)

A. Diabetes mellitus
B. Pancreatitis
C. Obesity
D. Myocardial infarction

9 RESPIRATORY QUOTIENT

$$RQ = \frac{\text{amount of } CO_2 \text{ produced}}{\text{amount of } O_2 \text{ utilized}}$$

- Value of RQ tells about the fuel used by body

RQ values

- Glucose (Carbohydrates) 00:01:12

$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

RQ (glu/carbs) = 6/6 = 1

- Fats = 0.7
- Proteins = 0.8
- Mixed diet = 0.85
- Alcohol = 0.66
- Exclusive carbohydrate diet = 1 00:02:52
- Excessive/High Carbohydrate diet > 1 (1.2)
  - Because in case of carbohydrate rich diet, carbohydrates are converted to fats.
  - Carbohydrates have high O_2 content as compared to fats. So, more O_2 containing compound (carbohydrates) is converted to less O_2 containing compound (fat), hence O_2 will be released in this process.
  - This O_2 released will be used for oxidation. So, less O_2 is required from outside. So, RQ increases.

How to remember

RQ of Fats and Proteins

- In Fats: 'F' resembles inverted '7' so, RQ = 0.7
- In Proteins: 'P' can be converted to '8' easily so, RQ = 0.8

00:04:50

Important Information

RQ in Diabetes

- In a normal person in fed state, main fuel for body is carbohydrates. But in a diabetic patient, main fuel for body is fats as carbohydrate/glucose is not entering the cells due to relative or absolute deficiency of insulin.
- So, RQ decreases in Diabetes as RQ of fats is less than the RQ for carbohydrates.
- But on giving insulin, RQ again rises as glucose starts entering cells through GLUT-4 and now cells will again start using glucose.

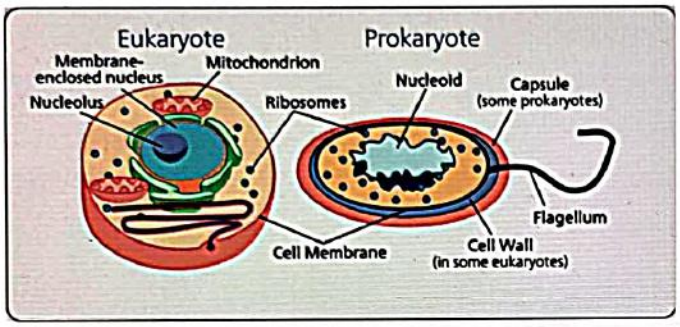
RQ decreases in	RQ increases in
• Fasting • Starvation • Diabetes • Alkalosis	• Exercise • Fever • Acidosis

Previous Year's Questions

Q. A person of 50 kg weight, his CO_2 & O_2 levels were measured from spirometry as CO_2 = 200 ml, O_2 = 250 ml. What is RQ in this case? (INI CET Nov 2020)

A. 4
B. 5
C. 0.8
D. 1.25

10 CELL ORGANELLES



Differences in prokaryotic and eukaryotic cells 00:00:39

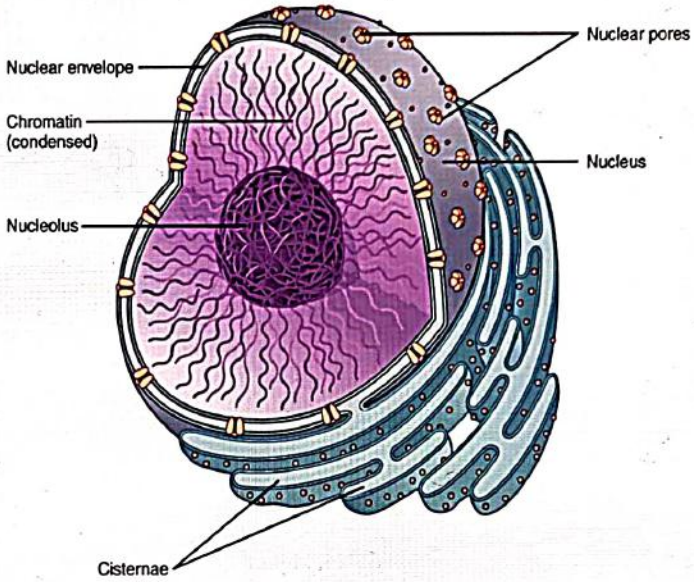
Prokaryotes	Eukaryotes
• Simple/primitive cell No organelle	• Complex/advanced cell • Membrane bound organelle
• Chemically complex Cell wall present	• Simpler Cell wall present in Fungi & Plant
• Plasma membrane do not have receptor	• Plasma membrane contain receptors
• Nucleoid (No membrane)	• Membrane Bound Nucleus with nucleolus
• Circular & double stranded DNA	• Linear & Double stranded DNA
• Transcription & Translation both occur simultaneously in cytoplasm	• Transcription occurs in nucleus & Translation occurs in cytoplasm
• Smaller Ribosomes	• Larger Ribosomes

CELL ORGANELLES 00:07:01

- 6 in number
  1. Nucleus (largest organelle)
  2. Mitochondria
  3. Peroxisomes
  4. Lysosomes
  5. ER
  6. Golgi apparatus

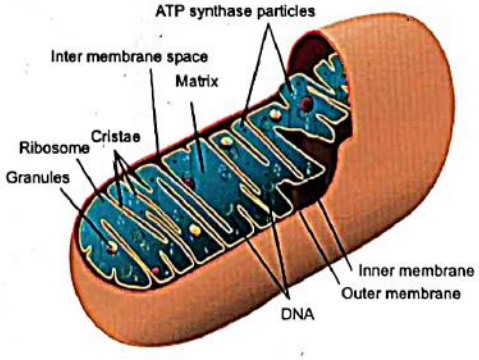
Nucleus 00:07:43

- Consists of two membranes
  - Inner and outer membranes
  - Joins to form big nuclear pores for easy movement of big molecules such as ribosomes, DNA polymerase, RNA polymerases etc.
- Condensed chromatin with histones proteins present in centre
- Contains Nucleolus
  - Rich in rRNA
  - Disappear during cell division



MITOCHONDRIA 00:09:25

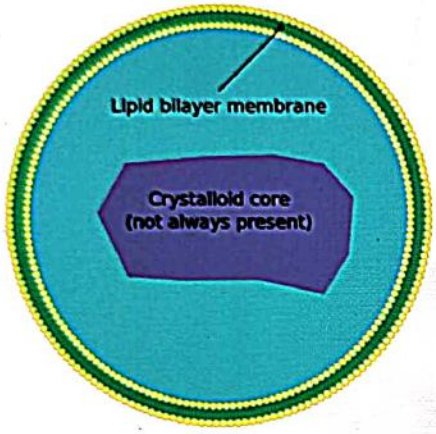
- Self-replicating
  - Contain their own DNA, ribosomes and protein synthesizing machinery
  - Synthesize some of their own proteins and 20% proteins of ETC also
- Multiple mitochondria per cell and number changes according to energy need of cells
- IMM is semi-permeable because of Phospholipid - Cardiolipin (4 Fatty Acids)
  - Normal Phospholipids has two fatty acids, so, cardiolipin is more non-polar



PEROXISOMES/GLYOXYSOMES/MICROBODIES

00:11:09

- Single membrane
- Peroxisomal pathways
  1. α- Oxidation of FA
  2. Oxidation of Very Long Chain Fatty Acid (VLCFA)
  3. Synthesis of Plasmalogen which is main Phospholipid in myelin sheath of brain
- No ATP production in any of peroxisomal pathways
- free radical H_2O_2 is regularly formed in most of Peroxisomal pathway
- Peroxisomal enzymes Catalase & Peroxidase destroy H_2O_2



Important Information

Peroxisomal Biogenesis Disorder (PBD)

- Defect in Formation of Peroxisomes
- All effect CNS
- Most severe PBD: Zellweger syndrome

Previous Year's Questions

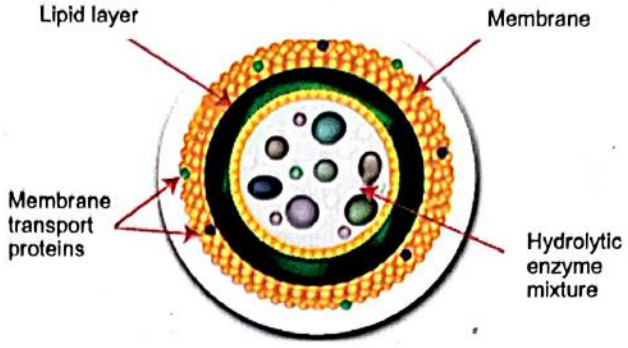
Q. In Zellweger syndrome, which of the following is absent? (NEET PG Jan 2019)

A. ER
B. Golgi apparatus
C. Mitochondria
D. Peroxisomes

LYSOSOMES

00:14:08

- Digestive Organelles
  - Break down Macromolecules by action of hydrolases (Optimal pH ~ 5)
- 1° Lysosome + vesicle containing macromolecule → 2° Lysosome



Lysosome

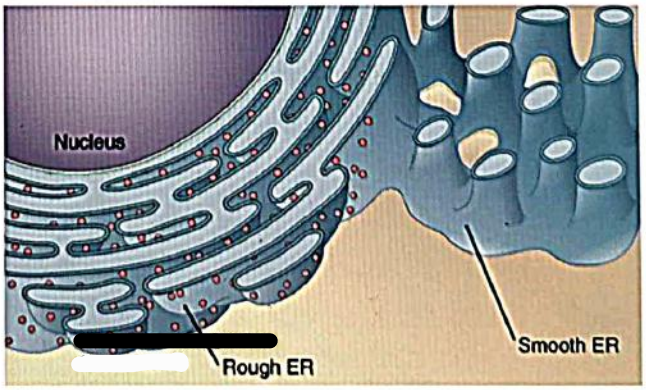
- **Lysosomal Storage Diseases :** Deficiency of one or more hydrolases causing accumulation of the substrate of that hydrolase in lysosomes

LSD name	Enzyme replacement therapy (ERT)
1. MPS (Mucopolysaccharidosis)	MPS type I, II and VI
2. I- cell Disease	NA
3. Pompe's Disease / Type II GSD	Available
4. Sphingolipidoses (SLP)	Gaucher's, Niemann-Pick and Fabry's
5. Wolman's Disease	Available
6. Cystinosis–S–containing Amino acids	NA

ENDOPLASMIC RETICULUM

00:19:13

- Continuation of nuclear membrane



Two Types

1. Rough Endoplasmic Reticulum (RER)
2. Smooth Endoplasmic Reticulum (SER)

RER	SER
• Attached to ribosome Functions • Synthesis of Protein which are exported outside the cell E.g. Insulin • Synthesis of Lipid required for outer Membrane of Nucleus, Lysosomes	• Not attached to ribosome Functions • Detoxification of drugs • Ca^{+2} Sequestration & Release • Sarcoplasmic Reticulum: SER in the myocytes of smooth & striated muscles

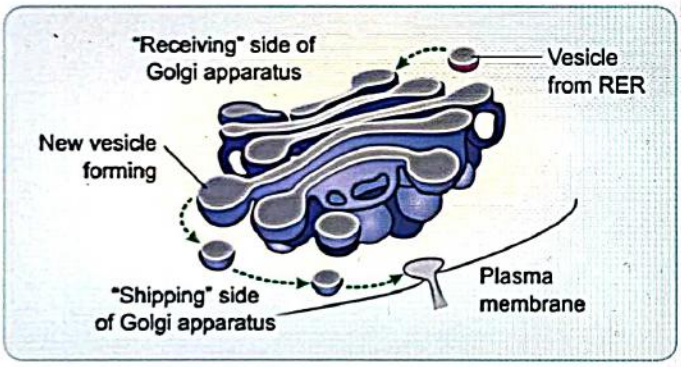
Microsomes : They are residues formed on disruption of ER under in vitro conditions.

GOLGI APPARATUS

00:22:17

Function

- Protein Packing
- Protein Glycosylation (O-Glycosylation only)
- Protein storage (sometimes)
- Phosphorylation of protein
- Tagging and Segregation
- Post translational modification



Important Information

Protein targeting disorders: defect in tagging of protein in Golgi apparatus causing defective targeting of that protein e.g.

1. I-Cell disease
2. Primary Hyperoxaluria
3. Familial Hypercholesterolemia
4. Zellweger Syndrome
5. Cystic Fibrosis

Previous Year's Questions

Q. 'I' cells disease is due to defect in:- (JIPMER Nov 2018)

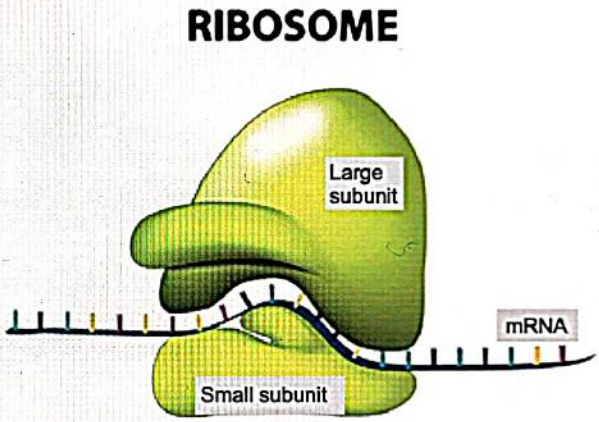
A. Peroxisome
B. Mitochondria
C. Lysosome
D. Golgi apparatus

RIBOSOME

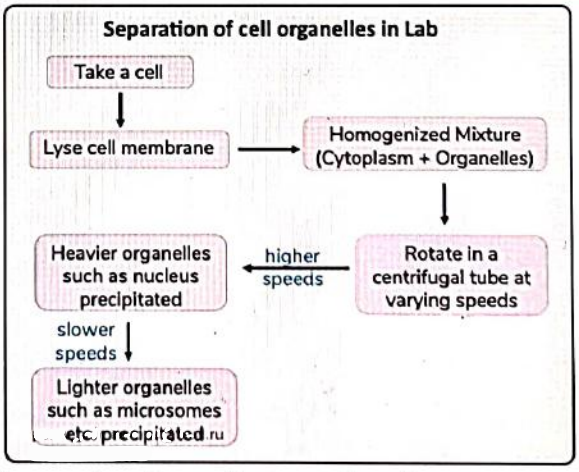
00:25:55

Free / Cytosolic ribosomes

- Not considered as organelles (no membrane present)
  - Needed for synthesis of proteins/enzymes involved in metabolism
  - Consists of large and small subunits
  - Made of RNA and Proteins (rRNA is marker for ribosomes)



00:27:39



- Sucrose density gradient centrifugation
  - Uses sucrose mixtures of different densities in centrifuge tube
  - Separation is based on size and weight

Markers for various Organelles 00:29:52

Cell Organelle	Marker
• Plasma Membrane	• Na^+K^+ ATPase, 5' Nucleotidase, Adenyl Cyclase
• Golgi Apparatus	• Galactosyl Transferase
• Cytosol	• Lactate DH
• Ribosome	• rRNA
• ER	• Glucose-6-Phosphatase
• Peroxisomes	• Catalase
• Nucleus	• DNA, RNA Polymerase
• Mitochondria	• Succinate DH, Glutamate DH
• Lysosome	• Acid Phosphatase

CLINICAL QUESTIONS

Q. In a drug efficacy study on artificially cultured liver cancerous cells, the cells are homogenized and passed through various steps of differential centrifugation to separate membranes, organelles and cytosolic homogenate with its proteins. Which of the following enzyme activities will be observed in this homogenate?

A. Carbamoyl phosphate synthase -I (CPS-I)
B. Transketolase
C. Isocitrate dehydrogenase
D. Pyruvate decarboxylase

Answer: B

Solution

Different biochemical reactions occur in different sites in the cell. Mostly the catabolic pathways such as beta-oxidation, citric acid cycle, parts of urea cycle generally occur in mitochondria and anabolic pathways such as fatty acid synthesis, gluconeogenesis etc. generally occur in the cytosol.

Transketolase is an enzyme involved in the pentose phosphate pathway which produce substrates NADPH and ribose 5-phosphate for anabolic pathways such as nucleic acid synthesis and all of reactions of this pathway occur in the cytoplasm. So, enzyme transketolase will be present in the cytosolic homogenate.

All the other enzymes are involved in reaction occurring in the mitochondria so, they will be removed by centrifugation.

Reference: Harper's 30th ed/pg. 198

11 BASICS OF CARBOHYDRATES & ENZYMES

CONSTITUENTS OF OUR DIET

00:00:38

1. 60% - 70% Carbohydrates
2. 15% - 20% Fats
3. Rest is proteins

Usage of different nutrients by body from diet (In Sedentary Lifestyle)

1. Carbohydrates

- 50% utilised for energy production
- 50% stored
  - 10% as Glycogen
  - 40% as Endogenous Fats (transported in the form of VLDL)

2. Dietary fats

- Aka exogenous fat that are transported in the form of chylomicrons

00:03:36

Important Information

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

Ans. VLDL. Excess carbohydrates in his diet will be converted to endogenous fat which are packed as VLDL. No chylomicrons will be formed because person is not taking any exogenous Fat.

- Atkin's diet is a low calorie, low carbohydrate diet
- Juice contains fructose that is most lipogenic sugar

Thermogenic Effect of Food/SDA (Specific Dynamic Action)

00:06:02

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

Previous Year's Questions

Q. Highest thermic effect is of which food? (INICET Nov 2020)

A. Carbohydrates
B. Fat
C. Protein
D. Mixed

ENZYMES (BASIC POINTS)

00:08:23

Classification of Enzymes

EC no.	Category name	Reaction catalyzed
1	Oxidoreductase	Transfer electrons or hydrogen atoms
2	Transferase	Transfer groups (Molecular formula is changed)
3	Hydrolase	Use H_2O to break
4	Lyase	Can make/ break [do not require H_2O / ATP]
5	Isomerase	Interconvert isomers (Molecular formula not changed)
6	Ligase	Use ATP to make

- Enzyme categories (MN: OTHLIL)
- **EC no.** = Enzyme commission number

00:13:21

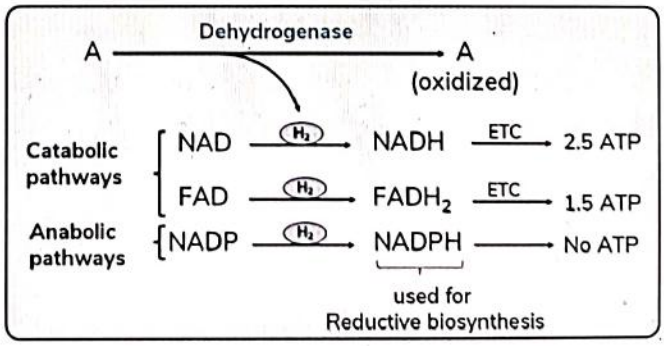
Important Information

Definition and terminology of oxidation and reduction

- Addition of O_2 → Oxidation
- Addition of H_2 → Reduction
- Addition of e^- → Reduction
- H_2/Hatom/reducing equivalent = e^-
- H^+ ion → Proton

DEHYDROGENASES

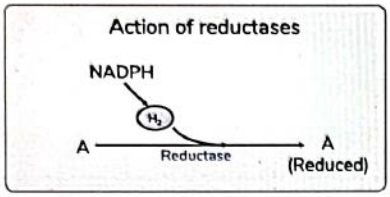
00:14:50



NADPH

00:17:34

- Produced from
1. HMP (major)
2. Malic Enzyme
3. Cytoplasmic Isocitrate Dehydrogenase (not in TCA)
- Used in
  - Reductive Biosynthesis / Anabolic Pathway



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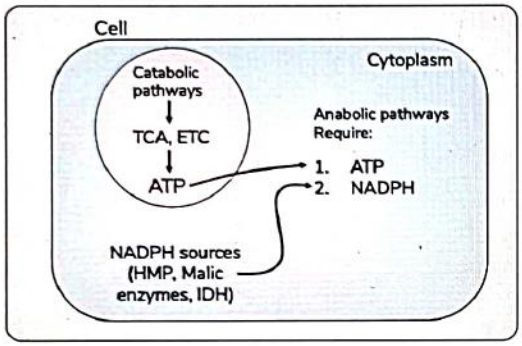


- **Dehydrogenase** (does Oxidation) and **Reductase** (does Reduction)
  - EC no. = 1

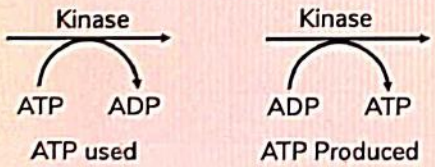
KINASE, PHOSPHORYLASE & PHOSPHATASE

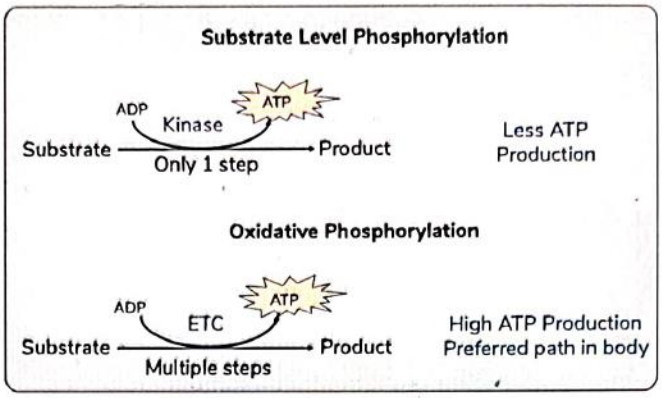
00:22:24

- **Kinase and Phosphorylase (transfer phosphate)**
  - EC no. = 2
  - Difference between Kinase and Phosphorylase
    - → Kinase transfer organic Phosphate (ADP, ATP) whereas inorganic Phosphate (Pi) is transferred by Phosphorylase
- **Phosphatase**
  - EC No. = 3
  - Removes phosphate with the help of H_2O

Important Information

- All Kinases, Phosphorylases & Carboxylases use Mg as co-factor but Pyruvate Kinase use K > Mg
- Whenever kinase enzyme is involved either an ATP is used or an ATP is produced as





SYNTHESIS

00:28:00

- Done by

Synthase	Synthetase
• no ATP Needed • EC no. = 4 (Lyase)	• ATP used • EC no. = 6 (Ligase)

- All Synthases are Lyases except
  - Nitric oxide Synthase (EC No. = 1)
  - Glycogen Synthase and Citrate Synthase (EC No. = 2)
  - ATP Synthase (EC No. = 3)

CARBOXYLATION

00:30:11

- Addition of Carbon dioxide
- Carried out by carboxylase
  - EC No. = 6
  - Requires (MN- "ABC")
    - → A = ATP
    - → B = Biotin (B_7)
    - → C = CO_2 and Mg^{2+}

DECARBOXYLATION

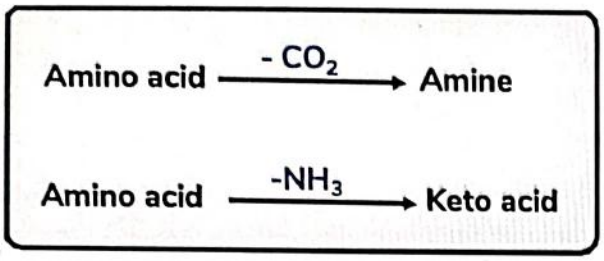
00:31:29

Types

1. Oxidative decarboxylation
- Requires Vitamin B1
- Enzyme is dehydrogenase (EC-1)
- e.g. link reaction, two TCA cycle reactions

2. Simple decarboxylation
- Requires Vitamin B6
- Enzyme is lyase (EC-4)
- e.g.
  - Histidine to Histamine
  - Glutamate to GABA
  - Tryptophan to Tryptamine
  - DOPA to Dopamine
  - Serine to ethanolamine
  - Cysteine to Beta- mercaptoethanolamine

- Glutamate to GABA
- Lysine to diamine cadaverine



00:37:21

Important Information

- In a reaction if
  - NAD used means Vit B_3 is used
  - FAD used means Vit B_2 is used
  - CoA used means Vit B_5 is used
- COOH in a compound ionises to COO^- and gives -ve charge
- NH_2 in a compound ionises to NH_3^+ and gives +ve charge

12 HIGH ENERGY COMPOUNDS

HIGH ENERGY COMPOUNDS

- **Definition**: compounds which release standard free energy on their hydrolysis.
  - Energy is released because products formed after hydrolysis are in more stable forms than the original compound.
- Reactions where energy is released are known as exergonic reactions.
- Currency of cell is: ATP aka energy currency of the cells.
- Endergonic reactions such as synthesis of proteins and nucleic acid needs energy which is supplied by exergonic reactions.

Examples of high-energy compounds are 00:02:21

Phosphates	Sulfur compounds
• Adenosine triphosphate (ATP) • Adenosine diphosphate (ADP) • Guanosine triphosphate (GTP) • Uridine triphosphate (UTP) • 1,3-bisphosphoglycerate • Creatine phosphate • Carbamoyl phosphate • PEP (Phospho Enol Pyruvate)	• S-adenosylmethionine (SAM) • Acetyl-CoA and other CoA derivatives

00:03:43

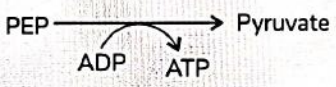
Metabolite	ΔG° (Kcal/mol)
Phosphoenol pyruvate	- 14.8
Carbamoyl phosphate	-12.3
1,3-Bisphosphoglycerate	-11.8
Acid phosphate	-11.2
Creatine phosphate	-10.3
Arginine phosphate	-7.6
ATP to ADP	-7.3
ATP to AMP + PPi	-7.7
ATP to AMP + Pi + Pi	-14.6

Function of High Energy Compounds 00:05:20

1. **Storage of energy**: e.g. ATP which can be stored and used for synthetic reaction whenever required

2. **For synthesis or metabolic pathways** e.g.
   - UTP → UDP glucose → for Glycogen metabolism, galactose metabolism, GAG synthesis, Disaccharide formation, glycolipid formation and bilirubin metabolism
   - CTP → used for phospholipid synthesis

3. **Transfer of energy:** e.g. energy released in conversion of PEP to pyruvate during glycolysis is transferred to ADP for ATP synthesis (substrate level phosphorylation) as:



4. **Quick source of energy:** e.g. creatine phosphate is synthesized and kept in muscles for emergency energy requirements such as flight and fight response.

Previous Year's Questions

Q. During exercise, most rapid way to synthesize ATP is: (AIIMS 2018)

A. Glycogenolysis
B. Glycolysis
C. Phosphocreatine
D. TCA cycle

LEARNING OBJECTIVES

UNIT II: CARBOHYDRATE CHEMISTRY

- Second unit deals with explaining the chemical and functional properties of carbohydrates that are major contributor to energy metabolism in the body. First chapter explains the basics concept of carbohydrate chemistry such as their molecular formula, symmetric and asymmetric carbons and functional groups in carbohydrates. Isomerism chapter explains the types of isomers such as structural and optical isomers of the carbohydrates with examples. Chapter 15 contains details on classification of carbohydrates as monosaccharides, disaccharides and polysaccharides with various examples along with their biological roles as storage polysaccharides and structural roles in case of mucopolysaccharides. A separate chapter "Mucopolysaccharidosis" deals with the frequently asked diseases that manifest as a result of defect in lysosomal degradation of these mucopolysaccharides due to enzyme deficiencies.

- Similarly, a short but important chapter on beta-galactosidase enzymes is included to tackle the clinical questions asked on lactose intolerance and Krabbe's disease. In the last chapter, active and passive mechanism of glucose transport in body is discussed. In this, various categories of glucose transporters (GLUTs and SGLTs) and their physiological role in different tissues is explained along with important points and tips to remember them. The disease which occur due to defect in these transporters are also given.

- **Major learning objectives**
  - To learn structure, chemistry and isomeric forms of carbohydrates
  - To know the classification of various carbohydrates and their function
  - To learn about the lysosomal storage disorders of mucopolysaccharides
  - To understand the process of glucose transport and transporters involved in this process

13 BASICS OF CARBOHYDRATE CHEMISTRY

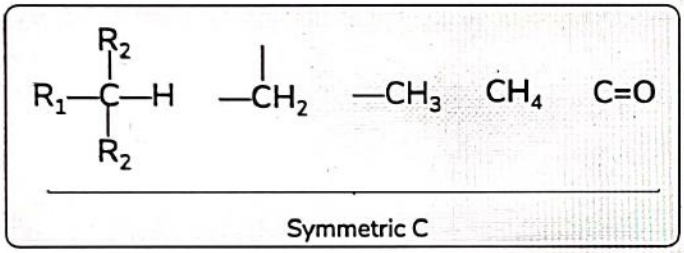
BASICS

00:00:25

- Carbohydrates = Carbon + hydrate
- Molecular/Empirical formula = $(CH_2O)_n$
  - Where n = no. of total carbons
- No. of possible isomers for carbohydrates = 2^n
  - Where n = no. of asymmetrical/chiral carbons
  - e.g. for glucose, n = 4 so, isomers = 2^4 = 16 isomers

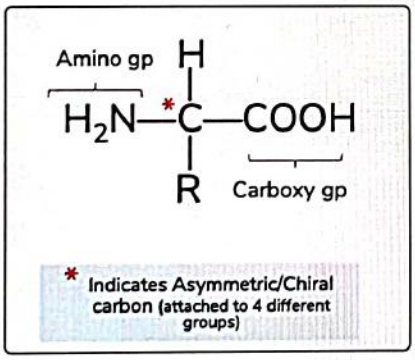
Symmetric carbon 00:02:42

- When any of 2/3/4 valencies are occupied by same atom/group of atoms. e.g.



Asymmetric carbon

- When carbon is attached to four different atoms or group of atoms,
- Whenever a compound has asymmetric carbon, that compound will show both structural & optical isomerism. e.g.
  - In amino acids central carbon is asymmetric, so, they show both Structural & Optical isomerism

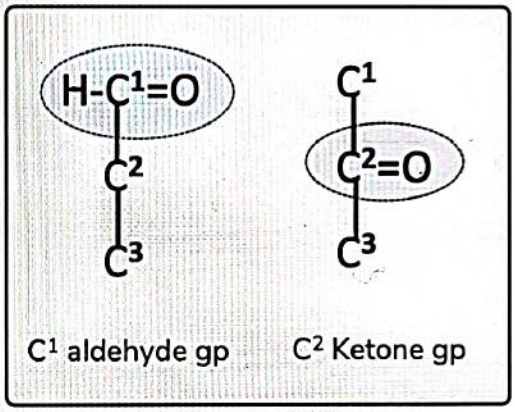


CARBOHYDRATES

00:05:30

Definition: Poly hydroxy Aldehyde or Ketones

- Polyhydroxy: Many OH groups
- OH group
  - Polar
  - Has tendency to bind phosphate e.g. Glucose to Glucose-6-phosphate conversion in cell
  - Suffix used for OH is '**ol**' e.g. Alcoh**ol**, Glycer**ol**, Cholester**ol** (Amphipathic: both polar & non-polar components)
- No. of OH groups are less than 1 the no. of carbon atoms. e.g.
  - 6C - 5 OH (Glucose)
  - 5C - 4 OH (Fructose)
- Aldehyde or ketone (Functional Groups) 00:09:14
  - Aldehyde group is always present at C1
  - Keto group is always present at C2
  - Functional carbon is symmetric but only in linear configuration.



14 ISOMERISM

ISOMERISM

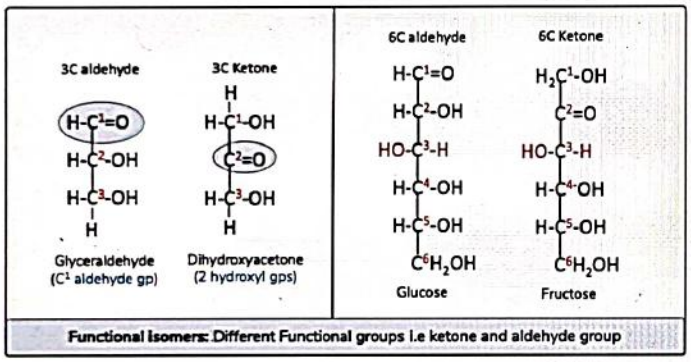
Isomers: Compounds having same molecular formula.

Types of isomerism 00:00:42

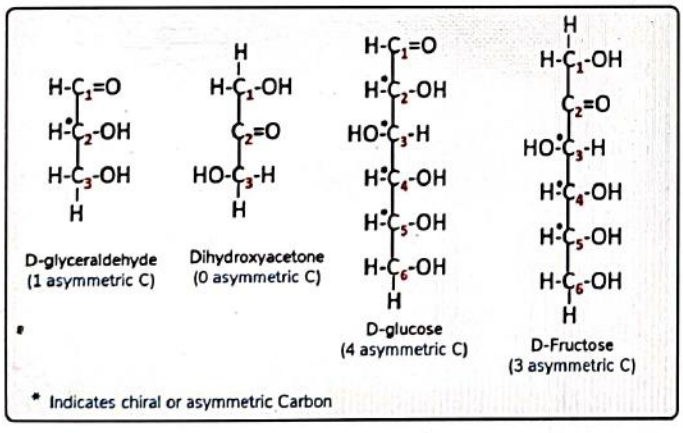
1. **Structural/Sterio isomerism:** Different structure
   1) Functional
   2) Enantiomerism
   3) Epimerism
   4) Anomerism
2. **Optical isomerism:** different Optical properties

1. Structural/Sterio isomerism 00:02:41

1) Functional isomerism: Different Functional groups [aldehyde or keto] (Don't assume -OH a functional group)



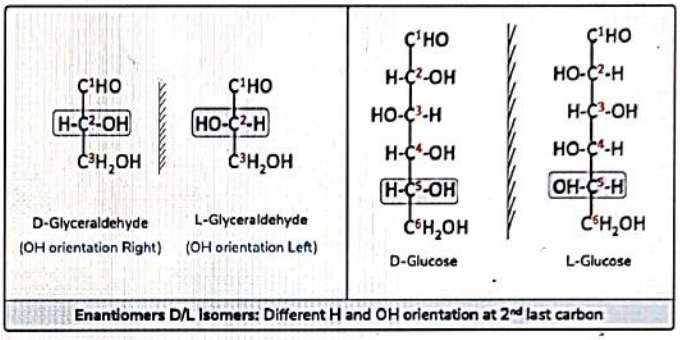
Number of asymmetric carbon in various carbohydrates



- Dihydroxyacetone is only carbohydrate with Zero assymetric carbon
- No. of assymetric carbons in ketone is always one less than number of assymetric carbons in corresponding aldehyde e.g.
  - Glucose = 4 assymetric C
  - Fructose = 3 assymetric C

00:11:54

2) Enantiomerism/ D-L isomerism/Mirror images: Different H & OH orientations around the Penultimate/ Reference/ 2nd last carbon



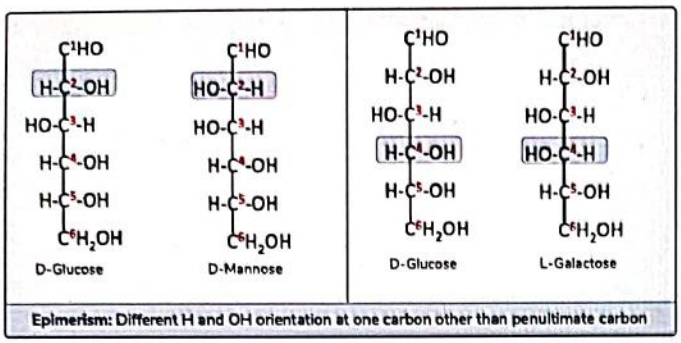
Important Information

- Abundant form of carbohydrates → D
  - Body → D
  - Cell → D
  - Plasma → D
  - Nature → D
- Abundant form of amino acids → L
  - In Proteins → L
  - Free AA → L or D e.g. D-Serine (found in Brain) and D-Aspartate

Q. Which form of amino acid is present in body?
Ans: D & L
Q. Which form of amino acid is abundant in body?
Ans: L
AA synthesized AA in body → L Form
Source of D-AA → Always Exogenous (obtained from diet)

00:18:52

3) Epimerism: Different H & OH orientation around only one carbon other than penultimate carbon

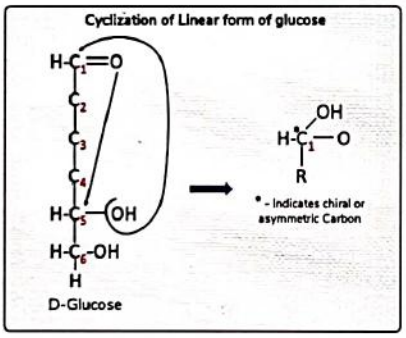


Epimerism: Different H and OH orientation at one carbon other than penultimate carbon

- Mannose is epimer of glucose at C2 whereas Galactose is epimer of glucose at C4
- Mannose & Galactose are not epimers of each other

4) Anomerism: Differenrt H and OH orientation around assymetric functional carbon 00:20:55
Linear Structures → Cyclic Structures
[Powder form] [Solution form]

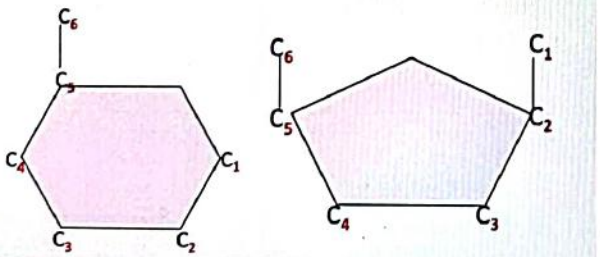
- When linear structute changes to cyclic structute, two carbons combine with each other.
  - Functional carbon always combines with 2nd last carbon
  - For aldoses C1 is functional Carbon and for ketoses C2 is functional carbon
  - The functional carbon changes form symmetric to assymetric (see fig)



  - Differenrt H and OH orientation around this assymetric functional carbon give rise to anomerism

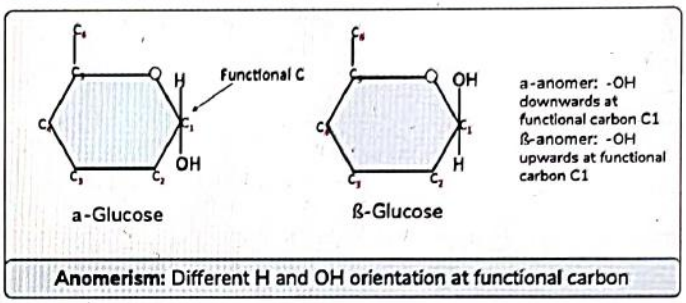
Cyclic structures 00:25:39

Pyranose	1. Furanose
• 6 Membered Ring	• 5 Membered Ring
• No. of carbons = 5	• No. of carbons = 4



Important Information

Glucose	→	99% Pyranose, 1% Furanose (mainly Pyranose)
Fructose	→	99% Furanose, 1% Pyranose (mainly Furanose)
Hexoses (6C)	→	Both Pyranose & Furanose exists
Pentoses (5C)	→	Only Furanose exits

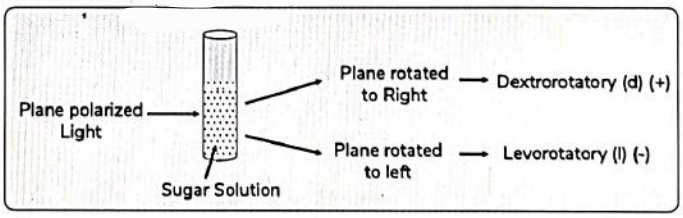


Anomerism: Different H and OH orientation at functional carbon

Summary of structural isomerism

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

2. **Optical isomerism:** Same molecular formula but different optical properties 00:32:15



Important Information

- Glucose is always d (+) whereas Fructose is always l (-)
- Levorotatory power of Fructose > Dextrorotatory power of Glucose
- Racemic mixture: equal amounts of d + l isomers, so, optically inactive.
- Racemase: Interconverts 'D' and 'L' isomers and not small 'd' and 'l', so, name racemase is a Misnomer (name does not tell correct function)

15 CLASSIFICATION OF CARBOHYDRATES

CLASSIFICATION OF CARBOHYDRATES

00:00:32

Name	No. of carbohydrates units
Monosaccharides	1
Disaccharides	2
Oligosaccharides	2/3-10
Polysaccharides	>10

MONOSACCHARIDES

00:02:50

- **No. of carbons = 3 to 9**
  NANA: N-AcetylNeuraminic Acid

No. of carbons	Aldehyde	Ketone
3C	Glyceraldehyde	Dihydroxyacetone
4C	Erythrose	Erythrulose
5C	Ribose	Ribulose
6C	Glucose	Fructose
7C	Glucoheptulose	Sedoheptulose
8C	x	x
9C	Sialic Acid (NANA)	x

Reactions of Monosaccharides

00:06:12

1. Oxidation

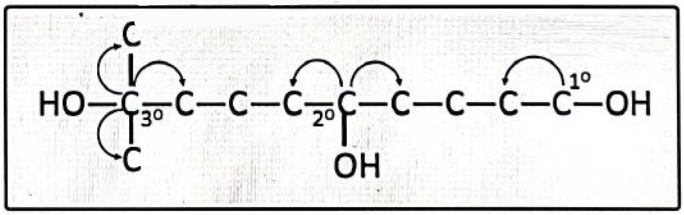
- Oxidation of various OH group of carbohydrates forms Acids

Position of Oxidation	General name of acid formed	Examples
C1 carbon	Aldonic acid	Gluconic acid from glucose
C6 carbon/1° alcohol	Uronic acid	Glucuronic acid and Iduronic acid • Present in GAGs • Epimers at C-5 • Iduronic acid is present in the Heparin
Both C1 and C6 carbons	Saccharic acid	• Glucose → Glucosaccharic acid • Galactose → Mucic acid • Mannose → Mannaric acid

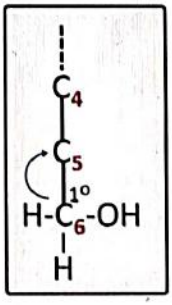
Nomenclature of various alcohol groups in carbohydrates

00:08:08

- Based on number of carbons attached to C to which OH is directly attached



- If C is attached to 1st carbon – 1° alcohol
- If C is attached to 2nd carbon – 2° alcohol
- If C is attached to 3rd carbon – 3° alcohol
- So, in case of carbohydrates consider C_6 as primary alcohol



2.Reduction 00:12:33

- **Reduction** of various OH group of carbohydrates give rise to alcohols aka Sugar Alcohols
- Sugar Alcohols
  - o They are hygroscopic in nature
  - o When present in excess in cell, cause cell swelling
  - o Glucose → Sorbitol/polyol → snow-flake cataract in diabetics
  - o Galactose → Galactitol/Dulcitol → Oil drop cataract in galactosemia
  - o Mannose → Mannitol → ↓ Intra Cranial Tension (ICT)

DISACCHARIDES 00:15:14

- 2 monosaccharides joined by O-glycosidic bond 0—0

Common Disaccharides

Disaccharides	Constituents	Glycosidic bond	Nature
Maltose	Glu + Glu	α (1→4)	Reducing
Isomaltose	Glu + Glu	α (1→6)	Reducing
Trehalose	Glu + Glu	α (1→1)	Non-Reducing
Sucrose	Glu + Fruc	α (1→2)	Non-Reducing
Lactose	Gal + Glu	β (1→4)	Reducing

All monosaccharides are reducing sugars (Functional group is free)

TESTS FOR CARBOHYDRATES 00:16:53

1. Molisch test

- General test given by all carbohydrates
- Condition: No. of carbons must be ≥ 5

2. Benedict's test

- Given +ve by reducing sugars

3. Barfoed's test

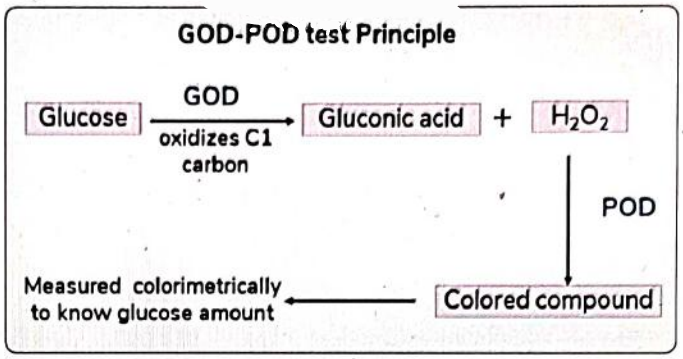
- Distinguishes b|w mono & Disaccharides
- Positive only in monosaccharides

4. Seliwanoff test

- Distinguish b|w keto & Aldehyde sugar
- Positive for keto sugar

5. GOD–POD (glucose oxidase-peroxidase) test

- Enzymatic test routinely done for accurate glucose measurement in blood



POLYSACCHARIDES 00:24:15

Classification

Homopolysaccharides	Heteropolysaccharides
• Made up of same carbohydrate units e.g. starch made of glucose only • Mostly Branched	• Made up of different carbohydrates units • Mostly unbranched

Homopolysaccharides

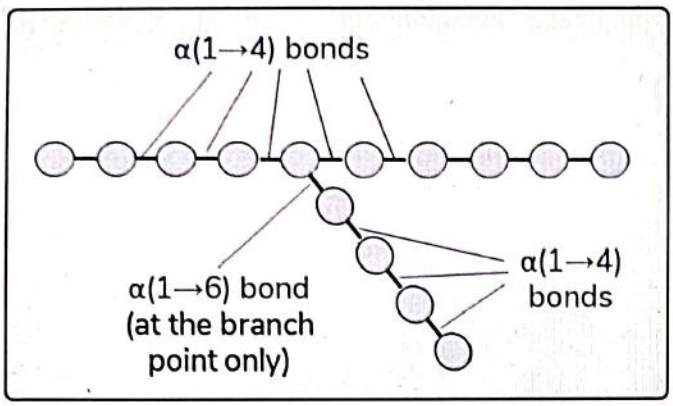
1. Starch
2. Glycogen
3. Dextran
4. Cellulose
5. Inulin
6. Chitin

1. & 2. Starch and Glycogen 00:25:30

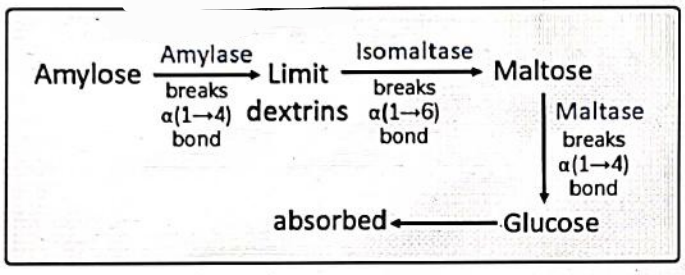
Differences	
Starch	**Glycogen**
• Storage carb in Plants	• Storage carb in animals
• Less branched	• More branched
o Branching point comes after 24–30 glucose residues	o Branching points comes after 8-12 glucose residues

Similarities

- Made up of α-glucose
- Has α(1→4) bonds in straight chain
- Has α(1→6) bonds at branch points



Digestion of starch 00:28:44



The sequence of digestion is Polysaccharide→ Oligosaccharide→ Disaccharide → Monosaccharide

Starch composition 00:31:49

- It is made up of
  1. Amylose
     - 20%
     - Unbranched
  2. Amylopectin
     - 80%
     - Branched

3. Dextran 00:33:11

- Made up of α–glucose
- Has α (1 → 4), α (1 → 6), α (1 → 2), α (1 → 3) bonds
- Highly branched structure
- Cannot be broken in our body
- High molecular weight structure

Importance and uses

1. I/v (intravenous) Dextran
   - Dextran will stay in the intravascular compartment
   - So, used as Plasma volume expander in hypovolemic shock
2. In Gel Filtration chromatography, Gel is dextran.
3. Dental plaques are network of Dextrans

4. Cellulose 00:36:14

- Unbranched
- Made up of β–Glucose
- Most abundant natural polysaccharide
- Acts as fibres in the diet (as β-bonds cannot be easily broken in body)

Fibres 00:38:36

Insoluble fibres	Soluble fibres
• Excreted unchanged from intestine • e.g. 1. Cellulose 2. Hemicellulose	• Absorbs H_2O & converted to Gel form, which is excreted • Better in preventing Constipation • Provide more osmotic load to intestine • e.g. 1. Pectins 2. Gums 3. Inulin

Previous Year's Questions

Q. Which is insoluble in water? (NEET 2020)
A. Lignin
B. Inulin
C. Amylose
D. Chitin

5. Inulin 00:40:09

- Made up of β-Fructose

Uses

- Ideal for measurement of GFR
  - Neither secreted nor absorbed in kidneys
- Acts as prebiotic (food for probiotic bacteria)
  - Not broken by our body so is used by bacteria as food

Important Information

In humans
- Cellulose cannot be broken due to beta-anomerism at C1
- Inulin cannot be broken due to beta-anomerism at C2

6. Chitin

00:42:12

- Unbranched
- Polymer of N-acetyl glucosamine
- Abundantly present in exoskeleton of insects and cell wall of fungi
- 2nd most abundant natural polysaccharide

Heteropolysaccharides

00:43:09

- Aka GAGs → Glycosa Amino Glycans (special carbohydrates with amino gp)
- Aka Mucopolysaccharides → Present in mucus secretions (for lubrication and cushioning)
  - contains more negative charge due to presence of groups such as SO_4^{-2}, COO^-, CH_3COO^-
  - Repulsion between these negative charges provides Slimy & Slippery nature
- **Definition:** Tandem repeats of AS–UA (Amino sugar-Uronic acid)
  - Tandem Repeat: Repeated one after another

  - All GAGs combine with proteins by covalent bond to form Proteoglycans
  - e.g. Chondroitin Sulphate (GAG) + Protein → Aggrecan (Proteoglycan)

00:48:09

Proteoglycans	Glycoprotein
• Carbohydrate >>> Protein • Carbohydrate is always Heteropolysaccharide • e.g. GAGs	• Protein >>> Carbohydrate • Carbohydrate is mono or oliosaccharide (never a polysaccharide) • e.g. Collagen, All plasma proteins except albumin

Examples of GAGs

00:50:20

Hyaluronic Acid	• Only GAG which is not Sulfated • Longest GAG • Has role in wound healing and cell migration • Located in Synovial Fluid & Vitreous humour
Chondroitin Sulfate (CS)	• Most abundant GAG • Present in Cartilage, Bone, Tendon
Dermatan Sulfate (DS)	• Found in Skin, Blood vessels, heart valves
Keratan Sulfate	• Only GAG without uronic acid • Most heterogenous GAG • Present in cornea & connective tissue • Responsible for Transparency of cornea
Heparin	• more sulphated than Heparan sulfate • Highest negative charge • Very good but costly anti-coagulant (prevent coagulation but do not dissolve clots) • Activates antithrombin III to slow down or delay the progression of DVT • Released from mast cell & liver
Heparan Sulfate (HS)	• Present on cell surfaces • Has a role in cell–cell adhesion e.g. retinal cell-cell attachment

Previous Year's Questions

Q. Transparency of corneal endothelium is maintained by: (NEET Sep 2021)

A. Heparan sulphate
B. Keratan sulphate
C. Chondroitin sulphate
D. Hyaluronic acid

Previous Year's Questions

Q. What is the mucopolysaccharide/proteoglycan present in glomerular basement membrane? (NEET 2020)

A. Heparan sulfate
B. Chondroitin sulfate
C. Hyaluronic acid
D. Keratan sulfate

Previous Year's Questions

Q. Synovial fluid contains? (FMGE Dec 2019)
A. Hyaluronic acid.
B. Chondrontoin sulphate
C. Keratan sulphate
D. Dermatan sulphate

DANAPAROID

- Mixture of CS, DS and HS
- Low MW compound
- acts as anticoagulant due to antithrombotic activity
- No effect on fibrinolytic system
- Prevents the formation of clot

D-XYLOSE ABSORPTION TEST

00:56:57

- Was used for diagnosis of malabsorption syndrome (not done anymore)
- Xylose is a pentose sugar, that just require intact intestinal mucosa for absorption
- Patient is given D-xylose for ingestion and later blood and urine sample checked for D-xylose
- Low levels of D-xylose in blood and urine indicates malabsorption

CLINICAL QUESTIONS

Q. A 54-year old women who was bed bound in a nursing home began to develop swelling of her left leg. She was diagnosed with Venous Doppler ultrasound and was found to have a deep vein thrombosis. She should be treated with which chemical to prevent the clot from enlarging.

A. Digitalis
B. Ouabain
C. Heparin
D. Heparan sulfate

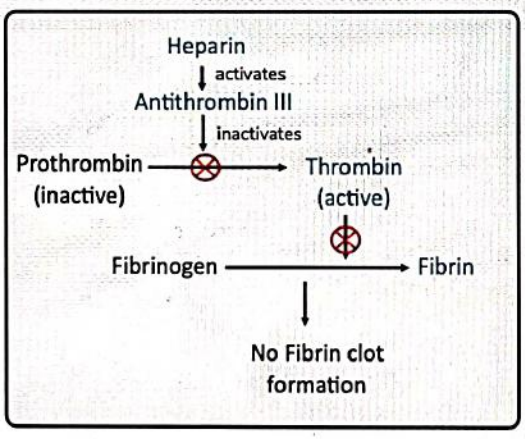
Answer: C

Solution

Heparin is a naturally occurring glycosaminoglycan or proteoglycan with the repeating disaccharide containing glucosamine (GlcN) and either of the two uronic acids.

Heparin is usually stored within the secretory granules of mast cells and released only into the vasculature at sites of tissue injury. Heparin binds specifically to lipoprotein lipase present in capillary walls, causing a release of this enzyme into the circulation.

Heparin is also used as anticoagulant that binds to antithrombin and activates it, which in turn inactivates thrombin. Thus, heparin will prevent the clot from enlarging in this patient.



Heparin is also used for laboratory techniques involving estimation of blood pH, Blood gases, Electrolytes, and Ionized calcium.

Regarding other options:

Digitalis: Drug of choice for treating congestive heart failure and arrhythmias

Ouabain: A plant-derived glycoside which inhibits active transport of Na^+ in the cardiac muscle. In lower doses, it can be used in treating arrhythmias.

Reference: Lippincott's 7th ed/pg. 164

16 MUCOPOLYSACCHARIDOSIS

MUCOPOLYSACCHARIDOSIS

- GAGs (mucopolysaccharides) are normally broken down in Lysosomes by hydrolases
- If there is defect in these hydrolases, GAGs accumulate in Lysosomes
- Aka Lysosomal Storage Diseases
- All are Autosomal Recessive (AR)
  - Except Hunter disease → X-linked recessive (♂)

General Clinical Features

00:02:08

- Coarse facial features
- Depressed nasal bridge
- Frontal bossing
- Copious nasal discharge
- Corneal Clouding
- Protuberant abdomen (due to Umbilical Hernia, Hepatosplenomegaly)
- Skeletal deformities
- Short stature (due to growth retardation)
- Clawing of hands

Other features

- MPS are found in urine
- Reilly body inclusions (especially in Type I)

Specific C/F

00:04:26

- If Dermatan Sulfate (DS) accumulation→Atherosclerosis
- If Heparan Sulfate (HS) accumulation→Mental Retardation

Important Mucopolysaccharidosis

00:05:16

Type	Disease name	Enzyme defect	GAG accumulated	Special features
I-H	Hurler disease	α-L-Iduronidase	DS + HS	• Inguinal hernias often present • Reilly body inclusion present
I-S	Scheie disease	α-L-Iduronidase	DS	• No mental retardation
II	Hunter's disease (clear vision)	Iduronate Sulfatase	DS + HS	• No corneal clouding • Exclusively males affected (XR)
VI	Maroteaux Lamy Syndrome	Aryl Sulfatase B	DS	• No mental retardation

ERT (Enzyme Replacement Therapy) for MPS

- Type I - Aldurazyme
- Type II - Elaprase
- Type VI - Naglazyme

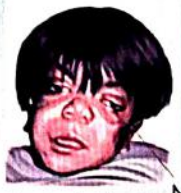
Names of other MPS

1. Sly syndrome
2. Morquio syndrome/disease
3. Sanfilippo syndrome

How to Distinguish between Hurler's and Hunter's Disease

00:11:22

- Both Have HS+ DS accumulation and similar c/f
- Distinguishing features

Hurler's Disease	Hunter's disease
 Hurler's disease — Corneal clouding	 Hunter's disease — Normal Cornea
• Corneal clouding present • AR • Severe • Inguinal Hernia +ve • Reily Body inclusion found	• Corneal clouding absent (Clear vision) • XR • k/a Mild Hurler with aggressive behaviour • Reily Body inclusion not found

Previous Year's Questions

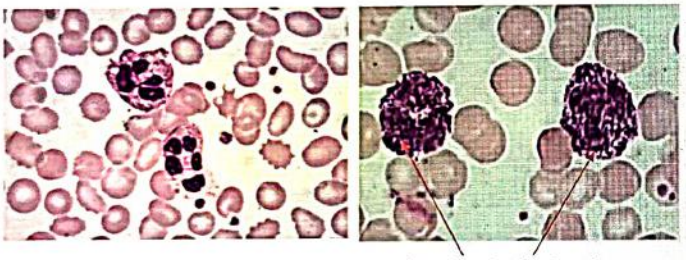
Q. A male child presented with coarse facies, protuberant abdomen, frontal head enlargement, thickening of cardiac valve, hepatosplenomegaly, hearing impairment. What is the most probable diagnosis? (AIIMS Nov 2018)

A. Hurler's disease
B. Hunter's disease
C. Fragile X syndrome
D. Tay Sach's disease

Reily Bodies

00:13:11

- Inclusions in granulocytes esp. in neutrophils
- Dark Purple coarse metachromatic granules present, surrounded by clear zones

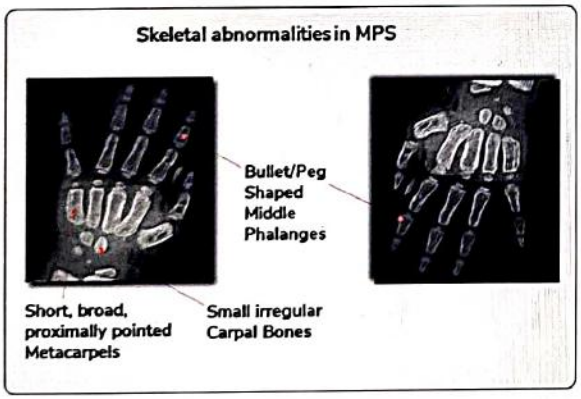


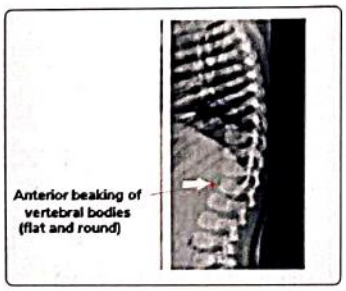
Normal neutrophils | Reilly body inclusions

Skeletal defomotmities in MPS

00:13:57

- Aka Dysotosis multiplex
- Occurs due to defective bone formation





I-CELL DISEASE/INCLUSION BODY DISEASE

00:15:50

Characteristics

- Not an MPS but c/f are same as MPS but more severe
- A Lysosomal storage disorder → protein targeting disorder
- A lysosomal storage disease but defect is in Golgi bodies.

Lysosomal Hydrolases
↓
Synthesized in RER
↓ Goes to
Golgi apparatus
↓ phosphorylation & transportation to
Lysosomes

Mannose is required on Hydrolases to reach lysosomes
↓
For transfer, Mannose must be phosphorylated to Mannose-6-Phosphate by enzyme N-Acetyl Glucosamine Phosphotransferase
↓
In I- cell Disease, N-Acetyl Glucosamine phosphotransferase is deficient
↓
So, Hydrolases do not reach lysosomes
↓
Inclusion Bodies formed in lysosomes due to accumulation of material

Diagnosis: Serum Lysosomal Enzymes ↑↑ (as lysosomal enzymes do not reach lysosomes)

Previous Year's Questions

Q. 'I' cells disease is due to defect in? (JIPMER Nov 2018)
A. Peroxisome
B. Mitochondria
C. Lysosome
D. Golgi apparatus

CLINICAL QUESTIONS

Q. A male child presented with coarse facies, protuberant abdomen, frontal head enlargement, thickening of cardiac valve, hepatosplenomegaly, hearing impairment but no corneal clouding. What is the most probable diagnosis?

A. Hurler's disease
B. Hunter's disease
C. Fragile X syndrome
D. Tay Sach's disease

Answer: B

Solution

Coarse facial features are a typical feature of Mucopolysaccharidosis (MPS). Hunter's disease is Type II Mucopolysaccharidosis, in which enzyme deficient is Iduronate Sulfatase. This is X-linked recessive, so occurs exclusively in males. There is no corneal clouding in Hunter's disease.

Regarding other options:

Hurler Syndrome is very similar to Hunter disease in most of the symptoms, but the main difference is presence of corneal clouding and riley body inclusion in the neutrophils in this disease.

Fragile X-Syndrome is a trinucleotide repeat expansion disorder in which patient has large face, large mandible, large testis, large everted ears and tall stature. It is the second most common cause of mental retardation.

Tay Sach's disease is because of increased GM_2 gangliosides due to deficiency of enzyme Hexosaminidase A. Clinical features are mental retardation, cherry red spots on macula and progressive neurodegeneration but no hepatosplenomegaly.

For other Mucopolysaccharidosis & their features refer to 1st table in text

Reference: - Harper's 30th ed/pg. 179

Q. A 6-year-old boy with coarse facial features was taken to a pediatrician due to concerns about his aggressive behavior and a loss of language skills. He also has recently experienced a seizure and becoming increasingly unsteady on his feet. Dermatan sulfate and heparin sulfate was found to be increased in the urine of this patient. This child most likely has a disorder related to which of the following processes?

A. Mobilization of glycogen
B. Salvage of purine bases
C. Gluconeogenesis
D. Degradation of glycosaminoglycans

Answer: D

Solution

Coarse facial features and accumulation of heparan sulfate and dermatan sulfate in urine suggests mucopolysaccharidosis (MPS) which is due to accumulation of glycosaminoglycan (GAGs) due to absence of lysosomal enzyme in various tissues. For general clinical features of MPS, go to 1st page of this chapter.

- **Regarding other options:**

  Main features of glycogen mobilization disorders are hypoglycemia and exercise intolerance.

  Disorders related to salvage pathways of purines such as HGPRT deficiency lead to accumulation of uric acid and gout.

  Disease related to gluconeogenesis will cause hypoglycemia and lactic acidosis.

Reference: Harper's 30th ed/pg. 638-639

17 BETA-GALACTOSIDASE

β-GALACTOSIDASE

- Generally, β-bonds are not broken in the body e.g. cellulose and inulin
- But when galactose is present on one side of β-bond, it can be broken.
- Any enzyme which break β-bond on the side of galactose is called as β- Galactosidase
- Two enzymes
  1. Lactase
  2. β-galactosyl ceramidase

1. Lactase Enzyme 00:02:07

- Breaks down Lactose (present in milk & milk products) to glucose and galactose

Lactose $\xrightarrow{\text{Lactase}}$ Glucose + Galactose

- Deficiency cause disease lactose intolerance
- C/F of lactose intolerance
  - Osmotic Diarrhoea
  - Distended Abdomen
  - Vomiting
  - Flatulence (gas formation by bacterial fermentation of lactose)

2. β-Galactosyl ceramidase 00:03:58

- Breaks down β-Galactosyl ceramide as galactose is joined to ceramide by beta bond
- Deficiency causes Krabbe's disease (a sphingolipidoses)

For details of Krabbe's disease, refer sphingolipidoses notes

Previous Year's Questions

Q. All of the following should be avoided by a patient with lactose intolerance except: (AIIMS May 2018)

A. Condensed milk
B. Ice-cream
C. Skimmed milk
D. Yoghurt

CLINICAL QUESTIONS

Q. A person after ingesting dairy products feels very uncomfortable with problems of flatulence and distension of his stomach due to gas formation. These symptoms do not appear when he completely avoids any dairy products in his diet. The most likely cause of these symptoms in this person is the deficiency of which of the following enzyme.

A. Alpha amylase
B. Beta galactosidase
C. Alpha glucosidase
D. Sucrase

Answer: B

Solution

The patient has abdominal discomfort & diarrhea after taking dairy products. So, diagnosis is Lactose Intolerance. Enzyme deficient in this patient is β-galactosidase aka lactase. It breaks down milk sugar lactose into glucose and galactose which can be easily absorbed.

Regarding other options:

α-Amylase digests starch while α-glucosidase or acid maltase is involved in a minor pathway of glycogen breakdown in lysosomes

If abdominal discomfort & diarrhea appear after taking sugar or sugarcane juice, then enzyme deficient is sucrase

Reference: Harper's 30th ed/pg. 520

18 GLUCOSE TRANSPORTERS

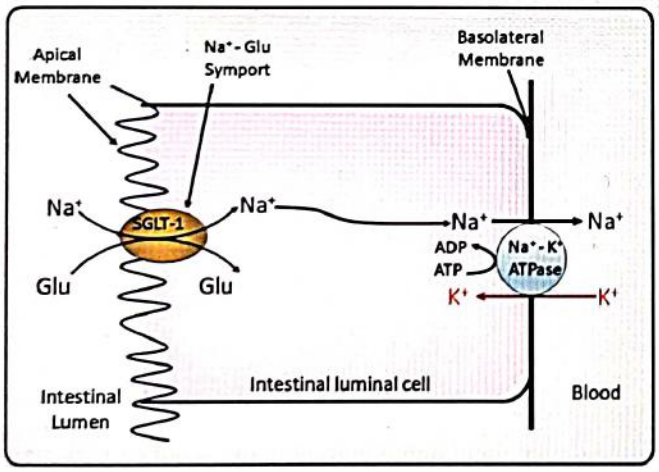
GLUCOSE TRANSPORTERS

- Glucose is a polar molecule due to presence of polyhydroxy groups. So, it cannot simply pass through the non-polar lipid bilayer membrane.
- Glucose transport is carrier mediated through specific channels in the membrane.
- Transport is of two types described as: 00:01:58

Facilitative diffusion	Active Transport
• Down conc. gradient • Bidirectional (can enter or leave) • GLUTs (GLUcose Transporters) • Na-independent	• Against conc. gradient • Unidirectional (can enter only) • Na-Glu symport/Na-dependent Glu Transport (SGLT)

ACTIVE TRANSPORT / Na-GLUCOSE SYMPORT/ SGLT

00:03:50



- Na and Glucose moving in same direction simultaneously (Na-Glu symport)
- Present only in two places

1. In Intestine
2. In Kidney

- ATP utilized by Na glucose symport is 'zero'
  - But Na^+ entering the cells need to be thrown out of cells to maintain electric neutrality
  - Done by $Na^+ K^+$ ATPase Pump using ATP
  - As energy is used indirectly so, it is also called as 2°/Indirect Active transport

Types of SGLTs

00:07:14

Transporter Name	Sugar transported	Location
SGLT-1	Glucose and Galactose	Both Intestine and kidneys
SGLT-2	Glucose only	Only Kidneys

- Mutation in SGLT1: Glu/Gal malabsorption
- Mutation in SGLT2: Renal glycosuria/ Familial renal Glycosuria/Glucosuria

Previous Year's Questions

Q. Method of transport of glucose in the intestine is: (AIIMS Nov 2018)

A. Primary active transport
B. Secondary active transport
C. Simple diffusion
D. Counter transport

FACILITATIVE TRANSPORT

00:08:57

- Na-independent
- Bidirectional, so, to avoid glucose exit from cell
  - Glucose is converted to Glucose–6-Phosphate as soon as it enters the cells
  - In other words, phosphorylation of glucose/ other monosaccharide is done for its entrapment inside the cells

Types of GLUTs

00:11:00

Name	Tissue Location	Function
GLUT-1	Brain, RBC, Placenta, Kidneys	Glucose uptake during fasting/ Basal Glucose Uptake
GLUT-2	Liver, Pancreas, Intestine, PCT	Liver- Glycogen formation Pancreas- Insulin release

GLUT-3	Brain (Neuronal), Placenta, Kidneys	Glucose uptake during fasting
GLUT-4	Skeletal & Cardiac muscles, Adipose tissues	Insulin stimulated Glucose uptake after meals
GLUT-5	Small intestine, Testis (Sperms)	Fructose transport
GLUT-6	WBC, Spleen	Not known (pseudogene that is not expressed into protein
GLUT-7	Liver Endoplasmic Reticulum	Glucose transporter in ER

Previous Year's Questions

Q. Which of the following is present in skeletal muscle? (FMGE Dec 2019)

A. GLUT 4
B. GLUT 5
C. GLUT 7
D. GLUT 2

00:19:15

How to remember

Formula to remember location of GLUTs 1, 2 and 3

$\text{Affinity} \propto \frac{1}{[S]}$ where [S] = substrate (glucose) concentration

- GLUTs 1 & 3 have high affinity
  - Less substrate is required (available in fasting)
  - So, active during fasting
  - Present in organs which always need glucose
- GLUT-2 have low affinity
  - More substrate is required
  - So, Active during fed state
  - Present in organ which store glucose

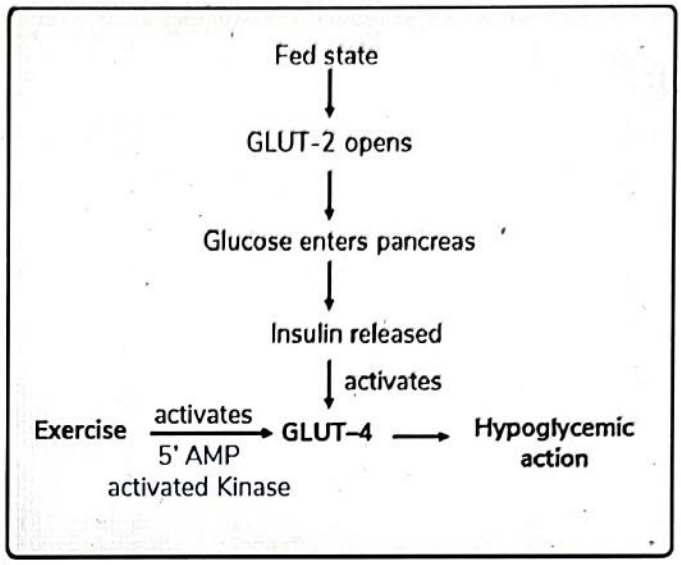
Q1. Are GLUT-1 and 3 dependent on insulin?

Ans: No, because these are active in fasting and there is no insulin present in fasting

Q2. Is GLUT-2 dependent on insulin?

Ans: No, As is clear from the flow chart below as GLUT-2 works even before insulin release.

00:20:05



- Only GLUT-4 depends on Insulin, so, it does not depend on affinity
- In Diabetic patient, due to relative or absolute deficiency of Insulin, GLUT-4 is not active & hypoglycaemic state is not achieved. They are advised to do exercise or brisk walking to decrease their requirement of insulin.

Glucose transporters in intestine and kidneys 00:22:09

Organ	Apical membrane	Basolateral membrane
Intestine	SGLT-1	GLUT-2
Kidneys	SGLT-1 and SGLT-2 (main)	GLUT 1, 2 or 3

Previous Year's Questions

Q. A child is presented with multiple times loose stools. He is managed with ORS. Glucose in ORS is absorbed via which transporter in GIT? (NEET Sep 2021)

A. SGLT-1
B. SGLT-2
C. GLUT-4
D. GLUT-2

FANCONI BICKEL SYNDROME

00:23:12

- Mutation in GLUT-2
- Aka Glycogen storage disease Type XI

- Hepatorenal glycogen accumulation occurs
- Other C/f
  - Growth retardation
  - Polyuria
  - Polydipsia
  - Fasting hypoglycaemia
  - Post-prandial hyperglycaemia
- As GLUT-2 is present in Liver and pancreas. A defect in GLUT-2 will result in to
- Post-prandial Hyperglycaemia due to

1. Glucose not entering liver after eating so, stays in blood causing hyperglycaemia
2. Glucose not entering pancreas and do not stimulate insulin release again causing hyperglycaemia due to no insulin as in diabetes

- Fasting Hypoglycaemia due to

1. As glucose formed from glycogen breakdown in fasting cannot come out of liver cell due to lack of GLUT-2, hence causing Hypoglycaemia
2. lack of reabsorption of glucose in Kidneys and loss of glucose in urine.

CLINICAL QUESTIONS

Q. A 6-month-old baby girl did not respond well to breast-feeding and was put entirely to a formula based on cow's milk at 4 weeks. In next few weeks, she was repeatedly admitted to hospital for complaints of screaming after feeding but was discharged without a specific diagnosis. Suspecting it to be lactose intolerance, her parents tried to eliminate milk from diet, but it did not improve her condition. It was then found that screaming bouts were initiated after the child drank juice which causes formation of gas and a distended abdomen. No enzyme deficiencies were found in liver needle biopsy and no abnormal growth findings were reported on physical examination. The biopsy of intestinal tissue from this patient would most likely reveal deficient or defective?

A. GLUT-2
B. Lactase
C. SGLT-1
D. GLUT-5
E. SGLT-2

Answer: D

Solution

As the patient's liver enzymes are normal and her symptoms are correlated to her intake of fruit juices, indicates a most likely problem in absorption of fructose present in fruit juices.

The primary transporter of fructose in the intestine is GLUT 5 and a deficiency in this transporter would lead to an inability to absorb fructose in the gut. The fructose will get accumulated in the intestine and upon fermentation by bacteria will produce various gases, including hydrogen, as well as organic acids to cause flatulence.

Regarding other options:

A lactase deficiency can be ruled out, as removal of cow's milk from her diet did not alleviate the symptoms.

SGLT-1 and SGLT-2 are secondary active transporters of glucose and galactose in intestine and kidneys, hence will not affect fructose absorption. Deficiency of SGLT-1 and SGLT-2 causes glucose–galactose malabsorption and familial renal glycosuria respectively

GLUT-2 is involved passive absorption of glucose in Liver, pancreas and intestine and its deficiency causes Fanconi Bickel Syndrome (Glycogen storage disease type XI) with clinical features such as hepatorenal glycogen accumulation, growth retardation, polyuria, polydipsia, fasting hypoglycemia but post prandial hyperglycemia resembling diabetes.

Reference: Harper's 30th ed/pg. 191

LEARNING OBJECTIVES

UNIT III: CARBOHYDRATE METABOLISM

- Unit III carbohydrate metabolism consists of topics discussing pathways involved in sequential oxidation of biological carbohydrates such as glucose, fructose and galactose to release energy that can be stored and used for biosynthetic processes. First chapter glycolysis gives the details of various reaction in the pathway categorized as energy utilizing and energy producing phases along with the important enzymes. Cori cycle and Cahill cycle for glucose circulation between liver and exercising and fasting muscle is also discussed. Pyruvate kinase deficiency and an alternative pathway in RBCs known as RL shunt is discussed in brief. Arsenic poisoning and its effect on glycolysis is talked about in a very short chapter 20. Pyruvate oxidation to acetyl CoA via link reaction and further degradation of acetyl CoA in TCA cycle is given in the next two chapters. The other important highlights are the PDH complex of link reaction and important enzyme of TCA along with the factors involved in their regulation. The fate of glucose in anaerobic conditions and in cancer cell is given in chapter "Pasteur's & Warburg's effect". NADH shuttles gives a brief summary of NADH entry into mitochondria and subsequent NADH an $FADH_2$ oxidation is discussed in chapter "ETC". Various component of ETC and mechanism of ATP generation is provided along with the discussion on uncouplers and inhibitors of ETC.

- Chapters 26-28 has information on gluconeogenesis that is activated in fasting/starvation states its regulation in fed vs fasting state (reciprocal regulation) and also in cancer cells known as "TIGAR". Chapters 29-31 gives a detailed discussion on storage carbohydrate glycogen metabolism and disorders related to its defective breakdown resulting in various glycogen storage disorders.

- Two minor pathways to glycolysis for glucose utilization one known as HMP other known as Uronic acid pathway are discussed in chapters 32 and 33 respectively. HMP give rise to synthetic precursors such as NADPH and ribose phosphates whereas uronic acid pathway give rise to uronic acid for GAG synthesis and phase 2 conjugation reactions. The last three chapters provide details of galactose and fructose metabolism and disease associated with congenital defects in enzymes involved in their metabolism.

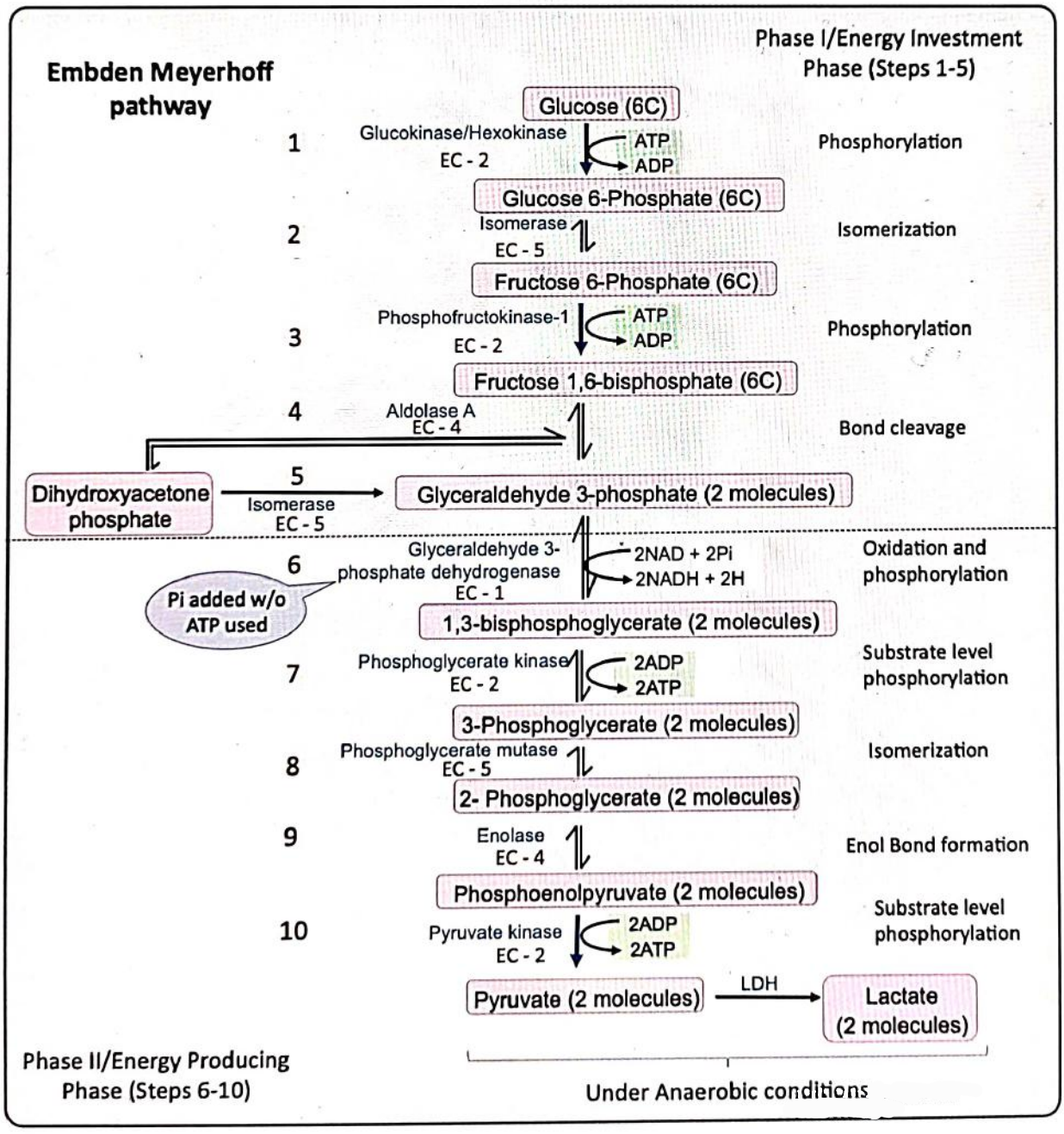
- **Major learning objectives**
  - To learn about the pathways involved in oxidative metabolism of carbohydrates
  - To know the regulation of carbohydrate metabolism under fed and fasting states
  - To learn about the disorders associated with defects in carbohydrate metabolism

19 GLYCOLYSIS

GLYCOLYSIS

00:00:40

- Also known as Embden Meyerhof Pathway after the name of scientist
- Major pathway of glucose metabolism compared to HMP which is a minor pathway for glucose oxidation.



Irreversible steps/ Regulatory steps	Substrate level phosphorylation (SLP)
1. Hexokinase 2. PFK 1 3. Pyruvate Kinase	1. PG Kinase 2. Pyruvate Kinase

Hence,

- all kinases except PG kinase are irreversible or regulatory step enzymes.
- Pyruvate Kinase is both an irreversible and a SLP enzyme

PYRUVATE KINASE

00:23:07

- Requires K > Mg
- 2nd most common human enzyme deficiency (1st mc is Glucose 6-Phospahte Dehydrogenase (G6PD) Deficiency of HMP pathway)
- Both Pyruvate Kinase & G6PD deficient patients presents with Hemolysis

Cause of Hemolysis

- **In PK deficiency**
  - Low energy in RBC is main reason for hemolysis as Glycolysis is major source of energy for RBC
  - PK deficiency→no glycolysis→low ATPs→Na^+-K^+ ATPase inhibited→ Hemolysis
- **In G6PD deficiency**
  - Oxidative Stress is main reason for hemolysis
  - G6PD deficiency→ ↓ NADPH production in RBC → ↓ reduced glutathione → ↑ H_2O_2 → cause cell membrane lysis by oxidation

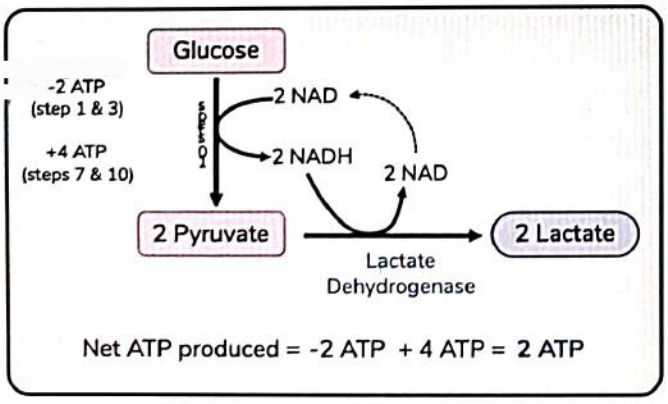
Distinguishing feature between these two is HEINZ BODIES which are only seen in G6PD deficiency

ENERGETICS OF GLYCOLYSIS

00:26:31

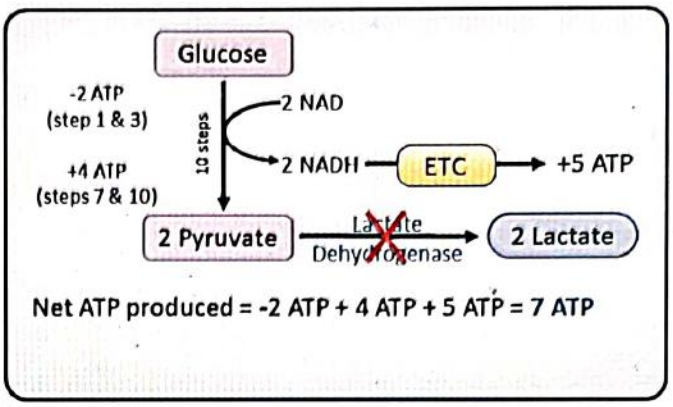
- Glycolysis is partial breakdown of glucose

Under Anaerobic conditions



Net ATP produced = -2 ATP + 4 ATP = **2 ATP**

Under Aerobic conditions



Net ATP produced = -2 ATP + 4 ATP + 5 ATP = 7 ATP

Important Information

- Purpose of extra step of anaerobic glycolysis is
  - regeneration of NAD to keep the glycolysis going.
  - If NAD supply stops oxidation reaction of glyceraldehyde 3-phoshate (Step 6 of glycolysis) by glyceraldehyde 3-phosphate dehydrogenase will stop as this step require constant supply of NAD.

Previous Year's Questions

Q. Importance of pyruvate to lactate formation in anaerobic glycolysis is production of:
(JIPMER Nov 2017)

A. FAD
B. NADH to NAD
C. ATP
D. NAD to NADH

In RBC

- No. of ATPs formed from Glycolysis under all conditions (Fed, fasting, starvation, aerobic and anaerobic): 2 ATP
  - As there are no mitochondria to perform aerobic glycolysis even if oxygen is present.

In Muscles

- Dead end of Glycolysis in muscle: Lactate
  - But this lactate can be utilized in other organs such as liver by following ways:
    1. Glucose lactate cycle (Cori Cycle)
    2. Glucose alanine cycle (Cahill cycle)

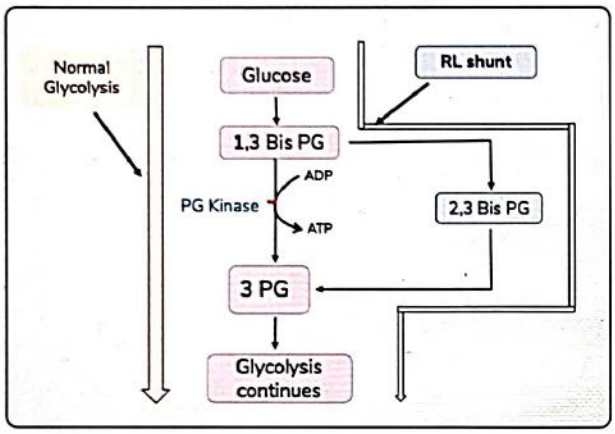
RL SHUNT / RAPAPORT LEUBERING SHUNT / CYCLE

00:32:15

- Occurs only in RBC's
- In RL shunt, one Substrate Level Phosphorylation (1,3 BPG to 3 PG conversion) do not occur
- So, net ATP formed is zero

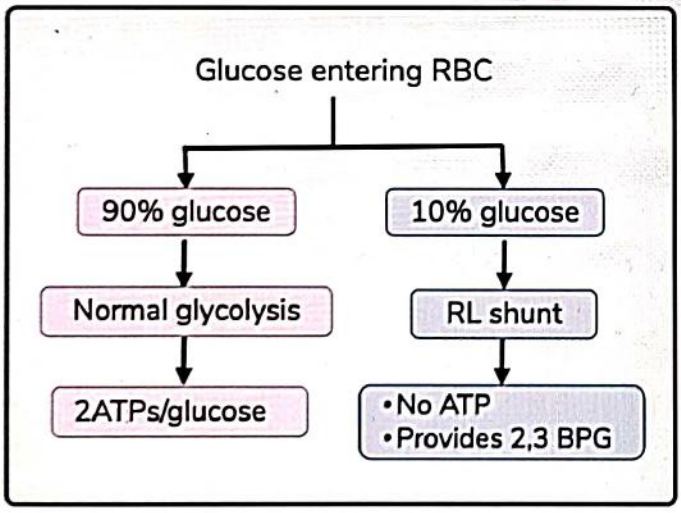
Important Information

If asked about ATP produced in RL shunt mark as zero ATP but if zero ATP option is not given then you can mark 2 ATP.



FULL STORY OF RBC

00:35:10



Significance of 2,3 BPG

2,3 BPG releases O_2 from HbA (α_2B_2)

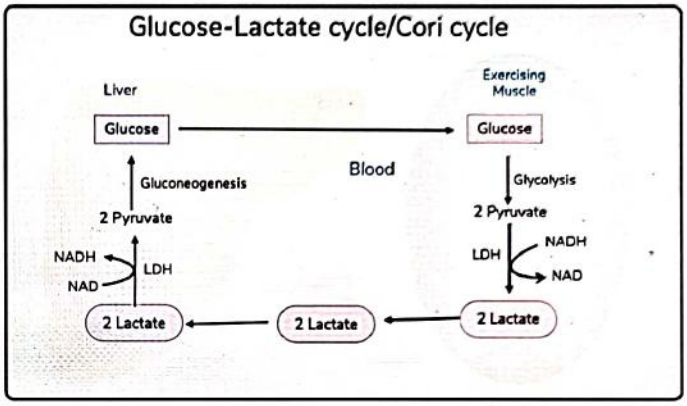
- 2,3 BPG binds to β-chain, so, it will not affect any other Hb. e.g. fetal haemoglobin (HbF) will not be affected as it is made of α and γ chains.
- Also, in fetus, the release of oxygen is not desired, instead it needs to absorb oxygen from mother's blood.

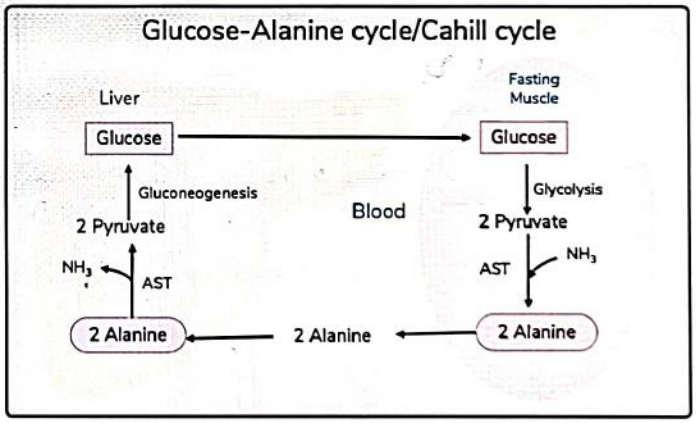
Previous Year's Questions

Q. Which of the following decreases affinity of oxygen with hemoglobin? (JIPMER May 2018)

A. Decreased H+ ions
B. 2,3 DPG
C. Increase in temperature
D. Decreased sorbitol

00:36:32





INHIBITORS OF GLYCOLYSIS

00:40:40

Compound	Enzyme Inhibited	Features
Iodoacetate	Glyceraldehyde 3–P dehydrogenase	• Glycolysis stops and Pyruvate is not formed • No ATP formed
Arsenite	Glyceraldehyde 3-P dehydrogenase	• Glycolysis continued & Pyruvate is formed • No ATP formed (explained in next chapter)

Na Flouride (NaF)	Enolase	• NaF used in blood sample collection vial • If NaF is not added to blood sample, glucose can be lost due to glycolysis leading to an underestimated value
Oxamate	Lactate dehydrogenase	

Previous Year's Questions

Q. Enolase is inhibited by: (FMGE June 2019)

A. NaF
B. Fluoroacetate
C. Iodoacetate
D. Potassium oxalate

CLINICAL QUESTIONS

Q. A 42-year-old man presented with symptoms of weakness, fatigue, shortness of breath, and dizziness. Blood tests reveal anemic condition with hemoglobin levels of 6.8 g/dl (normal > 13.5 g/dl) accompanied by an abnormally low level of lactate production in RBCs. Heinz bodies were not found in peripheral blood smear. This anemia in this patient is most probably due to deficiency of which one of the following enzymes.

A. G6PD
B. Pyruvate kinase
C. Hexokinase
D. Lactate dehydrogenase

Answer: B

Solution

This patient is suffering from deficiency of pyruvate kinase that is also the second most common enzyme deficiency causing hemolytic anemia. It is an autosomal recessive condition. Pyruvate kinase converts phosphoenolpyruvate (PEP) to pyruvate and in the process produces an ATP from ADP by substrate level phosphorylation (SLP). In its absence, there is low production of ATP in RBCs ultimately causing cell lysis as explained in the chapter.

Clinical features of PK deficiency

- Hydrops fetalis
- Prolonged neonatal jaundice
- Hemolytic anemia due to inability to maintain ion pumps in erythrocytes
- ↓ lactate production in the erythrocyte due to low pyruvate production

Reference: - Lippincott 7th ed/pg. 96

Q. A 50-year-old alcoholic had passed out in a street after heavy drinking. When his family members found him, he was rushed to emergency department. The physician gave him an injection of thiamine followed by an overnight parenteral glucose. The patient was alert and coherent next morning with normal serum thiamine and his blood glucose was 73 mg/dL (4 mM). He was discharged from the hospital soon after. Which of the following enzyme/proteins would have no significant physiologic activity in this patient soon after discharge?

A. Malate dehydrogenase
B. GLUT 1 transporter
C. Glucokinase
D. Phosphofructokinase-1

Answer: D

Solution

After an overnight fast (plasma glucose 73 mg/dL), the liver will be stimulated to produce minimal amount of glucose enough to maintain the blood glucose levels for vital organs such as Brain and RBCs. The activity of Glucokinase, an enzyme of glycolysis, would be insignificant as it requires high glucose concentration to be effective (high Km for glucose) and Moreover, as this enzyme is induced by insulin, its activity will be low in the absence or low concentration of insulin in the morning.

Regarding other options:

The other proteins-malate dehydrogenase, PFK-1 and GLUT-1 transporter are active all the time, their activity doesn't depend on fed or fasting state.

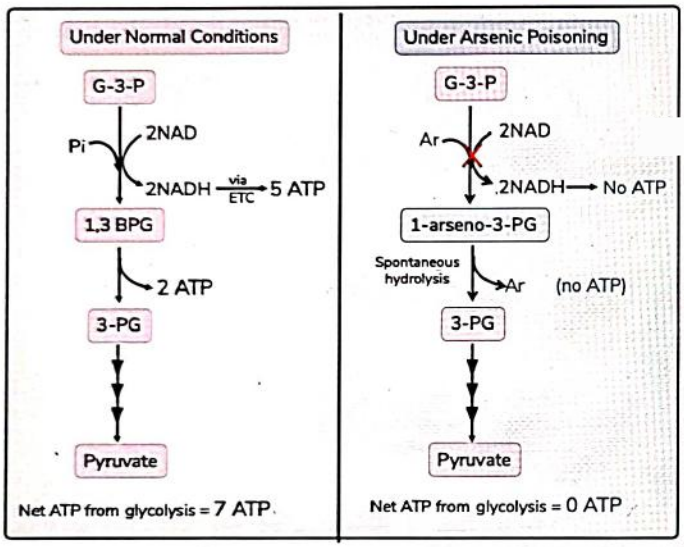
Reference: - Harper's 30th ed/pg. 148, 170

20 ARSENIC

ARSENIC POISONING

- Acts as a poison by inhibiting ATP formation from glycolysis
- Glycolysis pathway is not inhibited i.e. pyruvate is formed
- but no net ATP production from glycolysis (see fig below for mechanism)

00:00:59



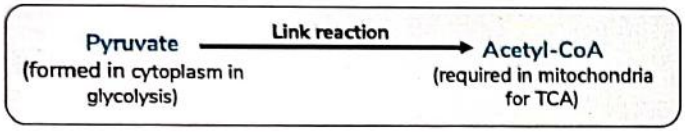
Important Information

- Arsenic also inhibits enzymes:
  1. Pyruvate dehydrogenase of link reaction
  2. α-ketoglutarate dehydrogenase of TCA cycle

21 LINK REACTION

LINK REACTION

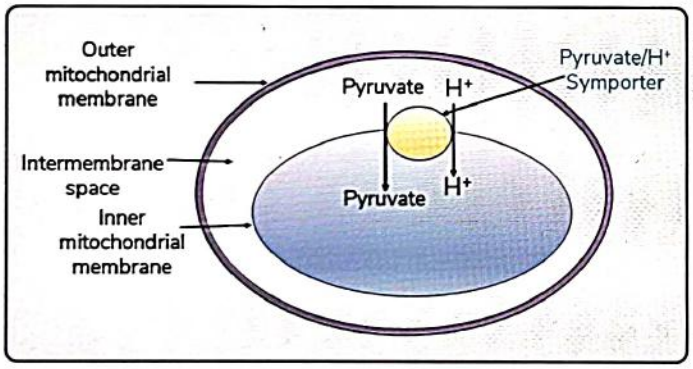
- Is a link b/w Glycolysis & TCA



- Is activated by Insulin (Occur in fed state)
- Occurs in Mitochondria by enzyme Pyruvate dehydrogenase (multienzyme complex) whereas Lactate dehydrogenase is involved in anaerobic glycolysis (present in cytoplasm)

Transport of pyruvate to mitochondria 00:02:22

- Pyruvate must enter mitochondria for conversion to acetyl CoA.
- Transport happens via Pyruvate/ H^+ Symporter that is present in IMM and carry out simultaneous movement of both H^+ and Pyruvate in mitochondria matrix as:



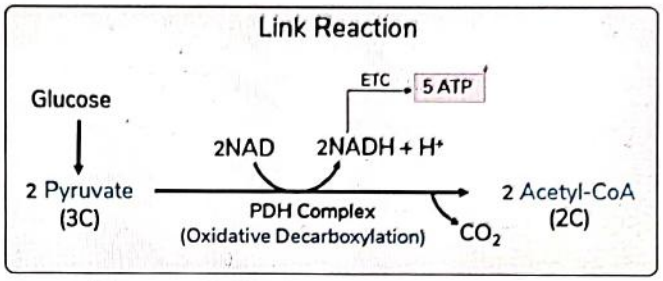
Pyruvate Dehydrogenase Complex [PDH Complex] 00:03:22

- Consists of 3 components
  - E_1 - Pyruvate dehydrogenase
  - E_2 - Dihydro lipoyl transacetylase
  - E_3 - Dihydro lipoyl dehydrogenase

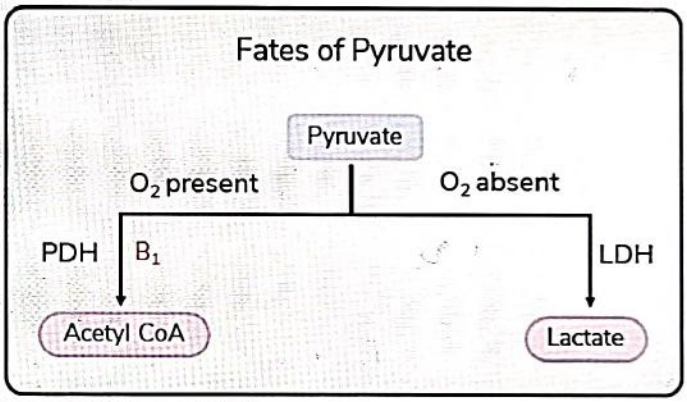
Important Information

- 5 coenzymes are required for link reaction & TCA: Lipoic acid, B_1, B_2, B_3, B_5.
- Lethargy (low energy) is mostly diagnosed due to low levels of these vitamins and multivitamins supplementations are recommended as a remedy

00:05:43



00:07:54



Important Information

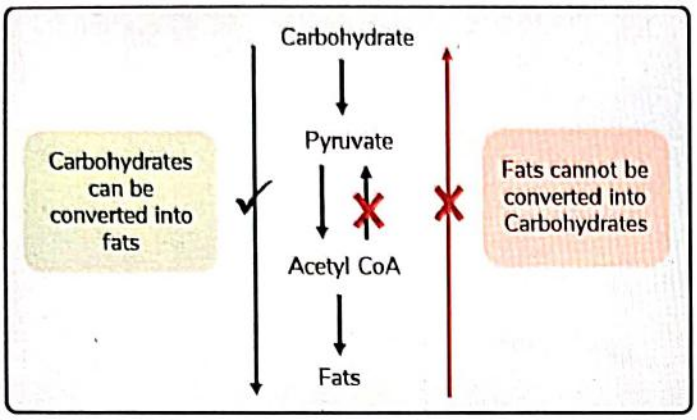
- In PDH or Vit B1 deficiency (Beri-Beri), pyruvate is diverted to lactate formation by LDH. Hence, lactate accumulates resulting in lactic acidosis

Previous Year's Questions

Q. Pyruvate consumption is hampered in which of the following condition(s): (PGI May 2019)

A. Pellagra
B. Beri-Beri
C. Scurvy
D. Rickets
E. Pernicious anemia

Link Reaction Is Irreversible 00:09:26



Exceptions

1. Glycerol (comes from breakdown of Triglycerides)
2. Propionic acid (comes from catabolism of odd chain fatty acids)

Both are breakdown products of fats and can give rise to glucose

Also, it can be very well said that Acetyl Co A is never Glucogenic

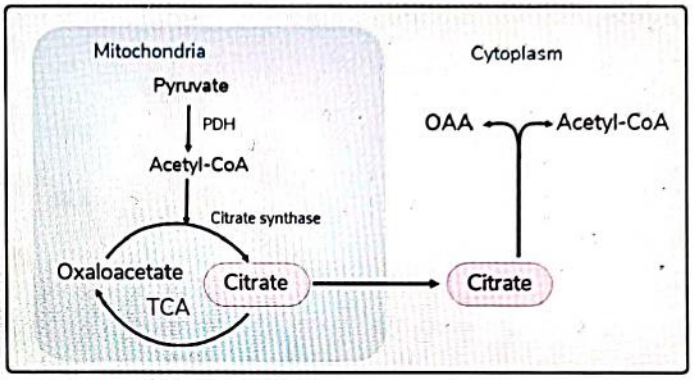
Regulation of PDH 00:12:03

- End-product Inhibition
  - Acetyl CoA (in Fasting) inhibits PDH.
  - Excess NADH inhibits PDH.
- Covalent modification
  - PDH is active in dephosphorylated state.
  - Dephosphorylation is done by Insulin. So, insulin activates PDH in fed state.

Fates of Acetyl CoA 00:14:15

- In Mitochondria
  1. TCA
  2. Activation of Gluconeogenesis
  3. Ketone Body Synthesis
- In Cytoplasm
  1. Fatty Acid/TG Synthesis
  2. Cholesterol Synthesis

Entry of acetyl CoA in cytoplasm is by Citrate Shuttle 00:15:45



CLINICAL QUESTIONS

Q. A full-term female infant during her neonatal period is not gaining weight and has metabolic acidosis. On physical examination at 6 months, she showed hypotonia, small muscle mass, severe head lag, a persistent acidosis (pH 7.0 to 7.2) with a failure to thrive. The blood examination showed highly elevated levels of lactate, pyruvate, and alanine. Thiamine administration did not alleviate the lactic acidosis. Which of the following enzyme is most likely deficient in this patient?

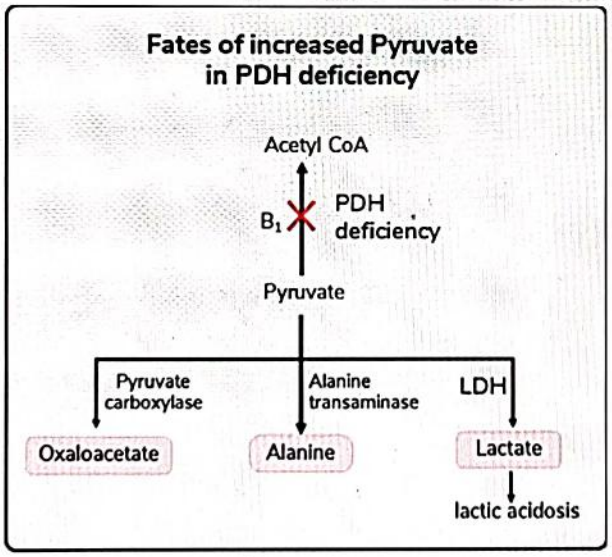
A. Alanine aminotransferase
B. Pyruvate dehydrogenase
C. Pyruvate carboxylase
D. Pyruvate kinase

Answer: B

Solution

The elevated levels of pyruvate, lactate, and alanine in blood indicate that there is a block in the pathway leading from pyruvate toward the TCA cycle. It shows a most probable deficiency in pyruvate dehydrogenase which can cause a buildup of pyruvate.

As the enzyme itself is deficient, increasing its cofactor will be of no use to treat the disease. So, thiamine administration cannot make up the enzyme deficiency.



Thus, on build-up of pyruvate, an increase in lactate and alanine would be expected if pyruvate dehydrogenase was deficient.

Reference: Harper's 30th ed/pg. 174

22 TCA CYCLE

TCA CYCLE

- Called as Tri Carboxylic Acid Cycle as there are 3 carboxyl (-COOH) groups present in first two intermediates citrate and Isocitrate

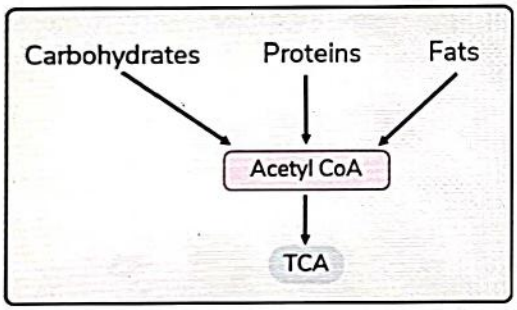
Other Names

- Kreb's cycle → TCA cycle
- Kreb's Henseleit cycle → Urea cycle
- Citric Acid cycle → First compound formed is citrate

Amphibolic cycle: both Anabolic & Catabolic roles

00:01:57

- **Catabolic role**
  - Different molecules broken down to acetyl CoA and enter TCA cycle

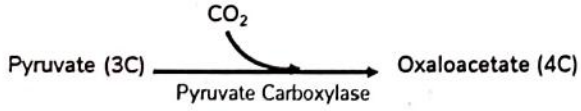


- **Anabolic role**
  - Anabolic means Synthesis
  - TCA intermediates are used for synthesis of various compounds e. g. Succinyl Co A → Haem

Vital cycle: No enzyme deficiency could be present in TCA i.e. any enzyme deficiency of TCA will be lethal

Anaplerotic reactions 00:03:43

- as the intermediates of TCA cycle are continuously used up for anabolic reactions, some other reactions known as anaplerotic reactions replenish or produce TCA Intermediates.
- e.g. OAA synthesis from pyruvate is the most important anaplerotic reaction



REGULATION OF TCA CYCLE

Q: TCA is activated by

A. Insulin
B. Glucagon
C. Both
D. None

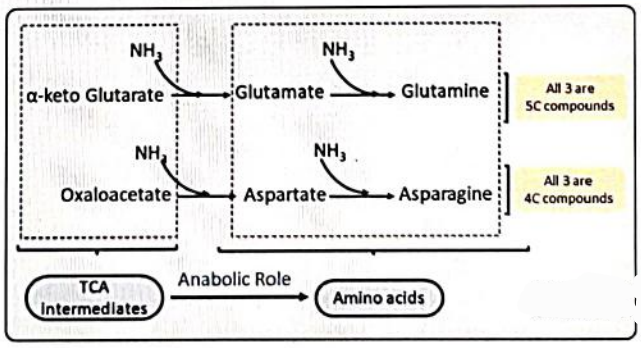
Ans: **D**

- TCA do not have any hormonal control. In other words, TCA does not depend on the fed or fasting state. It is always active irrespective of hormonal changes.
- But TCA cycle is not uncontrolled entirely and activity of TCA cycle is controlled and depends on certain factors.

- **TCA cycle is controlled by** 00:06:25
  1. Energy Status of the cell
     - Low cellular ATP → TCA activity High; High cellular ATP → TCA activity low.
  2. Availability of Oxaloacetate (not acetyl CoA)
     - OAA is regarded as carrier and 1st substrate of TCA Cycle
     - OAA also has catalytic role in TCA Cycle. OAA is regenerated in each round of TCA so, TCA is a considered as a Cycle and not a pathway

SOME IMPORTANT POINTS OF TCA CYCLE

00:09:10



- Succinate / Succinyl CoA is another intermediate of TCA which is a 4C compound.
- Malate (4C): Intermediate of TCA Cycle
  Malonate (3C): Inhibitor of TCA Cycle
- Malonate / Malonyl CoA is an inhibitor of
  1. TCA Cycle (Enz inhbited is Succinate Dehydrogenase)
  2. ETC [component inhibited is Complex II]
  3. Beta Oxidation of Fatty Acids (Enz inhibited is Carnitine Palmitoyl Transferase-1 (CPT-1))

Important Information

- All enzymes of TCA are present in mitochondrial matrix except Succinate Dehydrogenase
  - This enzyme is present in Inner Mitochondrial Membrane (IMM) and is a part of in complex II reaction of ETC

00:13:19

Refer figure 22.1

00:18:09

Important Information

- Thiokinase mostly produces: ATP
- but during starvation as the demand of GTP is high for performing glcuconeogenesis so thiokinase mostly produces: GTP

So, if question ask what thiokinase produces and there is also mention of liver, kidney or starvation than mark GTP otherwise always mark ATP

- Acetyl Co A is not the intermediate of TCA Cycle: all other compounds shown in the cycle are intermediates of TCA Cycle

ENERGETICS OF TCA CYCLE

00:21:24

From 1 Acetyl CoA

- 3 NADH = 7.5 ATP
- 1 $FADH_2$ = 1.5 ATP
- 1 ATP = 1.0 ATP
- Total = 10 ATP/acetyl CoA

Energetics of Complete Breakdown of Glucose

1 Glucose → 5 ATP
2 Pyruvate → 7 ATP
2 Acetyl CoA → TCA → 20 ATP
Total = 32 ATP/glucose

α-KetoGlutarate Dehydrogenase (α-KG DH) Complex and Pyruvate Dehydrogenase (PDH) Complex

- **Similarities**
  - Both are multi enzyme complex
  - Both require 5 coenzymes
    1. Lipoic acid
    2. TPP
    3. FAD
    4. NAD
    5. CoA
- **Difference:** α-KG DH is not regulated by Phosphorylation & Dephosphorylation whereas PDC is regulated by Phosphorylation & Dephosphorylation

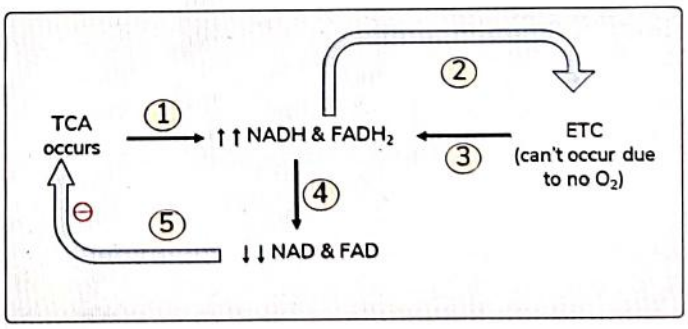
Previous Year's Questions

Q. Vitamins playing an important role in citric acid cycle (JIPMER May 2019)

A. Thiamine, riboflavin, niacin, lipoic acid
B. Thiamine, biotin, riboflavin, pantothenic acid
C. Thiamine, pyridoxine, riboflavin, niacin, pantothenic
D. Thiamine mecobalamin, pantothenic acid

TCA cycle occurs under aerobic conditions and can't occur under anaerobic conditions 00:23:34

- In Anaerobic condition If TCA occurs,
  1. NADH & $FADH_2$ will be produced from NAD, FAD
  2. But ETC will not operate as there is no O_2
  3. So, NADH & $FADH_2$ will keep on accumulating
  4. At the same time NAD & FAD will keep on depleting
  5. So, ultimately TCA Cycle will stop due to shortage of NAD and FAD



Rate Limiting Enzymes/Steps 00:26:32

- Generally single enzyme/step is rate limiting for most pathways
- but special case for TCA. There are 3 enzymes/steps which are rate limiting depending on conditions of cell
  1. Citrate synthase
  2. α-KG DH
  3. Isocitrate Dehydrogenase

Irreversible steps

1. Citrate Synthase (A)
2. α-KG DH

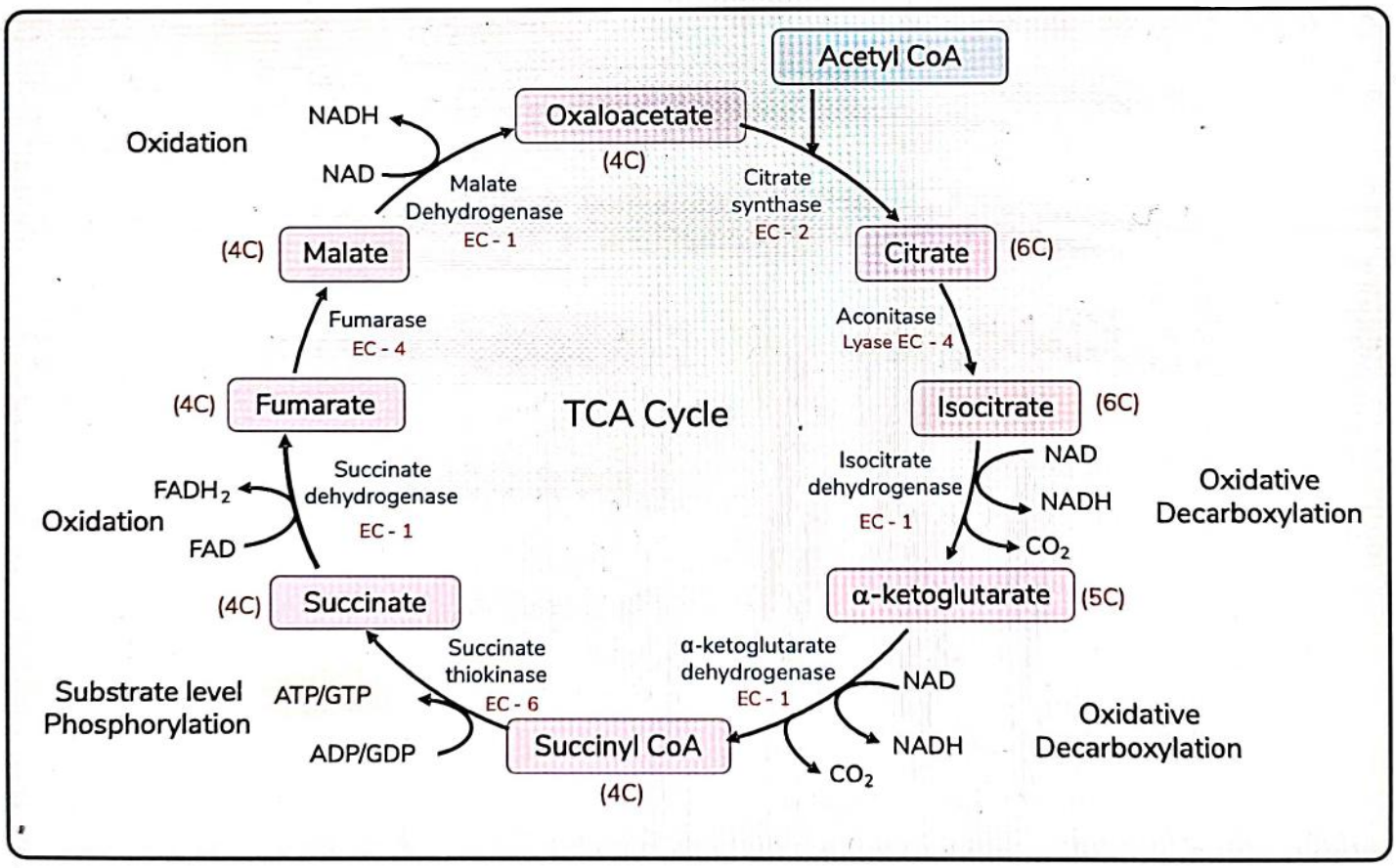
Note: if both are given as option and you need to choose one, then mark citrate synthase

Reaction catalysed	Name of dehydrogenase	Reduced product formed	Kinases of TCA
Oxidative decarboxylation	• α-KG DH • Isocitrate DH	NADH	Only one Kinase: Thiokinase
Oxidation	Succinate DH	$FADH_2$	
	Malate DH	NADH	

Q. CO_2 in TCA derived from?

- First Preferred answer is: Oxaloacetate (as the question mostly implied 1st cycle CO_2)
- If OAA not given in options, then mark acetyl-CoA

Figure 22.1



CLINICAL QUESTIONS

Q. A child of a farming family has accidentally ingested a pest control agent containing cyanide as one of the major components. He was immediately rushed to a hospital by her parents. The TCA compound which will get depleted first is which of the following?

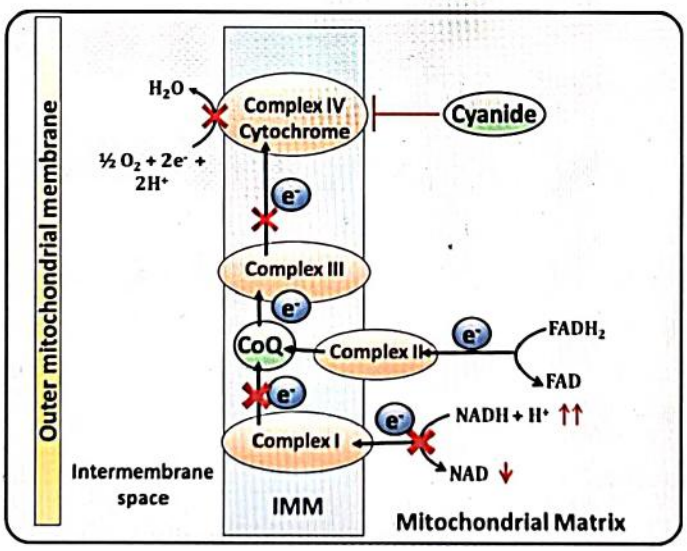
A. Aconitase
B. NAD
C. Citrate
D. Acetyl CoA

Answer: B

Solution

Cyanide inhibits complex IV of ETC. Cyanide is probably the most potent inhibitor of ETC. It exerts its effect by binding to Fe^{3+} of cytochrome oxidase (Complex IV) and blocking its function.

As ETC is involved in oxidation of majority of cellular NADH to regenerate NAD, So, if ETC is inhibited, NADH accumulates. If NADH accumulates, it means NAD depleted. So, TCA is affected because each TCA cycle requires 3 NAD (for Isocitrate Dehydrogenase, alpha-ketoglutarate dehydrogenase and Malate Dehydrogenase).



Regarding other options

Aconitase is a TCA cycle enzyme which catalyzes the isomerization of citrate to isocitrate without using any NADH. Fluoroacetate and fluorocitrate are competitive inhibitors of aconitase.

Acetyl CoA is starting material for TCA cycle and produced from pyruvate by dehydrogenation reaction also using NAD as electron acceptor. However, acetyl CoA can also be produced from other substrate such as amino acid catabolism and fatty acid oxidation. So, its production will not be affected much.

Reference: Harper's 30th ed/pg. 119

Q. A type-1 diabetes person on a trekking trip in mountains was unable to take his insulin shots for last 2 days. Depletion of Which of the following intermediates of TCA cycle in this person will cause suppression of TCA cycle?

A. Succinate
B. Malate
C. a-Ketoglutarate
D. Oxaloacetate

Answer: D

Solution

Remember that diabetic condition is similar to fasting/starvation (catabolic state) as body tissues does not get enough glucose. Hence gluconeogenesis and lipolysis are upregulated.

TCA suppression in diabetes occurs due to following reasons:

- Oxaloacetate is used up for gluconeogenesis, which is not available for TCA.
- Excess lipolysis leads to increased beta oxidation of fatty acids which lead to the formation of excess NADH, which further depletes NAD. This suppresses TCA.
- Acetyl CoA is diverted to ketone body synthesis.

Reference: Harper's 30th ed/pg. 149

23 PASTEUR's & WARBURG EFFECTS

BASIC CONCEPT

Energetics of Glucose 00:00:33

- Aerobic glycolysis → 32 ATP/glucose
- Anaerobic glycolysis → 2 ATP/glucose
  - 16 glucose to be used for 32 ATP
  - So, anaerobic glycolysis is wastage of Glucose

PASTEUR'S EFFECT 00:02:27

- Occurs in any normal cell
- In the presence of O_2, Anaerobic glycolysis is inhibited (saves the glucose wastage)

WARBURG'S EFFECT 00:03:31

- Occurs in cancer cells
- It is paradox from normal i.e. even in the presence of O_2, glucose is converted to lactate
- Hence it is known as "Aerobic glycolysis"
- Normally in aerobic glycolysis
  - End-product is Pyruvate and ATP formed = 7 ATPs
  - But here end-product is lactate and ATP formed = 2 ATPs
  - Lactate is the dead end of Glycolysis, so, no oxidative phosphorylation occurs further
- In conclusion, Warburg's Effect is Aerobic glycolysis with no oxidative phosphorylation
- Due to this effect, Cancer cells uses large amount of glucose to meet energy requirements
  - Responsible for weight loss of patient (Cachexia)

24 NADH SHUTTLES

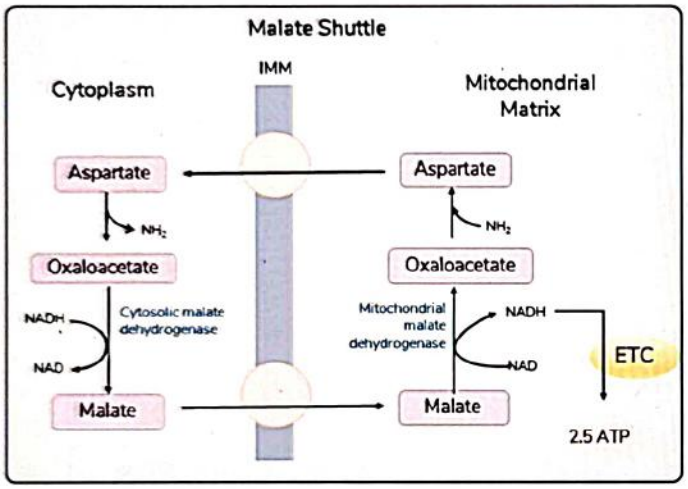
SHUTTLES

00:00:26

- Only Pyruvate, Malate and Aspartate can cross IMM whereas
- NADH and oxaloacetate are formed in cytoplasm and cannot cross IMM to reach mitochondrial matrix
- But NADH is required to enter ETC present in mitochondrial matrix to produce ATP
- Hence there are shuttles which transport NADH into mitochondria.

Two types of Shuttles

00:01:19



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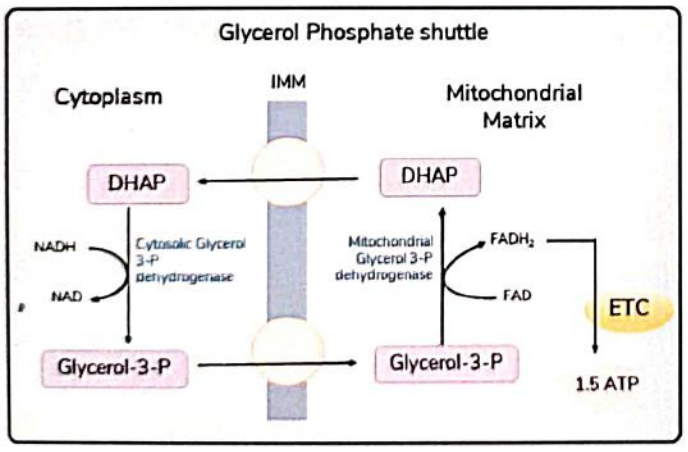


00:06:39

Important Information

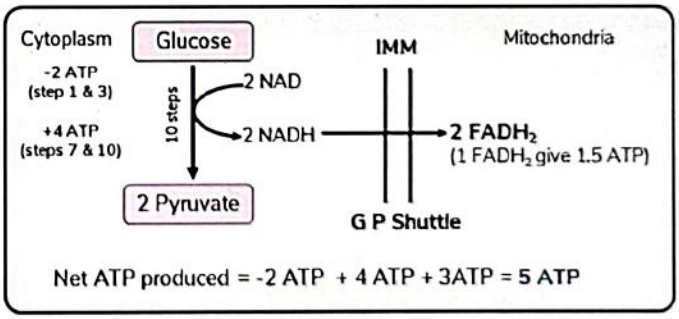
Q. Shuttle is required for
A. Glycolysis
B. Link Reaction
C. TCA
D. All

- NADH produced from TCA and Link reactions are produced inside mitochondria hence can directly enter ETC so, no shuttle is required.
- whereas glycolysis produces NADH in cytoplasm, so shuttle is required to transport this NADH into mitochondria

00:07:33

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

A. **5 ATPs**



	Malate Shuttle	Glycerol – P Shuttle
Organs where used	Liver & Heart	Skeletal Muscle & Brain
Main Enzyme involved	Malate Dehydrogenase	Glycerol – 3 – P Dehydrogenase
Significance	More ATP but slow due to more number of intermediates	Less ATP but quick source of ATP produced due to less intermediates

25 ETC

ETC

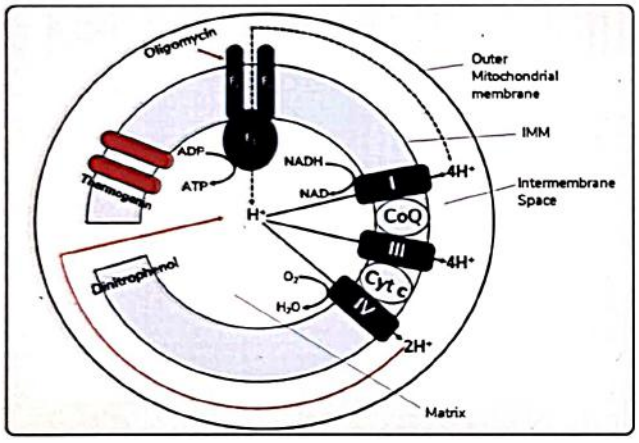
00:00:42

NADH ⟶ NAD + H^+/e^-

Electron flow sequence in ETC

1) NADH
2) Complex I
3) CoQ
4) Complex III
5) Cyt C
6) Complex IV
7) O_2

00:03:31



Energy from the flow of e^- is used to throw H^+ from matrix to intermembrane space by complexes

- No. of protons thrown out by different complexes
  - Complex I → 4
  - Complex III → 4
  - Complex IV → 2
  - 10 H^+/1 NADH

The excess H^+ in intermembrane space creates osmotic gradient (Chemiosmotic effect)

Excess H^+ from intermembrane space, enters the matrix via complex V

- Complex V has two portions

1. F_o	2. F_1
• Rolling gate • Proton Ion channel present on IMM • Attached to F_1	• Protruding towards the mitochondrial matrix • Has ATP synthase activity • Can convert ADP to ATP

- When they cross Fo (rolling gate), mechanical energy is created
- This mechanical energy is transferred to F_1 subunit and which in turn converts ADP to ATP

Names of Complexes

Complex I	NADH CoQ Reductase
Complex II	Succinate CoQ Reductase
Complex III	Cytochrome C Reductase
Complex IV	Cytochrome C Oxidase (Prosthetic group is Cu)

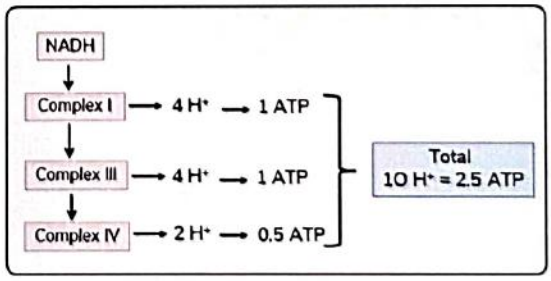
Components of ETC

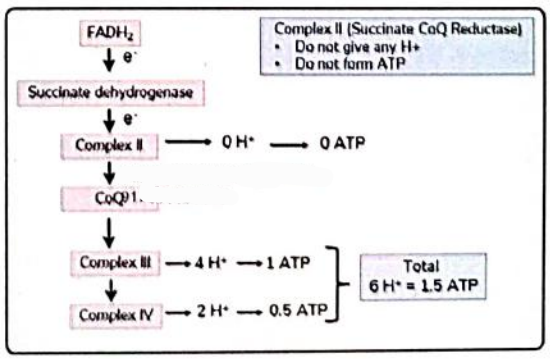
00:09:39

- All are present in IMM
- 5 protein complexes: Complex I to V
- Two mobile e^- carriers
  1. CoQ/Ubiquinone (only non-protein member)
  2. Cytochrome C → Peripheral membrane protein

Redox Potential

- Tendency to accept electron and get reduced
- Every successive substance has ↑affinity for e^-
  - NADH has Least redox potential
  - O_2 has highest redox potential

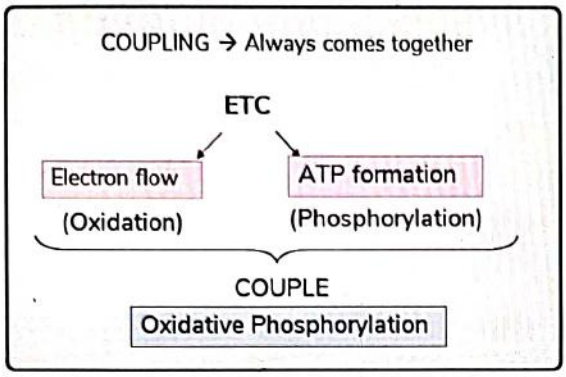




COUPLING AND UNCOUPLING

00:16:13

Coupling



Uncoupling

- Oxidation occurs but Phosphorylation do not occur
- **Uncouplers:** Substances which creates a hole in the IMM. Two Types e.g.

00:17:22

1. Drugs
   - Dinitrophenol
2. Natural / physiological uncouplers
   1. Thermogenin
      - Protein present in brown fat
      - It is a kind of proton Ion channel in IMM
      - Responsible for non-shivering thermogenesis
   2. Thyroxine
      - Thyrotoxicoses patient cannot tolerate heat

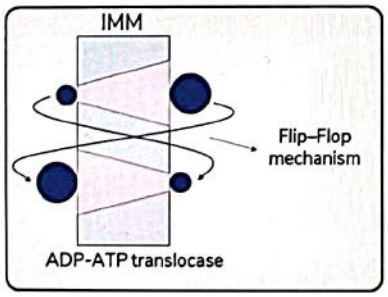
Oligomycin

- Inhibitor of complex V
- Not an uncoupler
- It ligates F_0 gate and closes it
- Inhibits both oxidation & Phosphorylation

ADP – ATP translocase

00:20:26

- Present in IMM
- Has 2 surfaces: Bigger & Smaller
- Transfer the substances by Flip- Flop Mechanism
  - Brings ADP In
  - Throws ATP Out



Atractyloside: Inhibitor of ADP–ATP Translocase
ADP to ATP conversion inhibited by →Oligomycin
ADP to ATP transfer Inhibited by →Atractyloside

Previous Year's Questions

Q. In an isolated mitochondria, in a medium containing Succinate, Fumarate, ADP and Pi, ATPs were produced. Later a compound was added, and oxidative phosphorylation was decreased. What's that compound? (NEET PG Sep 2021)

A. Oligomycin
B. 2,4 dinitrophenol
C. Antimycin
D. Rotenone

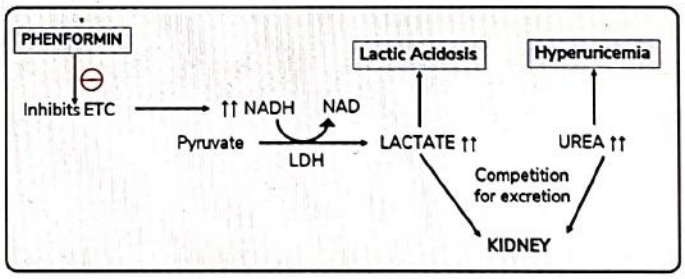
INHIBITORS OF COMPLEX I TO IV

00:27:21

Complex	Inhibitor
I	Rotenone, Phenobarbitone
II	Malonate (3C)
III	Phenformin
IV	CO, CN, H_2S

How to remember

- Complex I (ONE) - RotenONE, PhenobarbitONE



Excess alcohol cause same effects (lactic acidosis and hyperuricemia) by same mechanism.

Previous Year's Questions

Q. Dimercaprol inhibits (JIPMER May 2019)

A. ETC complex 1
B. ETC complex 2
C. ETC complex 3
D. ETC complex 4

CLINICAL QUESTIONS

Q. An obese young woman ingested a banned drug which was once used for weight reduction and she developed high fever. The drug is known to affect the ATP formation in electron transport chain. What could be that drug?

A. Barbiturate
B. Malonate
C. 2, 4 dinitrophenol
D. Rotenone

Answer: C

Solution

2,4 dinitrophenol is an ionophore which allows proton to pass through & dissipates the electrochemical gradient generated by proton pumps. It uncouples the oxidation from phosphorylation i.e. Oxidation continues without phosphorylation. So, energy of electrons is dissipated as heat which raises body temperature. It was once used for weight-reduction but discontinued later because of adverse effects

Regarding other options:

Rotenone is a fish poison. It is not over-the-counter drug.

Barbiturate poisoning leads to hypothermia, not fever.

Malonate is a competitive inhibitor of complex II (succinate dehydrogenase).

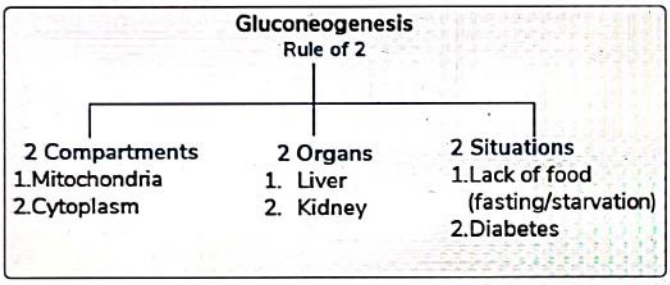
Reference: Harper's 30th ed/pg. 132

26 GLUCONEOGENESIS

GLUCONEOGENESIS

- Occurs in both mitochondria & cytoplasm
  - Any pathway occurring both in mitochondria & cytoplasm will first start from mitochondria
- Organ: occurs in Liver & Kidney
- As it occurs in Fasting state, so. enzymes will be active in Phosphorylated state.
  - All of its enzymes are activated by glucagon and inhibited by insulin

00:01:23



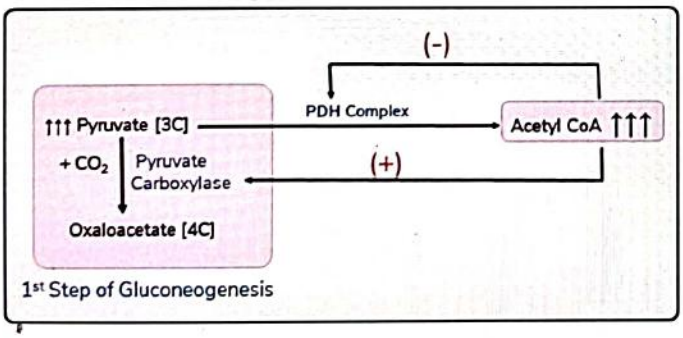
CONDITION IN FED AND FASTING STATE

In Fed State 00:03:06

- Insulin activates PDH complex
- Pyruvate → Acetyl CoA →Fat synthesis

In Fasting 00:03:42

- ß-oxidation of FA ⟶ Acetyl CoA ↑↑↑



1st Step of Gluconeogenesis

- Gluconeogenesis is not a reversal of glycolysis due to three irreversible reactions in glycolysis.
- These reactions need to be bypassed using enzyme different than glycolysis.

00:07:24

Glycolysis	Gluconeogenesis
1.Pyruvate Kinase	1.Pyruvate Carboxylase 2.PEPCK (Phosphoenol Pyruvate Carboxy Kinase)
2.PFK - 1	3.Fructose 1, 6 Bisphosphatase
3.Hexokinase	4.Glucose – 6 - Phosphatase

00:08:39

Refer Figure 26.1

Previous Year's Questions

Q. Enzyme present in both glycolysis and gluconeogenesis? (AIIMS June 2020)

A. PFK
B. PEPCK
C. Phosphoglycerate kinase
D. Pyruvate Kinase

Previous Year's Questions

Q. Enzymes used in gluconeogenesis are: (INICET Nov 2020)

A. Hexokinase
B. PEPCK
C. Pyruvate carboxylase
D. Glu 6-phosphatase
E. Pyruvate kinase

Transporters / channels

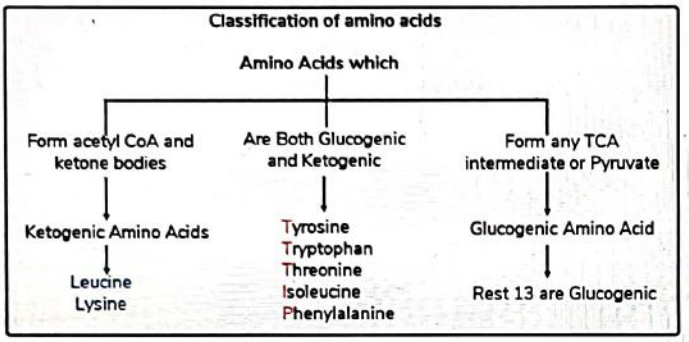
- T1 → For the entry of Glucose 6-P in ER
- T2 → For the exit of Glucose from ER
- T3 → For the exit of P_i

All these transporters are active during Fasting state only

Substrates of gluconeogenesis (mostly 3C) 00:19:07

1. Pyruvate (3C)
2. Lactate (3C)
3. Glycerol (3C)
4. Propionic acid (3C)
5. Glucogenic amino acids
6. Both Ketogenic & Glucogenic amino acids
7. Any TCA Intermediate (forms oxaloacetate which can enter gluconeogenesis)

00:21:35



Ketogenic AA = 2 + 5 = 7
Glucogenic AA = 13 + 5 = 18

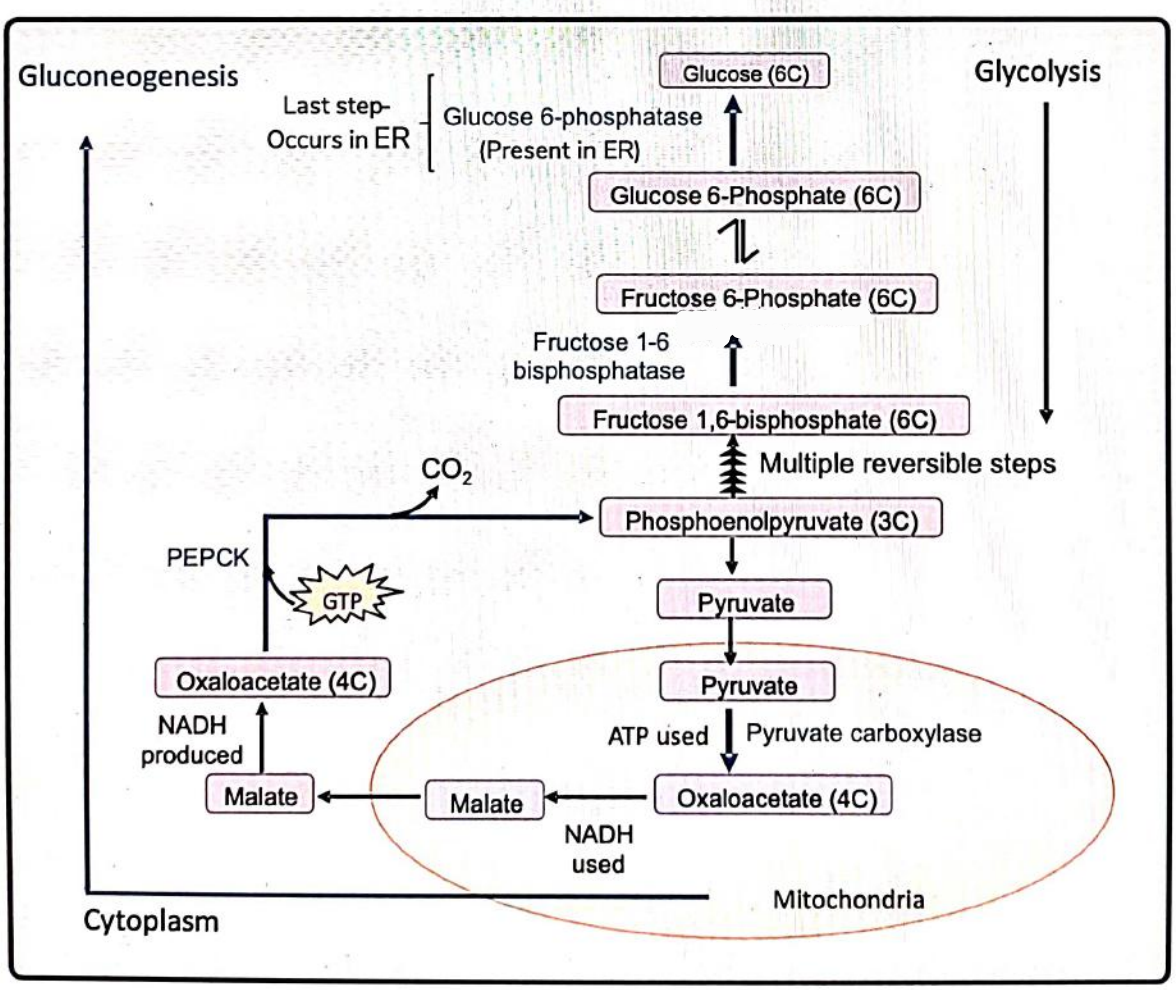
ENERGETICS FOR FORMATION OF 1 GLUCOSE

00:25:31

- **For each pyruvate**
  - Pyruvate carboxylase uses 1 ATP
  - PEPCK uses 1 GTP
  - PG Kinase uses 1 ATP

- 2 pyruvates are required for making 1 glucose (so, multiply by 2)
  - So, Total ATP/GTP used for 2 pyruvates = 4 ATP + 2 GTP
  - 6 High energy phosphates are used to make 1 Glucose from 2 molecules of Pyruvate
  - Similarly,
    - → 6 ATPs are used to make 1 Glucose form 2 molecules of Lactate
    - → 6 ATPs are used to make 1 Glucose form 2 molecules of Alanine

Figure 26.1



CLINICAL QUESTIONS

Q. A 3-month-old male infant developed seizures which progressively worsened. He is also showing hypotonia, psychomotor retardation, and poor head control. He had lactic acidosis and highly elevated plasma pyruvate levels. Pyruvate carboxylase activity in fibroblasts was found to be only 1 percent of the normal level. The best therapy for this patient will be oral administration of:

A. Alanine
B. Glutamine
C. Leucine
D. Lysine

Answer: B

Solution

Pyruvate carboxylase deficiency results in a reduction of amount of oxaloacetate (C4 acid), which is the acceptor of acetyl group from acetyl-CoA in first step of TCA cycle. So, C4 or oxaloacetic acid must be supplied continuously in order to keep the TCA cycle running efficiently.

Amino acids whose carbon skeletons feed into the TCA cycle and increase the C4 pool will accomplish this. Glutamine, which can get converted to α-ketoglutarate through action of glutaminase and glutamate transaminase, will lead to an increase in all of the C4 acids (succinate, fumarate, malate, and oxaloacetate). (Refer to TCA cycle diagram in chapter 22)

Regarding other options:

Alanine give rise to pyruvate by transamination and in case of the deficiency of pyruvate carboxylase will not increase the C4 pool.

Lysine and leucine are ketogenic amino acids and thus also do not increase the C4 pool. These amino acids do not form TCA intermediates.

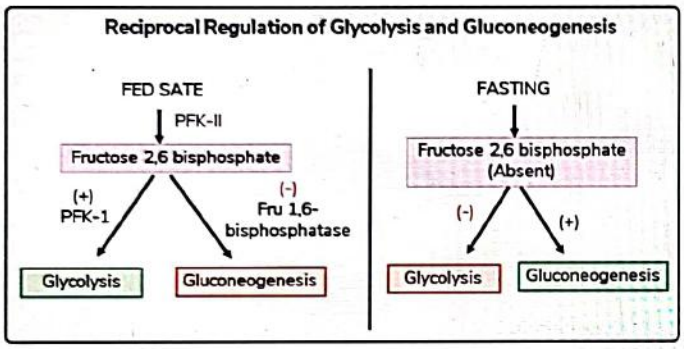
Reference: Harper's 30th ed/pg. 185

27 RECIPROCAL REGULATION

RECIPROCAL REGULATION

00:00:14

- To regulate the activity of two opposing pathways e.g. glycolysis and gluconeogenesis
- prevents a futile cycle and wastage of cell energy



Previous Year's Questions

Q. Gluconeogenesis is inhibited by? (FMGE Dec 2019)

A. Glucagon
B. Insulin
C. Cholecystokinin
D. 5 alpha hydroxylase synthase

00:04:42

Important Information

Formula: Regulator [activator / inhibitor] is more important than Substrate

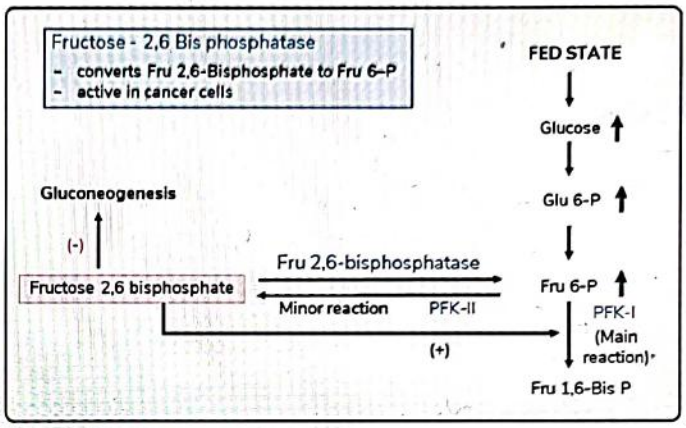
Q. No acetyl CoA. lots of pyruvate. Gluconeogenesis occurs or not?

Ans. Acetyl CoA is activator & pyruvate is substrate. so, No Gluconeogenesis occurs as activator is absent

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

Ans. Fructose 2,6 Bis (P) is inhibitor & Glycerol is substrate. So, No Gluconeogenesis occurs as one molecule of Fructose 2,6 Bis (P) inhibitor is more important than 1000 molecules of substrate glycerol

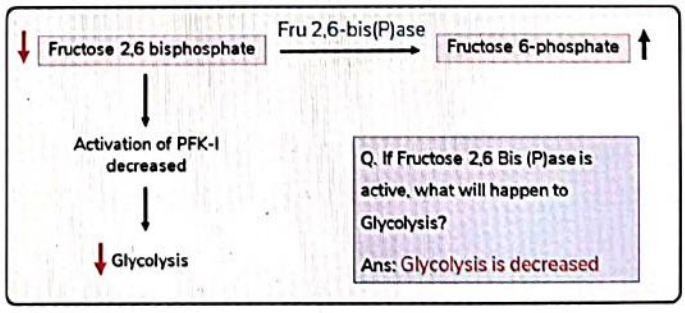
00:06:52



Important Information

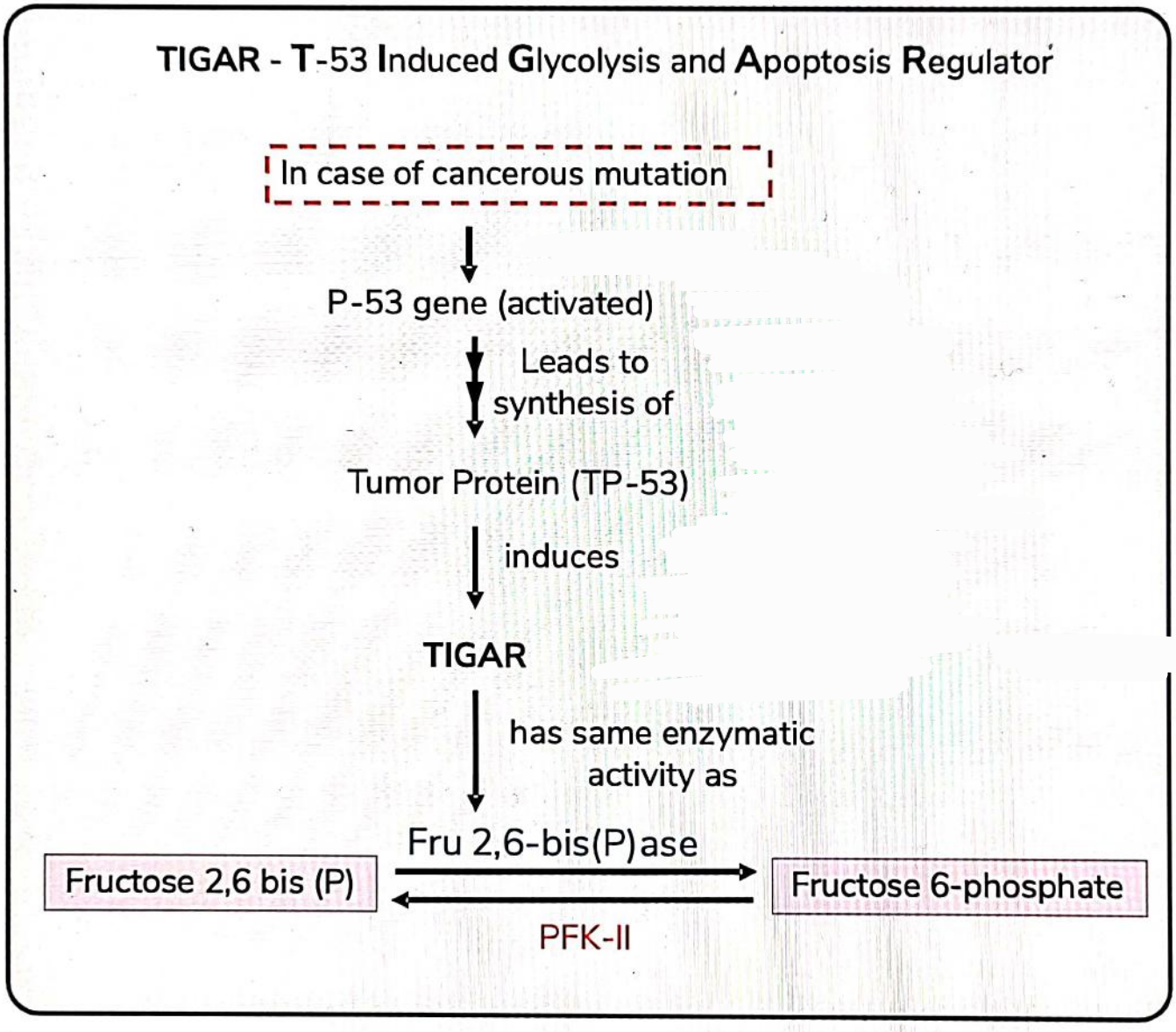
- Fructose 1,6-Bisphosphate → Glycolysis
- Fructose 1,6-Bis Phosphatase → Gluconeogenesis
- Fructose 2,6-Bisphosphate → Reciprocal Regulation of glycolysis and gluconeogenesis
- Fructose 2,6-Bis Phosphatase → Cancerous mutation (not in normal cells)

00:08:53



28 TIGAR

00:01:07



TIGAR (TP-53 Induced Glycolysis & Apoptosis Regulator)

00:03:12

- Activation of TIGAR leads to Decreased Glycolysis
  1. Cell replication do not occur due to low energy in cell.
  2. Cancer cell become dormant and Repair process begins and cell become normal
- However, If repair process do not begin for some reason, cell is killed by Apoptosis to prevent cancer

29 GLYCOGEN METABOLISM

GLYCOGEN

00:00:42

- Glycogen is stored in Liver & Muscle. So, most of glycogen metabolism occurs in these organs
  - By weight glycogen is more in Liver
  - By % glycogen is more in Muscle
- Glycogenesis → Synthesis → Occurs in Cytoplasm
- Glycogenolysis → Breakdown → Also occurs in Cytoplasm
  - Both Rate Limiting enzymes belong to Transferases (EC No. 2)

Storage Organ	Main Function	End-product
Liver	• Used to Maintain Blood Glucose	• Glucose
Muscle	• Used for Muscle contraction	• Glucose- 6– P o Cannot come out of muscle cells and used there only

Previous Year's Questions

Q. Glucose is stored in Glycogen form. why? (AIIMS June 2020)

A. Compact
B. Can be reduced from multiple branches/ends
C. Can be stored at multiple sites
D. Can provide glucose as much as needed and when needed for 1 week

00:02:40

Important Information

Q. If Muscle glycogen is used for anaerobic glycolysis, then how many ATPs obtained?

Ans: 3 ATPs

In anaerobic glycolysis, ATP is consumed at:

1. Hexokinase 2. PFK – 1

Net ATPs = 4 – 2 = 2

In Muscle Glycogen Metabolism,

- Glucose–6-(P) is the end-product which directly enter at glucose 6-P step saving one ATP in phase I.
- So, only 1 ATP is consumed at PFK – 1 Reaction

So, Net ATPs = 4 – 1 = 3

GLYCOGENESIS OR GYLCOGEN SYNTHESIS

00:05:35

- Primer for glycogen synthesis: glycogenin (protein)
- UDP–Glucose is Activated glucose
- Glycogen synthase transfers glucose from UDP–Glucose to Glycogenin. So, acts as Transferase (EC no. 2)

Refer Figure 29.1

GLYCOGENOLYSIS

Minor pathway of glycogenolysis

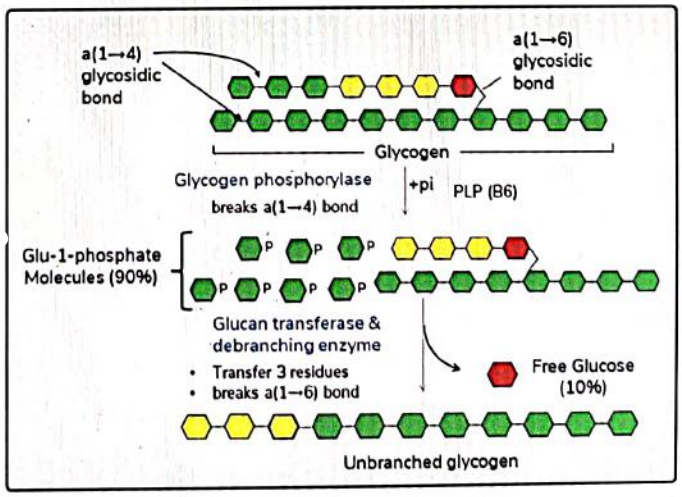
- Occur in lysosomes
- Acid maltase converts glycogen directly to glucose
- Pompe's disease
  - D/t deficiency of Acid Maltase
  - Only Glycogen Storage disease which is a Lysosomal Storage Disease

Major pathway of glycogenolysis 00:13:14

1. Glycogen Phosphorylase
- Transfers P_i, uses PLP (B_6)
- Breaks α (1 → 4) bonds from one end
- It breaks the bonds until 4 glucose residues are left at branch point
- End-product: Glucose 1–P (90%)

2. Glucan Transferase
- Transfers 3 residues to neighbouring straight chain

3. Debranching Enzyme
- Breaks α (1 → 6) bond
- End-product: free Glucose (10%)



Previous Year's Questions

Q. Which vitamin is required for glycogen Phosphorylase? (AIIMS Nov 2017)

A. PLP
B. TPP
C. Riboflavin
D. Lipoic acid

Important Information

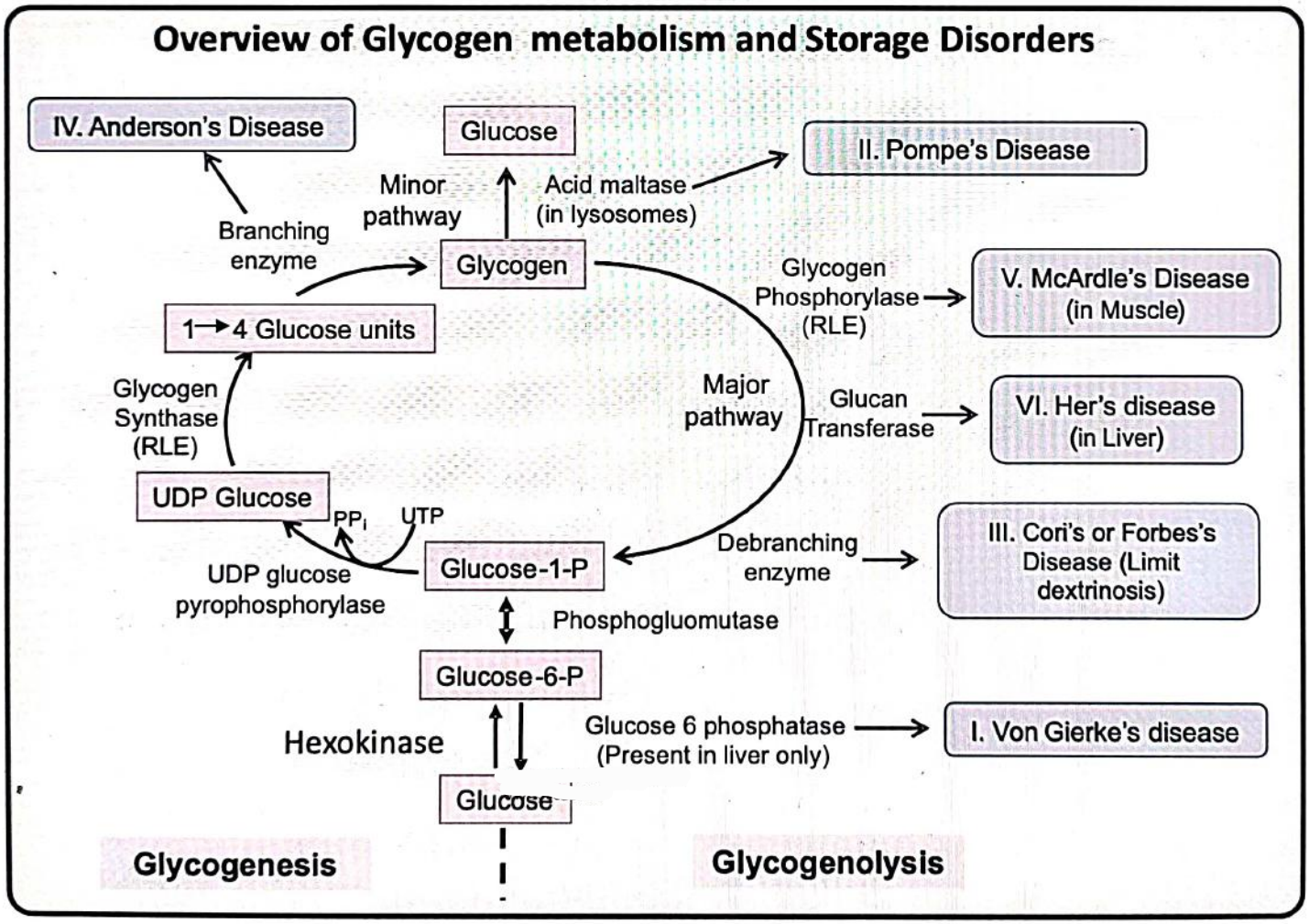
- Only in liver glucose 6-phosphate is converted to glucose by enzyme glucose 6-phosphatase
- This enzyme is absent in muscle, so, muscles cannot maintain blood glucose levels

Enzyme with Bifunctional Activity: Glucagon Transferase + Debranching enzyme (same protein with 2 enzymatic activities)

Common step for both Glycolysis & Glycogenesis: Hexokinase/Glucokinase step

Enzyme requiring vitamin B6/PLP as cofactor: Glycogen phosphorylase

Figure 29.1



30 GLYCOGEN STORAGE DISEASES

GLYCOGEN STORAGE DISEASES (GSDs)

Reason: Glycogen not getting broken down due to enzyme deficiency causing glycogen accumulation.

Main features 00:00:47

- Liver Glycogen Storage Disorders: Hypoglycemia
- Muscle Glycogen Storage Disorders: Muscle Cramps & Exercise Intolerance 00:03:13

Type	Disease	Enzyme Deficient	Affected Organ
I	Von Gierke's [MC]	Glucose 6-P ase	Liver
II	Pompe's [Lysosomal Dis]	Acid maltase	
III	Cori's/limit dextrinosis	Debranching enzyme	Liver, Muscle, Brain
IV	Anderson/ Amylopectinosis	Branching enzyme	
V	McArdle's	Muscle Phosphorylase	Muscle
VI	Her's	Liver Phosphorylase	Liver

Important Information

Patients of McArdle's Disease Don't have increased Lactate levels after exercise.

Previous Year's Questions

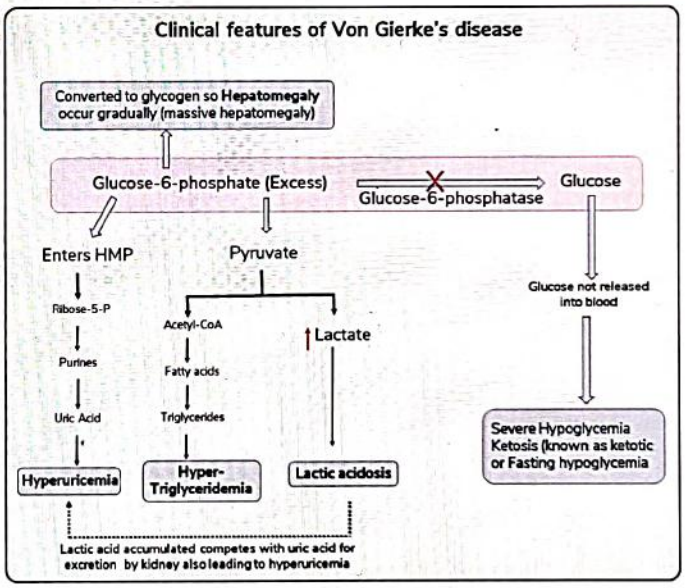
Q. An adolescent male patient came with pain in calf muscles on exercise. On biopsy, excessive amount of glycogen was found to be present in the muscle. What is the most likely enzyme deficiency? (AIIMS May 2018)

A. Muscle debranching enzyme
B. Phosphofructokinase-I
C. Glucose 6-phosphatase
D. Phosphorylase enzyme

00:10:17

Type I (Von Gierke's)	Type VI (Her's)
Similarities: No Glycogenolysis	
Differences	
• No Gluconeogenesis • Severe Hypoglycemia • Ketosis	• Gluconeogenesis occurs • Mild Hypoglycemia • No Ketosis

00:12:18



Clinical Features

1. Severe Hypoglycemia (most important)
2. Ketosis
3. Hepatomegaly
4. Hypertriglyceridemia
5. Lactic Acidosis
6. Hyperuricemia
7. Enlarged Kidneys
8. Doll like facies/moon like due to fat deposition

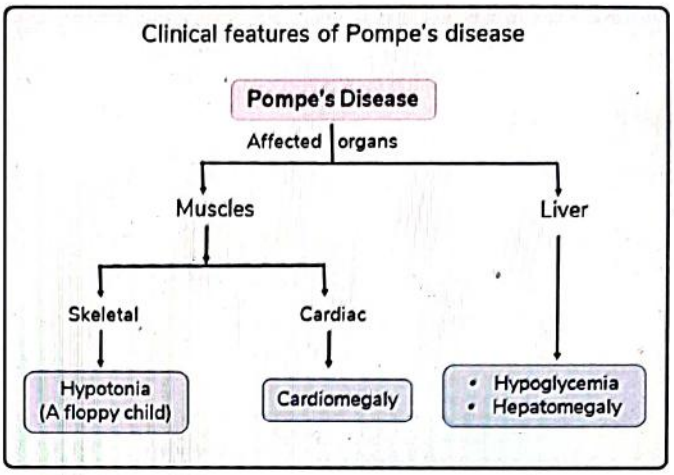
Rx

1. Small frequent meals
2. Corn starch diet

Previous Year's Questions

Q. A 10-year-old boy rapidly develops hypoglycemia after moderate activity. Blood examination reveals raised levels of ketone bodies, lactic acid & triglycerides. On examination, liver & kidneys were enlarged. Histopathology of liver shows deposits of glycogen in excess amount. What is the diagnosis?
(AIIMS Nov 2017)

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



00:15:59

Type I (Von Gierke's)	Type III (Cori's / Limit Dextrinosis)
Similarities	
Hypoglycemia, Hepatomegaly, Hyperlipidemia	
Differences	
• Hepatomegaly	• Hepatosplenomegaly
• Lactic acidosis	• No Lactic acidosis
• Severe Hypoglycemia	• Mild Hypoglycemia/early morning hypoglycemia
• Kidney enlarged	• No Kidney enlargement
• Normal glycogen accumulated	• Limit dextrins (mainly α–(1→6) bonds) accumulated
• On Glucagon administration, Blood glucose does not ↑ In fed or fasting state	• On Glucagon administration, Blood glucose ↑ only in fed state, not in fasting state

Important Information

- Most common GSD in children: Type I (Von Gierke's)
- Most common GSD in adults: Type V (McArdle's)

CLINICAL QUESTIONS

Q. Baby has hypoglycemia, especially early morning hypoglycemia. Glucagon given. It raises blood glucose if given after meals but does not raises blood glucose if given during fasting. Liver biopsy shows increased glycogen deposits. The most likely enzyme defect in this patient is?

A. Muscle phosphorylase
B. Glucose-6-phosphatase
C. Branching enzyme
D. Debranching enzyme

Answer: D

Solution

This patient has debranching enzyme deficiency, also known as limit dextrinosis or Cori's Disease. Debranching enzyme is a bifunctional enzyme with two activities:

1. Glucan transferase
2. Amylo α-1,6-glucosidase

It breaks alpha (1→6) glycosidic bond present at the branch point of glycogen in glycogen breakdown .(Refer to Glycogenolysis pathway in chp glycogen metabolism). Normally this enzyme releases just 10% glucose. So, the patient has only early morning hypoglycemia in its deficiency i.e. mild hypoglycemia. (No hypoglycemia during the daytime, when meals are taken).

Limit dextrins (a branched oligosaccharide having lots of alpha 1→ 6 bonds) are accumulated in this patient's liver during fasting, so, if glucagon given during fasting, phosphorylase (rate-limiting enzyme of glycogenolysis activated by glucagon) will be activated which cannot break limit dextrins. Phosphorylase enzyme can only break alpha 1→4 bonds. So, this will not raise blood glucose. In fed state glycogen is formed. So, if glucagon is given during fed state, phosphorylase will be activated which can break glycogen (has both alpha 1→4 & alpha 1→6 glycosidic bonds) & can raise blood glucose.

In von Gierke's disease, if glucagon is given, blood glucose will not be increased and in this disease, the patient has severe hypoglycemia as both glycogenolysis and gluconeogenesis cannot occur due to deficiency of Glucose-6-phosphatase, so, Option B is not the answer.

Reference: Harper's 30th ed/pg. 178

Q. A 20-year old boy patient who is suffering from congenital deficiency of muscle phosphorylase is getting examined by an ischemic forearm exercise test by squeezing a rubber ball. This patient would exhibit which one of the following as compared to a normal person performing the same test.

A. Exercise endurance
B. ↑ blood glucose in the blood drawn from the exercising forearm vein
C. ↑ blood lactate in the blood drawn from the exercising forearm vein
D. Relatively ↓ blood lactate in the blood drawn from the exercising forearm vein

Answer: D

Solution

Normal or low blood lactate and ↑ blood ammonia after an ischemic exercise test suggest a defect in the conversion of glycogen (glucose) to lactate. This is a case of McArdle's disease that occurs due to deficiency of muscle glycogen phosphorylase enzyme such that glycogen cannot be converted to glucose and further to lactate in exercising muscle. Hence, Normal or relatively low levels of lactate will be formed from muscle glycogen after exercise.

Another thing that happens is that due to lack of energy and ATP, muscle cells get damaged. Hence Myoglobin will be released in urine causing myoglobinuria.

Definitive diagnosis of this disease includes an enzymatic assay in muscle tissue and mutation analysis of the myophosphorylase gene.

Extra Information:

Abnormal exercise response also occurs with:

- deficiencies of muscle phosphofructokinase
- deficiencies of debranching enzyme (when the test is done after fasting).

Reference: Harrison 20th ed/pg. 433

Q. A young boy suffering from severe fasting hypoglycemia was brought to ER. Physical examination revealed hepatomegaly whereas blood examination indicated hyperlactic acidemia and hyperuricemia. A liver biopsy indicated that hepatocytes contained more than normal amounts of glycogen that was of normal structure. All these findings in the patient suggest most likely deficiency of which of following?

A. Glycogen synthase
B. Glucose 6-phosphatase
C. Glycogen phosphorylase
D. Amylo-α (1→6) - glucosidase

Answer: B

Solution

This diagnosis is glucose 6-phosphatase deficiency (Von Gierke's disease) that causes severe fasting hypoglycemia, hyper lactic acidemia, ketosis and hyperuricemia as shown in diagram in the text.

Regarding other options:

A deficiency of glycogen phosphorylase would result in decrease in glycogen degradation, causing fasting hypoglycemia, but not the other symptoms.

Glycogen synthase deficiency, which is rare, results in lower amounts of stored glycogen.

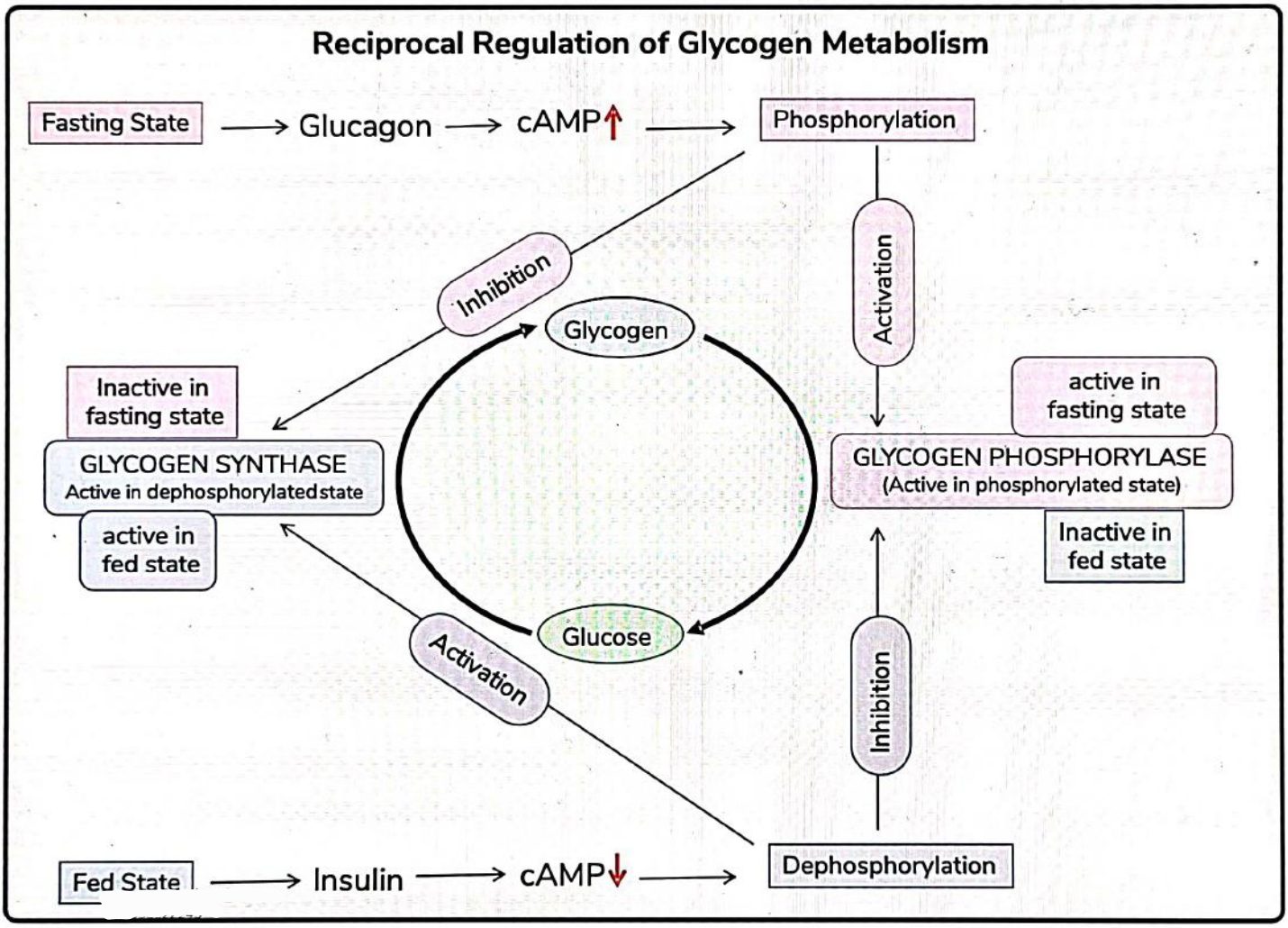
Amylo α (1→6)-glucosidase or Debranching enzyme removes single glucosyl residues attached to the glycogen chain through an α(1→6) glycosidic bond. Its deficiency causes Cori's disease and give rise to limit dextrins which were not found in this patient.

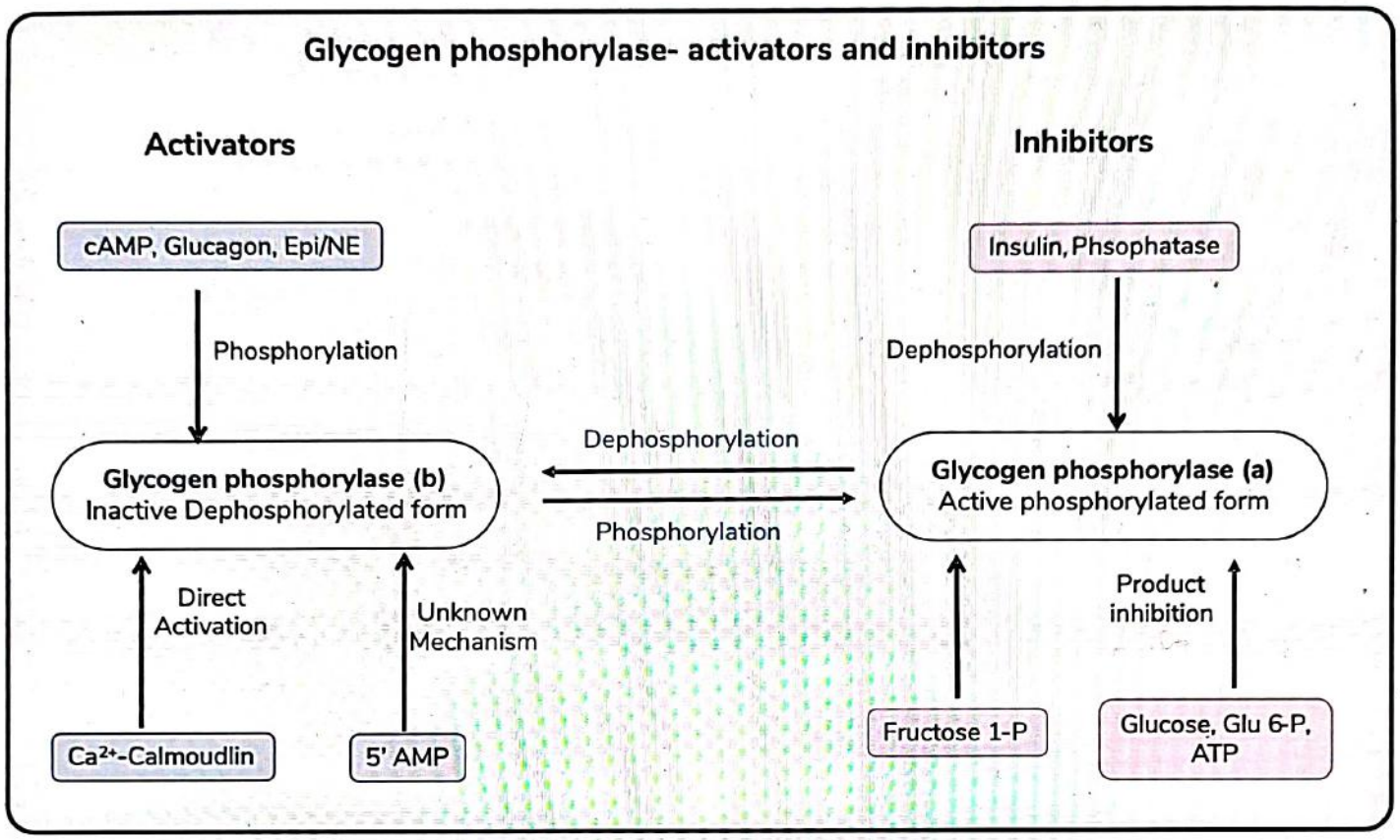
Reference: Harper's 30th ed/pg. 170, 186

31 GLYCOGEN REGULATION

REGULATION OF GLYCOGEN METABOLISM

00:00:35





Important Information

- Fructose 1-P accumulated in Liver in Hereditary Fructose Intolerance will
  - cause glycogen phosphorylase inhibition
  - result in Hypoglycemia

32 HMP

HMP (HEXOSE MONOPHOSPHATE PATHWAY)

- Glucose 6–P (a hexose phosphate) is the starting material hence the name Hexose Monophosphate Pathway
- It is a minor pathway for the oxidation of Glucose
- Other name is Pentose Phosphate Pathway (PPP) as Pentose Phosphate i.e. Ribose 5–P is synthesized only by this pathway.
- NADPH is also synthesized majorly by HMP but NADPH is also synthesized by other pathways.
- It is an Anabolic pathway occurring in cytoplasm.
- Occur in fed state, so, activated by Insulin and Inhibited by glucagon.
- No ATP are produced in HMP

Previous Year's Questions

Q. Pentose phosphate pathway occurs in: (PGI May 2019)

A. Cytosol
B. Mitochondria
C. Lysosome
D. Golgi apparatus
E. Endoplasmic reticulum

00:03:09

Important Information

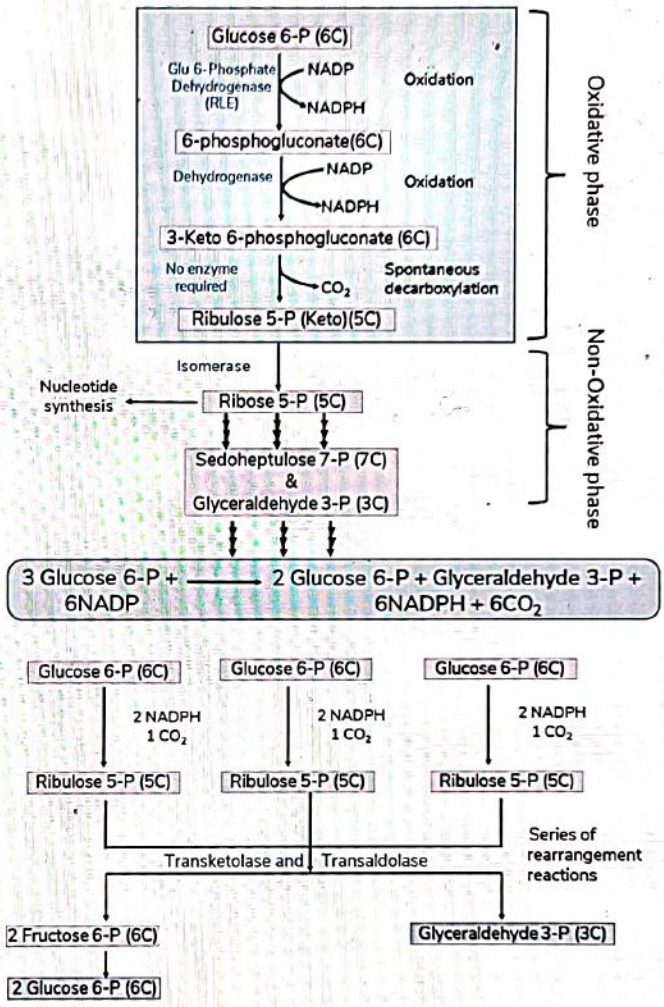
Q. Pathways which do not produce any ATPs

- RL Shunt
- α – Oxidation
- Oxidation of very long chain Fatty Acids
- Arsenic Acid poisoning in Glycolysis
- Uronic Acid pathway

2 Phases 00:03:57

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

00:05:56



- Glyceraldehyde 3–P is intermediate as well as end-product of HMP
- Glucose 6–P is substrate as well as product of HMP

Keep in Mind HMP is a cycle, not a pathway as Glu 6-P is regenerated

Major Sites of HMP

- Liver, Adipose tissue
- Lactating mammary gland
- Adrenal cortex
- Gonads
- Placenta
- RBCs

Never the sites for HMP

- Non-Lactating Mammary Glands

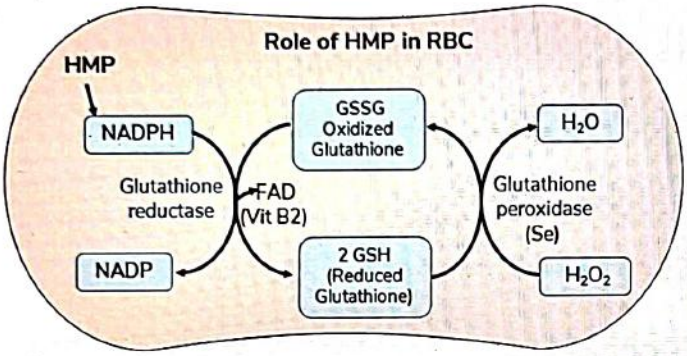
Low HMP activity is found in

- Skeletal muscles
- Skin

ROLE OF HMP IN RBC

00:14:58

- HMP → NADPH → Reduced glutathione → Destroys H_2O_2

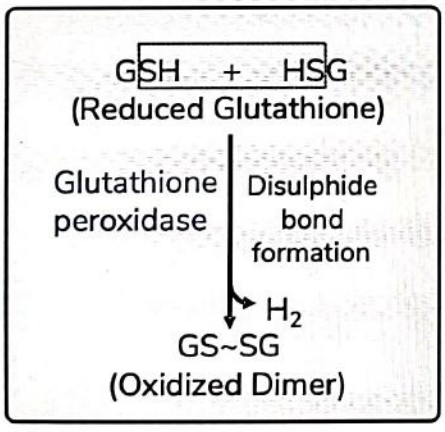


Glutathione

- It is a tripeptide made of 3 amino acids (Glutamate, Cysteine and Glycine) joined by 2 Peptide bonds

How to remember

GLUTA-THI-ONE
GLUTA – Glutamate
THI - Cysteine THIOL (Sulfur group (SH) containing AA)
ONE- Glycine (simplest smallest AA)



Previous Year's Questions

Q. What is seen in G6PD deficiency? (NICET July 2021)

A. Decrease membrane lipid peroxidation
B. Decrease generation of reduced glutathione
C. Decrease NADH generation
D. Decrease scavanging of RBC by macrophages
E. Hemolysis

00:18:00

Important Information

- Enzyme transketolase in HMP Phase II requires vitamin B_1 and Mg as cofactors
- So, Marker of Vitamin B1 deficiency: RBC Transketolase Activity (not quantity of this enzyme)
- Marker for Vitamin B_2 deficiency: RBC Glutathione Reductase Activity

Procedure

To test Vit B_1 and Vit B_2 deficiency in suspected patient:

- Serum sample is taken
- enzyme activity assay of above-mentioned enzymes is done
- Low activity is a confirmation of the deficiency of these vitamins

Previous Year's Questions

Q. Which of the following shows functional assessment of B1 deficiency: (AIIMS May 2019)

A. RBC Transketolase
B. RBC Glutathione reductase
C. Serum Thiamine levels
D. RBC Glutathione Peroxidase

CLINICAL QUESTIONS

Q. A 30-year-old man on a drug therapy with a potent oxidizing agent has self-limiting episode of hemolysis, back pain, and jaundice. The peripheral blood smear reveals a non-spherocytic, normocytic anemia, and Heinz bodies are seen in some of his erythrocytes. The anemia in this patient is a result of:

A. A lowered concentration of oxidized glutathione
B. A lowered concentration of reduced glutathione
C. Increased production of NADPH
D. An increase in the production of glucose 6-P

Answer: B

Solution

The patient has glucose 6-P dehydrogenase deficiency and cannot generate NADPH from glucose 6-P. In the red blood cells, which lack mitochondria, this is the only pathway through which NADPH can be generated.

In the absence of NADPH, the molecule which protects against oxidative damage (glutathione) is oxidized preferentially to protect membrane lipids and proteins. There is only limited glutathione in the membrane, so once it is oxidized, it needs to be converted back to reduced glutathione (the protective form). The enzyme that does this, glutathione reductase, requires NADPH to supply the electrons to reduce the oxidized glutathione (see figure in text).

In the absence of NADPH, the glutathione cannot be reduced, and the protection offered by reduced glutathione is eliminated, leading to membrane damage and cell lysis.

Reference: Harper's 30th ed/pg. 198

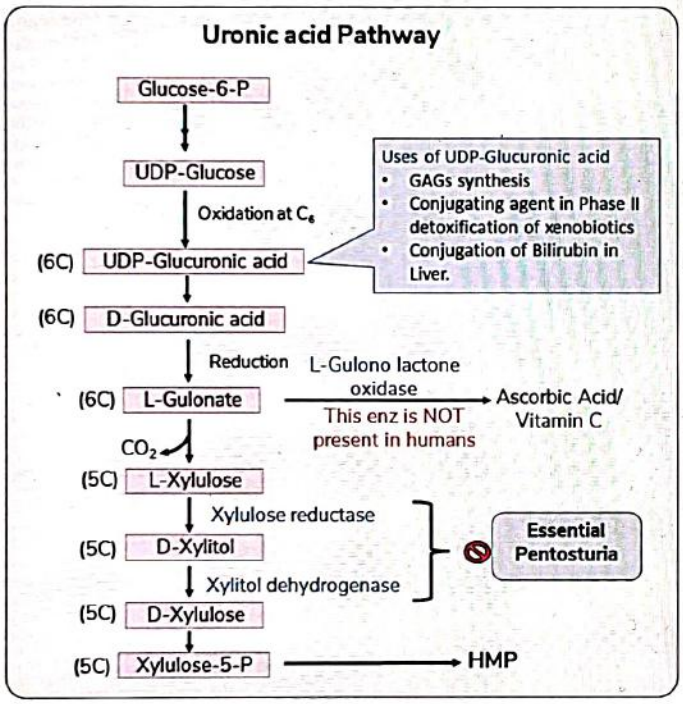
33 URONIC ACID PATHWAY

URONIC ACID PATHWAY

Similarities b/w Uronic Acid Pathway & HMP Pathway

- Minor pathways for oxidation of Glucose
- Both occur in cytoplasm
- Main organ involved: Liver
- Starting material: Glucose-6-P
- No ATP formed

00:01:20



Uses of Uronic acid Pathway

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

Previous Year's Questions

Q. Due to which of the following enzyme deficiency, vitamin C cannot be synthesized in humans?
(AIIMS May 2018)

A. L-Glucuronic acid oxidase
B. L-Gulonic acid reductase
C. L-Gulonolactone oxidase
D. L-Gulonolactone reductase

Essential Pentosuria

00:06:29

- Inherited disorder
- D/t deficiency of either Xylulose Reductase or Xylitol dehydrogenase
- L-xylulose gets accumulated & excreted in urine
  - Monosaccharide (reducing substance)
  - Benedict's test is positive (+)
  - Glucose oxidase test strip is negative (-)
- Benign condition, but should be differentiated from diabetes

Pentosuria

- Can also occur in normal situations like consumption of large amount of fruits.
- One of the components of Garrod's tetrad

00:08:30

How to remember

Garrod's tetrads: 4 diseases (MN-CAAP)

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

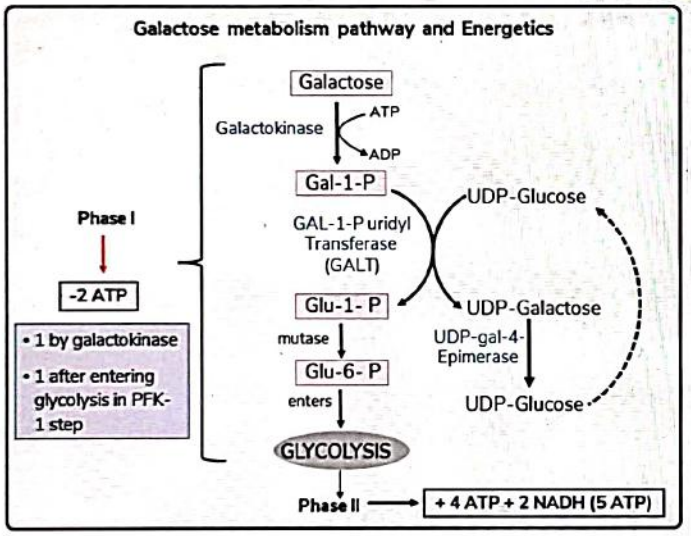
34 GALACTOSE METABOLISM

GALACTOSE METABOLISM

Monosaccharides in diet

- Glucose (major)
- Galactose: Milk and milk products
- Fructose: Fruits and other minor sources such as honey
- Most of the enzymes of glycolysis are also used for glucose and fructose metabolism.
- Three extra enzymes each for galactose and fructose metabolism are present which convert these sugars to intermediates which can enter glycolysis to produce energy

00:04:00



Important Information

Three main enzymes

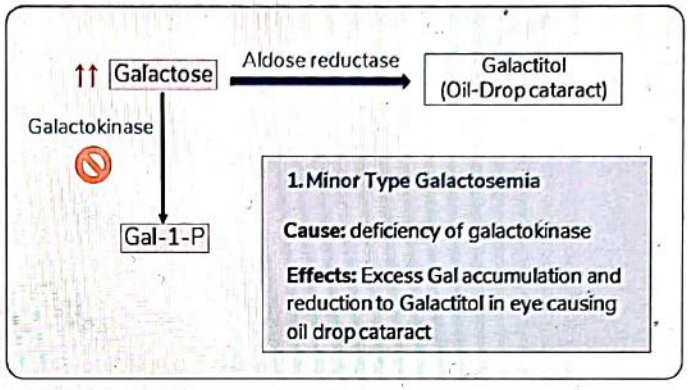
1. Galactokinase
2. GAL-1-P uridyl Transferase (GALT)
3. UDP-gal-4-Epimerase

Energetics of galactose metabolism:

- Galactose metabolism uses galactokinase instead of hexokinase, rest all same as glucose i.e
  - Galactose under Aerobic Glycolysis = 7 ATP
  - Galactose on complete breakdown = 32 ATP

DEFECTS IN GALACTOSE METABOLISM- GALACTOSEMIAS

00:09:48



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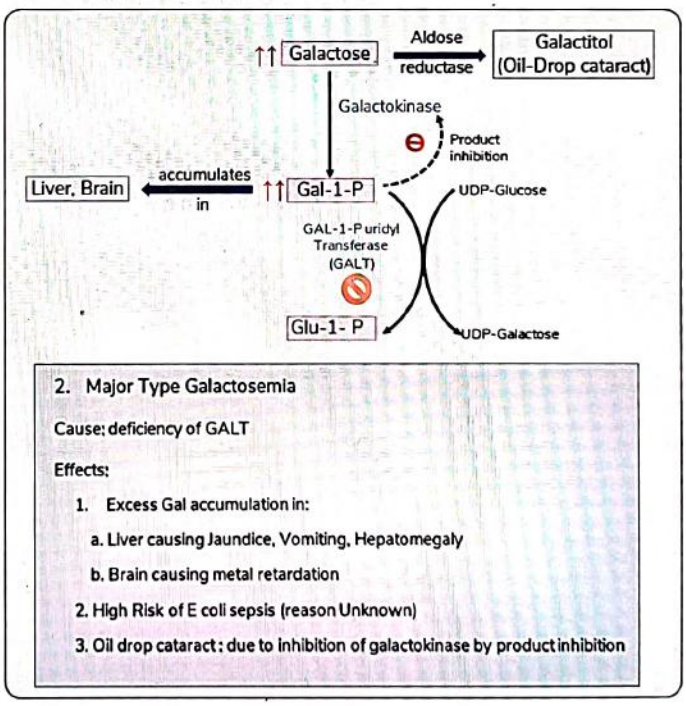


- Galactosemia presents itself in 1 week of birth with symptoms of jaundice which also coincides with physiological jaundice
- In developed countries, screening is done for all types of Galactosemias so galactosemic patients are treated differently from physiological jaundice patients.
- However, in developing countries, new-born infants with jaundice are mostly considered as having

physiological jaundice so given phototherapy.

- It is due to lack of resources to screen for galactosemia.
- But if photo therapy does not work, then the patients must be tested for galactosemia and should be treated accordingly to prevent mental retardation.

Previous Year's Questions

Q. The enzyme deficient in Galactosemia is:
(NEET May 2018)

A. Sphingomyelinase
B. Hexosaminidase
C. Galactose 1- phosphate uridyl transferase
D. Glucocerebrosidase

CLINICAL QUESTIONS

Q. A 3-year old male child presents with hepatomegaly and bilateral lenticular opacities. Laboratory examination revels normal blood glucose levels by GOD-POD assay but positive Benedict's test in urine. Physician suspected it to be a case of galactosemia. Deficiency of which one of the following enzymes will cause such features.

A. Glucokinase
B. Galactose 1-phosphate uridyl transferase
C. Aldose reductase
D. Aldolase A

Answer: B

Solution

Galactose is produced in intestine from degradation of milk sugar lactose by enzyme lactase. The patient symptoms suggest it to be a case of classic galactosemia which is due to deficiency of enzyme Galactose 1-phosphate uridyl transferase of galactose metabolism. In this disease there is excess Gal accumulation in Liver causing Jaundice, Vomiting, Hepatomegaly and in Brain causing mental retardation.

Other clinical features include high risk of E. coli sepsis (reason unknown) and Oil drop cataract due to inhibition of galactokinase by product inhibition as shown in the figure in text.

Regarding other options:

Glucokinase and aldolase A are enzymes of glucose metabolism.
Aldose reductase converts galactose to galactitol which accumulates in eye causing oil drop cataract.

Reference: Lehninger's 6th ed/pg. 562-563

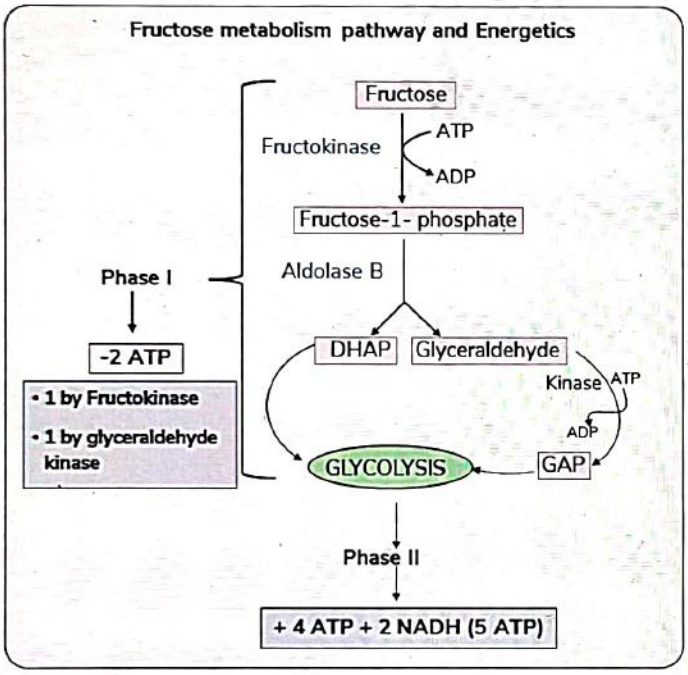
35 FRUCTOSE METABOLISM

FRUCTOSE METABOLISM

00:00:22

- **Organ:** Mainly Liver
- **Sources of fructose**
  - Table sugar Sucrose (made of Glucose + fructose)
  - Sugarcane juice, Honey, High fructose corn syrup.

00:01:28



00:04:10

Important Information

Three main enzymes

1. Fructokinase
2. Aldolase B
3. Kinase

Energetics of Fructose metabolism

00:04:32

- Energetics of Fructose & Glucose are same (as both utilize two ATPs in Phase I)
- Hence, same as glucose:
  - Fructose under Aerobic Glycolysis = 7 ATP
  - Fructose on complete breakdown = 32 ATP

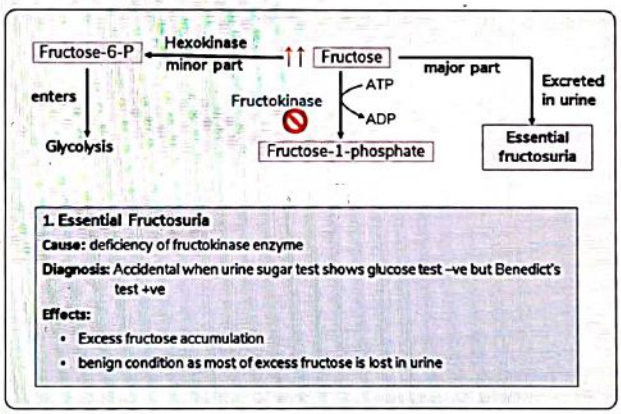
00:06:22

Important Information

- Fructose is the most rapidly metabolized monosaccharide because
  - It by-passes PFK-I Step (rate limiting enzyme of glycolysis and a time-consuming step)
  - Fructose rapidly forms Pyruvate → Acetyl CoA → Fats. That's why Fructose is also the Most Lipogenic Sugar

DEFECTS IN FRUCTOSE METABOLISM

00:08:23



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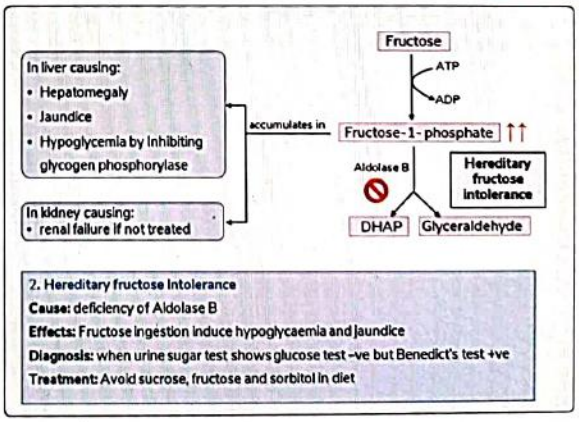


Fructose entry into cells, unlike glucose, is insulin independent

CLINICAL QUESTIONS

Q. A 3-year old boy was brought to the emergency department after several episodes of vomiting and lethargy. He was also found to have hypoglycemia. His pediatrician was concerned about possible hepatic failure along with recurrent episodes of vomiting and lethargy. A detailed counseling of the parents revealed that these symptoms appear every time after ingestion of sweets or fruits. What is the most likely diagnosis?

A. **Hereditary Fructose Intolerance**
B. Glycogen storage disease type III
C. Galactosemia
D. Fructosuria

Answer: A

Solution
Hereditary fructose intolerance

- Autosomal Recessive
- Due to deficiency of aldolase B.
- Asymptomatic until patient ingest fructose
- Patient has jaundice, hepatomegaly and hypoglycemia.
  - Fructose-1-phosphate is accumulated, which inhibits glycogen phosphorylase causing fasting hypoglycemia.

Regarding other options:
Galactosemia

- accumulation of galactose in blood due to defect in galactose metabolism.
- The most common enzyme which is deficient is GALIPUT (Galactose-1-Phosphate Uridyl Transferase).

Glycogen storage disease type III

- also known as GSD-III or Cori's disease
- deficiency of debranching enzyme,
- Limit dextrins are accumulated in liver→ hepatomegaly.

Fructosuria

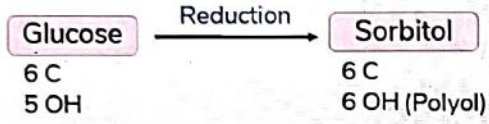
- benign condition
- enzyme deficient is fructokinase.

Reference: Harper's 30th ed/pg. 188-191

36 SORBITOL PATHWAY

- All monosaccharides on reduction forms alcohols which are hygroscopic in nature e.g. glucose to sorbitol conversion. Due to 6 OH and 6C, it is also called as polyol.

Polyol formation by reduction of monosaccharides



- Sorbitol pathway is used for the synthesis of fructose in the body from glucose which is different from fructose obtained from diet.

00:01:57

Sorbitol/Polyol Pathway

Aldose Reductase

- Converts glucose to sorbitol by reduction using NADPH as coenzyme
- Present in almost all tissues of the body
- Has low affinity for glucose, so acts only when high amount of glucose is present in body.

Sorbitol Dehydrogenase 00:03:16

- Converts sorbitol to fructose by dehydrogenation using NAD as coenzyme
- Presents in few cells of the body as fructose is required in only some of the cells such as seminal vesicle (as sperm cells use fructose as energy source). It is also present in liver

00:04:46

Important Information

Clinical significance of polyol pathway:

- In patient with uncontrolled diabetes, excess blood glucose enters insulin-insensitive tissues such as peripheral nerves, renal glomeruli, lens and retina and leads to the formation of excess sorbitol by aldose reductase. But sorbitol dehydrogenase is absent in most of these cells and sorbitol cannot be converted to fructose. Hence, sorbitol accumulation occurs.
  - Sorbitol is hygroscopic and will absorb water causing cell swelling ultimately leading to snow-flake cataract in diabetic patients.
  - Also, sorbitol can also cause other complications in diabetic patients such as retinopathy, neuropathy and nephropathy.
- As excess NADPH is used up, so, reduced glutathione (antioxidant) will be decreased leading to increased chances of oxygen free radical damage.

CLINICAL QUESTIONS

Q. A 58-year-old man with type I (IDDM) diabetes went to an ophthalmologist for assessment of developing a cataract. The results of his blood work are shown below:

Fasting blood glucose	180 gm/dl
Hemoglobin A	15 mg/dl
Hemoglobin A1c	10% of total Hb
Urine ketones	Positive
Urine glucose	Positive

The enzyme responsible for cataract formation in this patient is which of following.

A. Glucokinase
B. Aldose reductase
C. Aldolase B
D. Galactokinase
E. Aldolase A

Answer: B

Solution

- The blood examination with high fasting glucose (>126 mg/dl) and HBA1c >6.5% along with urine ketone bodies and urine glucose indicates a diabetic condition.
  - In diabetic patients, excess blood glucose enters insulin insensitive tissues and leads to the formation of sorbitol by aldose reductase (see fig in text). But sorbitol dehydrogenase is absent in most of these cells and sorbitol cannot be converted to fructose. Hence, sorbitol accumulation occurs.
  - As sorbitol is hygroscopic and will absorb water causing cell swelling ultimately leads to snow-flake cataract in diabetic patients.

Regarding other options:

Galactokinase is an enzyme of galactose metabolism. Its deficiency causes galactosemia. In galactosemia, this same enzyme converts excess galactose to galactitol, creating oil drop cataracts.

Glucokinase and aldolase A are enzymes of glycolysis and defects in these enzymes generally manifest as hemolysis and anemia.

Aldolase B is involved in fructose metabolism and its deficiency causes fructose intolerance with symptoms such as hepatomegaly, jaundice and hypoglycemia but no cataract.

Reference: Harper's 30th ed/pg. 202-203, 205

LEARNING OBJECTIVES

UNIT VI: ENZYMES

- Unit IV entails important topics on enzymes such as their structure, classification and kinetics. The first two chapters gives some basics of enzyme structure and properties related to enzyme catalysis. The third chapter discuss the clinically important isozymes and their use as a diagnostic tool. "Enzyme classification" chapter summarizes the 6 standard categories and subcategories of enzymes with examples of important enzymes from exam point of view.

- Enzyme kinetics chapter gives you a comprehensive account on the various factors which affect the enzyme activity. It also details the mathematical equations that is used to study derive important enzyme kinetic parameters such as K_m and V_{max} along with some numerical problem to better understand these equations. Finally, it includes a discussion on effect of different types of inhibitors on kinetic parameters. The next chapter "Enzyme Inhibitors" provides a brief introduction along with mechanism and important examples of different types of enzyme inhibitors.

- A short chapter "Enzyme uses" tells the enzyme used for diagnostic and therapeutic purposes. The last chapter provides a detail on the various mechanism which are employed to regulate the enzyme activity according to the physiologic needs of cells and tissues.

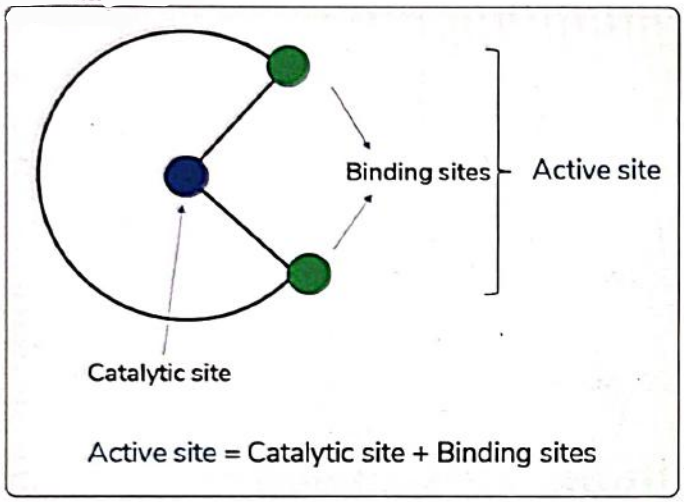
- **Major learning objectives**
  - To learn basics of enzyme properties, cofactors and isozymes forms
  - To know various classes of enzymes with examples
  - To gain understanding on enzyme kinetics and effect of different kind of inhibitions on kinetic parameters
  - To study about different ways of enzyme regulation
  - To learn diagnostic and therapeutic use of enzymes

37 ENZYME BASICS

ENZYMES

00:00:22

- All enzymes are proteins except Ribozyme (RNA acting as enzyme)



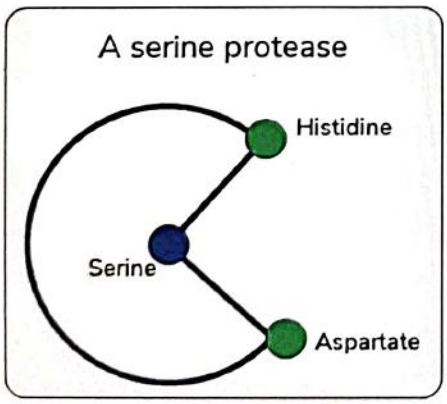
Chymotrypsin

00:02:45

- Protein breaking enzyme
- Aspartate & Histidine binds the substrate
- Serine is responsible for cutting the substrate
- So, also called as Serine Protease

Serine proteases

- All have same Catalytic triad containing
  1. Histidine
  2. Aspartate
  3. Serine



Examples

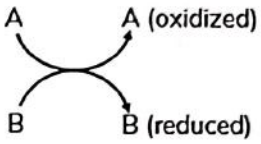
1. Trypsin
2. Elastase
3. Plasmin
4. Thrombin
5. Complement proteins
6. Clotting factors X & XI
7. PSA (Prostate Specific Antigen)

- Serine proteases have role in Tumor metastasis

BI–BI REACTION

00:05:17

- 2 Substrates, 2 Products Reaction



Types

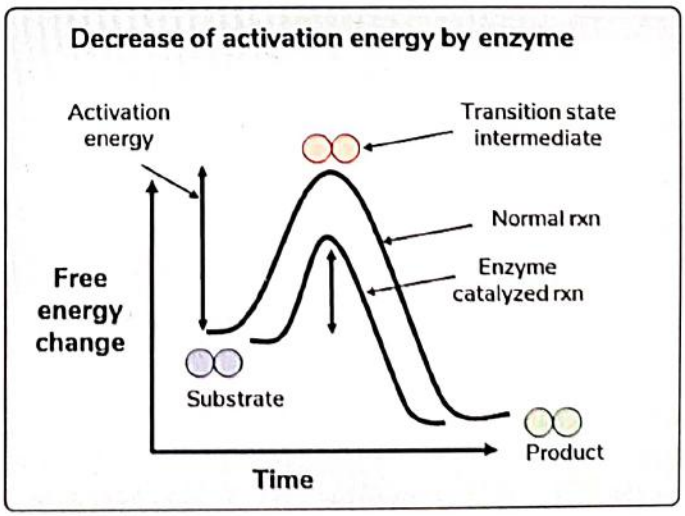
1. Ordered by dehydrogenases mostly
2. Random by kinases mostly
3. Ping Pong Reaction

- Serine proteases
- Amino Transferases e.g. SGOT and SGPT

PROPERTIES OF ENZYMES

00:06:44

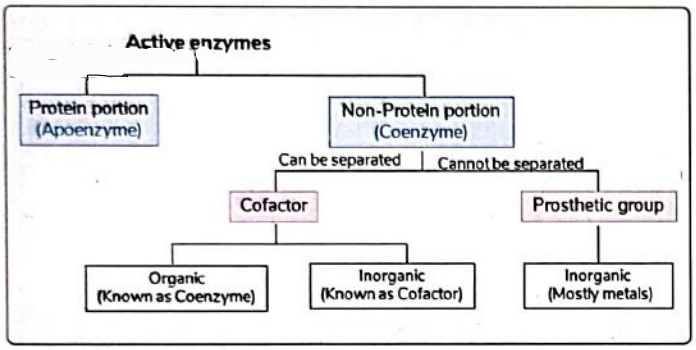
- Not used in the reaction
  - ↑ Rate of reaction (Speed up rxn /decrease the time of rxn)
- ↓ Activation energy (see fig)
- Do not change the equilibrium of reaction
- Do not change the free energy of substrate/product



38 COFACTORS

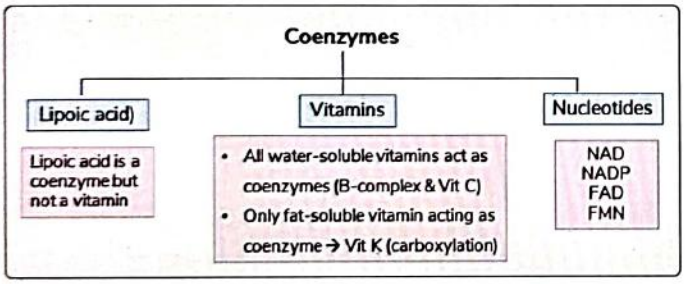
COFACTORS & PROSTHETIC GROUPS

00:00:25



Important Information

- All coenzymes are cofactors
- Metals are cofactors but not coenzymes
- Enzymes using metals as cofactor are known as metal activated enzymes.
- Enzymes using metals as prosthetic groups are known as Metalloenzymes.



Previous Year's Questions

Q. All are co-factors for De-hydrogenase except: (FMGE June 2019)

A. NADP
B. FAD
C. NAD
D. Lipoic acid

Nucleotide Derivatives but not co-enzymes 00:04:48

- SAM (S–Adenosyl Methionine) → Methyl donor
- PAPS (Phospho Adenosyl Phospho Sulfate) → Sulfate donor

Cu is required for all Oxidases, e.g. 00:05:50

- Cyt c Oxidase
- Tyrosinase
- Ascorbic Acid Oxidase
- Amino Acid Oxidase
- Lysyl Oxidase
- Cytoplasmic SOD (Super Oxide dismutase)

Exception

- Mitochondrial SOD requires Manganese (Mn)
- Xanthine oxidase and Sulphite oxidase do not require copper, they require Molybdenum (Mb)

Previous Year's Questions

Q. Copper is a cofactor for: (JIPMER Nov 2018)

A. Glutathione peroxidase
B. Tyrosinase
C. Prolyl oxidase
D. Carbonic anhydrase

Previous Year's Questions

Q. Cofactor for Mitochondrial SOD? (FMGE Aug 2020)

A. Mn
B. Cu
C. Zn
D. Mb

39 ISOENZYMES

ISOENZYMES

Def: distinct form of enzymes which catalyse same biochemical reaction

Same between isoenzymes

- Reaction
- Enzyme
- Species

If species is different but same function than k/a Alloenzyme

Different in isoenzymes

- Structure
  - e.g. LDH–1 (HHHH) and LDH–2 (HHMM)
- Genes
- K_m, V_{max}
- Electrophoretic mobility

LDH ISOENZYMES

00:03:34

Isoenzyme	Subunit	Location
LDH-1	HHHH	Heart
LDH-2	HHHM	Blood (WBC) → Functional Plasma Enzyme
LDH-3	HHMM	Brain, Kidney, Lungs (Main organ) and Pancreas
LDH-4	HMMM	Muscle and Liver
LDH-5	MMMM	

Important Information

Q. Predominant form of LDH in Liver?
Ans: LDH – 5 (though 4 & 5 both are present)

- In normal persons: LDH 2 > LDH 1
- In case of Myocardial Infraction: LDH 1 > LDH 2 (Known as Flipped Ratio)

Previous Year's Questions

Q. Which of the following flipped pattern of LDH is seen in myocardial infarction? (NEET May 2018)

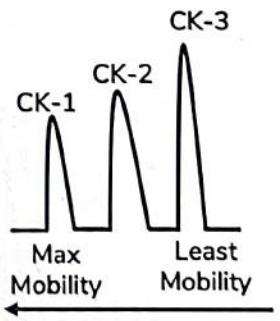
A. LDH 1>2
B. LDH 2>1
C. LDH 3>4
D. LDH 5>4

CREATININE KINASE (CK) ISOENZYME

00:08:01

Isoenzyme	Subunit	Location
CK-1	BB	Brain
CK-2	MB	Heart (raised in MI)
CK-3	MM	Muscles

Electrophoretic pattern of CK isozymes



How to remember

- Isozyme number inversely related to mobility. i.e.
  - Least number → moves maximum
  - Highest number → moves least

ENZYMES AND PROTEINS RAISED IN MI

00:09:04

Enzymes raised in MI	
CK–2/CK–MB	4 – 6 hrs
AST/SGOT	6 – 8 hrs
LDH–1	8 – 10 hrs
Proteins raised in MI	
Myoglobin	2 – 6 hrs (earliest marker, but nonspecific)
Troponin T & I	3 – 6 hrs

TROPONINS

- Regulatory proteins in cardiac muscle

Name	Function
Troponin C	Calcium binding
Troponin I	Actin Myosin inhibitory ATPase (most specific cardiac marker)
Troponin T	Tropomyosin binding

Other Markers

- BNP (Brain Natriuretic Peptide) → Marker for cardiac failure
- IMA (Ischemia Modified Albumin) → New cardiac biomarker

Previous Year's Questions

Q. A heart patient suddenly has chest pain. Which isoenzyme of LDH enzyme is elevated?
(FMGE Aug 2020)

A. LDH-1
B. LDH-5
C. LDH-2
D. LDH-4

HEXOKINASE

00:12:29

Types

- I
- II (Most abundant)
- III
- IV (aka Glucokinase)

Hexokinase	Glucokinase (Hexokinase IV)
• Phosphorylates all hexoses	• Phosphorylates only Glucose
• Present in all cells	• Present in liver and Pancreas (same properties as GLUT-2)
• High affinity • less [S] required • active in fasting state • low K_m • low V_{max}	• Low affinity • more [S] required • active in fed state • high K_m • high V_{max}
• Feedback inhibition by Glucose 6-P	• Induced by Insulin

CLINICAL QUESTIONS

Q. A 60-year-old man had a myocardial infarction 8 hour ago. Measurement of which of the following pair of enzymes in plasma will be of highly relevant for diagnosis in this patient?

A. CK-MB and LDH
B. AST and ALT
C. CK-MB and AST
D. AST and LDH

Answer: C

Solution

According to the table on enzyme raised in MI (see text), CK-MB and AST will be released in first 8 hours and will be most important for diagnosis. LDH will be raised after 8 hours, so, its levels will be low at 8-hour time period.

Reference: Harper's 30th ed/pg. 62

40 ENZYME CLASSIFICATION

CLASSIFICATION OF ENZYMES

EC no.	Category name	Reaction catalyzed
1	Oxidoreductase	Transfer electrons or H atoms
2	Transferase	Transfer groups (Molecular formula is changed)
3	Hydrolase	Use H_2O to break
4	Lyase	Can make/break a bond (do not require H_2O / ATP)
5	Isomerase	Interconvert isomers (Molecular formula not changed)
6	Ligase	Use ATP to make a bond

- **EC no.** = Enzyme commission no./Code no. 00:00:40

How to remember

- Enzyme categories **(MN: OTHLIL)**

Refer Table 40.1

EXAMPLES OF ENZYME 00:02:09

Oxygenases

1. **Monooxygenases**: add one oxygen [O]
- also called as Mixed function oxidases because when O_2 is added; One atom is given to substrate and other is given to H_2 to form H_2O
- RH → ROH (hydroxyl gp) so, also called as Hydroxylases
- Examples

1. Phenylalanine Hydroxylase (convert Phenylalanine to Tyrosine)
2. Cyt P_{450} (Hydroxylation of Steroid hormones)
3. Nitric Oxide Synthase (NOS) (Synthases belong to EC no. 4 but NOS is EC no.1 as exception)

Important Information

Most hydroxylases are monooxygenases But Prolyl & Lysyl Hydroxylase are Dioxygenases

2. **Dioxygenases:** add 2 oxygens [O-O] 00:06:31
- Homogentisate Dioxygenase (deficiency causes Alkaptonuria)

Hydrolases 00:08:11
- Whenever a macromolecule is synthesized, H_2O is removed
- Whenever a macromolecule is broken down, H_2O is added to break bond by Hydrolases

Carbohydrates breaking	Protein Breaking
• Amylase • Lactase • Sucrase • Maltase • Isomaltase	• Protease • Peptidase • Arginase • Urease

Lipid Breaking	Nucleic Acid Breaking
• Lipase Esterase • Phospholipase	• Nuclease • Exonuclease • Endonuclease • Restriction endonuclease

Table 40.1

1. OXIDOREDUCTASES	Transfer of electron (Hydride (H^-) ions or H atom)
• Oxidases	• Use Oxygen as an electron acceptor e.g. Cytochrome C Oxidase, Tyrosinase
• Dehydrogenases	• Use molecules other than oxygen (NAD, FAD, NADP) as an electron acceptor
• Peroxidases	• Use H_2O_2 as an electron acceptor e.g. Glutathione Peroxidase, Catalase
• Oxygenases	• Directly incorporate O_2 into the substrate e.g. Mono and dioxygenase
2. TRANSFERASES	Carry group transfer reactions
• Methyl Transferase	• Transfer one carbon units between substrates
• Amino Transferase	• Transfer amino groups
• Kinase	• Transfer phosphate from ATP to a substrate
• Phosphorylase	• Transfer phosphate from inorganic phosphate to a substrate
3. HYDROLASES	Hydrolysis of substrate using water
• All digestive enz	• Enzymes which break down macro-molecules
• Phosphatase	• Remove phosphate from a substrate
4. LYASES	Addition of groups to double bonds or formation of double bonds by removal of groups
• Synthases	• Link two molecules without involvement of ATP
• Aldolases	• Produce aldehydes via elimination reactions
• Decarboxylases	• Produce CO_2 via elimination reactions e.g. simple decarboxylase (not oxidative decarboxylases (EC-1)
• Hydratase/Hydrase	• Removes water to make double bond or add water across a double bond, without breaking bond e.g. Enolase, Aconitase, Fumarase, PEPCK.
5. ISOMERASES	Transfer of group within same molecule to form isomers
• Racemase	• Interconverts L and D stereo isomers
• Mutase	• Transfer groups between atoms within a molecule
• Epimerase	• Interconvert Epimers e.g. Glucose and Galactose
6. LIGASE	Formation of C-C, C-N, C-O, C-S bonds by condensation reaction using ATP
• Carboxylase	• Use CO_2 as a substrate
• Synthetase	• Link two molecules using ATP

41 ENZYME KINETICS

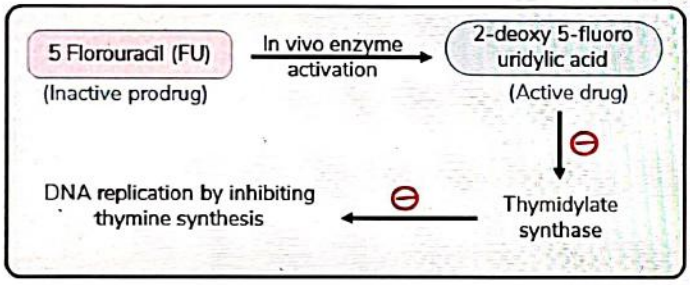
ENZYME KINETICS

Def: study of velocity and rate of enzyme reaction

Uses of enzyme kinetics study 00:01:47

1. To understand mechanism of action of enzyme
2. To know various steps in a reaction

- The information obtained can be used for disease analysis, diagnosis and treatment
  - Using enzymes as target for drugs e.g.
    - → Allopurinol is used for gout treatment by inhibition of enzyme
    - → Xanthine oxidase by stopping inhibition of Uric Acid.
  - Designing of prodrugs based on enzyme activating it e.g.



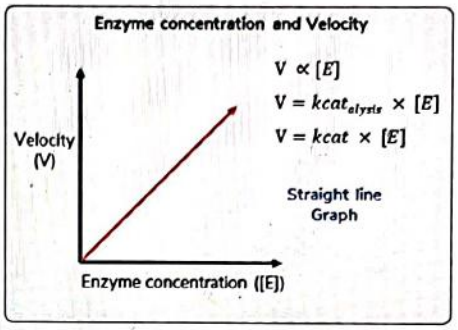
EFFECT OF VARIOUS FACTORS/VARIABLES ON THE VELOCITY

- Effect of variables are studied in the form of X-Y graphs
- Variable is on Y axis and velocity on X-axis
- Variables studied

1. Enzyme concentration
2. Substrate concentration
3. Temperature
4. pH
5. Inhibitors

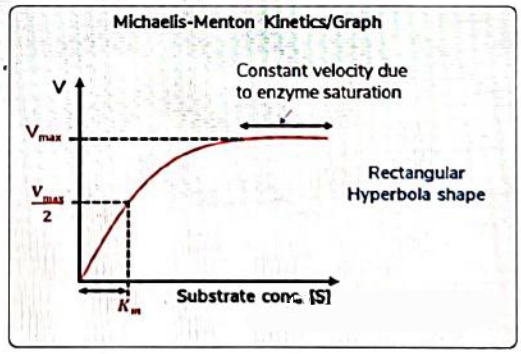
1. Velocity vs enzyme concentration [E] 00:06:20

- Linear or straight-line graph
  - As the enzyme concentration increases velocity also increases or we can also say that Velocity is directly proportional to enzyme concentration



2. Velocity vs substrate concentration [S] 00:08:05

- Rectangular hyperbola graph
  - Initially as substrate concentration increases, velocity increases proportionally,
  - After that it reaches a saturation point after which the velocity remains constant.
  - This occurs because the enzyme is kept constant in this experiment
- Michaelis-Menton constant [K_m] 00:11:03
  - K_m can't be equal to V_{max} /2 (as the units of two parameters are different)
  - K_m is defined as that substrate concentration at which velocity of reaction is half of V_{max}
  - Units of K_m is mM/L or M/L



Important Information

Q. Velocity at K_m is:
a) ½ of [S] b) ½ of V_{max}
A. ½ of V_{max}

Q. K_m is:
a) Association constant b) Dissociation constant
A: None of these (K_m is just Michaelis Menton constant)

- $K_m \propto \frac{1}{\text{affinity}}$
  - If K_m Is large → less affinity → More Substrate required to attain V_{max}
  - If K_m is less → More affinity → less Substrate required to attain V_{max}
- K_m does not change with change in either enzyme or substrate concentration
  But in case of competitive inhibition, affinity ↓ so K_m ↑
- K_m is Signature of Enzyme
  - It is constant value for a particular enzyme
  - It is a different value for different enzymes e.g LDH-1 & LDH-2 have different K_m values
    → Mutation can change the K_m value of an enzyme
- Michaelis-Menton Equation 00:21:00
  - Rate equation for a one substrate enzyme catalyzed reaction
  - Velocity depend on both enzyme as well as substrate concentration

  → $V_o = \frac{V_{max} \times [S]}{K_m + [S]}$

  → But $V_{max} = K_{cat} \times [E]$

  → So, putting value of V_{max} in above equation, we get

  → $V_o = \frac{K_{cat} \times [E] \times [S]}{K_m + [S]}$

  → Proves that velocity is dependent on both enzyme as well as substrate concentration

Important Information

1. When [S] <<< K Then $K_m + [S] = K_m$

   In that case, $V_o = \frac{V_{max} \times [S]}{K_m}$

   or $V_o \propto [S]$ as (V_{max} and K_m are constant)

   Also known as first order kinetics

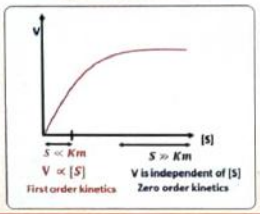
2. When $[S] = K_m$

   In that case, $V_o = \frac{V_{max} \times [S]}{[S] + [S]}$ or $V_o = \frac{V_{max} \times [S]}{2[S]}$ or $V_o = \frac{V_{max}}{2}$

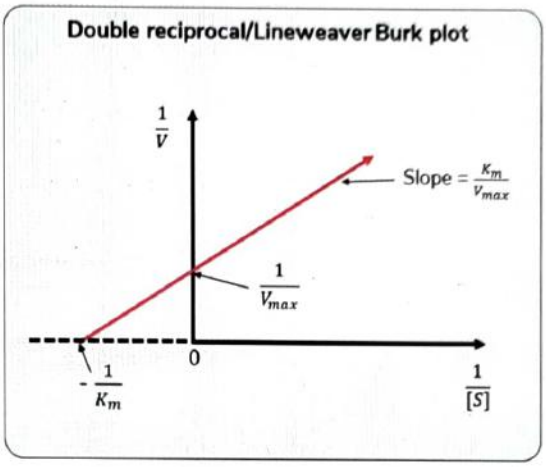
3. When [S] >>> K Then $K_m + [S] = [S]$

   In that case, $V_o = \frac{V_{max} \times [S]}{[S]}$ or

   $V_o = V_{max}$ (i.e. velocity independent of substrate conc.)



- Double Reciprocal Curve / Lineweaver Burk Graph [Plot] 00:26:59
  - Modification in MM graph can be done to make it Straight line graph
  - Easier to plot and do calculations



Lineweaver Burk equation

$$V_o = \frac{V_{max} \times [S]}{K_m + [S]} = \frac{V_{max} \times [S]}{K_m} + \frac{V_{max} \times [S]}{[S]}$$

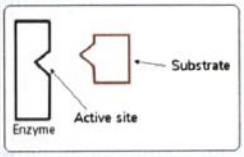
$$V_o = \frac{V_{max}}{K_m} \times [s] + V_{max}$$

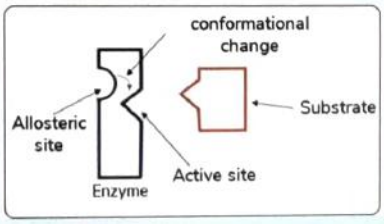
Taking reciprocal on both sides

$$\frac{1}{V_o} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

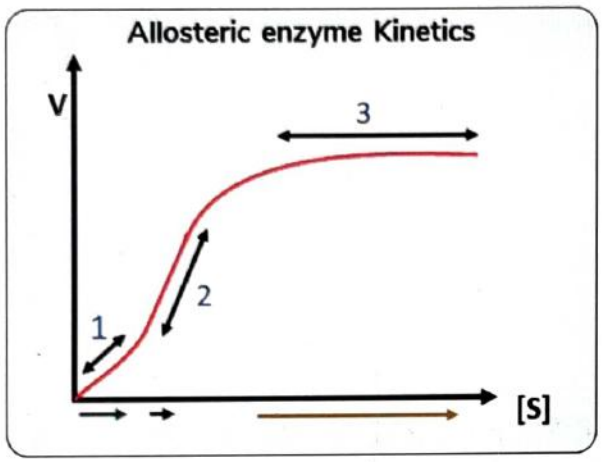
Simple Enzymes	Allosteric/Regulatory enzymes

• Most enzymes
• V vs [S] is Rectangular hyperbola graph
• Only one site called as active site
  ○ Substrate bind to active site and reaction happens
	• Few Enzymes
	• V vs [S] is Sigmoidal/S shape graph
	• active site + regulatory/ allosteric site
	  ○ regulator (activator or inhibitor) binds to allosteric site
	  ○ induce conformational change in active site so that substrate can or cannot bind.
	  ○ In other words, substrate binding is affected by presence of activator or absence of inhibitor





Sigmoidal Graph for allosteric enzymes

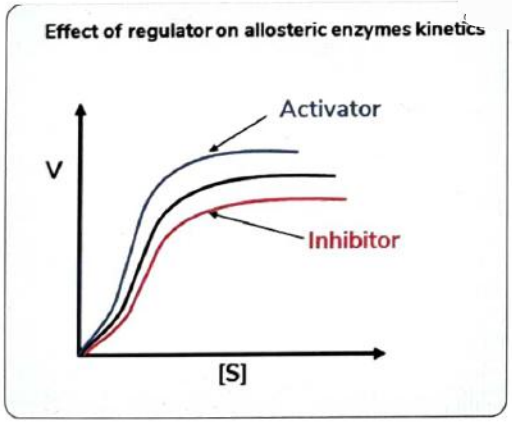


1. linear increase in V
- Increase in V proportional to [S]

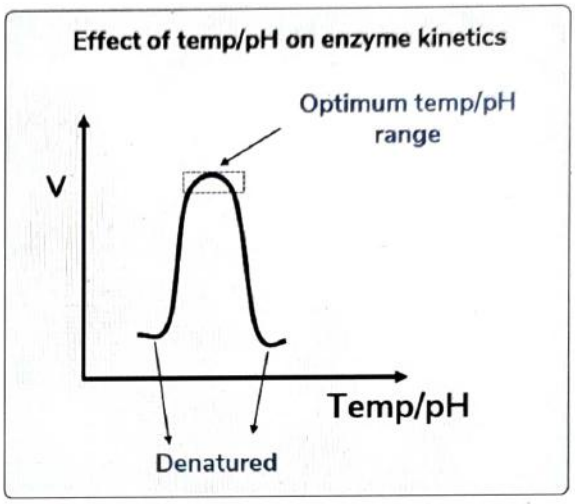
2. Exponential increase in V
- Small increase in [S] resulted in large increase in V due to Cooperativity

3. Constant velocity
- No increase in V despite increase in [S]

- **Allosteric/Regulatory Enzymes** 00:35:34
  - Multi subunit enzymes
  - Activator shifts the curve to the left while inhibitor shifts the curve to the right
  - Shows co operativity
    → One substrate binding to enzyme increases the affinity for other substrates for enzyme
  - Allosteric modifiers do not bind in concentration dependent manner
  - Adding more substrate to enzyme reaction cannot displace modifier

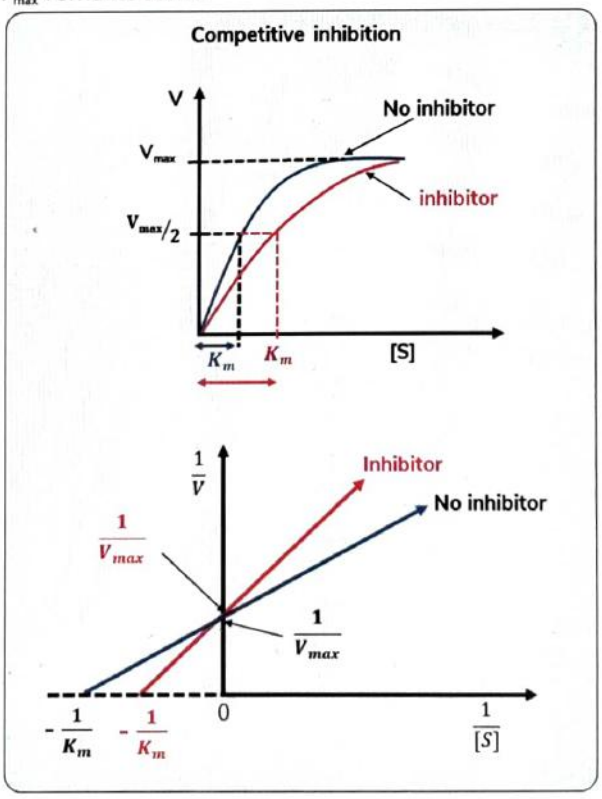


3 & 4. Velocity vs Temp /pH 00:42:21
- Bell shaped curve for temp/pH vs V
- At extremes of temperature and pH, enzyme/proteins are denatured so velocity is negligible.
- Velocity is maximum at optimum temperature & pH only.
- For human enzymes:
  - Optimum temp = 45- 55 °C
  - Optimum pH = 5-9

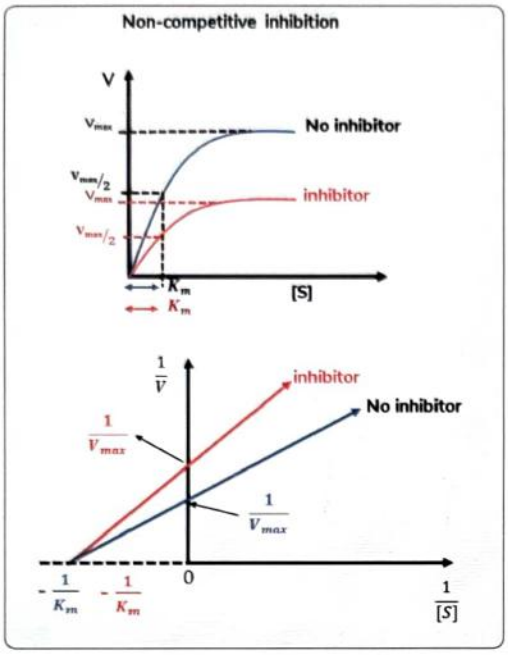


5. Enzyme Inhibitors 00:43:38
1. Competitive
2. Non-competitive inhibitors

1. Competitive Inhibition
- Affinity ↓ so K_m ↑
- V_{max} remains same

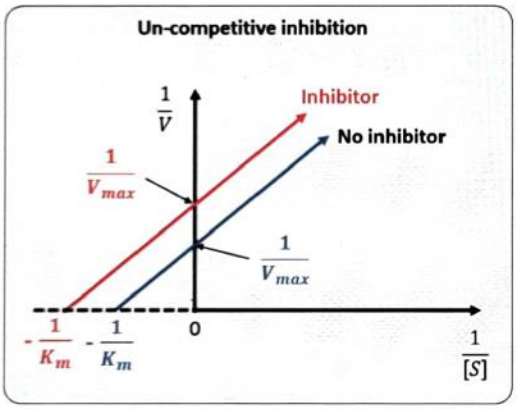


2. Non-Competitive Inhibition
- K_m remains same
- V_{max}↓



Un-Competitive Inhibition

- Both K_m and V_{max} ↓



Previous Year's Questions

Q. In Non-competitive inhibition: (NEET 2020)

A. V_{max} decreases, K_m remains constant

B. V_{max} remains same, K_m increases

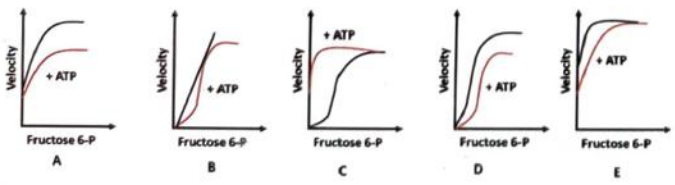
C. Both V_{max} and K_m are decreased

D. Km is usually increased, V_{max} is reduced

IMPORTANT QUESTIONS ON ENZYME KINETICS

00:49:05

Q. Which of the diagram illustrated below best represent the effect of ATP on hepatic PFK-1?



Ans: D

- As ATP is an allosteric inhibitor of PFK-1. So, it will cause a shift of sigmoidal curve to down which is shown in option D only.
- In option A, B C and E the kinetics shown is MM kinetics which is not true for PFK-1 alone or in the presence of its inhibitor ATP, so these options are automatically excluded.

Consider a reaction that can be catalysed by one of the two enzymes A & B, with the following kinetics:

	K_m (M)	V_{max} (mM/min)
A	5×10^{-6}	20
B	5×10^{-4}	30

Q1. At the substrate concentration of 5×10^{-6}M, what will be the velocity of the reaction catalysed by enzyme A?
When $[S] = K_m$, $V_o = V_{max}/2$
So, velocity (V_o) for A = 20/2 = 10 mM/min

Q2. At the substrate concentration of 5×10^{-4}M, what will be the velocity of the reaction catalysed by enzyme B?
Applying same logic as Q1.
Velocity for B = 30/2 = 15 mM/min

Q3. At the substrate concentration of 5×10^{-4}M, what will be the velocity of the reaction catalysed by enzyme A?
As $5 \times 10^{-4} >>> 5 \times 10^{-6}$ or $[S] >>> K_m$
And when $[S] >>> K_m$ Then $K_m + [S] = [S]$

- In that case $V_o = \frac{V_{max} \times [S]}{[S]}$ or $V_o = V_{max}$

V_o = 20 mM/min

Previous Year's Questions

Q. An enzyme catalysed reaction was carried out with the initial substrate concentration 1000 times greater than the K_m for that substrate. After 9 minutes, 1/3 of the substrate had been converted to the product, and the amount of product was 12mmol. In a separate experiment, one-third as much enzyme and twice as much substrate is combined, how lont it would take for the same amount (12mmol) product to be formed? (AIIMS May 2018)

A. 13.5 mins

B. 27 mins

C. 8 mins

D. 9 mins

As given $[S] >>> K_m$ so, $K_m + [S] = [S]$

- In that case $V_o = \frac{V_{max} \cdot [S]}{[S]}$ or $V_o = V_{max}$ or $V_o = K_{cat} \times [E]$

As K_{cat} is a constant so we can say $V_o \propto [E]$

or $\frac{V_1}{V_2} = \frac{E_1}{E_2}$

Let's take E1 = x, so, E2 = 1/3x (as per question statement)

or $\frac{V_1}{V_2} = \frac{E_1}{E_2} = \frac{x}{x/3} = 3$ or $V_2 = \frac{V_1}{3}$

As velocity is decreased 3 times so the reaction will take 3-fold more time.

So, Time taken will be 9 X 3 = 27 minutes

42 ENZYME INHIBITORS

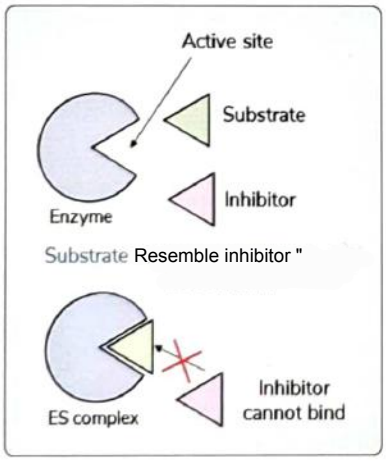
ENZYME INHIBITIONS TYPES

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

COMPETITIVE INHIBITION

00:01:11

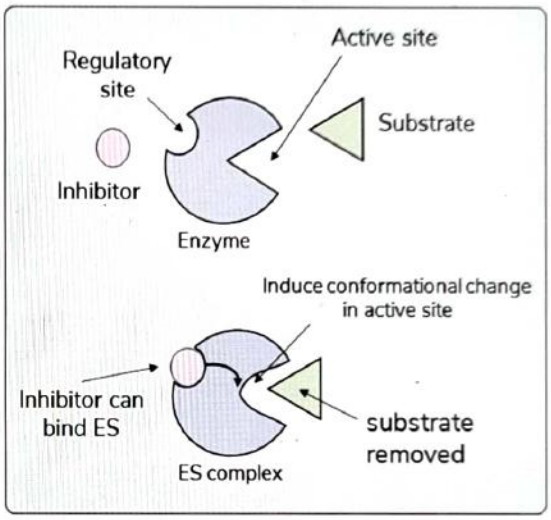
- Inhibitor resembles substrate in structure.
- Inhibitor and substrate compete for binding at active site.
- So, in competitive inhibition the affinity b/w enzyme and substrate decrease because now enzyme has affinity for both substrate and inhibitor
- And as $Km \propto \frac{1}{\text{affinity}}$ So, K_m increases
- As K_m increases [S] ↑
- [S]↑ will ensure more probability of substrate binding to active site compared to inhibitor.
- So, it keeps the velocity same and hence V_{max} remains same
- Inhibitor cannot bind the ES complex as active site is already occupied by substrate.



NON – COMPETITIVE INHIBITION

00:04:09

- Substrate do not resemble inhibitor in structure
- Inhibitor binds at regulatory or allosteric site. When inhibitor binds at regulatory site, it changes the shape of active site, so that substrate cannot bind.
- V_{max} is decreased
- Affinity of active site for substrate is not affected. So, K_m is same
- As K_m is same so Affinity is same
- Inhibitor can also bind with ES complex, and change shape of active site so, that substrate will be thrown out of active site.
- This inhibition is mostly irreversible.



Previous Year's Questions

Q. In non-competitive antagonism which of the following is correct: (AIIMS Nov 2018)

A. V_{max} decreases
B. K_m decreases
C. No change in V_{max}
D. Both K_m and V_{max} increases

Examples of Competitive inhibitors 00:07:39

Inhibitor	Enzyme inhibited	
Arsenate	Glyceraldehyde 3-P DH	Glycolysis enzymes
Oxamate	Lactate DH	
Malonate (3C)	Succinate DH	TCA enzymes
Fluorocitrate	Aconitase	
Sulfonamide (anti-bacterial)	PABA analogue → inhibits Folic acid synthesis in Bacteria	

Methotrexate (anti–cancerous)	Inhibits DHF Reductase → THF (vital reaction in human)
Dicumarol (anticoagulant)	Vlt K analogue → inhibits Coagulation
Ethanol	Alcohol Dehydrogenase • used in treatment of Methanol Poisoning
Statins	HMG CoA Reductase (RLE of Cholesterol Synthesis)

Previous Year's Questions

Q. Dicumarol inhibits vitamin K by? (AIIMS June 2020)
A. Competitive Inhibition
B. Non-competitive Inhibition
C. Suicide Inhibition
D. Allosteric inhibition

Examples of Non–competitive Inhibitors 00:13:01

Inhibitor	Enzyme inhibited	
Iodoacetate	Glyceraldehyde 3–P DH	Glycolysis enzymes
NaF	Enolase	
Fluoroacetate	Aconitase	TCA enzymes
Arsenate	α–Ketoglutarate DH	
Cyanide	ETC (Complex IV)	
Heavy Metals	Inhibits SH group (present at the active site of many enzymes in the body)	
Dimercaprol	Complex III of ETC	
Disulfiram (Antabuse)	Aldehyde DH	
Di–Isopropyl Fluorophosphate	Serine Proteases	

Previous Year's Questions

Q. Fluoroacetate inhibits which metabolic pathway. (AIIMS May 2018)
A. TCA cycle
B. Glycolytic pathway
C. Oxidative phosphorylation
D. ETC

UN-COMPETITIVE INHIBITION (ANTI-COMPETITIVE) 00:15:50

- Both K_m and V_{max} decreases.
- Inhibitor can only bind to ES complex
- Uncompetitive Inhibitors: Acetylcholine inhibits Placental ALP (Alkaline Phosphatase)

Summary of effect of different Inhibitors

Type of Inhibition	K_m	V_{max}
Competitive Inhibitors	Increased	Same
Non-competitive Inhibitors	Same	decreased
Uncompetitiv Inhibitors	decreased	decreased

ALLOSTERIC INHIBITION 00:17:24

- Similar to non-competitive inhibition in that the allosteric regulator will come and bind a regulatory site and change the shape of active site.

Difference between allosteric and Non-competitive inhibition

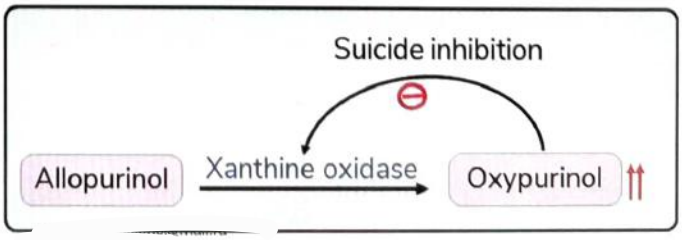
Allosteric	Non-competitive
• Activation or inhibition • Occur naturally in body e.g. o During fed state, gluconeogenesis is allosterically inhibited o During fasting, gluconeogenesis is allosterically activated	• only inhibition • Occur due to unnatural substances such as Drugs

SUICIDAL INHIBITION/ MECHANISM BASED INHIBITION 00:18:54

Example: Allopurinol Inhibits Xanthine oxidase (XO) by a proper mechanism

Suicide Mechanism

- Allopurinol first make oxypurinol using XO which will then inhibits XO itself.
- It's like oxypurinol is suicidal i.e. it's inhibiting the enzyme responsible for its formation



Another example: Aspirin, which is suicidal inhibitor of cyclooxygenase

Previous Year's Questions

Q. **Allopurinol inhibits which enzyme?** (FMGE Dec 2019)
A. Xanthine oxidase
B. Kinase
C. Carboxylase
D. Carbonic anhydrase

FEED BACK INHIBITION / END PRODUCT INHIBITION

00:20:04

- End-product itself inhibits the reaction.
- e.g.
  - Cholesterol inhibits HMG CoA Reductase
  - Haem inhibits ALA Synthase (RLE) & stops haem Synthesis

Important Information

- Feedback Inhibition is a natural phenomenon occurring in body. It is normally observed in regulation of enzymes and pathways.
- Suicidal Inhibition is unnaturally occurring phenomenon e.g. Drugs

CLINICAL QUESTIONS

Q. Aconitase, one of the enzymes of TCA, is non-competitively inhibited by fluoroacetate. The change of kinetic parameters in the presence of fluoroacetate will be reflected as:

A. decrease in V_{max}
B. decreases in K_m
C. No change in V_{max}
D. Increase in both K_m and V_{max}

Answer: A

Solution

Non-competitive inhibition: The non-competitive inhibitor can bind with free enzyme and induce a change in its configuration so that substrate cannot bind, thereby preventing the reaction from occurring.

Effect on V_{max}: Non-competitive inhibition cannot be overcome by increasing the concentration of the substrate. That decrease the apparent Vmax of the reaction.

Effect on K_m: Non-competitive inhibitors do not interfere with the binding of substrate to enzyme. So, the enzyme shows the same K_m in the presence or absence of the non-competitive inhibitor.

Reference: Lippincott's 7th ed/pg. 121,122, 123.

43 ENZYME USES

Diagnostic uses

- For diagnosis of diseases e.g. SGOT, SGPT are marker for Liver diseases

Therapeutic uses

00:01:11

Sr.	Name of enzyme	Disease/ condition treated	Mechanism of action
1	Lactase	Lactose Intolerance	Breakdown milk sugar Lactose to glucose and Galactose
2	Lactamase	Penicillin Allergy	Breakdown of β-lactam ring of penicillin
3	Urokinase / Streptokinase	Intravascular clots	Converts Plasminogen to Plasmin → plasmin can then be used for lysis of clots
4	Trypsin / chymotrypsin	for pain & inflammation in chronic back pain and sprain patients	Proteases → cause breakdown of proteins present at site of inflammation → relieve or reduce inflammation
5	Collagenase	Scars of Skin ulcers	Reduce the formation of scar tissue by breaking collagen fibres present in it.
6	Pepsin	Pancreatic insufficiency & chronic indigestion	Help in digestion of dietary proteins
7	Asparaginase/ Glutaminase	ALL (Acute Lymphoblastic Leukaemia)	Cancer cells has high demand for Asparagine & Glutamine → These enzymes breakdown these amino acids hence reducing cancer cell growth and ultimately causing their death.
8	Uricase	Gout	Breakdown uric acid
9	α1–Anti trypsin	Emphysema	Deficiency of α1–Anti trypsin can be made up by supplying external enzyme

Previous Year's Questions

Q. Liver enzyme showing liver obstruction? (NEET May 2018)

A. ALP
B. AST
C. ALT
D. Aminotransferase

Previous Year's Questions

Q. Which of the following enzymes/diagnostic marker is incorrectly paired? (INICET July 2021)

A. Beta Glucocerebrosidase - Von Gierke's
B. Ceruloplasmin - Hepatolenticular degeneration
C. High LDH1/LDH2 – Myocardial Infarction
D. Lipase – Pancreatitis

44 ENZYME REGULATION

ENZYME REGULATION

00:00:51

Various ways of Enzyme Regulation

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

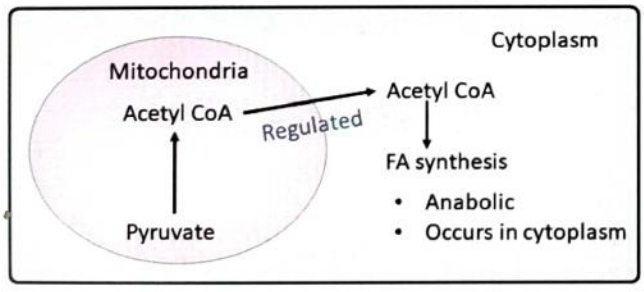
1. **Allosteric:** Refer to topic regulatory enzymes discussed under enzyme inhibitors.

2. **Covalent modification** 00:01:27

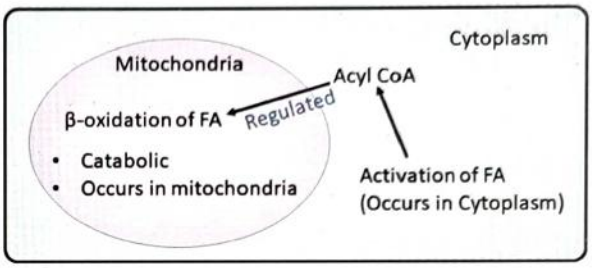
- most common covalent modification of enzymes is phosphorylation & dephosphorylation for regulation of their activity. Examples:
  - in fed state, Insulin will cause dephosphorylation of anabolic enzyme and activate them
  - In fasting state, glucagon cause phosphorylation of catabolic enzymes and activate them.

3. **Compartmentalization of Cells** 00:02:19

- Different enzymes are kept in different compartments of cell for regulating their use for various pathways depending on the conditions of the cell or tissues. example
  1) Acetyl CoA is required for FA synthesis in cytoplasm while the substrate acetyl-CoA is produced inside mitochondria.
  - So, according to the demand of FA synthesis, acetyl CoA is either allowed to go into cytoplasm or kept inside mitochondria



  2) Another example is beta oxidation where the activation of FA (first step) occur in cytoplasm and other steps occur in mitochondria.
  - So, according to energy demands of cells either acyl CoA is allowed to go inside or just kept outside in cytoplasm



00:03:32

4. **Rate of synthesis of enzymes:** Synthesis of enzymes is regulated at the level of genes. Accordingly, there are two types of genes:

Housekeeping/ Constitutive Genes	**Inducible Genes**
• always active e.g. TCA cycle enzymes genes are always expressed in a cell as TCA cycle is required to function under all conditions.	• induced to express whenever required e.g. Gluconeogenesis enzyme genes are induced to express only during fasting and are repressed under normal conditions using various hormonal mechanisms.

5. **Rate of degradation of enzymes** 00:05:17

- Depending upon whether an enzyme is required for longer or shorter period in a cell, its degradation can be either slowed down or increased respectively.
- This way total activity of that enzyme can be either increased or decreased in the cell according to situation.
- Two modes of enzyme degradation are:

1) Ubiquitin Proteasome Pathway

- Specific/specialized pathway
- requires ATP
- Regulated pathway
- occurs in cytoplasm and nucleus
- proteins undergoing degradation is first tagged with Ubiquitin (a protein which is highly conserved during evolution)

2) Lysosomal Pathway

- Non-specific
- ATP Independent

- enzyme involved is Acid hydrolase (Can breakdown many protein & other macro molecules non-specifically)

6. Synthesis of Inactive Zymogens 00:06:48

- Enzymes are synthesized as inactive enzymes at the site of production.
- These inactive enzymes are activated only at the site of action.
- e.g. Proteolytic enzymes produced by Pancreas for digestion of food need to be in activated form in the intestine only where actual digestion happens. So, these enzymes are synthesized in inactive form in pancreas and activated in intestine.
  - Chymotrypsinogen (Inactive form) → Chymotrypsin (active form)
  - Similarly, Trypsinogen → Trypsin

CLINICAL QUESTIONS

Q. Trypsinogen, a protease present in pancreatic secretion is an inactive form of enzyme trypsin. Which of the following process will activate trypsinogen to its active form trypsin?

A. Removal of amino group
B. Removal of carboxyl group
C. Phosphorylation
D. Removal of part of protein

Answer: D

Solution

Many enzymes (mainly digestive enzymes) are first synthesized as inactive precursors, known as zymogens or proenzymes. In a zymogen, part of the protein blocks the active site of the enzyme. Cleaving off this peptide activates the enzyme. e.g. Trypsinogen to Trypsin.

Trypsinogen → Trypsin + peptide fragment

Reference: Lippincott's 7th ed/pg. 175

LEARNING OBJECTIVES

UNIT V: AMINO ACIDS & PROTEINS

- Unit V amino acids and proteins is designed to discuss the structure, properties and metabolism of amino acids and their polymers (proteins). First two chapters gives a brief explanation on basics of amino acid structure, their classification based on polarity and brief overview of metabolism of different categories. Next four chapters talks about the details on metabolism of specific amino acids that are most frequently asked amino acids in PG exam. These chapter also include details on important inborn and acquired diseases occurring due to defect in the metabolism of these amino acids.

- The next set of chapters (51-55) are devoted to catabolism of amino acids. It includes the breakdown of carbon skeleton of amino acid and its incorporation into other pathways such as TCA and release of ammonia by transamination and its transport in blood and finally to liver in the form of glutamate and glutamine. Urea cycle chapter discuss the formation of urea from the released ammonia in the liver.

- A short chapter on secondary messenger nitric oxide discuss its synthesis, mechanism of action and functions. Chapters 57 and 58 talks about the formation of peptide bond, various levels of protein structure and their properties, conditions which can denature proteins and cause their precipitation.

- Chapters 59 and 60 discuss the method for purification and identification of amino acids and proteins by chemical reaction which give various colored compound with amino acid and techniques such as chromatography and electrophoresis which utilizes chemical and physical properties of molecules to achieve their separation.

- Fibrous protein chapter discuss synthesis and structure of two abundant fibrous proteins collagen and elastin and disorder associated with defect in their biosynthesis and post translational modifications. Chapters 62-64 discuss haem synthesis and catabolism and disease associated with enzyme defects in haem synthesis aka porphyria. A short last chapter talks about the protein which assist in protein folding known as chaperones.

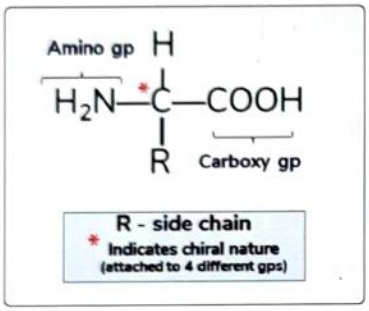
- **Major learning objectives**
  - To learn basic chemical structure and ionization behavior of amino acids
  - To know chemical structure of 20 amino acids and their classification based on polarity
  - To study pathways involved in metabolism of biologically important amino acids and disorders associated with defect in these pathways
  - To understand catabolism of amino acid, nitrogen excretion via urea cycle
  - To learn urea cycle disorders and consequences of Hyperammonemia
  - To know the structure and function of various protein and technique for their purification and identification
  - To study the metabolism of haem and disorders due to defective haem synthesis

45 AMINO ACIDS: BASICS

AMINO ACID

00:00:30

- Amino group is always on left side
- Acid group is always on right side
- Central carbon atom is Asymmetric
  - So, can show both Optical & Structural Isomerism



- All aa have 1 asymmetric carbon
- Exceptions
  - Glycine: Zero asymmetric carbon
  - Isoleucine & Threonine (both essential AA): 2 asymmetric carbons

Important Information

Q. Which AA is semi-essential out of Arginine and histidine?

Ans. Arginine (more essential than His)

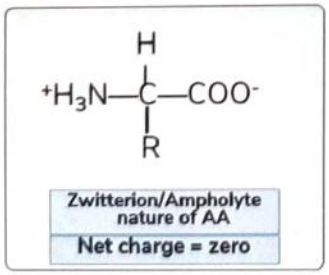
Q. AA that is essential in children but not in Adults?

Ans. Histidine

Solubility

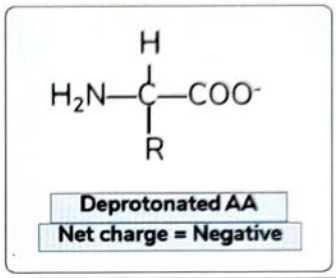
00:04:34

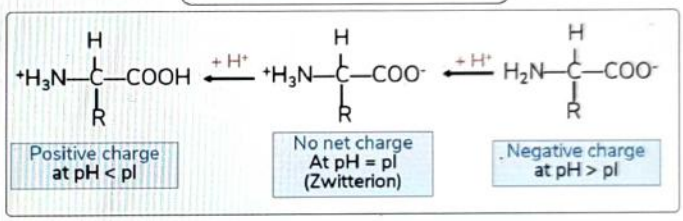
- Solubility is due to charges present on molecule
- Anything in body is ionised
- pI = Isoelectric pH (where zwitterion exits)
  - At pH = pI, Precipitation occurs (no net charge)
  - If pH < pI, Acidic pH, protein has positive charge
  - If pH > pI, Alkaline pH, protein has negative charge



Deprotonated form of AA

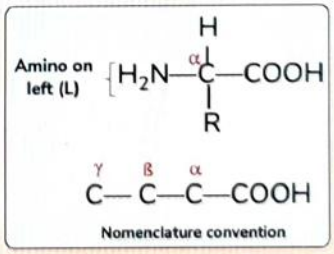
- proton is absent on both the functional groups
- happens at alkaline pH
- AA has negative charge on it





Important Information

- **Acidic AA: Negatively charged**
- **Basic AA: Positively charged**
- **All AA are L – α amino acids**
  - **L means NH_2 group is on left side**
  - **α means α-carbon is attached to amino group**



Forms of amino acids

00:12:19

- In Proteins: Always α
- Free AA:
  - α
  - β e. g ß-Alanine (catabolic end-product of pyrimidines)
  - γ
- In Proteins: always L
- Free AA: L or D e.g. D-Serine (Found in Brain) and D-Aspartate

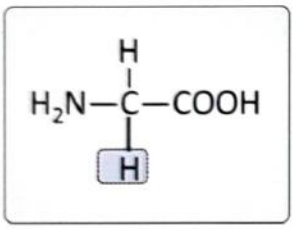
46 CLASSIFICATION AND METABOLISM OF AMINO ACIDS

CLASSIFICATION OF AMINO ACIDS 00:01:10

1. **Aliphatic amino acids**
   1. Glycine
   2. Alanine
   3. Valine
   4. Leucine
   5. Isoleucine

GLYCINE 00:01:51

Characteristics



- Side chain: Simple hydrogen atom
- Non-essential AA
  - Tip to memorize: Simple side chain so easy to make
- Smallest & Simplest AA
- No isomers

Uses of Glycine 00:03:45

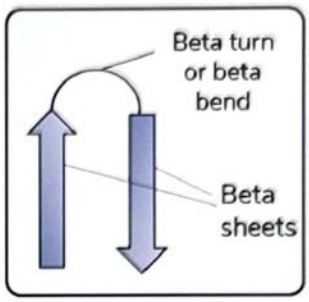
- Haem synthesis (used in the first step of haem synthesis)
- Glutathione synthesis
- Creatine synthesis
- Serine synthesis
  - Serine is a non-essential AA i.e. can be synthesized in our body from glycine 00:04:30

Important Information

Q. Which AA is responsible for the flexibility of proteins?

Ans: Glycine

- Has smallest side chain → Can fit in a small space → creates 'Bends' in proteins
  - Ability to bend is known as Flexibility
- Found in ß-turn or ß-bend
  - mostly Glycine & Proline can be found in these structures
- Never found in α – helix
  - will destabilize the structure by creating bends



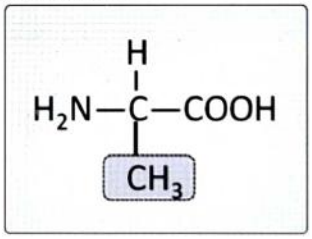
Previous Year's Questions

Q. Bends in alpha helix structure are formed by which amino acid? (JIPMER Nov 2017)

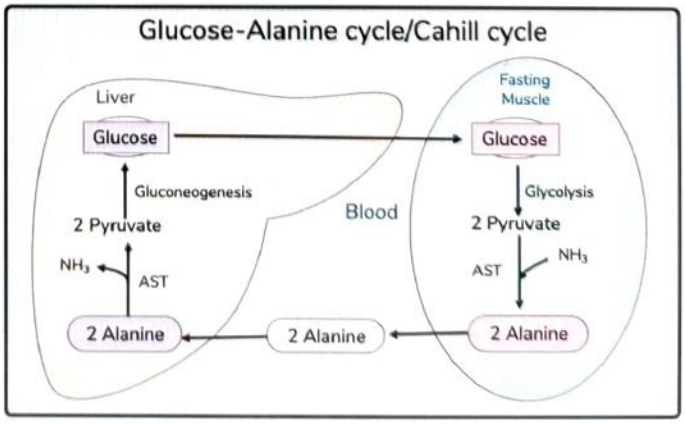
A. Glycine
B. Lysine
C. Methionine
D. Glutamine

ALANINE 00:07:18

Characteristics



- Side chain: Simple methyl group
- Non-essential AA
- 3C compound (Good substrate for Gluconeogenesis)
- Most Glucogenic AA



LEUCINE, VALINE, ISOLEUCINE

00:08:22

Characteristics

- Branched chain amino acids
- Essential AAs
- Some diseases are associated with defect in their catabolism

Maple syrup urine disease 00:09:50

- Defect in catabolism of branched chain AA
- Oxidative Decarboxylation do not occur
- Enzyme involved: α–Keto acid Dehydrogenase or α–Keto acid Decarboxylase
- C/F
  - Burnt Sugar like odour from urine
  - Ketosis
  - Mental Retardation
  - Abnormal muscle tone
  - Coma, Death (High mortality Rate)

Previous Year's Questions

Q. Maple syrup urine disease is due to deficiency of: (FMGE June 2019)

A. α-keto acid decarboxylase
B. α-keto acid carboxylase
C. α-keto acid desulphate
D. α-keto acid chain oxidase

Isovaleric aciduria /acidemia 00:14:08

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

00:15:13

Important Information

Polarity of aliphatic amino acids

- All aliphatic AA are non-polar
- Isoleucine (Most non-polar) > Valine > glycine (Least non-polar) [controversy]

Q1. Which is Polar?	Q2. Which is Polar?
1. Glycine	1. Glycine
2. Alanine	2. Alanine
3. Valine	3. Aspartate

How to Answer?

In the options,

- if glycine is given with other highly non-polar amino acids (Q1.) then you consider glycine as polar
- Whereas if one of the other options include a highly polar amino acids (aspartate) (Q2.) then consider glycine as non-polar

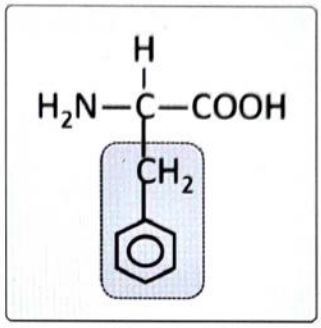
2. Aromatic amino Acids 00:18:49

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

PHENYLALANINE

00:19:24

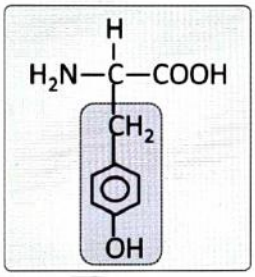
Characteristics



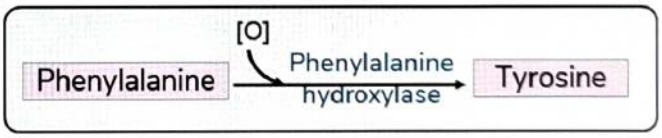
- Essential AA
- Tip to memorize: Complex side chain so hard to make
- Non-polar

TYROSINE

Characteristics



- Non-essential AA (can be made from phenylalanine)

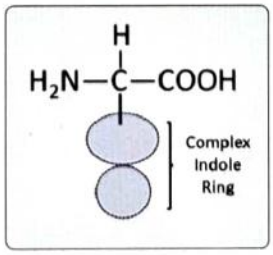


- Polar (controversy)

TRYPTOPHAN

00:22:10

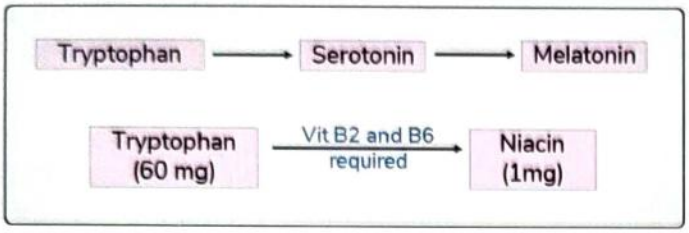
Characteristics



- Essential AA
- Tip to memorize: Complex side chain so hard to make
- Non-polar

- Never found in α-helix

Uses



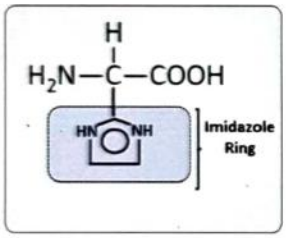
- Vit B_2 & B_6 deficiency will also leads to Vit B_3 deficiency
- Vit B_3 (Niacin): Atypical vitamin (formed in the body)
- Atypical vitamins: Vitamin D & vitamin B_3

Hartnup's disease 00:24:36

- Autosomal recessive
- Failure to reabsorb tryptophan from kidneys
- C/F
  - Amino Aciduria (Tryptophan coming in urine)
  - Pellagra like symptoms (due to Niacin deficiency as tryptophan is required for niacin synthesis)

HISTIDINE 00:26:24

Characteristics



- Side chain contains imidazole ring
- Histidine is essential but semi-essential (some amount can be made in body but rest has to be acquired from the diet.
- Histidine has max. buffering capacity among all amino acids

BASIC AA 00:27:41

- Arginine, Lysine and Histidine
- All are positively charged so, all are Polar
- Comparison of Polarity
  - Arginine > Lysine > Histidine
- Essential amino acid category
  - Arginine (semi-essential)
  - Lysine (essential)
  - Histidine (semi-essential)

ACIDIC AA

- Aspartate, Asparagine, Glutamate and Glutamine
- All are negatively charged so, all are Polar
- All are Non-essential AA Because all of them can be made from TCA intermediate as
  - OAA → Aspartate → Asparagine
  - α-KG → Glutamate → Glutamine

Important Information

Q. Which of the following moves fastest towards cathode?

A. Valine → Non-polar
B. Aspartate → Negatively charged
C. Histidine → Positively charged
D. Arginine → Highly positive (+++)

Ans D. As arginine has highest positive charge so it will move fastest toward negatively charged cathode.

Cation → (+)
Anion → (-)
Cathode → (-)
Anode → (+)

-OH CONTAINING AA 00:31:08

Serine

- Non-essential AA
- Polar

Threonine

- Essential AA
- Only AA with 2 Asymmetric carbons
- Polar

Tyrosine

- Non-essential AA
- Polar

Important Information

Q1. Which AA with max. tendency to bind phosphate?
Q2. Which AA which is site for covalent modification?
Q3. Which AA responsible for O – Glycosidic bonds?

Answer for all of these 3 questions is OH containing AA.

- So, in option choose option of OH containing AA.
- If 2 or more OH containing AA are given, Serine is best option to mark.

Q. Which AA responsible for N – Glycosidic bonds?
Ans: Asparagine (has CONH which can provide N for N-glycosidic bond)

SULFUR CONTAINING AMINO ACIDS 00:34:29

Cysteine

- Has Sulfhydryl group
- Polar
- Non-essential
- Can be made from methionine

Methionine

- Sulfur is attached to 2 carbons with strong bond (C–S–C)
- Non-polar
- Essential AA
  - **Tip:** difficult to attach sulphur with 2 Cs

Important Information

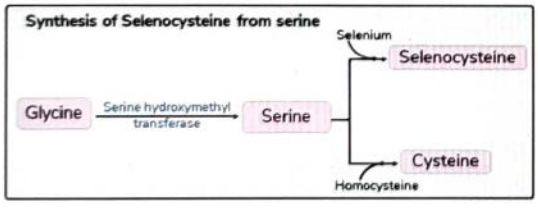
Q. Cysteine become essential in methionine deficiency same as tyrosine become essential in tryptophan deficiency

IMINO ACID – PROLINE 00:36:28

- NH_2 is not free
- Not found in α helix
  - As it will disrupt this secondary structure of proteins and hence not preferred.
- So, mostly found in ß turns
- Non-polar
- Non-essential

SELENOCYSTEINE AND PYRROLYSINE 00:38:12

- 21st AA → Selenocysteine → Given by UGA
- 22nd AA → Pyrrolysine → Given by UAG
- UGA and UAG are stop codons and usually do not code for aa
- Exceptions: Selenocysteine & Pyrrolysine
  - These AA are formed by Co-translational (during translation) modification (not by Post-translational modification)



Previous Year's Questions

Q. Which amino acid does not include post translational modification? (AIIMS Nov 2017)

A. Selenocystiene
B. Triiodothyronine
C. Hydroxy-proline
D. Hydroxy-lysine

SELENO PROTEINS 00:40:27

- Enzymes which require Selenocysteine at catalytic site
- Mainly Reductases & Peroxidases
- e.g.
  1. Glutathione Peroxidase
  2. Thioredoxin Reductase
  3. Iodothyronine deiodinase

Classification of amino acid based on polarity 00:41:33

Polar charged	Polar uncharged
1. Basic amino acids (+ charge) • Arginine • Lysine • Histidine 2. Acidic amino acids (- Charge) • Glutamate • Aspartate	1. Cysteine (Sulphydryl group) 2. -OH containing amino acids • Serine • Threonine • Tyrosine 3. Amides of Acidic amino acid (CONH gp) • Glutamine • Asparagine

DERIVED AMINO ACIDS 00:42:50

- Not coming from codons but obtained from other AAs
  - Seen in proteins
    → Hydroxyproline, Hydroxy lysine
  - Not seen in proteins
    → present as free amino acids e.g. Ornithine, Citrulline (Involved in Urea cycle)
    → Homocysteine (Involved in Methionine metabolism)

Previous Year's Questions

Q Essential amino acids is/are: (PGI May 2019)

A. Threonine
B. Phenylalanine
C. Alanine
D. Methionine
E. Cysteine

CLINICAL QUESTIONS

Q. A child presents with a history of irritability, developmental delay and urine with burnt sugar odor to a pediatrics OPD. Excess of all of the following amino acids can be tested in the urine of this child except:

A. Leucine
B. Phenylalanine
C. Isoleucine
D. Valine

Answer: B

Solution

Burnt sugar like odor is a characteristic feature of Maple Syrup Urine Disease (MSUD) which occurs due to the inherited deficiency of branched Chain Keto-acid Decarboxylase resulting in defect in oxidative phosphorylation and catabolism of branched chain amino acid -Valine/Isoleucine/leucine. As a result, these amino acids are excreted in urine.

For clinical features of MSUD refer to text.

Reference: Harper's 30th ed/pg. 309

Q. The transporter protein present in blood for the transport of lipid-based hormones such as testosterone or estrogen must be soluble in blood. Which of the following amino acid if present on the surface of these proteins will provide the necessary hydrophilic property to these proteins?

A. Tryptophan
B. Methionine
C. Glutamic acid
D. Isoleucine

Answer: C

Solution

Glutamate/glutamic acid is a negatively charged or acidic amino acid. Rest all are non-polar in the options. (refer to table and text in the notes). Based on the principle of like dissolves like, the polar amino acid glutamate will provide the necessary hydrophilic property to these proteins.

Reference: Harper's 30th ed/pg. 17

47 GLYCINE METABOLISM

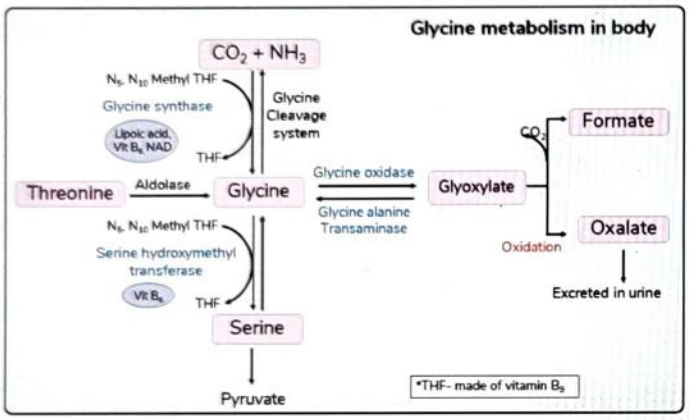
GLYCINE

00:00:30

- Simplest & smallest AA
- Non-polar (controversy)
- Non-essential AA
- Glucogenic AA

GLYCINE METABOLISM

00:00:54



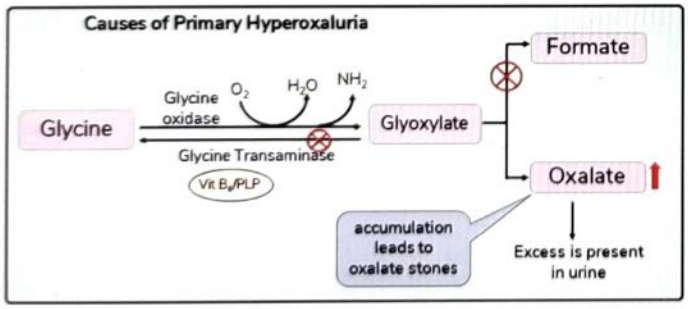
GLYCINE METABOLISM DISORDERS

00:03:37

Hyperoxalurias

1. Primary hyperoxaluria

- Protein targeting disorder
- Oxalate stones in kidneys
- oxalate depositions in extra renal tissues



Rx:

- Hydration to prevent oxalate stones.
- Restriction of Oxalate rich foods such as Green leafy vegetables, beetroot & tea is advised

2. Secondary hyperoxaluria

- Occur due to some reason other than mentioned for primary hyperoxaluria.
- Causes
  1. Vit C toxicity
  2. Vit B_6 Deficiency
  3. Ethylene Glycol poisoning
  4. Methoxy Flurane
  5. Bariatric surgery

Glycinuria

00:06:28

- Occur due to defective reabsorption of 2 AA
  1. Glycine
  2. Proline
- Transporter for both is same
- Disease occurs d/t defect in this transporter
- C/F
  - Serum glycine levels are normal
  - Has ↑ risk of oxalate stone but urine oxalate is normal

Non-Ketotic Hyperglycinaemia

00:08:02

- Occurs due to defect in Glycine cleavage system (glycine catabolism)
- Glycine increases which is a neurotransmitter
- C/F
  1. Mental retardation
  2. Seizures
  3. Lethargy
  4. Apnea↑
  5. glycine in blood, CSF & urine
  6. aka glycine encephalopathy
  7. Ketone bodies not increased
  8. No effective treatment

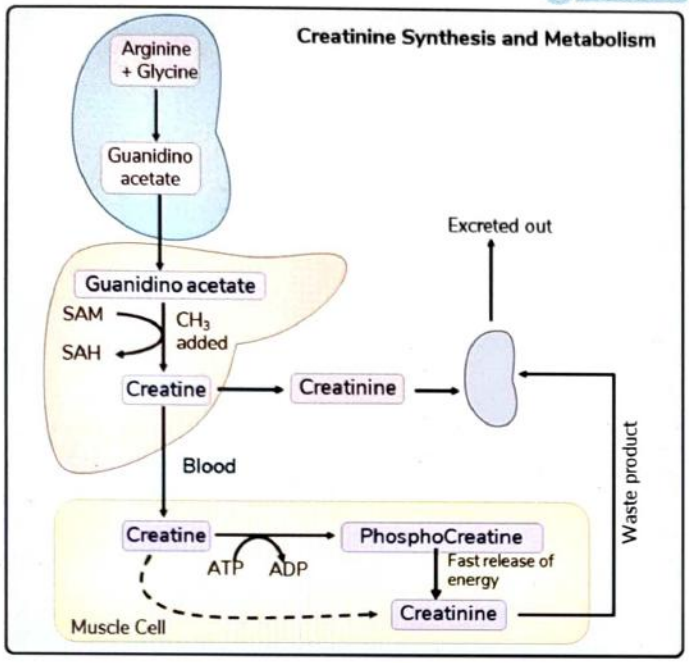
Remember that Ketotic hyperglycinemia occurs in propionic acidemia

USES OF GLYCINE

1. Haem synthesis
2. Part of Glutathione
3. Purine rings formation
4. Serine biosynthesis
5. Conjugating agent
6. Neurotransmitter
7. forms creatinine

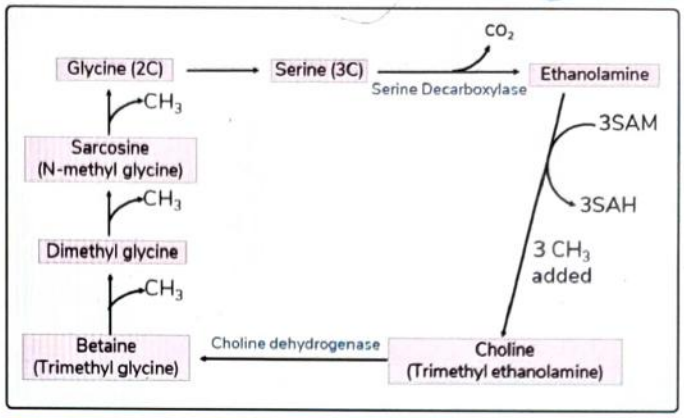
Creatine synthesis

00:09:36



Formation of choline

00:12:31



Important Information

- Glycine metabolism is interlinked with folate metabolism
- Choline & Betaine metabolism is also linked to tetrahydrofolate metabolism

CLINICAL QUESTIONS

Q. A middle-aged man visited to a physician with severe pain in his back, which later turned out to be due to kidney stones. The chemical analysis of stone indicated a buildup of oxalic and glyoxylic acids. Problem with which of the following amino acid metabolism can cause development of these stones?

A. Alanine
B. Tryptophan
C. Glycine
D. Glutamine

Answer: C

Solution

Defect in glycine transaminase when associated with impaired glyoxylate to formate conversion, will divert excess glyoxylate to form oxalate, leading to the formation of oxalate stones in kidneys. This is known as primary hyperoxaluria. (Refer to fig in the text)

Regarding other options:

Defect in tyrosine metabolism causes various types of tyrosinemias and alkaptonuria.

Defective absorption and metabolism of isoleucine and tryptophan can cause Hartnup's disease also known as pellagra like dermatosis.

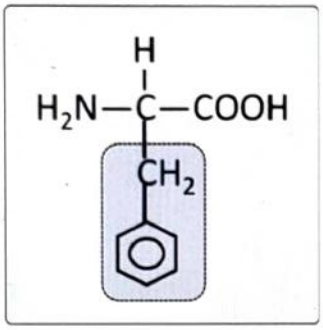
Accumulation of glutamine can result in increase of ammonia concentration in body leading to symptoms of hyperammonemia such as cerebral edema, vomiting, fine tremors, lethargy and slurred speech etc.

Reference: Harper's 30th ed/pg. 301

48 PHENYLALANINE & TYROSINE METABOLISM

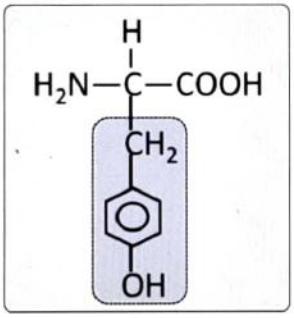
PHENYLALANINE

00:00:30



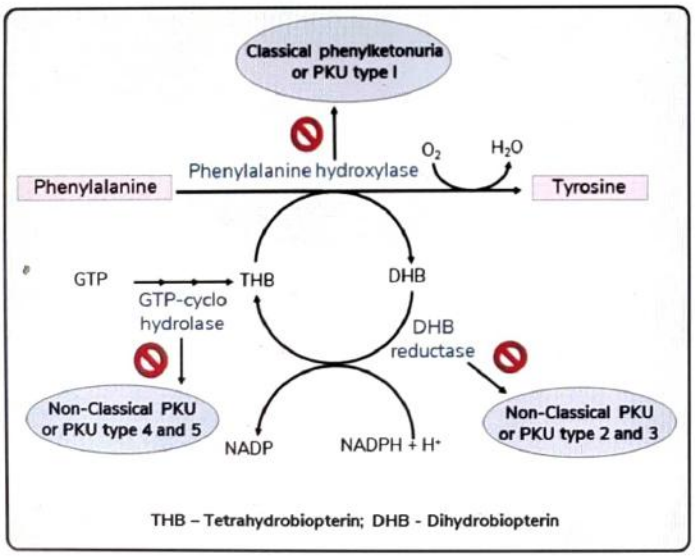
- Essential AA
- Tip to memorize: Complex side chain so hard to make
- Non-polar (as benzene ring is non-polar)

TYROSINE



- Non-essential AA (can be made from phenylalanine)
- Polar (controversy)

00:01:09



3 Aromatic AA hydroxylases

1. PAH
2. Tyrosine Hydroxylase
3. Tryptophan Hydroxylase

All these three enzymes require:

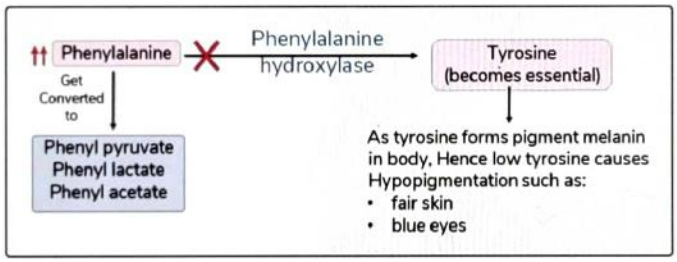
1. Tetrahydrobiopterin
2. NADPH

00:05:24

PHENYLALAINE METABOLISM DISORDERS

Phenylketonuria

- Mc metabolic disorder of AA
- Autosomal recessive
- PAH deficient



- C/F
  - mousy/musty body odour (due to phenylacetate)
  - severe MR (due to Phenylalanine)

Diagnosis

1. $FeCl_3$ urine test → Green colour → positive d/t Phenyl pyruvate (3 C keto acid) in urine. Name: [Phenyl-keto-uria → phenyl- keto acid - in urine]
2. DNPH Test → Positive
3. Bacterial Guthrie's test/bacterial (*Bacillus subtillis*) inhibition test → positive

Important Information

Screening for PKU should be done after 2-3 days of birth because at birth Phe levels are normal d/t maternal enzymes. Levels increases after a few days of birth.

Maternal PKU

- Occurs d/t lack of proper diet in pregnancy
- Child have
  - Microcephaly
  - MR

- Growth retardation
- Congenital heart defects

Important Information

Q. In Phenylketonuria, why Brain is affected?
It is due to:

1. ↓ Neurotransmitter
   - ↑↑ Phenyl alanine in blood → taken into brain cells so that Tyrosine & Tryptophan unable to reach brain
   - ↓ catecholamines and ↓ serotonin synthesis as they are made from Tyrosine and tryptophan respectively
2. ↓ Thyroxine

Previous Year's Questions

Q. Which of the following is a feature of Phenylketonuria? (NEET Jan 2018)

A. Loss of deep tendon reflexes
B. Mental retardation
C. Macrocephaly
D. All

Treatment of Phenylketonurias

1. Lifelong Restriction of phenyl alanine in diet
   - Aspartame (artificial sweetener) is contraindicated as aspartame is a Dipeptide made of Aspartate + Phenylalanine
2. Give tyrosine & tryptophan
   - Because Tyrosine becomes essential in this condition
   - These amino acids are required in the brain cells in PKU as discussed earlier.
3. Tetra hydro Biopterin Supplementat^{n} specially for non-classic PKU
   - THB load test: distinguish classic & non-classic PKU

Previous Year's Questions

Q. An infant presented to the OPD with a history of vomiting and malnutrition. Patient has blue eyes, blonde hair & fair skin. On investigation, Guthrie test was found to be positive. All are true regarding this disease EXCEPT: (AIIMS May 2018)

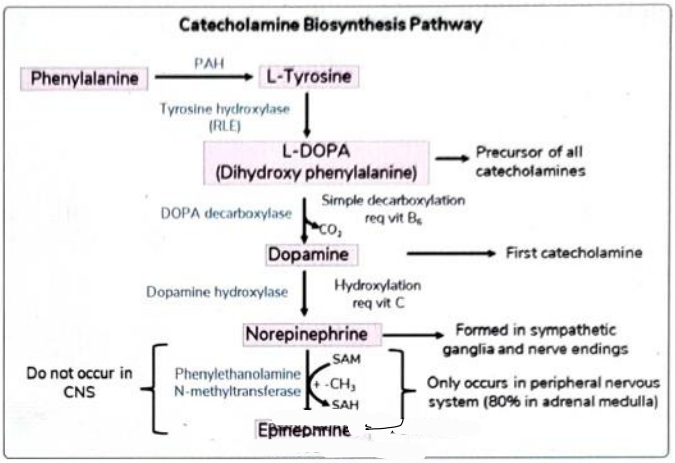
A. Due to PAH enzyme defect
B. White patch of hair due to tryptophan deficiency
C. Phenyl acetate positive in urine
D. Mental retardation is present

USES OF TYROSINE

Tyrosine is required for the synthesis of

1. Catecholamines
2. Thyroid hormones
3. Melanin pigment

00:16:57



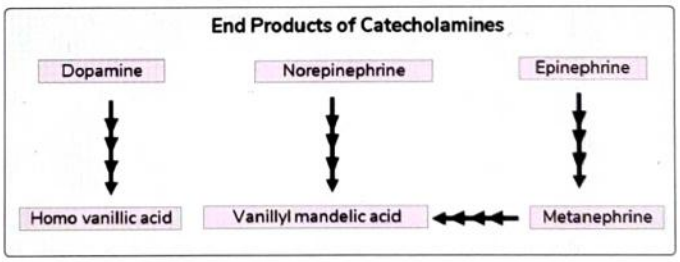
Important Information

First catecholamine synthesized: Dopamine
Catecholamine with methyl group: Epinephrine

Catecholamine pathway has organ specific termination i.e. the end-product can be epinephrine or norepinephrine depending on the organ where the pathway is occurring.

Catabolism of Catecholamines

- **It is done by two enzymes:**
  1. COMT (Catechol–O–Methyl Transferase)
  2. MAO (Mono Amine oxidase)



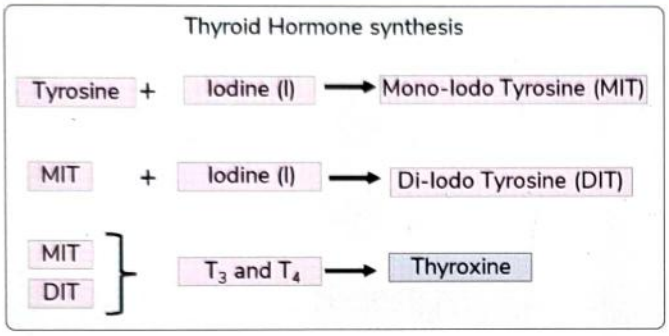
Vanillyl Mandelic Acid (VMA) 00:20:32

1. ↑ in Pheochromocytoma (Tumor of adrenal gland)
   - Headache
   - Palpitation
   - Profuse sweating
2. ↑ in Neuroblastoma of Adrenal gland

Diagnosis: 24hr urine samples collected & VMA levels measured (Normal VMA levels = 2-6 mg/day)

Previous Year's Questions

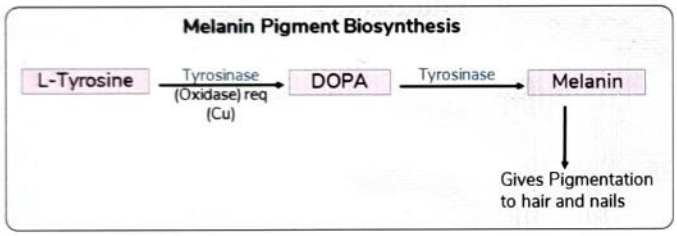
Q. VMA is excreted in urine in: (NEET Jan 2018)
A. Alkaptonuria
B. Phenyl ketonuria
C. Diabetic ketoacidosis
D. Pheochromocytoma



DISORDERS OF TYROSINE METABOLISM

1. Albinism

00:13:03



C/F

- Milky white skin
- White hair
- Red eye colour

Important Information

Vitiligo

- Tyrosinase is normal
- Lack of melanoblasts in regional areas

Previous Year's Questions

Q. Melanin is derived from which amino acid? (FMGE Dec 2019)

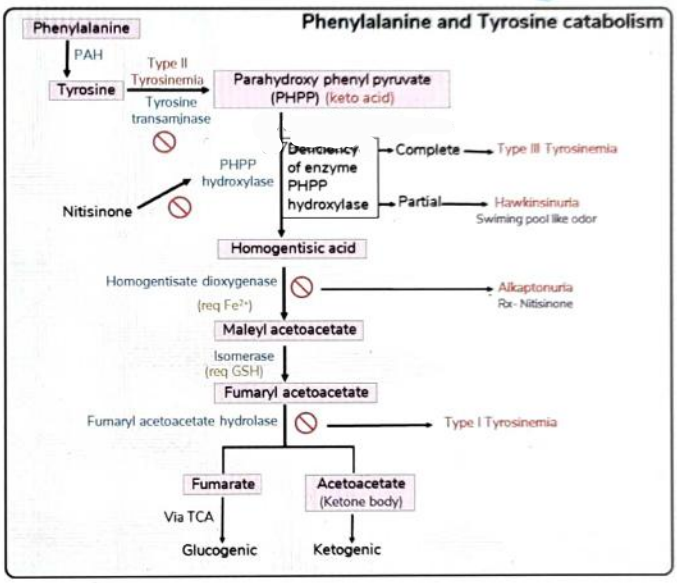
A. Tyrosine
B. Tryptophan
C. Phenylalanine
D. Alanine

Tyrosine Hydroxylase	Tyrosinase
• Present in adrenal medulla, sympathetic ganglia & nerve endings	• Synthesized in melanosomes present in melanocytes of skin and hair

Important Information

The only function of Phenylalanine in our body is synthesis of tyrosine
Diet → Phenyl alanine → tyrosine → catabolized

00:21:54



2. Tyrosinemias

00:24:43

Type I/Tyrosinosis/ Hepatorenal Tyrosinemia

- Most common
- Fumaryl acetoacetate hydrolase is deficient

Type II/ Oculocutaneous Tyrosinemia

- Tyrosine transaminase deficient
  - Eyes affected → corneal ulcers
  - Skin affected → Hyperkeratotic plaques

Types III/Neonatal Tyrosinemia

- Deficiency of PHPP hydroxylase

3. Alkaptonuria

00:27:35

- D/t deficiency of Homogentisate dioxygenase (requires iron)
- Rx: Nitisinone
- Homogentisic acid accumulated → oxidized → Black colored compound in urine

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

- Part of Garrod's Tetrad:
  1. Cystinuria
  2. Alkaptonuria
  3. Albinism
  4. Pentosuria
- Age of onset: 30 - 40 yrs
- Presents with lower back pain
- No MR
- Homogentisic acid gets polymerized in to alkapton bodies which accumulates in:
  1. Cartilages (Nose, Ear Pinna, IV discs): Arthritis
  2. Connective tissue: Bluish black colour
- Collectively, accumulation of homogentisic acid in all body parts is termed as Ochronosis and arthritis as Ochronotic arthritis.
- Benedict's test is positive d/t Homogentisic Acid (Reducing substance)
- Diagnosis is by $FeCl_3$ urine test → positive

Previous Year's Questions

Q. A 25-year old patient presented with dark urine, which further blackens on exposure to air. What's the enzyme defect in this case? (NEET Sep 2021)

A. Fumaryl acetoacetate hydrolase
B. Di Hydroxy Phenyl Acetate Dioxygenase
C. Homogentisate Dehydrogenase
D. Phenyl alanine hydroxylase

CLINICAL QUESTIONS

Q. A young male is who has newly moved in your neighborhood has white hair, white skin, and nystagmus. A defect in the metabolism of which one of the following compounds is responsible for this presentation?

A. Histidine
B. Tryptophan
C. Methionine
D. Tyrosine

Answer: D

Solution

The patient has the signs of albinism, a lack of melanin. Melanin is produced from tyrosine by enzyme tyrosinase which is an oxidase. Tyrosinase deficiency causes albinism, due to non-production of pigment melanin from tyrosine causing light or no color of skin and hair. Refer to fig melanin pigment synthesis in the text.

This is a melanocyte-specific genetic deficiency.

Reference: Harper's 30th ed/pg. 317

Q. Parents of a 5-year-old boy who are a first-degree consanguineous couple accidentally noticed that the urine of their child turned black when it was left standing in air. They went to their family physician to seek advice on this. Further counselling reveals that child has no other medical problems with normal childhood growth and development. His sibling is also normal. The physician should order a test of which of following compounds in this patient urine?

A. Methylmalonate
B. Homogentisate
C. Phenylpyruvate
D. Homocysteine

Answer: B

Solution

The child is suffering from alkaptonuria which is an autosomal recessive disorder due to inborn error in metabolism of tyrosine due to deficiency of enzyme homogentisic acid oxidase. This enzyme converts one of the intermediates of tyrosine catabolism in to methylacetoacetate. (See fig in text)

As a result of this enzyme deficiency, substrate homogentisic acid accumulates and excreted in urine. Urine turns black on exposure to air due to oxidation of homogentisic acid causing dark staining of diapers. It is a benign condition in childhood but over the years homogentisic acid gets polymerized in body forming alkapton bodies, which gets accumulated in cartilages such as nose, ear pinna, intervertebral disc and connective tissue which has bluish black colour. Patient develops arthralgia & arthritis. This condition is known as Ochronosis and arthritis is also known as ochronotic arthritis.

Urine gives positive Benedict's test as homogentisic acid is a Reducing substance.

Regarding other options:

Homocysteine accumulation occur due to error in methionine metabolism such as deficiency of enzyme cystathionine beta synthase or cystathionase or due to vitamin B_9 or Vitamin B_{12} deficiency.

References: Harper's 30th ed/pg. 304

Q. A newly born child was declared negative for PKU in newborn screening done on blood sample taken from umbilical cord so, pediatrician did not impose any dietary restriction. Parents brought the child to pediatrician again at 12 month of age and this time the child was reported to have signs of severe mental retardation, so blood and urine tests are ordered again that showed elevated phenylalanine in blood and phenylacetate so, finally a diagnosis of PKU is made. The reason for wrong diagnosis at birth is that:

A. cord blood is not a good source of fetal blood
B. the test was performed too early
C. Guthrie test used in diagnosis has low sensitivity
D. maternal blood should have been used

Answer: B

Solution

The phenylalanine concentration in PKU affected infants is usually normal at birth due to phenylalanine degradation using maternal enzymes but it increases rapidly in 1-3 days after birth. So, a test done right after birth will most probably give a false negative test.

So, the best way to remove false negative is to do the test on 2nd to 3rd day after birth by taking blood sample from the infant's heel.

Regarding other options

- Mass spectrometry instead of Guthrie test is used widely nowadays for newborn screening.
- Maternal blood will not show any elevated phenylalanine as there is no defect in mother enzymes.

Reference: Harper's 30th ed/pg. 304

Q. A 2-year-old boy is brought to clinic because of poor development as well as vomiting, irritability, and a skin rash. The boy's mother also notes that his urine has a strange "mousy" odor. Physical examination reveals the child has an eczema-like rash, is hyper reflexive, and has increased muscle tone. He has a surprisingly fair-skinned complexion compared to the rest of his family. What is the most likely diagnosis?

A. Tay-Sachs disease
B. McArdle disease
C. Phenylketonuria
D. Pyruvate dehydrogenase deficiency

Answer: C

Solution

In PKU, phenylketone i.e. phenylpyruvate is found in urine of patients (pyruvate is a keto-acid). Affected children are normal at birth but fail to reach developmental milestones

Clinical features:

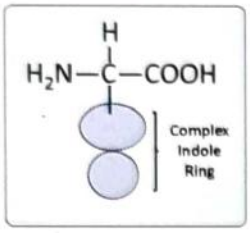
- Body odor: mousy or musty because of phenylacetate.
- Severe mental retardation due to excess Phenylalanine.
- Tyrosine becomes essential.
- Deficiency of pigment melanin (formed from tyrosine), leads to fair skin, blue eyes and light hair color.

Other features: Microcephaly, rash, hypertoma, seizures, hyperactivity, exaggerated tendon reflexes, wide-spaced teeth, enamel hypoplasia.

Reference: Harper's 30th ed/pg. 286

49 TRYPTOPHAN METABOLISM

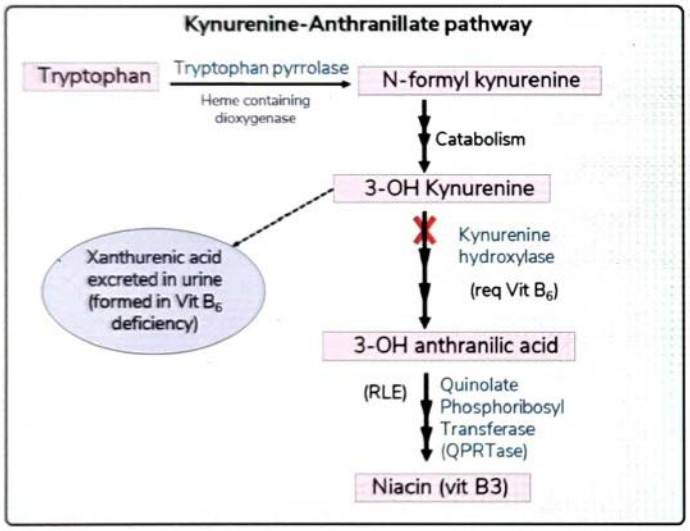
TRYPTOPHAN



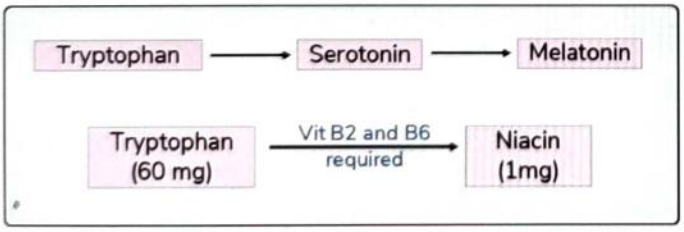
- Essential AA
- Tip to memorize: Complex side chain so hard to make
- Non-polar
- Never found in α helix (Bulky AA)

Niacin synthesis

00:00:40



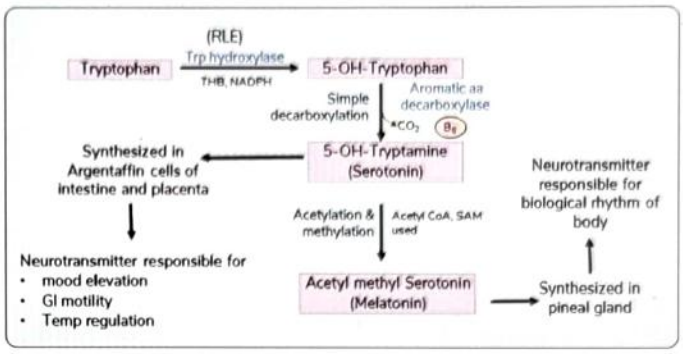
Uses



- Vit B_3 (Niacin) Atypical vitamin (formed in the body)
  - Atypical vitamins: Vitamin D & vitamin B_3
- Donor of formyl group to 1 carbon pool of Body
  - C_2 of purines
  - formyl methionine formation [prokaryotes]

Synthesis of Serotonin & Melatonin

00:03:27



Excretory end-product of serotonin is 5-Hydroxy Indole Acetic Acid (5-HIAA)

Previous Year's Questions

Q. Serotonin is also known as? (NEET Jan 2018)

A. 5-hydroxytryptamine (5-HT)
B. N-methyl phenylamine
C. 3-Methoxytyramine
D. Phenethylamine

DISORDERS OF TRYPTOPHAN METABOLISM

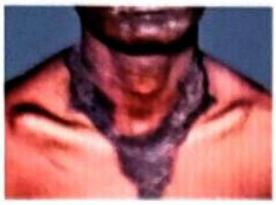
00:06:21

Hartnup's Disease

- Autosomal recessive
- Failure to reabsorb & absorb neutral AA & tryptophan due to defective transporter present in intestine and kidney
- C/F
  - Amino Aciduria [Tryptophan coming in urine]
  - Pellagra like symptoms (due to Niacin deficiency as tryptophan is required for niacin synthesis)
    - → Tryptophan (that is not absorbed from intestine) → Bacterial action → Indoxyl compounds formed → Blue color diaper
    - → In intestine, Indican compounds are formed → detected by Obermeyer test [positive]

Previous Year's Questions

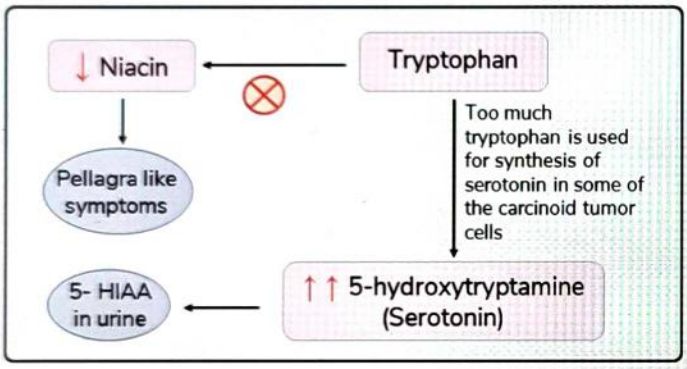
Q. The given below clinical features occurs due to deficiency of:



A. Tryptophan
B. Phenylamine
C. Cysteine
D. Glutamine

Carcinoid Syndrome

00:08:11



C/F

- Profuse sweating
- Flushing
- GI motility
- 5-Hydroxy Indole Acetic Acid (HIAA) comes in urine

Previous Year's Questions

HIAA is excreted in urine in: (NEET Jan 2018)

A. Alkaptonuria
B. Carcinoid syndrome
C. Albinism
D. Phenylketonuria

Previous Year's Questions

Q. Pellagra is/are caused by: (PGI May 2019)

A. Niacin deficiency
B. Hartnup disease
C. Tryptophan excess
D. Rifampicin use
E. Carcinoid syndrome

CLINICAL QUESTIONS

Q. A child diagnosed with classic PKU was advised for supplementation of large doses of tryptophan, leucine, and tyrosine in his diet. The reason behind such treatment is which one of the following?

A. To inhibit PAH activity
B. To activate PAH activity
C. To restore neurotransmitter synthesis
D. To stimulate protein synthesis

Answer: C

Solution

A child with classic PKU has a deficiency in PAH, which blocks the conversion of the essential amino acid phenylalanine to tyrosine. This leads to phenylalanine accumulation in the blood, and the high phenylalanine levels outcompete the absorption of tryptophan, leucine, and tyrosine in brain. This will lead to low neurotransmitter synthesis in brain causing neuronal dysfunction.

By increasing the levels of neutral amino acids, the tryptophan, leucine, and tyrosine, the competition for absorption of these amino acids is again restored and neurotransmitter synthesis can proceed normally.

Regarding other options:

High levels of tryptophan, leucine, and tyrosine do not alter the activity of PAH, nor do they allosterically stimulate, or inhibit, protein synthesis.

Reference: Dinesh Puri 3rd ed/pg. 286-287

Q. A 6-year-old boy experienced episodes, where he develops patches of skin rashes which go away in a week to 10 days. A pediatrician told the boy's parents to give him niacin the next time this will occur, along with a high-protein diet. The rash resolved in a day or two when they did this. This child has which one of the following diseases based on this information?

A. Cystinuria
B. Hartnup's disease
C. HHH syndrome
D. Alkaptonuria

Answer: B

Solution

Hartnup's disease aka pellagra-like dermatosis is an autosomal recessive metabolic disorder affecting the absorption & reabsorption of nonpolar amino acids (particularly tryptophan). Tryptophan is used to synthesize serotonin, melatonin, and niacin. Thus, deficiency of niacin leads to pellagra.

Regarding other options:

Cystinuria is a different transport defect, which affects dibasic amino acid transporter.

Ornithine translocase deficiency, also called hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, is a rare autosomal recessive, urea cycle transporter defect, which causes ammonia to accumulate in the blood.

Alkaptonuria is due to a defect in homogentisic acid oxidase.

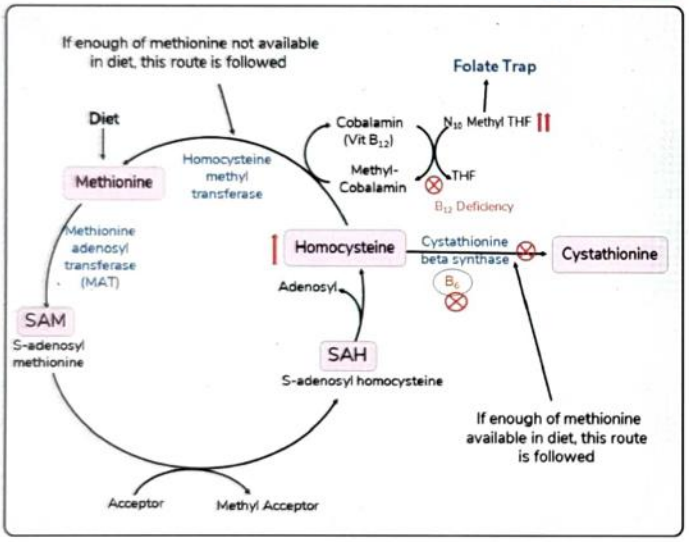
Reference: Harper's 30th ed/pg. 308, 557

50 METHIONINE & CYSTEINE METABOLISM

Characteristics of Cysteine and Methionine

Cysteine	Methionine
• S bonded to 1 C (H - S – C)	• S bonded to 2 C (C - S – C)
• Polar (due to -SH group)	• Non-polar
• Non-essential AA	• Essential AA
• Glucogenic AA	• Glucogenic AA

00:00:35



Important Information

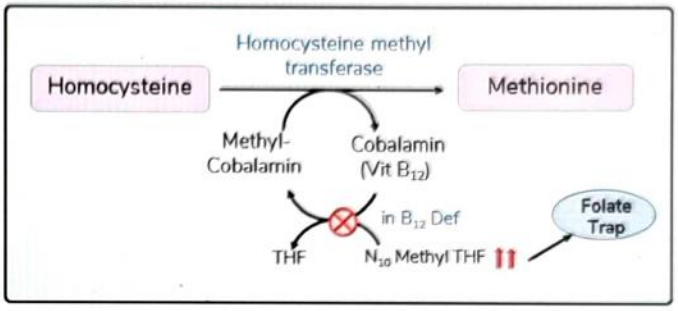
MAT Type I & 3: present in Liver
MAT type 2: present in extra hepatic tissues
Reaction requiring Methyl groups

1. 7-methyl guanosine cap of mRNA
2. NE → Epinephrine
3. Cephalin [ethanolamine] → Lecithin [Choline]
4. Guanidinoacetate → creatine
5. Acetyl serotonin → melatonin

FOLATE TRAP

00:04:36

- Due to deficiency of Vit B_{12}, Methyl THF to THF conversion is blocked. So, folate accumulates in the form of methyl THF
- It is also known as Functional deficiency of folate i.e. functional form of folate (THF) not available in body.



Previous Year's Questions

Q. Which B_{12} dependent enzyme is involved in amino acid metabolism? (INICET Nov 2020)

A. Methyl malonyl CoA isomerase
B. Homocysteine synthase
C. Folate reductase
D. Methionine synthase
E. Beta hydroxy butyrate synthase

HOMOCYSTINURIA (HCU)

00:07:26

Cause

- Homocysteine accumulation occurs in case of B_6, B_9, B_{12} deficiency (refer to first figure)
- In blood, homocysteine accumulates but the compound homocystine comes out in urine as two homocysteine (-SH) molecules joins to form homocystine (S~S)

Dangerous effect of Homocysteine accumulation

- Irritation of endothelium of blood vessels causing stroke, atherosclerosis, pulmonary embolism & MI
- **Other C/F**
  1. MR
  2. Ectopia lentis
  3. Seizures
  4. Osteoporosis
  5. Marfanoid habitus
     → Similar to Marfan syndrome except Lens is dislocated outwards & upwards in Marfan syndrome whereas Lens is dislocated

downwards & inwards in homocystinuria

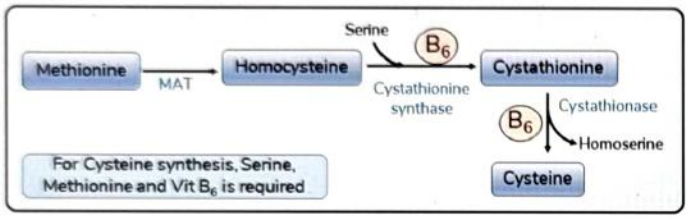
Types of HCU

1. **Genetic**: d/t enzyme deficiency
2. **Acquired**: d/t vitamin deficiency (B_6, B_9, B_{12})

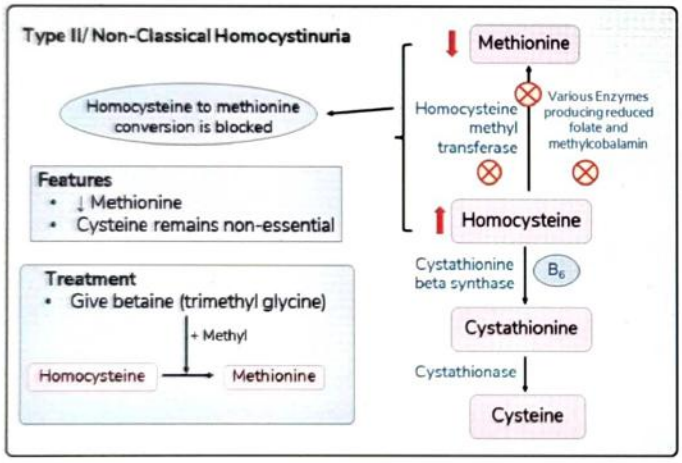
Acquired HCU

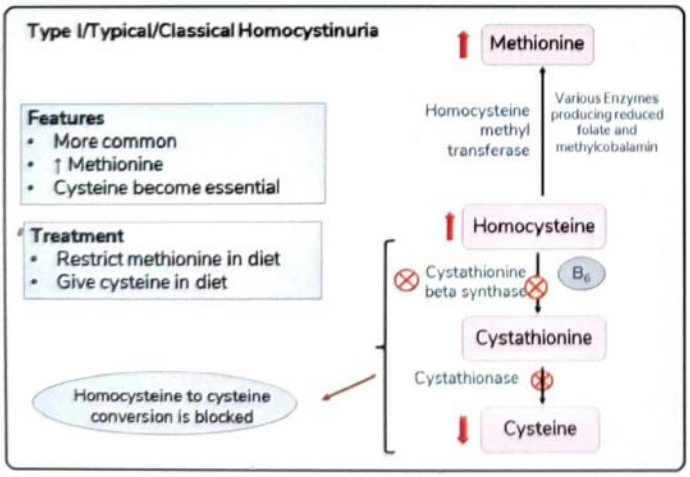
- In case of a young patient with conditions such as stroke or atherosclerosis, patient's homocysteine levels should be checked, as it can easily be a case of vitamin deficiency and homocystinuria.

Cysteine is non-essential as it is derived from Methionine. But It becomes essential, once methionine is deficient in diet (See fig below)



Genetic HCU





Previous Year's Questions

Q. Which of the following vitamin needs to be supplied in the diet of patients with Cystathionine beta synthase defect? (NEET Sep 2021)

A. Cysteine
B. Methionine
C. Serine
D. Homocysteine

Diagnosis of HCU 00:11:30

Cyanide Nitroprusside Test (CNT): this test is positive for

1. Cysteine
2. Cystine
3. Homocystine
4. Homocysteine

- Principle of test
  - CN breaks the S ~ S bond → SH is liberated → reacts with Na Nitroprusside to give Magenta/ Red purple colour

Homocystinuria	Cystinuria	Cystinosis	Cystathioninuria
• AR • CNT ⊕	• AR • CNT ⊕	• LSD • CNT ⊕	• CNT ⊖

CYSTINURIA 00:15:09

- d/t defective dibasic Amino Acid Transporter present in intestine & kidney
- causes defective absorption & reabsorption of four amino acids **C**ystine, **O**rnithine, **L**ysine, **A**rginine

How to remember

- In cystinuria, there is defective absorption & reabsorption of four amino acids:

Mnemonic - COLA
Cystine
Ornithine
Lysine
Arginine

Main clinical feature: presence of cystine stone in kidney

Treatment of Cystinuria

- Aim is to ↑ solubility of cystine stones. It can be done by:

1. Good hydration
2. Alkalisation of urine
3. Chelating agents like penicillamine (Penicillamine + cystine → soluble complex → excreted)

Previous Year's Questions

Q. In Cystinuria, all of the following amino acids are excreted in urine, except: (NEET Jan 2018)

A. Cystine
B. Ornithine
C. Leucine
D. Arginine

CYSTINOSIS

- Generalised Lysosomal storage disease (LSD)
- Due to defect in cystine transporter (cystinosin) in lysosomes
- Cystine deposits occur in various tissues such as bone marrow, cornea, liver & kidney etc.

Rx: Cysteamine → forms complex with cystine (No role of penicillamine)

CYSTATHIONINURIA

- Defect in enzyme cystathionase, so, cystathionine accumulates
- So, CNT test is negative as there is no S ~ S bond in cystathionine for the reaction.

CLINICAL QUESTIONS

Q. A 40-year-old male whose brother recently had heart attack at 45-year age is worried and visited a physician to consult about the risk of him having a heart attack. Doctor ordered blood test such as HbA1C, lipid profile and homocysteine level. The report revealed normal HbA1c but elevated homocysteine levels. These findings suggest that the amino acid that is not metabolized properly in the patient blood is:

A. Alanine
B. Phenylalanine
C. Methionine
D. Glutamate

Answer: C

Solution

Refer to 1st figure for methionine metabolism and reason for homocysteine accumulation in body.

- A defect in either the enzyme that forms methionine (methionine synthase) or the enzyme that forms cystathionine (cystathionine ß-synthase) will lead to elevated homocysteine levels.
- Also deficiency of cobalamin and/or folate can also lead to homocysteine accumulation.
- As this patient has no genetic enzyme deficiencies so the most probable cause of his homocystinuria is deficiency of vitamins which can be treated by vitamin supplementation.

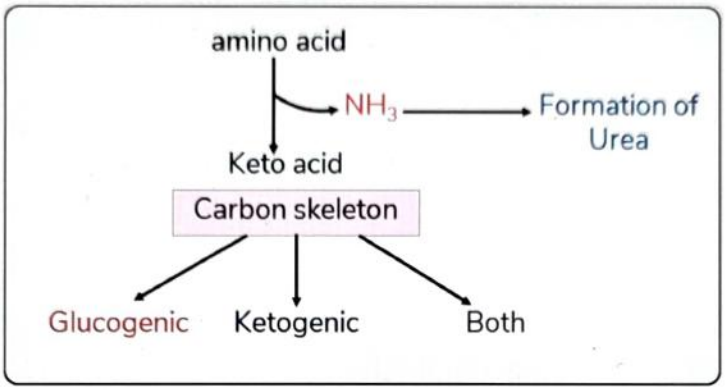
All other amino acids given in options do not require homocysteine for their synthesis.

Reference: Harper's 30th ed/pg. 308-309

51 CATABOLISM OF AMINO ACIDS

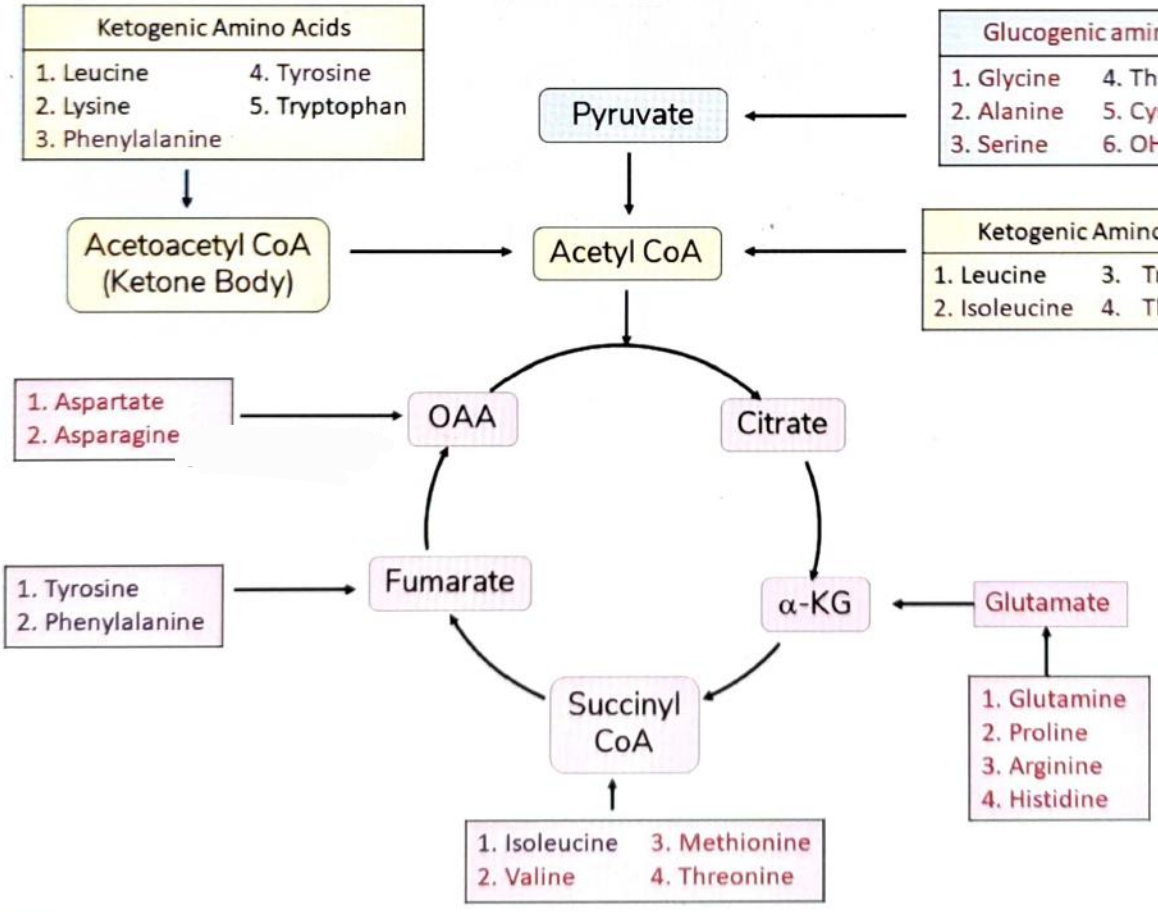
CATABOLISM OF AMINO ACIDS

00:00:42



00:02:39

Entry of Catabolic end-product of carbon skeleton of various amino acids in TCA



Important Information

- Any AA forming intermediate of TCA cycle is glucogenic
- Glutamate is formed as an end-product of carbon skeleton of proline by reactions other than transamination (discussed later)

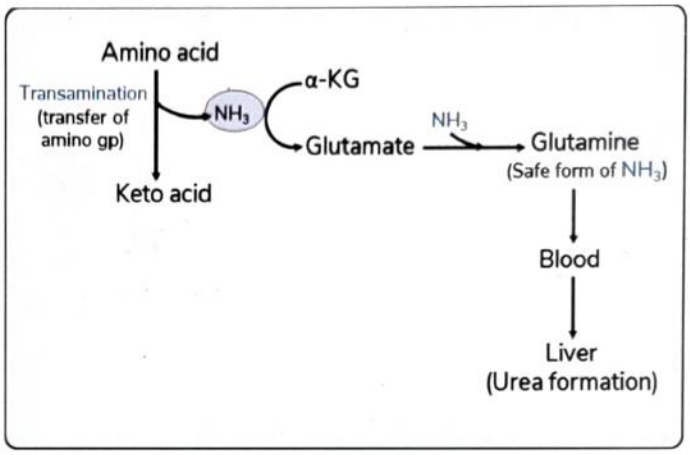
Previous Year's Questions

Q. In aerobic condition, amino acids which are seen at the level of pyruvate? (FMGE Dec 2019)

A. Alanine and aspartate
B. Alanine and Glycine
C. Arginine and histidine
D. Leucine and isoleucine

NITROGEN EXCRETION

00:05:48

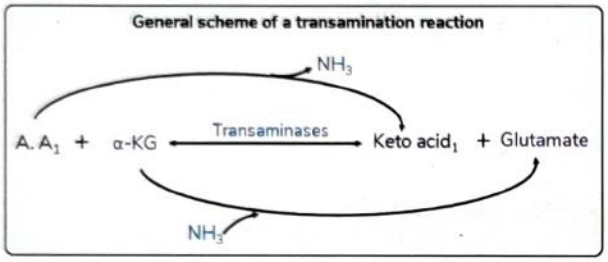


52 TRANSAMINATION

TRANSAMINATION REACTION

00:00:20

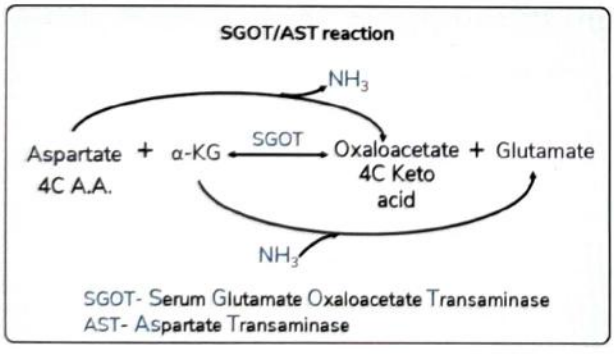
- 1st reaction in the catabolism of AA
- Reversible
- Requires B_6/PLP (pyridoxal phosphate)
- Occur through covalent catalysis i.e. strong covalent bond formed
- Common AA involved: Glutamate

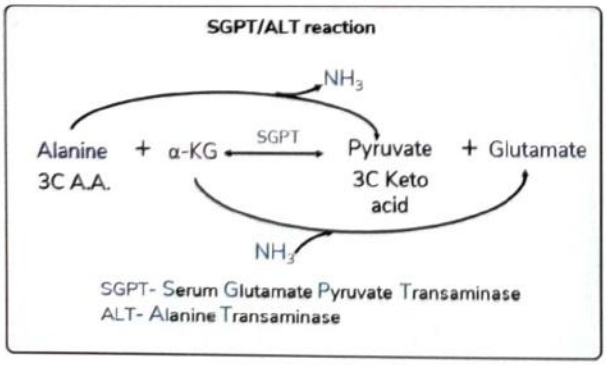


Transaminases

00:01:56

- **Specific for 1 pair of substrates** e.g. SGOT only used for OAA and Aspartate and no other aa pairs.
- Name is given after the AA from which amino group is removed
- Important for the synthesis of non-essential AA. e.g.
  1. OAA → Aspartate
  2. Pyruvate → Alanine
  3. α-KG → Glutamate
  4. Glyoxylate → Glycine

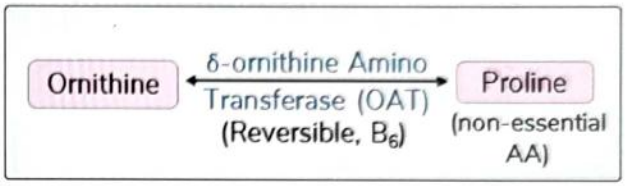




- Only α-amino group can take part in transamination
  - Exception is δ(delta) amino group of ornithine

δ-Ornithine Amino Transferase (OAT)

00:06:44



- Present in liver kidney, retina, brain
- Deficiency: Gyrate atrophy of choroid & retina
- C/F
  - Rare disease
  - autosomal recessive
  - ↑↑ ornithine
  - Proline not formed
  - Vision loss, cataract
- Rx
  1. Give Vit B_6 (as enzyme requires B_6)
  2. Restrict Arginine (as arginine is a source of ornithine)
     - Arginine broken down by arginase to ornithine and urea
- 17 amino acids can take part in transamination i.e. 17 amino acids can form glutamate
- Rest 3 Amino acids can't take part in transamination or cannot form glutamate are proline (OH-proline), Lysine and Threonine

How to remember

3 Amino acids that can't take part in transamination

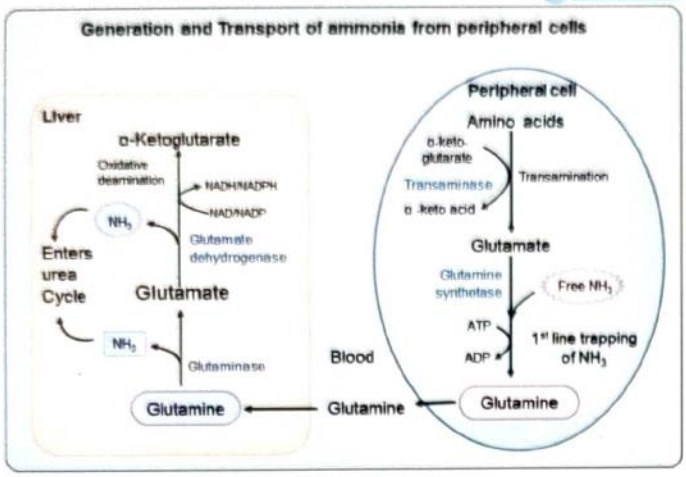
MN: POLYTHENE

PO Proline, OH-proline

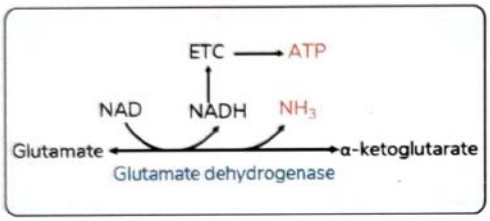
LY Lysine

THENE Threonine

00:10:38



Glutamate dehydrogenase (GDH)



- This reaction occurs only in Liver
- Glutamate is the only AA, that can undergo oxidative deamination to release amino group in the liver

Activators of GDH	Inhibitors of GDH
• NAD • ADP • GDP	• ATP • GTP • NADH

Glutamine synthetase vs Asparagine synthetase

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

Important Information

Q. Transport form of NH_3?
- From body and most tissues - Glutamine
- From brain - Glutamine
- From Muscles - Alanine (via Cahill cycle)

Previous Year's Questions

Q. Ammonia formed in brain is transported into blood by conversion to which of following (FMGE Dec 2019)

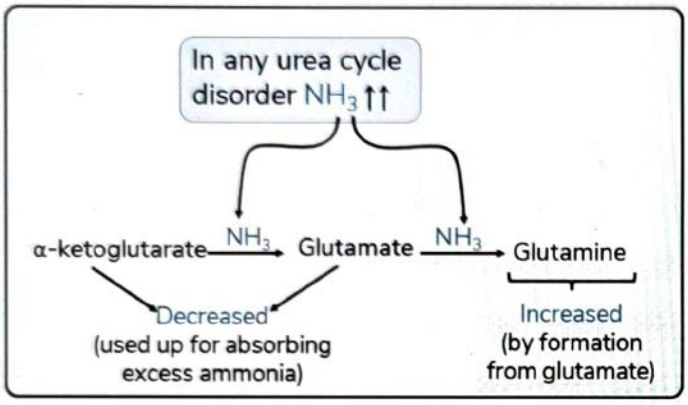
A. Glutamine
B. Glycine
C. Cysteine
D. Urea

Transdeamination: Transamination (All organs) + Oxidative deamination (liver)

53 HYPERAMMONEMIA

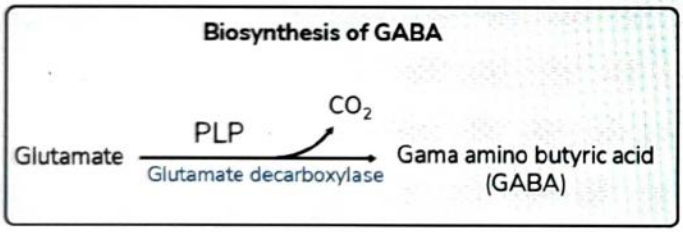
HYPERAMMONEMIA

00:01:09



1. Depletion of α-KG
- TCA cycle affected & ATP not produced
- Brain affected First

2. Depletion of Glutamate



- Excitation in Brain causing Fine Tremors

3. ↑ Glutamine: Osmotically active
- ↑ conc. in Blood and ↑ CNS → Cerebral edema

C/F of Hyperammonemia

00:04:31

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

Previous Year's Questions

Q. Ammonia formed in brain is transported into blood by conversion to which of following (FMGE Dec 2019)

A. Glutamine
B. Glycine
C. Cysteine
D. Urea

54 UREA CYCLE

UREA CYCLE / KREB'S HANSELEIT CYCLE / ORNITHINE CYCLE

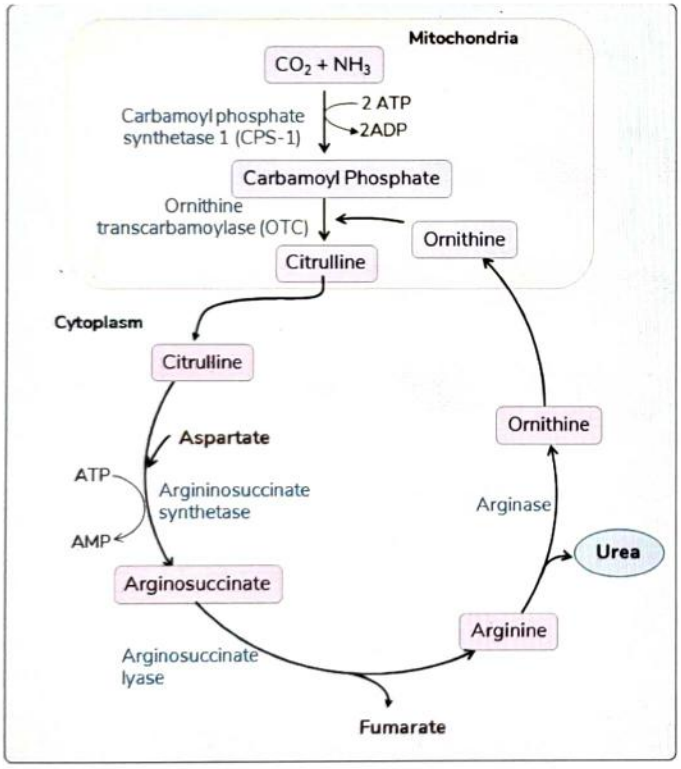
Characteristics

- As Ornithine is regenerated, hence name Ornithine cycle
- **CPS-I** (Carbamoyl phosphate synthetase - I)
  - RLE or Pacemaker enzyme
  - Carry out Committed step of urea cycle

Remember that: CPS–II is involved in Pyrimidine synthesis

- **Organ**: only liver
- **Compartments:** Both in mitochondria & cytoplasm

00:01:40



Enzymes of Urea Cycle

1. OTC (Ornithine TransCarbamoylase)

- Absent in brain
- OTC Deficiency is most common urea cycle defect

2. Arginase

- Absent in kidneys
- So, End-product of urea cycle in kidneys: Arginine
- So, Major source of semi-essential AA Arginine in body: Kidney

Energetics (controversy) 00:06:36

- In CPS–I reaction: 2 ATP → 2 ADP (2 ATP or 2 high energy phosphates)
- In AS synthetase: ATP → AMP (1 ATP or 2 high energy phosphates)

Total energetics: Either 3ATP or 4 high energy phosphates

Important Information

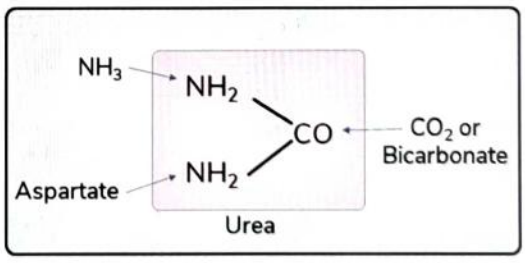
Urea Cycle Energetics

Q1. Total ATP used in Urea Cycle	Q2. Total ATP used in Urea cycle:
A. 2 ATP	A. 1 ATP
B. 3 ATP	B. 2 ATP
C. 4 ATP	C. 3 ATP

How to Answer?

- If both 3ATP and 4ATP options are given (Q1.) then choose 4 ATP as best answer because 1 ATP can be considered equivalent to one high energy phosphate and total 4 high energy phosphates are equal to 4ATP
- but if 4ATP option is not given (Q2.) then choose 3ATP.

Source of different atoms and groups in Urea 00:07:37

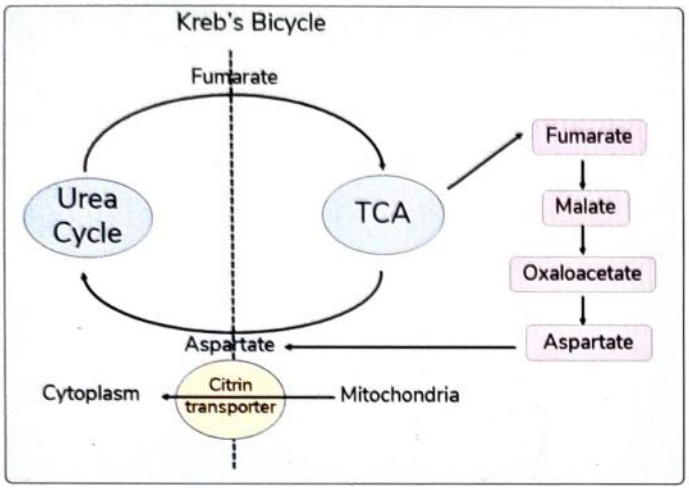


00:09:59

CPS I	CPS II
• Present in Mitochondria • Involved in Urea cycle • Source of ammonia is free ammonia	• Present in Cytoplasm • Involved in pyrimidine synthesis • Source of ammonia is glutamine

For details on CPS-II refer notes of Pyrimidine synthesis in chapter nucleotides

Kreb's Bicycle 00:10:47



Citrin transporter

- Mainly present in mitochondria of liver cells
- Transport glutamate also
- Also needed in Malate-Aspartate NADH Shuttle
- Defect causes Citrullinemia type II

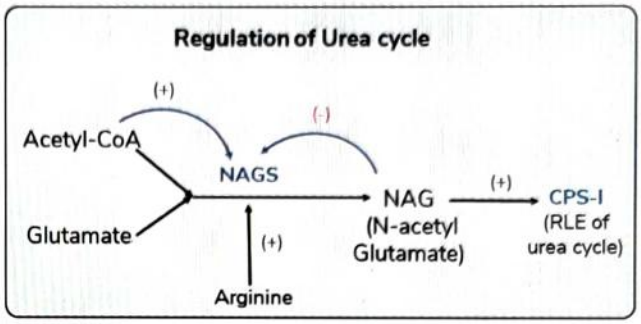
Previous Year's Questions

Q. Amino acid linking Kreb's cycle & urea cycle?
(NEET Jan 2019)

A. Aspartate
B. Fumarate
C. Alanine
D. Arginine

Regulation of urea cycle 00:12:36

- During starvation, cycle activity increases d/t ↑protein catabolism
- Protein rich diet → ↑ Urea cycle activity



- without activation by NAG, CPS will not work
- So, Deficiency OF NAG synthase ≡ Deficiency of CPS–I

55 UREA CYCLE DISORDERS

UREA CYCLE DISORDERS

00:06:32

Refer Table 55.1 00:03:13

Important Information

Urea Cycle Disorders

- Most Severe UCDs: **Hyperammonemia Type I and Type II**
  - **As NH_3 is present in inorganic form**
- Mild UCDs: **Citrullinemia type I, Arginosuccinic aciduria, Hyperargininemia**
  - **As NH_3 is present in organic form**
- **All urea cycle disorders are autosomal recessive Except**
  - OTC Deficiency → **X linked Recessive → Most Common**

Previous Year's Questions

Q. Enzyme deficient in Hyperammonemia type I: (JIPMER May 2019)

A. Ornithine transcarbamoylase
B. Arginase
C. Carbamoyl phosphate synthase I
D. Arginosuccinate synthase

Previous Year's Questions

Q. Glutamine is increased in CSF, blood & urine in which defect: (NEET Jan 2019)

A. Arginosuccinate synthetase
B. OTC
C. CPS-I
D. Arginase

AS Lyase deficiency	Arginase deficiency

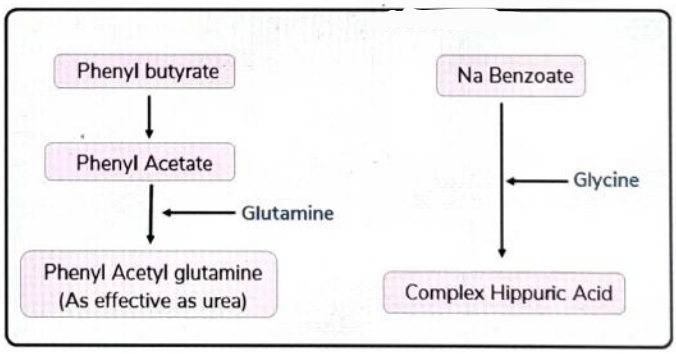
• Trichorexis nodosa
  ○ Brittle hair
  ○ Tufted hair
  ○ Habitual plucking of hair
	• Has least hyperammonemia
	• Milder symptoms (misdiagnosed as cerebral palsy)
	• Spasticity: progressive spastic diplegia
	• Scissoring of the gait

Treatment of UCD 00:10:41

1. Arginine supplementation (1st line treatment)

- Arginine is
  - An essential AA
  - Source of ornithine
  - Activator of 6th enzyme (NAG synthase)
  - C/I only in arginase deficiency (as already high amount of arginine present in body)

2. Acylation therapy or NH_3 scavenging agents



3. Restrict protein intake to 50%

Previous Year's Questions

Q. In urea cycle disorder, which of the following substance can be used to reduce the levels of ammonia? (AIIMS Nov 2019)

A. L-carnitine
B. Phenylbutyrate
C. Isoleucine
D. Glutamate

Table 55.1

Disease name	Enzyme Deficient	Substrate accumulated
Hyperammonemia Type I	CPS – I	NH_3
Hyperammonemia Type II (MC UCD)	Ornithine transcarbamoylase (OTC)	NH_3, OMP, UMP, Orotic acid
Citrullinemia type I	Arginosuccinate synthetase	NH_3, Citrulline
Arginosuccinic aciduria	AS lyase	NH_3, Argininosuccinic Acid
Hyperargininemia	Arginase	NH_3, Arginine
Urea cycle transporter defects		
Citrullinemia type II	Citrin (Asparate-Glutamate Transporter)	Citrulline
HHH Syndrome (Autosomal Recessive)	Ornithine transporter	Hyperammonemia Hyperornithinemia Homocitrullinuria

CLINICAL QUESTIONS

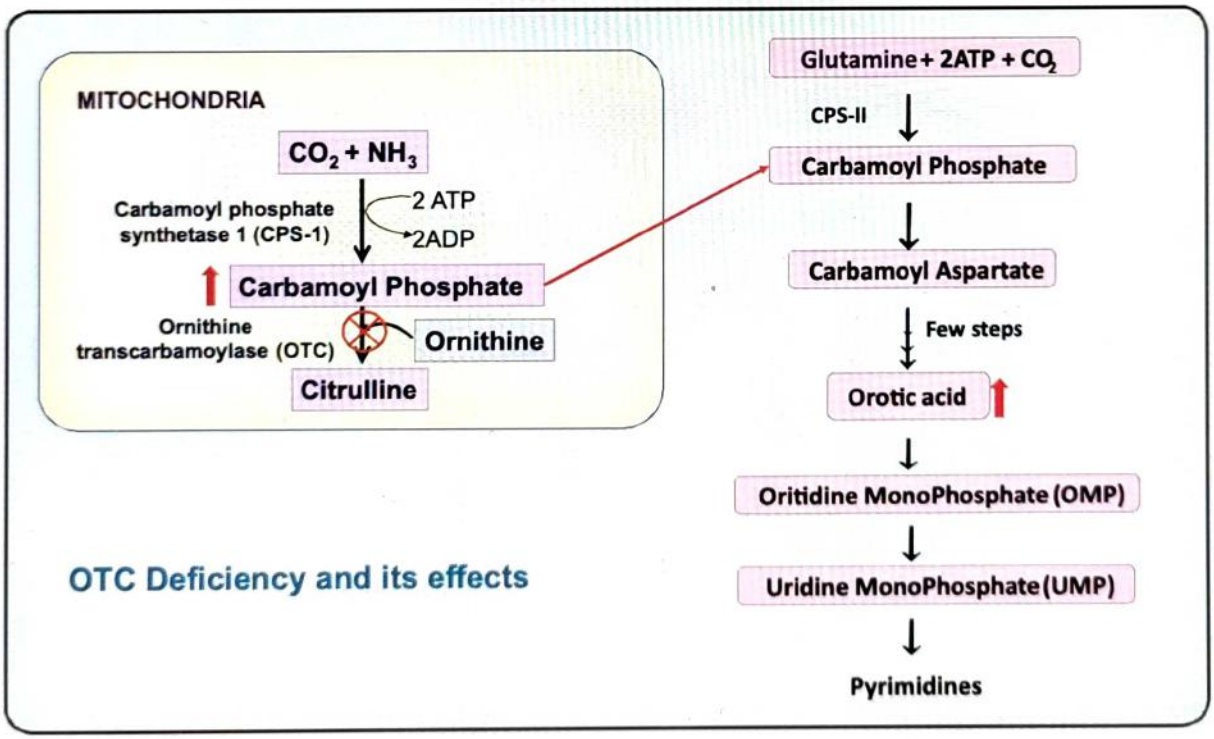
Q. A patient presented to casualty with nausea, vomiting. Intravenous glucose was given & the patient recovered. After few months, patient presented with same complaints. Blood glutamine was found to be increased. Also, uracil levels were raised. What is the diagnosis?

A. CPS-I deficiency
B. Arginino succinate synthetase deficiency
C. CPS-II deficiency
D. Ornithine transcarbamoylase deficiency

Answer: D

Solution

Increase in blood glutamine indicates hyperammonemia, which occurs in all enzyme defect of urea cycle. But Increased uracil indicates Ornithine trans carbamoylase deficiency. In OTC deficiency, excess carbamoyl phosphate from mitochondria enters cytoplasm and takes part in pyrimidine synthesis. Excess pyrimidine gets degraded resulting in increase in orotic acid, OMP, UMP. (See fig)



Regarding other options:

- If citrulline is increased, then it is Argininosuccinate synthetase deficiency.
- CPS-II (carbamoyl phosphate synthetase-II) is enzyme involved in pyrimidine synthesis, not in urea cycle.

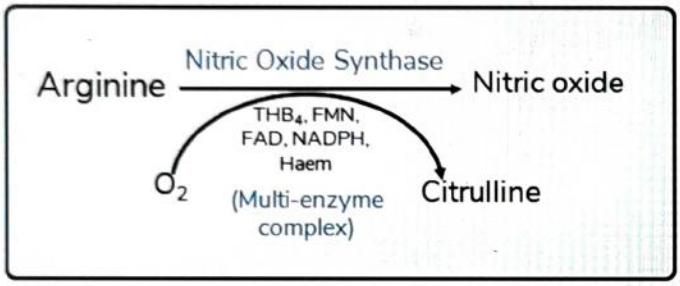
Reference: Lehninger's 7th ed/pg. 688, 690

56 NITRIC OXIDE

NITRIC OXIDE

- Also known as **EDRF** (Endothelium Derived Relaxing Factor)
- Synthesized from arginine in endothelial cells

00:00:49



Nitric oxide synthase 00:02:38

- Three isoforms:
  1. i - inducible
  2. n - neuronal
  3. e - endothelial

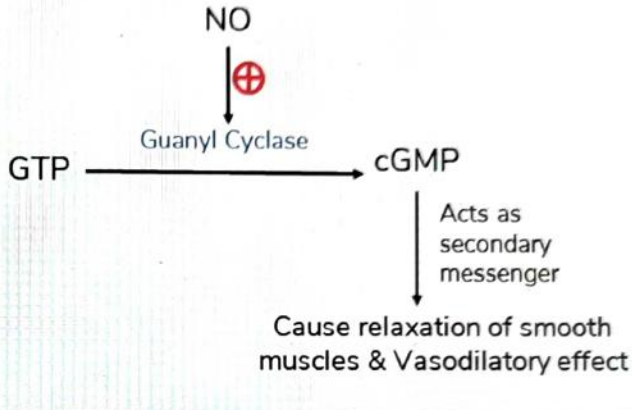
Roles of NO 00:03:11

1. Vasodilator
2. Anti-atherogenic
3. Anti-aggregator

- Vasodilator nitroglycerin also acts through NO

00:03:57

Biochemical mechanism of Action of NO



Previous Year's Questions

Q. Nitric oxide is synthesized from (NEET 2018, 2019, 2020)

A. Ornithine
B. Alanine
C. Aspartic acid
D. L-arginine

CLINICAL QUESTIONS

Q. A 45-year old patient with a family history of coronary artery disease visited a physician to consult about the occasional episodes of chest pressure behind the sternum when doing some exertional activity but also sometimes at rest. A small number of luminal irregularities without any major obstructions are observed in his coronary angiography. A normal dilation of epicardial blood vessels were observed on administration of acetylcholine for this procedure. The reaction responsible for this effect involve which of the following amino acid?

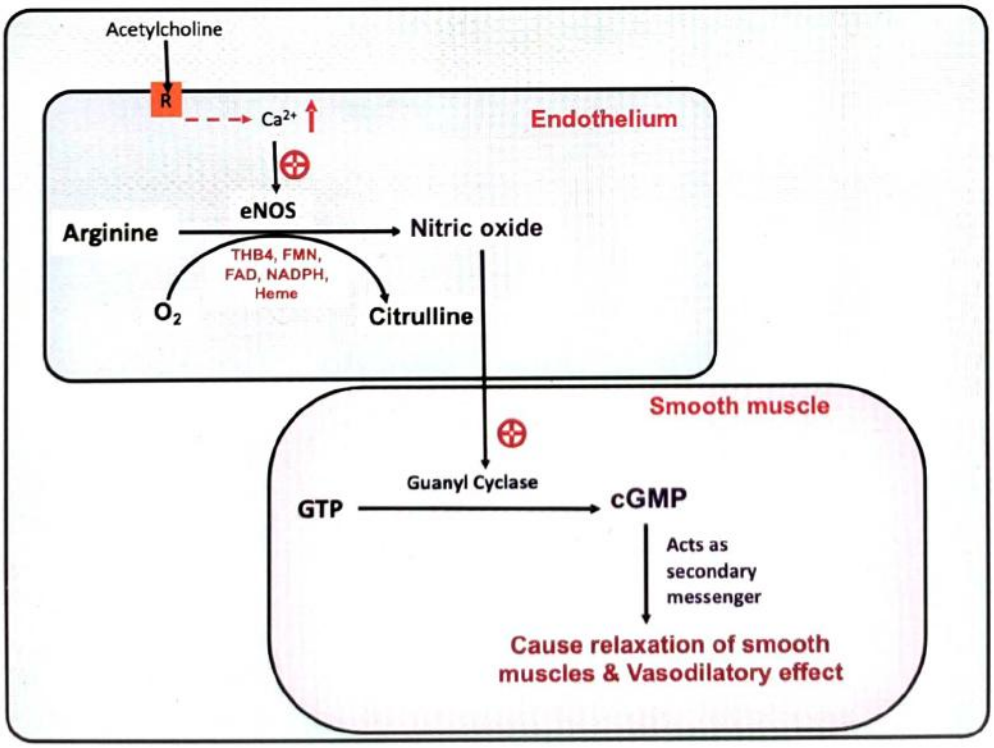
A. Tyrosine
B. Histidine
C. Glycine
D. Arginine

Answer: D

Solution

Substance such as acetylcholine, serotonin and bradykinins mediate the process of vasodilation by attaching to specific membrane receptors present on endothelial cells. This binding causes calcium ion influx into endothelial cells and activates endothelial nitric oxide synthase (eNOS). This enzyme generates nitric oxide from arginine using O_2 and cofactors such as NADPH, FAD, FMN etc.

Nitric oxide diffuses into smooth muscles and activates enzyme guanyl cyclase and trigger the formation of secondary messenger cGMP from GTP as shown in fig. High levels of cGMP activate protein kinase G causing reduction of cytosolic calcium levels and relaxation of smooth muscle cells.

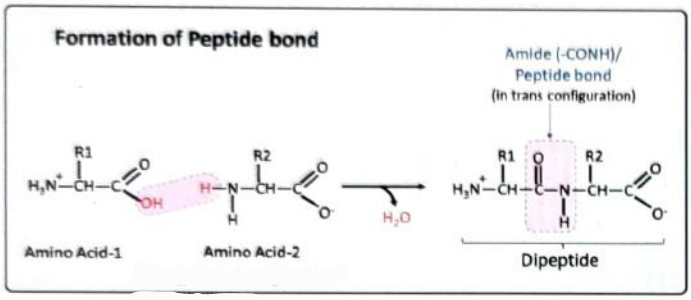


Reference: Lehninger's 6th ed/pg. 909-910

57 PROTEIN BONDS AND STRUCTURES

PEPTIDE BOND

00:00:58



- If amide bond is present in protein, it is known as Peptide Bond
- Covalent bond (on denaturation, this bond does not break (primary structure)]
- Has double bond character
  - Double bond is in 'trans' configuration

Remember that In unsaturated fats, double bond is in 'cis' configuration

PROTEIN STRUCTURE

00:04:24

Name	Definition
1° Structure	Sequence of amino acids
2° Structure	Obtained from folding of 1° structure • α-Helix • β-Sheets • β-Turns
3° Structure	Further folding → fully folded structure
4° Structure	> 1 polypeptide chain e.g. Hb (four polypeptide chains)

Monomeric protein: Those proteins which have only one monomer, they do not have Quaternary structure

α-Helix

- Symmetrical helical structure
- Secondary structure
- MC helix found in the body
- Right-handed

00:07:20

Important Information

AA not found in α-helix

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

Previous Year's Questions

Q. In proteins, the amino acids are folded. These folded units are further folded as dimers-Homodimers and heterodimer. These dimeric structures are called? (PGI 2019)

A. Primary
B. Secondary
C. Tertiary
D. Quaternary
E. Denatured proteins

Refer Table 57.1

00:08:15

- Ionic bond is also known as electrostatic bond
- S~S stands for disulphide bond

Previous Year's Questions

Q. Which structure of Protein is not denatured after heating up to 100 degrees? (FMGE Dec 2019)

A. Primary
B. Secondary
C. Tertiary
D. Quaternary

Table 57.1

Features	1°	2°	3°	4°
Bond	Covalent/ Peptide/ Amide	Hydrogen bond	• S~S • Hydrophobic • Hydrogen • Ionic	• Hydrophobic • H-bond • Ionic bond
Functional activity	Absent	Absent	Present	Present
Denaturation	Retained because peptide bond is very strong	Lost	Lost	Lost
Detection	Mass spectrometry, Edman's Technique	**X-ray crystallography** -Best for crystallizable proteins **NMR spectrometry** -Best for non-crystallizable proteins		

How to remember

Bonds present in 3° & 4° Structure
(MN- HHI)

- **Hydrophobic**
- **H-bond**
- **Ionic bond**

CLINICAL QUESTIONS

Q. A researcher while studying a protein involved in signal transduction found that it has multiple alpha-helical regions. These regions were found to be rich in stretches of 20 amino acids primarily containing valine, alanine and isoleucine. This region most likely performs which of following function?

A. Binding to extracellular ligand
B. Interacting with metal ions in transporter proteins
C. Binding to intranuclear DNA
D. Anchoring to cell membrane

Answer: D

Solution

This given part of protein is rich in hydrophobic amino acids such as valine, alanine and isoleucine which is highly indicative of it being associated with hydrophobic cell membrane.

Most of the membrane proteins discovered till date contain regions rich in hydrophobic amino acids and these regions are mainly inserted in the membrane through hydrophobic interactions and help in anchoring of protein in the membrane.

Regarding other options:

As the DNA (option c) or Metal ions (option b) interact with proteins through charge-charge interactions, the regions of protein which will bind to them most probably will contain polar amino acid residues.

The receptor part of a protein binding to an extracellular ligand (option a) generally contain hydrophilic amino acid (due to hydrophilic extracellular environment) and also have glycosylated residues which are generally serine and threonine.

Reference: Harper's 30th ed/pg. 481

58 PROTEIN PRECIPITATION

PROTEIN PRECIPITATION

- Refer to amino acid basic notes for information on relation between amino acid pI and pH.

Uses of protein precipitation 00:00:40

- Purification of enzymes / Proteins
- Preparation of protein free filtrate (PFF) for various biochemical tests

Precipitation reactions of proteins 00:01:37

- Any factor which
  1. causes denaturation
  2. Neutralizes charge
  3. causes dehydration
- Will lead to protein precipitation

Various methods 00:02:44

Method	Mechanism of precipitation
1. Heat 2. strong mineral acids	• Cause precipitation by denaturation
3. Heavy metal salts in alkaline medium	In alkaline medium, • Proteins are negatively charged while Heavy metals are positively charged • So, Neutralization of charges occur which leads to precipitation known as positive ion precipitation
4. Alkaloidal reagents (Trichloroacetic acid, phospho-tungstic acid, sulfosalicylic acid]	• Alkaloidal reagents are negatively charged • In acidic medium, proteins are positively charged • So, Neutralization of charges occur & leads to negative ion precipitation

Previous Year's Questions

Q. Which of the following is not used for protein precipitation? (INICET July 2021)

A. Heavy metals
B. Alcohol & acetone
C. Change in pH other than isoelectric pH
D. Trichloroacetic acid

Salting out 00:06:12

- When salt is used for protein precipitation → Salting out
  - e.g. Heavy metal salts, Neutral salts e.g. Ammonium sulfate
- Albumin & Globulin can be precipitated by ammonium sulfate salt
  - Albumin are precipitated by Full saturation
  - Globulins are precipitated by half saturation

00:07:36

Globulins
↓
More molecular weight, large size
↓
Less surface area
↓
Less hydration
↓
Less salt required for precipitation

00:08:13

Albumins
↓
Less molecular weight, smaller size
↓
More surface area
↓
More hydration
↓
More salt required for precipitation

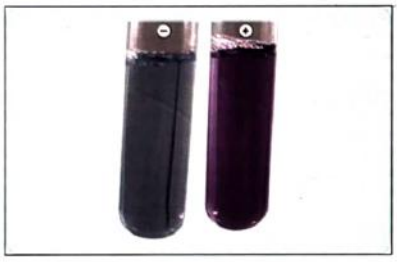
59 COLOR REACTIONS OF PROTEINS & AMINO ACIDS

COLOUR REACTIONS 00:00:31

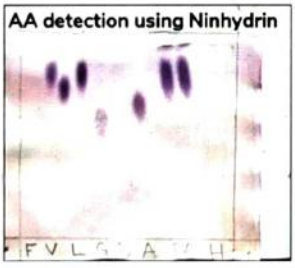
- Chemical reagents react with Proteins and Amino acid to give colour.
- These reactions are used for qualitative and quantitative detection of amino acids and proteins.

Ninhydrin test 00:01:31

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

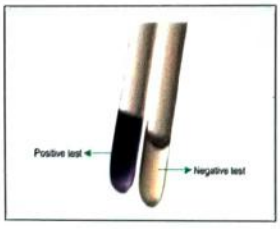


- Proline & Hydroxyproline give yellow colour with Ninhydrin
- Uses
  - in chromatography of amino acid for their detection
  - Fingerprinting using Ninhydrin Spray
    → Ninhydrin reacts with amino acids in sweat to give purple colour



Biuret test 00:03:25

- Positive: Purple colour
- Minimum 2 peptide bonds are required for a positive test
- Tripeptides & protein will give this test positive.
- Dipeptides (one peptide bond) and free amino acids do not give this test positive

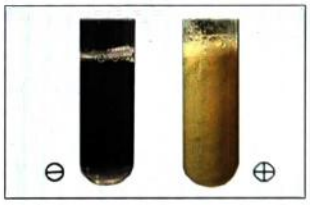


TESTS FOR AROMATIC AMINO ACIDS 00:04:29

Name of Test	Amino acid detected
1. Xanthoproteic test	• For all aromatic amino acids Except Phenyl Alanine • Positive test: yellow colour
2. Hopkin Cole's test	• Positive for Tryptophan • Positive test: blue colour due to indole ring
3. Obermeyer test	• +ve for Tyrosine • Positive test: Red colour
4. Millon's test	
5. Pauly's test	• +ve for Histidine & Tyrosine • Positive test: Red colour

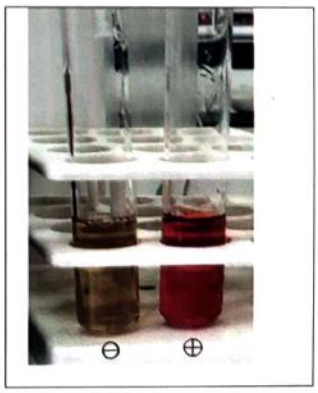
Xanthoproteic reaction 00:06:31

- Positive: Yellow colour
- Nitric acid is used. (reaction of nitric acid with skin leaves a yellow colour due to this rxn.)



Sakaguchi Test 00:07:12

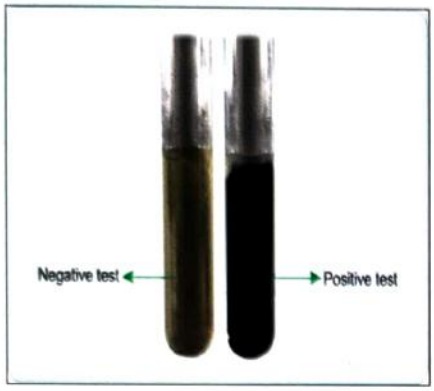
- Positive : Red colour
- +ve for Arginine (basic Amino acid)



TEST FOR SULFUR CONTAINING AA 00:07:28

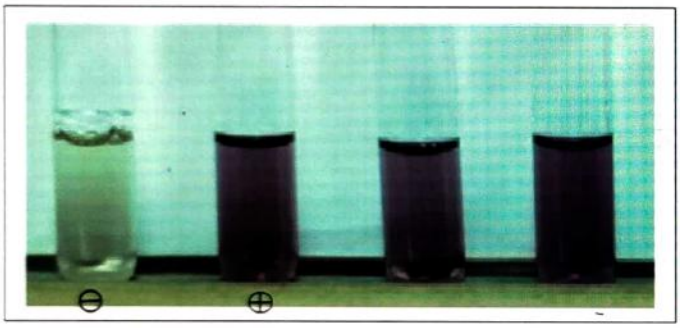
1. Sulfur Test/Lead Sulfide Test

- +ve for all S containing amino acid
- Exception: methionine (due to C – S – C in methionine)
- Positive : Black or Brownish colour



2. Cyanide NitroPrusside (CNT) Test 00:08:11

- Positive for any compound having SH or S~S group.
- Positive in cystinuria, homocystinuria, cystinosis,
- Reddish purple/ Magenta colour in positive test



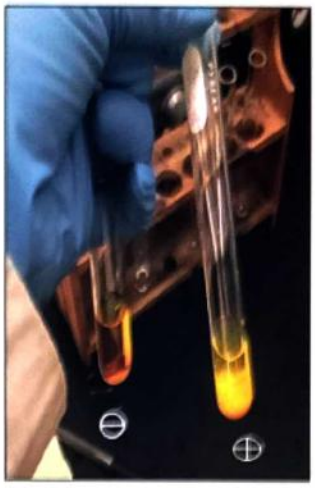
OTHER TESTS

Ferric Chloride Test 00:08:57

- Ferric chloride – Give various colours depending upon the substance
- Detects phenylpyruvate and branched chain amino acids (BCAA)
- Positive for
  - Phenylketonuria (presence of phenylpyruvate)
  - Maple Syrup Urine Disease (presence of BCAA)

Dinitro Phenyl Hydrazine Test (DNPH Test) 00:09:37

- Used for screening of PKU & MSUD patients
- Detects α-keto acids in urine
- +ve test - Orange crystalline derivatives



Guthrie's test or Bacterial inhibition assay 00:10:38

- Detects phenylpyruvate, phenyl-lactate and phenyl-acetate in serum
- Positive in PKU

VMA spot urine test 00:11:07

- For detection of pheochromocytoma
- Colourless reaction but still can be detected by spectrophotometer

5-HIAA test (Hydroxy Indole Acetic Acid) 00:11:26

- For carcinoid syndrome (HIAA is raised in this disease)

Previous Year's Questions

Q. An infant presented to the OPD with a history of vomiting and malnutrition. Patient has blue eyes, blonde hair & fair skin. On investigation, Guthrie test was found to be positive. All are true regarding this disease Except: (AIIMS May 2018)

1. Due to PAH enzyme defect
2. White patch of hair due to tryptophan deficiency
3. Phenyl acetate positive in urine
4. Mental retardation is present

60 CHROMATOGRAPHY & ELECTROPHORESIS

CHROMATOGRAPHY

00:00:28

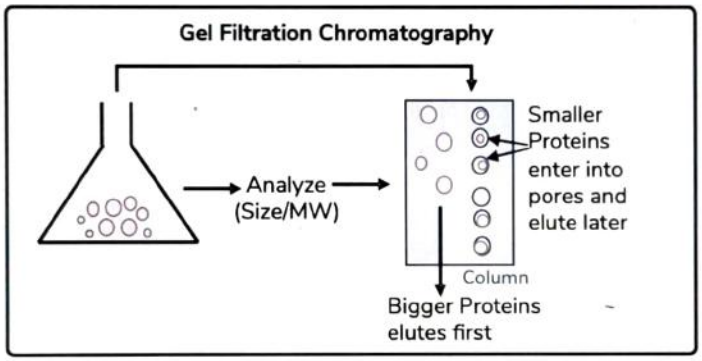
Stationary Phase: Column
Mobile Phase: Mixture to be separated

Types of column chromatography

1. Based on Size / MW → Gel Filtration Chromatography
2. Based on Charge → Ion Exchange Chromatography
3. Based on Affinity → Affinity Chromatography

1. Gel filtration chromatography / Size-exclusion chromatography

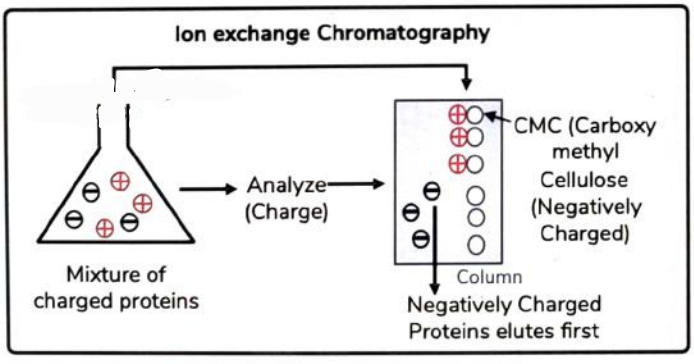
00:01:59



- Gel: Sephadex (Biochemically Dextran)
  - Dextran is also used as plasma expander
- Gel in the column do not allow bigger proteins
- Bigger proteins cannot enter the gel so, Elute first
- Smaller proteins enter the gel, & elute out later

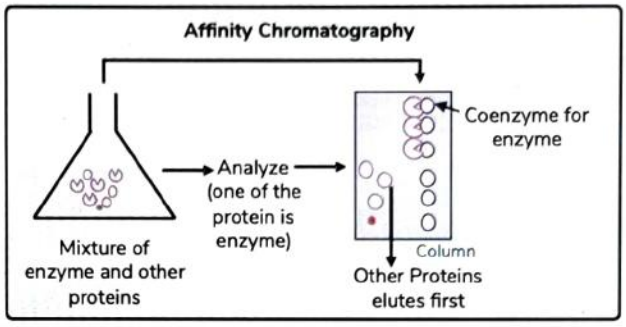
2. Ion-exchange chromatography

00:06:30



3. Affinity chromatography

00:09:04



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

Previous Year's Questions

Q. Glycosylated Hb (Hb-Alc) is best detected by
(INICET July 2021)

A. Ion exchange
B. Affinity chromatography
C. Isoelectric focussing
D. Electrophoresis

Other types of chromatography

4. Paper chromatography

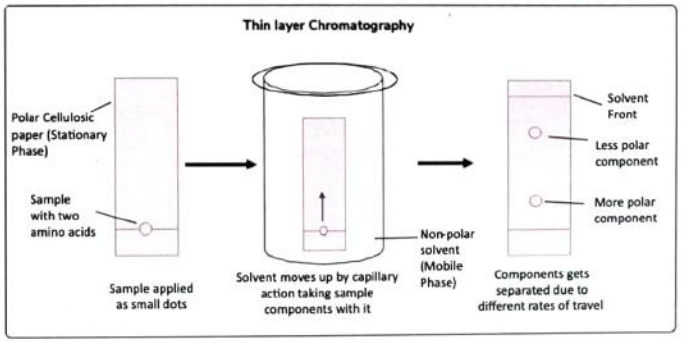
- Older & cheaper
- Used for teaching purpose
- Stationary phase: Paper

5. Thin layer chromatography

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

Common principle

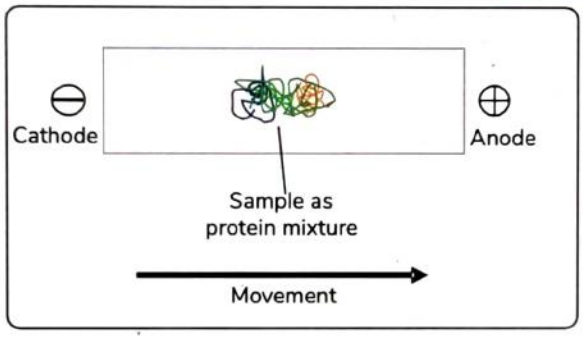
00:13:01



- On paper, mixture of amino acids taken at one end & marking present at other end
- It is placed in non-polar solvent
- Solvent moves up on the paper, once it touches marked line, then it is removed & staining done
- Staining indicates the polarity of AA with respect to the Solvent
  - Early Staining: Polarities of AA & Solvent are different (polar)
  - Late Staining: Polarities of AA & Solvent are same (non-polar)
  - Mobile phase: Mixture of amino acid + solvent

ELECTROPHORESIS

00:18:18



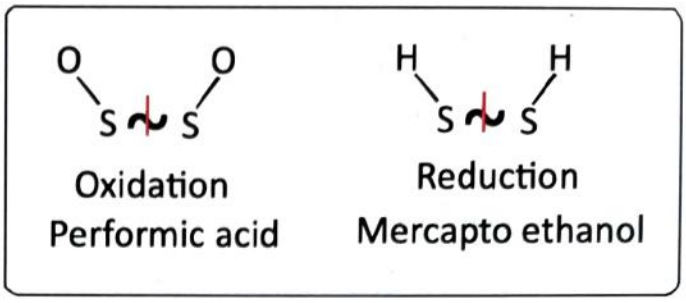
- Movement in electric field
- Depends on
  - Charge (main factor)
  - Size
  - Shape

SDS – PAGE

00:19:56

- Depends only on size
- **PAGE** stands for Poly Acryl amide Gel Electrophoresis
- **SDS is** Sodium Dodecyl Sulfate (Salt derivative of Lauric Acid (12C))

- **Properties**
  1. Denature proteins → 2° 3° 4° Structures lost (no shape)
  2. Anionic detergent → Coat all the proteins with negative Charge
  3. 1.4 gm of SDS binds to 1 gm protein
  4. SDS cannot break disulfide bond (disulfide bond present in 3° Structure)
     - Disulphide bond can be broken by
       a. Oxidation using Performic acid
       b. Reduction using Mercapto ethanol



Previous Year's Questions

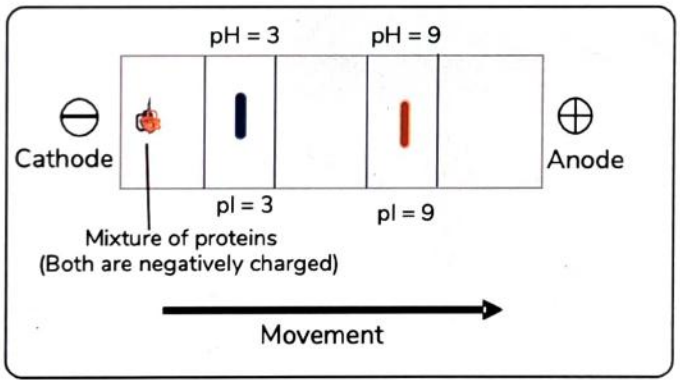
Q. Which of the following will be more towards the negative pole in gel electrophoresis. (PGI May 2018)

A. 5bp
B. 50 kbp
C. 160 bp
D. 550 bp
E. 50000 bp

Isoelectric focussing

00:25:58

- Separation is done using Iso electric pH (pI) of a protein, in an electrophoresis gel with pH gradient



CLINICAL QUESTIONS

Q. A 13-month old child showing developmental delay with coarsened facial features and hepatosplenomegaly is diagnosed with I-cell disease. It is a protein targeting disorder in which targeting system that attach mannose 6-phosphate to enzymes for their targeting lysosomes is not working correctly. As a result, affected individuals lack multiple enzymes in their lysosomes. Which of the following techniques could be used to purify the putative lysosomal membrane protein from cell lysate that recognizes mannose 6-phosphate groups and transports enzymes into lysosomes?

A. Ion exchange chromatography
B. Affinity chromatography
C. Electrophoresis
D. Gel filtration chromatography

Answer: B

Solution

Out of all the given technique, only affinity chromatography uses the high affinity of proteins for specific chemical groups or specific interaction of antibodies to isolate their proteins binding partners. For methodology and principle refer to text.

In the questions above, the mannose 6-P can be covalently attached to column material which will bind with the putative binding proteins in the cell lysate.

Regarding other options:

Ion exchange chromatography separates proteins with an overall charge of one sort from proteins with an opposite charge (e.g. negative from positive) by using ion exchange resins and buffer with proper pH which provided a certain charge to the desired protein.

Gel filtration chromatography and Electrophoresis separates proteins based on size. In electrophoresis charge on protein is used to aid in faster movement of protein through the gel using an electric field.

Reference: Lehninger's 6th ed/pg. 89-92

61 FIBROUS PROTEIN

FIBROUS PROTEINS

00:00:22

Collagen	Elastin
• Is a Glycoprotein • cannot be stretched • Contains OH–Lysine • ~ 28 types • Intramolecular crosslinks-Aldol condensation	• Is a Protein • can be stretched • no OH-lysine present • Only 1 type • Intramolecular crosslinks-Desmosine condensation

Collagen

00:03:01

- Most abundant of all human proteins.
- 1° Structure → repeated unit is $(Gly-X-Y)_n$
  - so, every 3rd amino acid residue is glycine
  - X, Y can be either amino acids out of Proline or OH-Pro and Lys or OH-Lys
  - glycine plays a special role in providing structure to collagen due to its small size and ability to create bends in secondary structure.

Important Information

Q. Collagen has which of following?
(a) Proline
(b) Phenylalanine
(c) OH – Proline
Both a and c are correct. But because most of proline and lysine residues in collagen are hydroxylated, hence the best answer is OH-Proline

Previous Year's Questions

Q. Collagen is rich in which amino acid? (FMGE June 2019)

A. Glycine
B. Arginine
C. Phenyl-alanine
D. Tyrosine

Types of Collagen

00:05:13

Type	Mnemonic	Location
I	S	Skin (most abundant)
II	C	Connective tissue
III	A	Arteries and CVS
IV	B	Basement membrane of Glomerulus
VII		Junction of dermis & epidermis

Previous Year's Questions

Q. Which of the following type of collagen is present in healing and granulation tissue? (AIIMS May 2018)

A. Type I
B. Type II
C. Type III
D. Type IV

Previous Year's Questions

Q. Type of collagen maximum in skin? (NEET 2019)

A. Type I
B. Type II
C. Type III
D. Type IV

Collagen Associated Diseases

00:07:17

- Ehler-Danlos Syndrome (EDS)
  - A heterogeneous group of disorders characterized by stretchy skin + loose joints
  - EDS type IV affects CVS, so, is the most severe type
- Alport Syndrome
  - C/F: Hematuria and End State Renal Disease (ESRD)
- Epidermolysis Bullosa
  - C/F: Skin Blisters

Post Translational Modifications (PTM) of Collagen 00:09:10

- PTMs are done after the protein synthesis to make extra bond to provide extra strength and stability.

1. Hydroxylation of proline and lysine residues
   - -OH group is added which will help in creating numerous extra H-bonds in secondary structure to provide extra strength and rigidity.

Hydroxylation of collagen residues

Proline —(Prolyl hydroxylase; Require vit C & Fe^{2+})→ Hydroxyproline

Lysine —(Lysyl hydroxylase; Require vit C & Fe)→ Hydroxylysine

Important Information

Clinical Significance: The deficiency of vitamin C will hinder these reactions resulting in fragile collagen of blood vessels of gums which can be easily bruised and bleed during brushing causing scurvy.

2. Glycosylation 00:11:15
   - Addition of Carbohydrates in protein which helps in making aldol condensation of collagen
   - Enzyme required is Lysyl oxidase which require Copper (Cu) as cofactor.

Menke's disease

- Dietary deficiency of Cu
- All oxidases including lysyl oxidase is affected
- C/F
  1. Kinky hair
  2. Greying of hair
  3. Growth Retardation

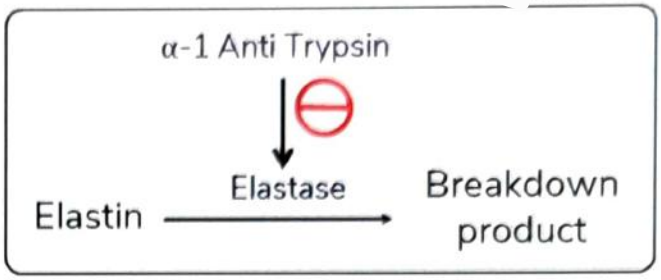
Important Information

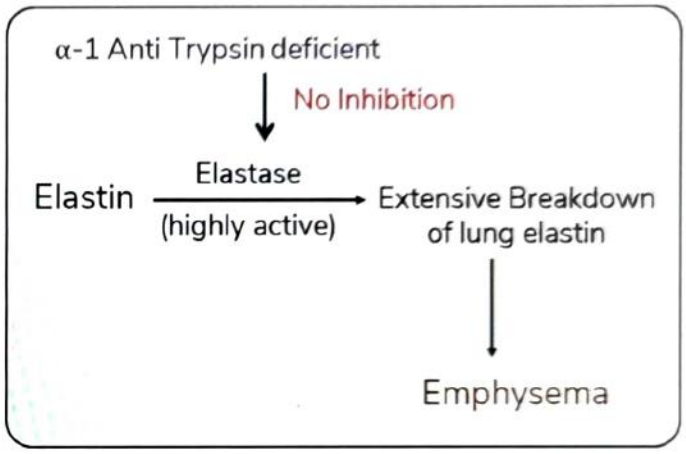
PTM of Collagen requires:

- **Three enzymes**
  1. Prolyl hydroxylase
  2. Lysyl hydroxylase
  3. Lysyl oxidase
- **Two metal ions:** Cu and Fe
- **One vitamin:** Vitamin C

Elastin 00:13:25

- Present in tissue requiring elasticity such as Skin, Lungs, Elastic Ligaments, Vascular tissue, Large arterial blood vessel.
- Intramolecular cross links present are called Desmosine (4 Lysine residues involved)





Marfan Syndrome 00:15:43

- AD disease due to Mutation in Fibrillin-1
- Fibrillin-1
  - Glycoprotein (Structural component of microfibrils)
  - Helps in deposition of elastin
  - Mutation will adversely affect bones, heart and eyes and elastin will also be affected
- C/F
  - Tall stature
  - long limbs
  - lens dislocation
  - arachnodactyly
  - media of large arteries is weak. (defective elastin)
  - Death occurs due to Rupture of dilated aorta

Keratin 00:17:50

- Present in nails, hair outer layer of skin
- Rich in cysteine (SH) residues.
  - So, there are many S~S disulfide bonds in Keratin which give strength to the keratin.
  - More the (S~S) bonds, harder is the keratin.

CLINICAL QUESTIONS

Q. A patient complaint of bleeding gums and loosening teeth. His dietary history revealed that he has been consuming only fast food and no vegetable or fruit in his diet for the past 6 months. The symptoms in this patient is most likely due to which one of the following.

A. Reduced synthesis of collagen
B. Reduced hydrogen bond formation in collagen
C. Increased hydrogen-bond formation in collagen
D. Reduced disulfide-bond formation in collagen

Answer: B

Solution

The patient developed scurvy owing to a lack of vitamin C in his diet. Vitamin C plays the role of a coenzyme in hydroxylation of proline and lysine while protocollagen is converted to collagen. The hydroxylation reaction is catalyzed by lysyl hydroxylase (for lysine) and prolyl hydroxylase (for proline). This reaction is dependent on vitamin C and molecular oxygen. (See fig in text)

The lack of cofactor vitamin C will reduce the activity of these enzyme hence reduce the number of hydroxyproline reduces which in turn will cause low strength and stability of the collagen due to reduced hydrogen-bond formation within the collagen triple helix.

Regarding other options:

The vitamin C does not have any role in disulfide-bond formation, which is required to initiate triple-helix formation within the cell.

Reference: Harper's 30th ed/pg. 631

Q. A 32-year-old man was admitted to the ER with a large amount of blood and protein in his urine. He also has misshaped lenses (anterior lenticonus) and sensorineural hearing loss in his teenage years. The physician suspected it to be a case of Alport syndrome and ordered a genetic test. The tests would most likely to show a defect in which of the proteins?

A. α1-Antitrypsin
B. Collagen type IV
C. Fibrillin
D. Collagen Type VI

Answer: B

Solution

In Alport syndrome, there is defect in type IV collagen that alters the basement membrane composition of kidney glomeruli. The absence of a functional basement membrane interferes with proper filtration of waste by kidneys resulting in accumulation of waste products in blood and both blood and proteins can enter the urine.

Type IV collagen is also important for hearing (it is found in the inner ear) and for the eye.

Regarding other options:

A mutation in α1-antitrypsin will lead to emphysema while mutations in fibrillin lead to Marfan syndrome.

Reference: Harper's 30th ed/pg. 631

62 HAEM SYNTHESIS

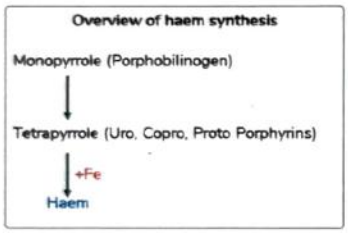
HAEM

00:00:21

- Hb = Haem (prosthetic group) + globin (Protein)
- Haem = Fe^{+2} + Porphyrin
- Porphyrin = 4 Pyrrole rings are joined together
- Depending upon different side chains, there are various isomeric form of porphyrins
  - I → Negligible amounts
  - III → Belongs to IX series (also called as porphyrin IX) – Most common
  - Type II and IV does not exist in nature

HAEM SYNTHESIS

00:02:26



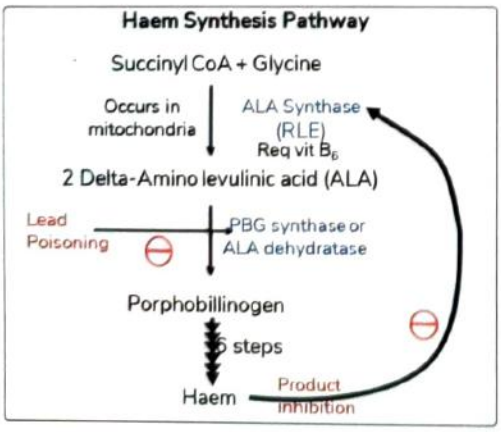
Uses of Haem in body

- Synthesis of Hb/ Mb/ Cytochromes
- As a part of enzymes
  - Tryptophan Pyrrolase
  - NOS (Nitric Oxide Synthase)
  - Catalase

Haem Synthesis

00:04:41

- As cytochromes are part of ETC hence haem is synthesized in all cells of body except Mature RBCs because there are no mitochondria in them
- Compartment: Mitochondria (First Step) + Cytoplasm (Next 7 steps)



There are two types of ALA Synthase

00:07:13

1. Type I
   - present in all Tissues (mainly Liver)
   - Inducible enzyme
   - Inhibited by free Haem

2. Type II
   - present in Erythroid tissues
   - does not require regulation as haem synthesis occur only once in a lifetime in these cells.
   - Synthesis depend on availability of globin.

00:09:49

Important Information

Vit B_6 deficiency will cause anemia as it will inhibit first step of haem synthesis.

Q Enzyme decreased in lead poisoning?
Ans: PBG synthase/ ALA Dehydratase

Q. Enzyme increased in lead poisoning?
Ans: ALA Synthase

Q. Compound excreted in urine in lead poisoning?
Ans: ALA

Previous Year's Questions

Q. Which TCA intermediate is a used in haem synthesis? (AIIMS May 2019)

A. Alpha ketoglutarate
B. Fumarate
C. Succinyl CoA
D. Malate

63 PORPHYRIA

PORPHYRIAS

00:03:21

Refer Flow Chart 63.1

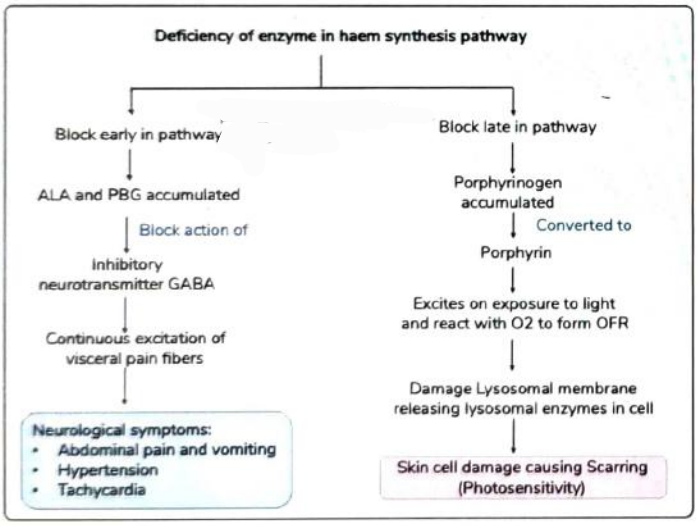
Porphyria is when Haem is not getting formed. It is of two types:

1. **Genetic porphyria**
- Due to Enzyme deficiencies other than ALAS
- ALAS Deficiency causes X-linked Sideroblastic Anemia
- ALAS – Gain of function mutation (induced expression of ALAS) → X linked Protoporphyria because of limited amount of Fe in body

2. **Acquired porphyria**
- Lead Poisoning (mc of acquired porphyria)
- Fe deficiency

Reason behind symptoms in various Porphyrias

00:08:58



Inheritance pattern of Porphyrias

00:13:40

- All are AD except:
  - X–linked Proto Porphyria
  - Congenital Erythropoietic Porphyria
  - Erythropoietic Proto P → most common in children
  - Genetic ALA dehydratase deficient Porphyria

00:14:43

Aggravation of Porphyria by drugs

Drugs like Barbiturates, Phenytoin, Griseofulum

↓

Metabolized using cytochromes

↓

Increased synthesis of Cyto P450 enzymes

↓

Increased Haem used causing free haem decrease

↓

ALA synthase induced

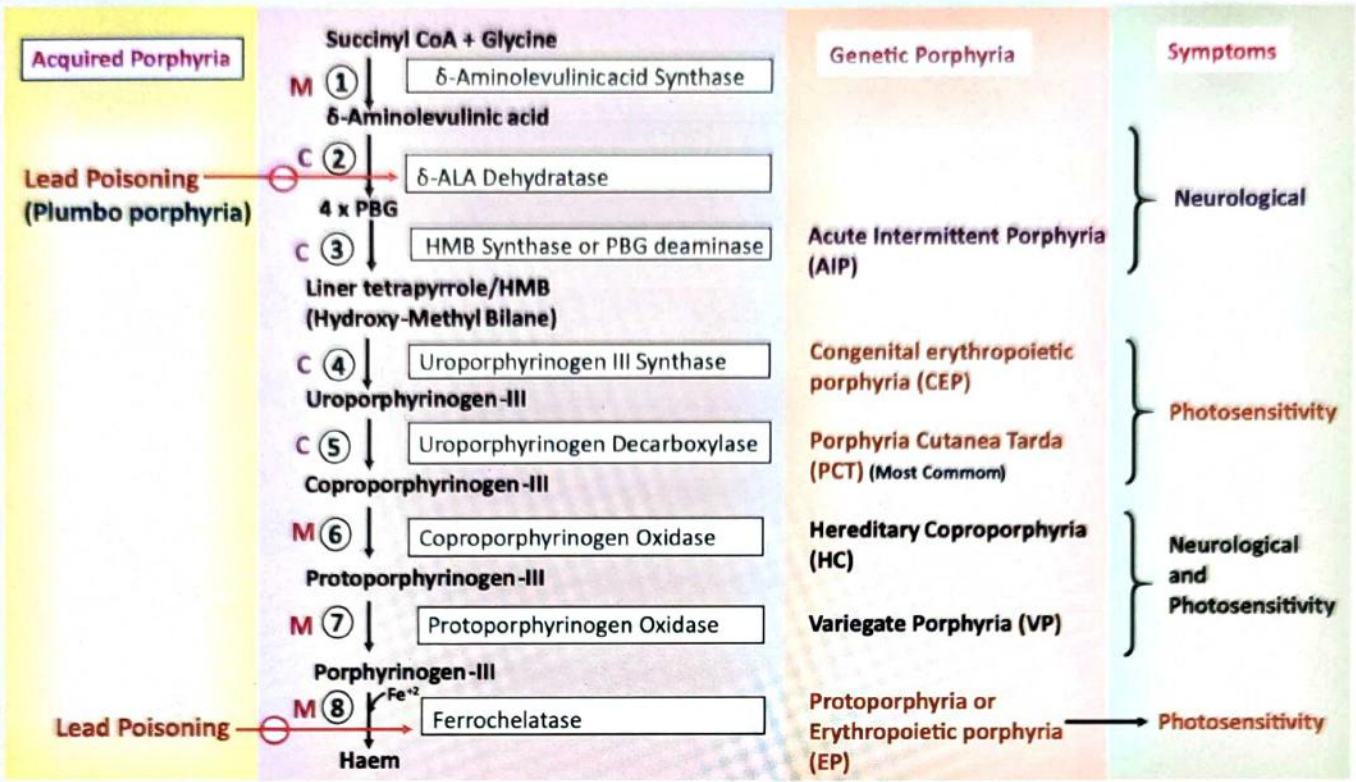
↓

Increase in synthesis of Haem Pathway intermediates

↓

Aggravate Porphyria attack

Flow Chart 63.1



M – occur in Mitochondria (Steps-1, 6, 7, 8) C – occur in Cytoplasm (Steps-2, 3, 4, 5)

CLINICAL QUESTIONS

Q. A 35-year-old man has a history of intermittent abdominal pain and episodes of confusion and psychiatric problems. High amounts of δ-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. Variegate porphyria

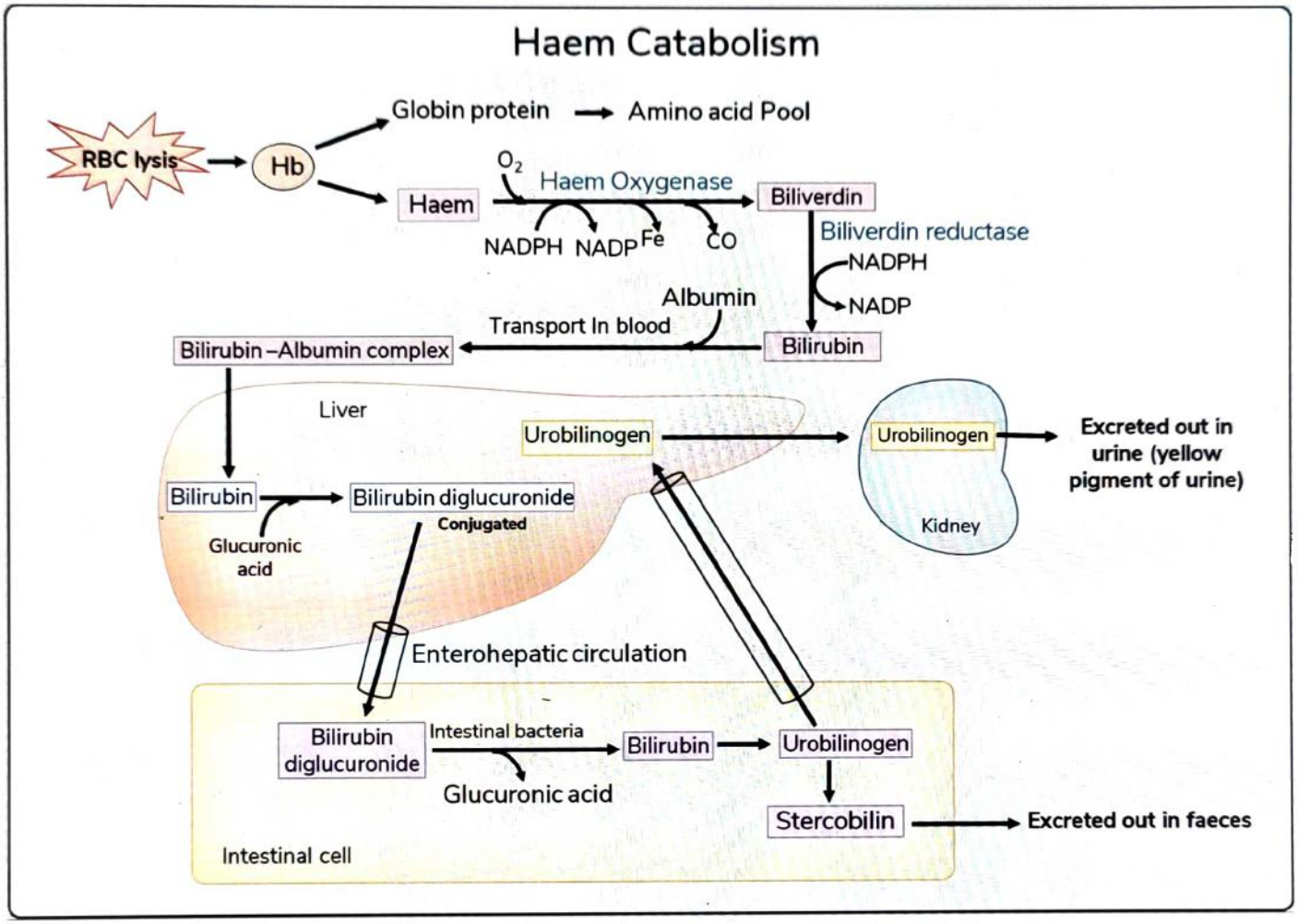
Answer: B

Solution

Acute intermittent porphyria (AIP) is a common form in humans characterized by acute abdominal pain and neurological symptom due to partial deficiency of porphobilinogen deaminase (PBGD). The porphyrin precursors, porphobilinogen (PBG), and delta-aminolevulinic acid (ALA) accumulates in the body. The reason behind the symptoms are shown in figure in text.

Reference: Harper's 30th ed/pg. 328

64 HAEM CATABOLISM



- Albumin-bilirubin complex is called as Unconjugated Bilirubin
  - It cannot be excreted in urine due to large molecular weight.
- Conjugated bilirubin formed in liver can be excreted out in urine

Important Information

- If in a disease conjugated bilirubin is increased, it means bilirubin will be coming in urine
- In a normal person, urine urobilinogen is present but urine bilirubin will be absent

65 CHAPERONES

CHAPERONES

- Definition: Proteins which help in protein folding.
- Mostly present in RER but also found in cytoplasm and mitochondria
- Have associated ATPase activity to produce energy used in protein folding process

CLASSES OF CHAPERONES

00:01:52

1. HSP (Heat Shock Proteins)
   - Major class
   - Produced in response to heat, toxins, radiations, stress, heavy metals, free radicals etc.
   - Examples
     - HSP-10 and HSP-70 (numbers represent the molecular weight in KD)
     - HSP-40 (co-chaperone)
     - HSP-60 (Chaperonins): help chaperons in protein folding and act late in the process
2. ERp (Endoplasmic Reticulum proteins)
3. GRP (Glucose Regulated Proteins)
4. BiP (Immunoglobulin heavy chains binding proteins)
5. Calnexin
6. Calreticulin

Be aware that Calbindin is a calcium binding protein but not a chaperone

Enzymes which help in Protein Folding

00:05:13

1. PDI (Protein Disulphide Isomerase)
   - Helps in formation of correct disulphide bonds
2. PPI (Peptidyl Prolyl cis-trans Isomerase)
   Interconverts cis and trans peptide bonds of proline residues
   ↓
   So, corrects orientation of prolyl peptide bond
   ↓
   Hence assists in protein folding

Previous Year's Questions

Q. Which of the following groups of proteins assist in the folding of other proteins? (NEET PG 2018)

A. Proteases
B. Proteasomes
C. Templates
D. Chaperones

LEARNING OBJECTIVES

UNIT VI: LIPIDS

- Lipid unit consists of topic in lipid structure, lipid-protein complexes known as lipoproteins, fatty acid and cholesterol metabolism. Lipid chemistry chapter gives a brief classification of lipids and discuss the structure of major biological lipids such as triglycerides, phospholipids and sphingolipids. A clinically important group of lysosomal storage diseases of lipids known as sphingolipidoses are discussed in second chapter. A short chapter on essential fatty acids explains the role of nutritionally essential polyunsaturated fatty acids.

- The transport of endogenous and exogenous fats by lipoproteins are discussed in chapter 69. It gives details of structure of various lipoproteins such as LDL, VLDL, HDL and chylomicrons and their transport mechanism in blood. Fredrickson classification of disorders due to defect in various components of the lipoprotein components and metabolism is given briefly along with tips on how to recognize the symptoms. Two short chapters one on high density lipoprotein (HDL) and other on lipotropic factors discuss their mechanism of synthesis and function including their cardioprotective role.

- The next three chapters discuss the synthesis and breakdown of major body fuel reserves i.e. fats or fatty acid metabolism. FA synthesis chapter consists of topics on FA synthase complex, pathway of FA synthesis and reaction of triacylglycerol synthesis. Pathways of synthesis and utilization of major fuel molecules during fasting and starvation i.e. ketone bodies are given in chapter "KB pathways". "Beta-oxidation of fatty acids" chapter has information on transport of fatty acids into mitochondria and their breakdown to acetyl CoA and mechanism of reciprocal regulation of FA synthesis and breakdown in fed and fasting state. The disease occurring due to defects in FA dehydrogenases and minor pathways of FA oxidation are also given in the end.

- The last two chapters provide knowledge on cholesterol structure and its synthesis pathway and formation of primary and secondary bile acids from cholesterol and their function.

- **Major learning objectives**
  - To learn basic chemical structure of major biological lipids
  - To know lysosomal storage disorders of sphingolipids
  - To learn the chemistry and function of PUFAs
  - To understand the structure and function of various lipoproteins and associated disorders
  - To study synthesis and catabolism of fatty acids
  - To understand metabolism of cholesterol and bile acids

66 LIPID CHEMISTRY

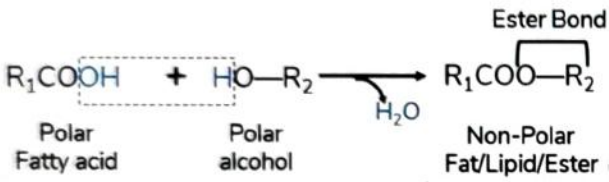
BASICS

Lipid: Any compound which is insoluble in water & soluble in non-polar organic solvent

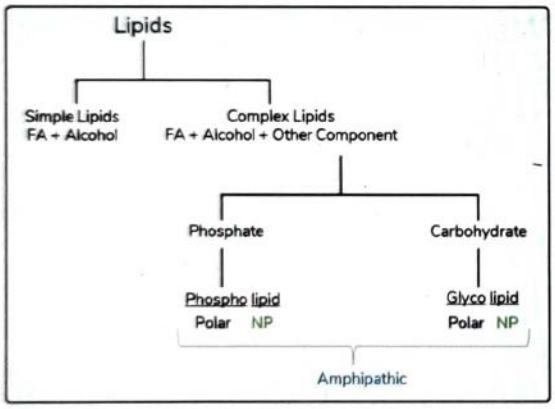
Fatty acids (FA) 00:01:21

- Polar (as any acid contain polar carboxyl group (-COO$^-$)

Conversion of polar fatty acid to non-polar fat by esterification

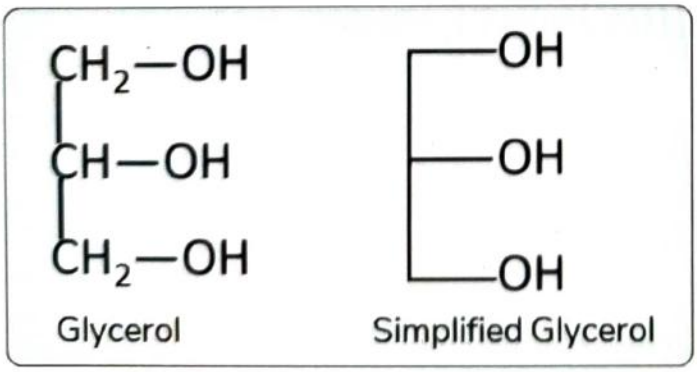


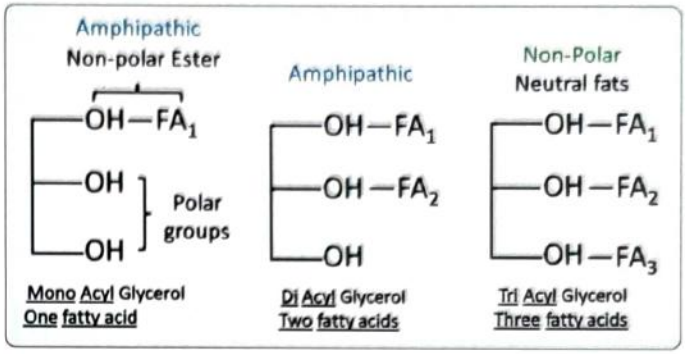
CLASSIFICATION OF LIPIDS 00:03:51



Simple Lipids 00:06:34

- Base alcohol is glycerol

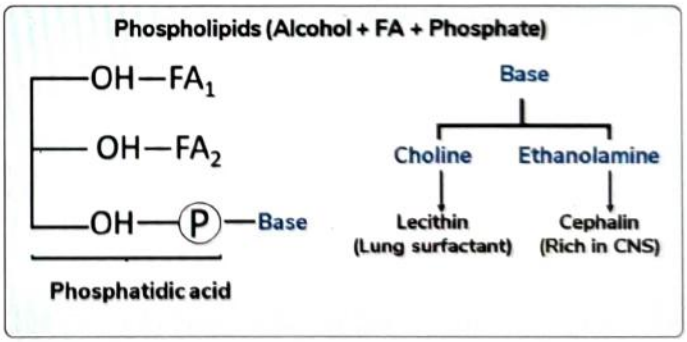




- Triacylglycerols (TGs) are main storage form of lipids in our body.
- 'Acyl' is another term used to represent FA

Keep in mind that acetyl CoA and acyl CoA are two different compounds. In Acetyl CoA, Acetyl refers to 2 carbon acid i.e. acetic acid whereas Acyl usually refers to any long chain fatty acid (>C16)

Phospholipid (PL) 00:12:16

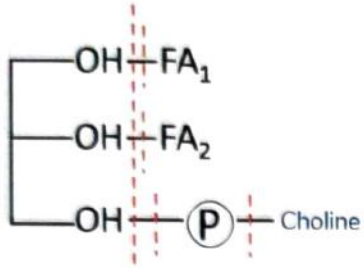


How to remember

- Lecithin starts with 'L' present in Lungs that also Start with 'L'
- Cephalin starts with 'C' present in CNS that also Start with 'C'

00:14:27

Products Of Hydrolysis of Lecithin



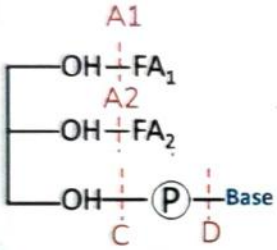
Lecithin breakdown gives:

1. Choline +
2. 2 FAs +
3. Phosphate +
4. 1 Glycerol

Phospholipases: Enzymes which hydrolyse phospholipids at different places

00:15:51

Product released from phospholipids by action of phospholipases



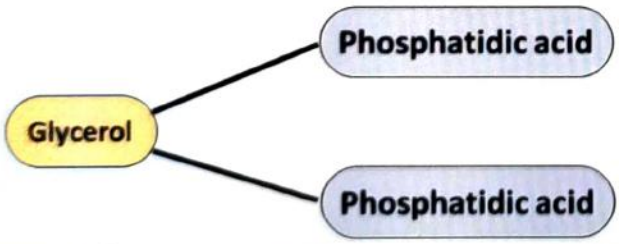
A_1 → FA_1
A_2 → FA_2
D → Phosphatidic Acid + Base
C → Diacyl glycerol + Base with (P)

Cardiolipin

00:17:44

- Complex phospholipid
- Present in inner mitochondrial membrane

Simplified Cardiolipin Structure



- Product of hydrolysis of cardiolipins = 3 glycerol + 4 FA + 2 P
- Can be antigenic (due to its complex nature)
  Remember normally PL are not antigenic

Disease related to Cardiolipins

1. Barth Syndrome

00:19:37

- Rare disease
- X-linked
- Defect in cardiolipin remodelling
- C/f
  - Muscle weakness
  - Neutropenia
  - Cardiomyopathy

2. Anti Phospholipid Syndrome

00:21:31

- Occurs due to Anticardiolipin antibodies formed in body
- C/f
  - A thrombotic condition
  - Disease females has history of recurrent infection

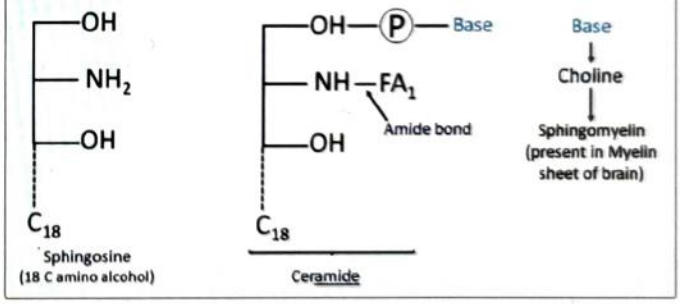
Phospholipids Types

00:22:00

1. **Glycero Phospholipids**
   Parent alcohol: Glycerol
2. **Sphingo Phospholipids**
   - Parent alcohol: Sphingosine
     - Sphingosine is an unsaturated 18 carbon amino alcohol

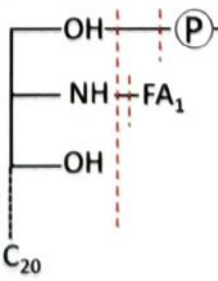
00:23:07

Sphingo Phospholipids (Alcohol + FA + Phosphate)



00:26:26

Products of hydrolysis of Sphingomyelin?



Sphingomyelin hydrolysis

1. Choline +
2. 1 FA +
3. 1 Phosphate +
4. 1 Sphingosine

Glycolipids 00:26:58

- Made of Alcohol + FA + Carbohydrate
- Phosphate & base is never present
- Alcohol glycerol is never present
- Alcohol used is sphingosine (so, aka glycosphingolipids)

Types

1. **Glucosylceramide / Glucocerebroside**

- Sphingosine + FA + glucose
  - Sphingosine + FA = ceramide
  - Ceramide + Glucose = Glucosylceramide or Glucocerebroside
- Never found in CNS but always found in extra neural tissues

2. **Galactosylceramide/ Galactocerebroside**

- Sphingosine + FA + Galactose
- Ceramide + Galactose = Galactosylceramide/ Galactocerebroside
- Always found in CNS

67 SPHINGOLIPIDOSES

SPHINGOLIPIDOSES

- Sphingolipids are recycled in the cells as any other macromolecule by hydrolysis in lysosomes.
- In case of lysosomal enzyme deficiency these lipids will accumulate in lysosomes (Lysosomal storage diseases).
- This condition is called as sphingolipidoses (SLP)
- Sphingolipidoses are of various types depending on type of Sphingolipid accumulated

00:01:57

Refer Figure 67.1

- Other Sphingolipidoses

1. GMI gangliosidosis due to def of β-Galactosidase
2. Metachromatic leukodystrophy due to def of enzyme Cerebroside Sulfatase

00:07:05

Important Information

- **All Sphingolipidoses are autosomal recessive except Fabry's which is X- linked recessive**
- **All Sphingolipidoses have mental retardation except Gaucher's disease**
- **All Sphingolipidoses have hepatosplenomegaly except Tay Sach's & Krabbe's disease**

00:07:42

Important Information

- **All Sphingolipidoses have cherry red spot except Fabry's & Gaucher's disease**
- **Sphingolipidoses with angiokeratoma: GMI gangliosidosis & Fabry's disease**
- **Krabbe's disease aka Globoid cell Leukodystrophies**
- **SLP resembling Rheumatoid arthritis: Farber's Disease**
- **SLP resembling sickle cell crisis: Fabry's Disease**

Previous Year's Questions

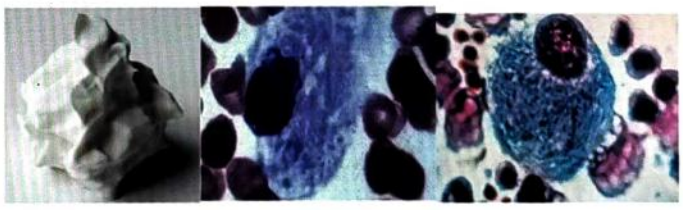
Q. Enzyme deficient in Tay Sach's disease is?
(FMGE - Aug - 2020)

A. Hexosaminidase A
B. Glucocerebrosidase
C. G6PD
D. Hexosaminidase A&B

GAUCHER'S DISEASE

00:09:39

- Most common LSD
- Enzyme Replacement Therapy (ERT) available for Gaucher's disease
- **C/F**
  - Bony pain, pathological fracture
  - No MR (as beta-glucosylceramide is not present in brain)
  - Hepatosplenomegaly present
  - Large amounts of glucosylceramide accumulated in macrophages causing:
    →Eccentric nucleus
    →Cytoplasm: Crumpled tissue paper appearance or wrinkled appearance



Important Information

Q. A patient having organomegaly, who bruise easily and have bony pain?
Ans: Gaucher's Disease

WOLMAN'S DISEASE

00:14:35

- Not a sphingolipidosis but a lysosomal storage disease
- Enzyme deficient is Acid Lipase

- ↑↑ Ch esters and TG (aka cholesterol ester storage disease)
- C/f
  - Watery green diarrhoea
  - Relentless Vomiting and failure to thrive
  - Hepatosplenomegaly
  - Calcification of adrenals is pathognomonic feature

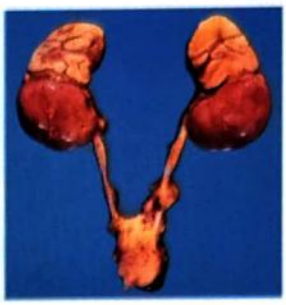
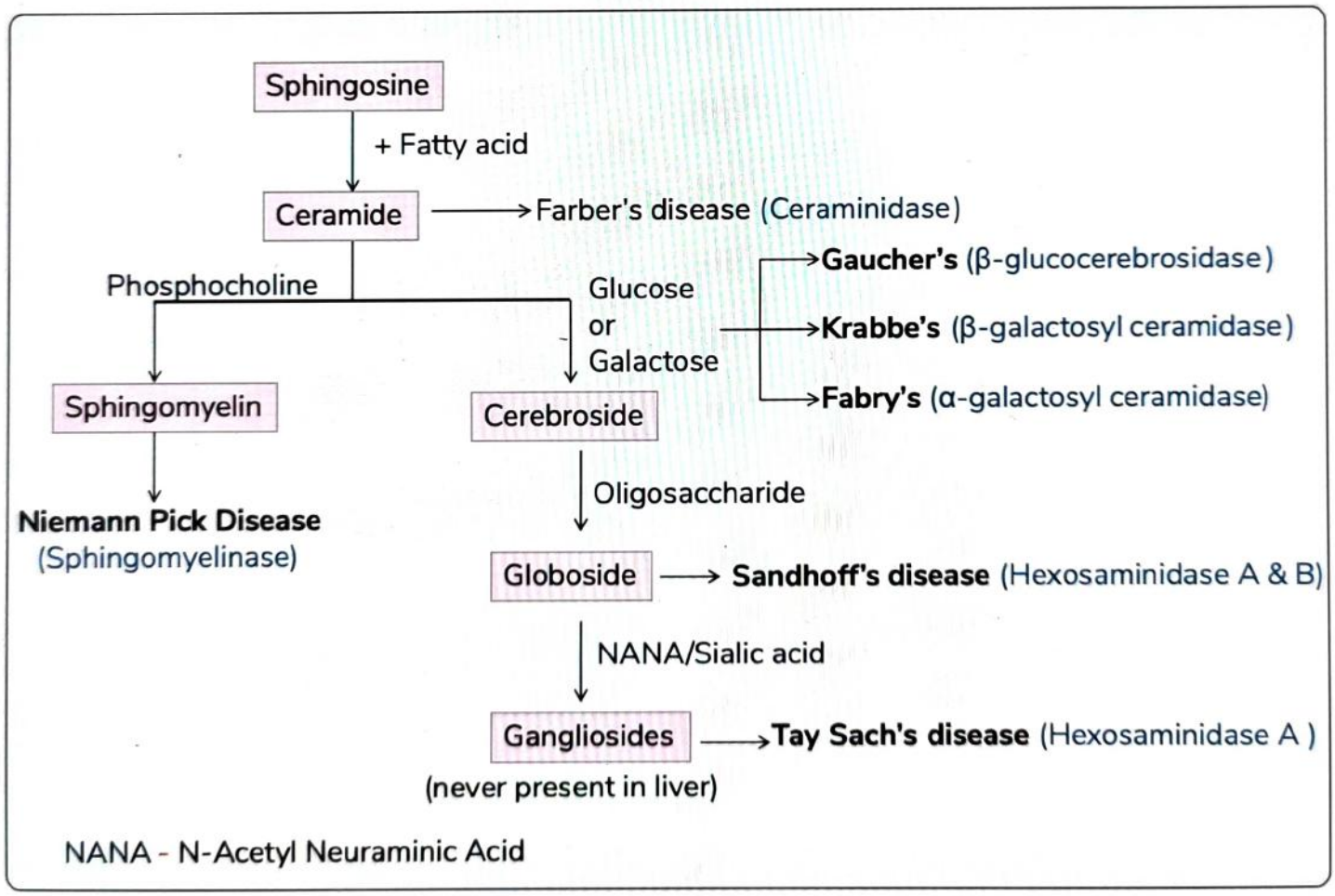


Figure 67.1



CLINICAL QUESTIONS

Q. A 10-month-old child had normal growth and development for the first few months, but growth started deteriorating gradually along with symptoms such as deafness, blindness, atrophied muscle, inability to swallow, and seizures. A cherry red macula was also noticed in both eyes during the fundus examination. 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's disease
B. Tay Sach's Disease
C. Gaucher's disease
D. Fabry's disease

Answer: A

Solution -

The child is exhibiting the symptoms of either Tay–Sachs or Sandhoff 's disease, both of which are sphingolipidoses. However, in Tay Sach's disease only gangliosides will be increased and globosides degradation should be normal. But in Sandhoff's disease both globosides and gangliosides will accumulate. (see fig in text)

Gm2 Gangliosidoses			
Disease	**Deficient enzyme**	**Lipid accumulating**	**Clinical features**
Tay-Sachs disease	Hexosaminidase A	Gangliosides	• Mental retardation, • Blindness, Muscular weakness, • Cherry-red spot
Sandhoff's disease	Hexosaminidases A and B	Golobosides and gangliosides	• Macrocephaly • Hyperacusis • Cherry-red spot

Reference: Harper's 30th ed/pg. 202

Q. A one-week old infant presented with relentless vomiting, watery green diarrhea, abdominal distension and failure to thrive. Further examination showed hepatosplenomegaly and increased levels of cholesterol esters and TG in plasma. X-ray showed calcification of adrenals. The child most probably has defect in which of the following enzyme?

A. Acid Maltase
B. Acid phosphatase
C. Alkaline phosphatase
D. Acid lipase

Answer: D

Solution

The calcification of adrenals is highly diagnostic of Wolman Disease. It is a cholesterol ester storage disease and occurs due to lysosomal acid lipase deficiency. As a result of lipase deficiency excess triglycerides will not be degraded and accumulates in cells.

Other clinical features of this disease are vomiting, watery green diarrhea, hepatosplenomegaly. Enzyme replacement therapy is available for this disease.

Regarding other options:

Deficiency of acid maltase causes Pompe's disease (a glycogen storage disease) with features such as hypotonia, cardiomegaly in muscles, hepatomegaly and hypoglycemia in liver.

Total lysosomal acid phosphatase (LAP) deficiency is an autosomal recessive disorder with clinical features such as intermittent vomiting, lethargy, hypotonia, opisthotona, and terminal bleeding in early infancy.

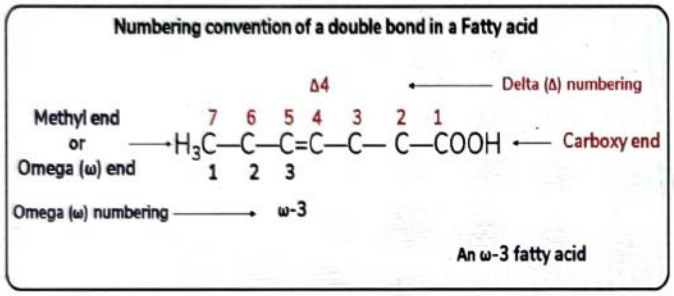
Reference: Harper's 30th ed/pg. 234

68 PUFAs

Poly Unsaturated Fatty Acids (PUFAs) means FAs having ≥ 2 double bonds

- Aka Essential FAs (EFA): They cannot be synthesized in our body and needs to be essentially taken in diet

00:01:23



Categories of PUFAs

00:03:45

Omega-3 category	Omega-6 category
1. Cervonic acid/ DHA (DocosaHexaenoic Acid)	1. γ – Linolenic acid
• 22 C (docosa) & 6 = (hexaenoic)	• 18 C & 3 =
2. α-Linolenic acid	2. Linoleic acid
• 18 C & 3 =	• 18 C & 2 =
3. Timnodonic acid/EPA (EicosaPentaenoic Acid)	3. Arachidonic acid
• 20 C (eicosa) & 5 = (pentaenoic)	• 20 C & 4 =

How to remember

- Any fatty acid starting from "L" have same number of carbons e.g. α-Linolenic acid, γ-Linolenic acid and Linoleic acid all have 18 carbons
- Smaller Name - Linoleic = 2 double bonds
- Bigger name - Linolenic = 3 double bonds

Important Information

- DHA:
  - Is required for brain development of first 2-3 years of life so, Health drinks are fortified with DHA.
  - Breast milk always contains DHA.
- α-Linolenic acid is precursor of ω-3 category means if α-Linolenic acid is taken in diet, other two ω-3 FAs can be made form it in the body.
- α-Linoleic acid is precursor of ω-6 category and can be used to make other two ω-6 FAs in the body
- Most Essential FA is - Linoleic acid as it can make arachidonic acid which is required for PGs and Leukotrienes synthesis.

00:09:18

Important Information

Q. PUFAs which are cardioprotective:

Ans. ω-3 PUFAs. i.e. low ω-6/ω-3 ratio is desirable for preventing coronary heart diseases, obesity and other chronic western lifestyle diseases.

How ω-3 PUFAs are cardioprotective?

Omega-3 PUFAs:

- Affect membrane fluidity in a positive way
- Help in transfer of cell signal molecules
- Many of ω-3 metabolites are anti-inflammatory
- Prevents conversion of ω-6 PUFAs into pro-inflammatory eicosanoids by acting as alternate substrate for enzymes such as lipoxygenase and cyclooxygenase involved in these conversions

Previous Year's Questions

Q. Which of the following is an essential fatty acid?
(FMGE JUNE - 2018)

A. Citric acid
B. Linoleic acid
C. Stearic acid
D. Palmitic acid

CLINICAL QUESTIONS

Q. A teenager, concerned about his weight, attempts to maintain a fat-free diet for a period of several weeks. If his ability to synthesize various lipids were examined, he would be found to be most deficient in his ability to synthesize:

A. Triacylglycerol
B. Phospholipids
C. Sphingolipids
D. Prostaglandins

Answer: D

Solution

Prostaglandins are synthesized from arachidonic acid. Arachidonic acid is synthesized from linoleic acid, (an essential fatty acid obtained by humans from dietary lipids). So, if his diet is not providing him linolenic acid, he would not be able to synthesize prostaglandins as their synthesis is totally dependent on the external supply of linoleic acid.

But the synthesis of other compounds mentioned above does not depend totally on external lipids supply. So, their synthesis will be affected less. In other words, the teenager would be able to synthesize all other compounds, but in somewhat depressing amounts.

Reference: Harper's 30th ed/pg. 224

69 LIPOPROTEINS

LIPID TRANSPORT

00:00:25

- Polar substance is soluble in polar medium and non-polar substance is soluble in non-polar medium
- Transport medium in our body is blood and Blood is water based, hence it is polar
- So, carbohydrate & proteins which are polar can easily dissolve in blood and thus can be easily transported from one place of body to another
- But dietary Lipids due to their non-polar nature cannot be dissolved directly in blood and need special transport structures known as Lipoproteins e.g. HDL, LDL, VLDL

LIPOPROTEINS

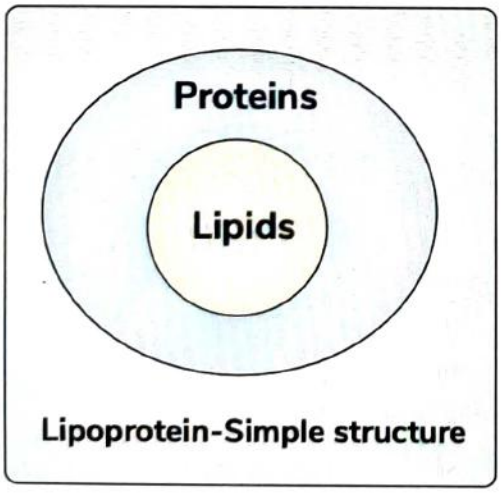
Structure

00:01:45

- In Lipoproteins, Lipid is present towards core surrounded by proteins in periphery.

Lipids present in Lipoproteins

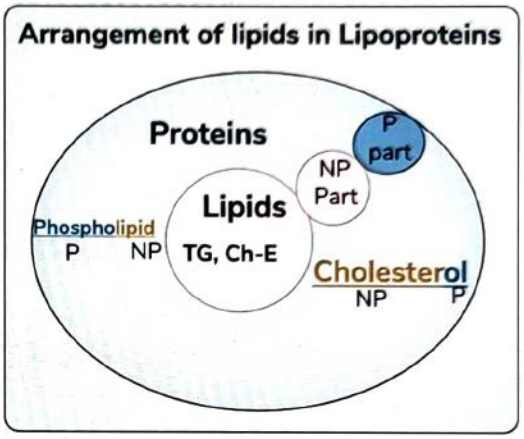
- Triglyceride NP
- Phospholipid Amphipathic
- Cholesterol Amphipathic
- Cholesterol ester (cholesterol + FA) → NP
- Proteins are called Apo-proteins



Lipoprotein-Simple structure

Arrangement of lipids in Lipoprotein

- Non-polar Lipids (TG, cholesterol ester)
  - Present embedded in core
- Amphipathic Lipids (Cholesterol, phospholipids)
  - Polar portion will be towards outside
  - Non-polar portion will be towards inside



00:04:57

Density

Chylomicron
Chylomicron remnant
VLDL (Pre β-LP)
VLDC remnant / IDL (Broad β-LP)
LDL (β-LP)
HDL (α-LP or Lipoprotein A)

Relation of Density with lipoprotein contents

$$Density \propto \frac{1}{Size\ of\ LP}$$

$$Density \propto \frac{1}{TG\ content}$$

$$Density \propto \%\ of\ protein$$

Important Information

Q. Which is the largest of all the lipoproteins?
Ans: Chylomicron
Q. Which lipoprotein has maximum TG content?
Ans: Chylomicron
Q. Which lipoproteins has maximum protein content?
Ans: HDL

EXOGENOUS FAT

00:07:26

Refer Figure 69.1

- Chylomicron Transport Exogeneous Fat or Exogeneous TG from intestine to peripheral Tissue.
- Chylomicron remnant formed is transported back to liver.
- In circulation, HDL donates apo C and apo E to chylomicron so that it is converted to chylomicron remnant.
  - Apo C is required for activation of LPL for TG breakdown of chylomicrons.
  - Apo E is required for uptake of chylomicron remnant by liver.
- Liver processes the component of chylomicron remnant according to its requirements.

Lipoprotein lipase vs Hormone sensitive lipase

Lipoprotein Lipase (LPL)	Hormone sensitive lipase (HSL)
• Present in endothelium cells	• Present inside the adipose tissue cells
• Anabolic enzyme	• Catabolic enzyme
• Activated by insulin only in capillary bed of adipose tissue	• Inhibited by insulin but activated by glucagon, epinephrine and thyroid hormones
• Breaks TG present in chylomicrons and VLDL when they pass through adipose tissues.	• Break TG into fatty acid and glycerol but cannot break FA at second position of TGs.

Activators of HSL	Inhibitors of HSL
• Glucagon	• Insulin
• Thyroxin	• Nicotinic acid (high doses of niacin are used as anti-hyperlipidemic drug)
• Catecholamine	• Prostaglandin E (PGE)
• ACTH	
• TSH	

Important Information

Q. Which lipid is present in chylomicron:
Ans: TG (as relatively negligible amount of cholesterol as compared to TG)
Q. Which lipid is present in chylomicron remnant?
Ans: TG + Cholesterol (as relative cholesterol amount has increased considerably after TG breakdown by LPL)

ENDOGENOUS FAT

00:10:53

Refer Figure 69.2

- VLDL Transport Endogenous TG from Liver to Peripheral Tissues
- VLDL moves in circulation keep losing TG and gets converted to IDL
- IDL further moves in circulation and keep giving TG to peripheral tissues such that only cholesterol is left in the end. This structure is called LDL.

Functions of LDL

- Cholesterol is required for membrane, vitamin D and steroid hormone synthesis in peripheral cells
- LDL provide cholesterol to peripheral tissues
- LDL carry both

1. Exogenous cholesterol
  - Cholesterol present in chylomicron remnant → liver → VLDL → LDL)

2. Endogenous cholesterol
  - synthesized in body and transported to liver

Important Information

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

HIGH DENSITY LIPOPROTEINS (HDL)

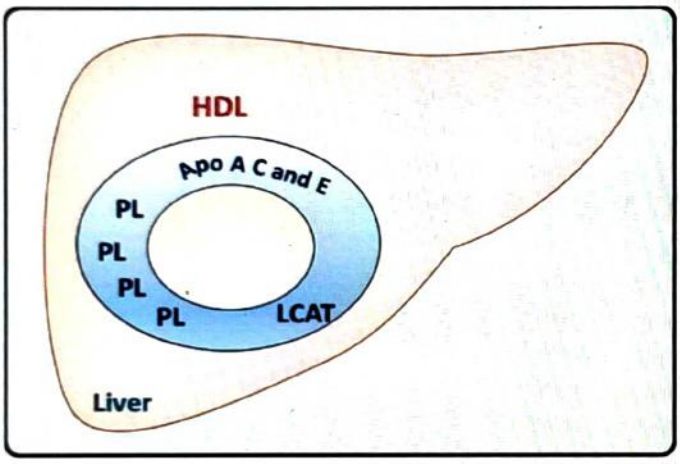
00:13:12

- HDL is mainly synthesized is Liver (some amount in intestine)

Structure of HDL

- **HDL consists of**

1. Phospholipids (PL)
   - HDL is Lipoproteins with maximum PL
2. Apo A, C and E proteins synthesized by liver.
   - Apo C and E has no role for HDL.
   - They are given to VLDL and chylomicrons
3. LCAT enzyme which converts Cholesterol to Ch-ester



Functions

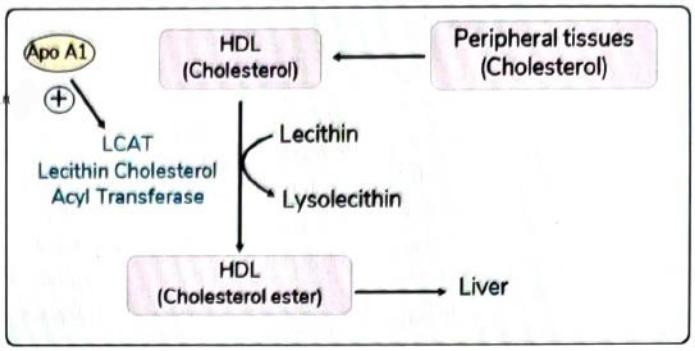
- HDL performs Reverse cholesterol Transport i.e. transport of excess cholesterol from peripheral tissues and blood vessels to Liver

Important Information

Q. Exogenous TG is transported to peripheral tissues by?
Ans: Chylomicron.
Q. Endogenous TG is transported to peripheral tissues by?
Ans: VLDL
Q. Exogenous Cholesterol is transported to peripheral tissues by?
Ans: LDL
Q. Endogenous Cholesterol is transported to peripheral tissues by?
Ans: LDL

How HDL adds Fatty acid

00:16:52



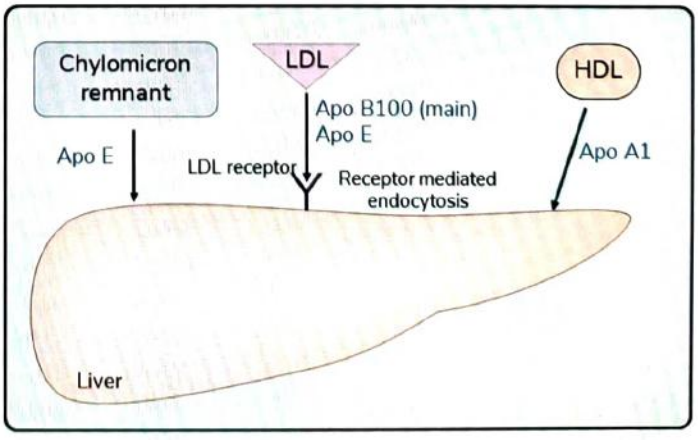
COMPOSITION OF VARIOUS LIPOPROTEINS

Lipoprotein	Lipid present	Protein/Apoprotein present
Chylomicron	TG	Apo B-48
Chylomicron Remnant	TG + Ch	Apo B-48 + Apo E
VLDL	TG	Apo B-100
VLDL Remnant (IDL)	TG + Ch	Apo B-100 + Apo E
LDL	Ch	Apo B-100 + Apo E
HDL	Ch-ester	Apo-A, Apo-C and Apo-E

LIGANDS ON LIPOPROTEINS FOR UPTAKE BY LIVER

00:20:10

- Ligands are proteins present on lipoprotein by which liver will recognise particular Lipoprotein



ROLE OF APOLIPOPROTEINS

Structural Role	
Apo B-48 & Apo B-100	Provide hydrophilic exterior in periphery. Cannot be removed
Enzyme Activators and Inhibitors	
Apo C-I and C-II	LPL activator (main is C-II)
Apo C-III	LPL inhibitor
Apo A-I	LCAT activator
Apo A-II	LCAT inhibitor
Ligands for Receptors	
Apo E	Ligand for remnants, minor LDL receptor ligand
Apo B-100	LDL receptor ligand

LCAT

LCAT vs ACAT

LCAT	ACAT
• Lecithin Cholesterol Acyl Transferase • Present in HDL in blood vessels	• Acyl-CoA Cholesterol Acyl Transferase • Present inside the adipose tissue cells

LCAT deficiency

00:22:10

Complete deficiency	Partial deficiency
• Norum's Disease • Severe hemolytic anemia and End State Renal Disease (ESRD)	• Fish-eye Disease • c/f are same as Norum's disease but No hemolytic anemia or ESRD

Norum's Disease

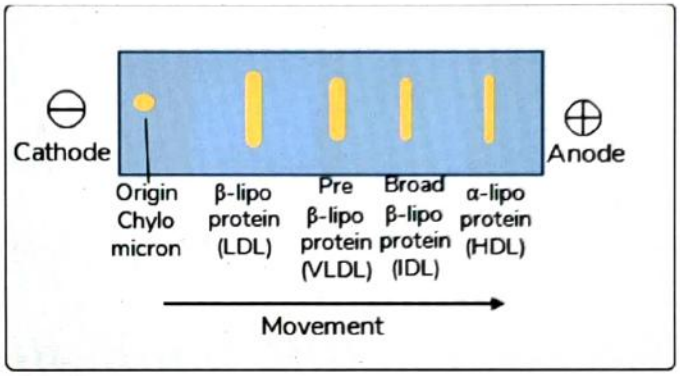
Cholesterol + lecithin $\xrightarrow[\text{absent}]{\text{LCAT}}$ Ch-ester + lysolecithin
(both Increased) (both Decreased)

C/f

- Causes ESRD
- Excess free cholesterol deposited in cornea causing Opacification
- Hemolytic anemia

00:25:24

ELECTROPHORESIS OF LIPOPROTEINS



00:23:48

Important Information

HDL = α – Lipoprotein or Lipoprotein 'A'

- Prevent atherosclerosis

Lipoprotein 'a' = LDL + apo 'a'

- Cause atherosclerosis

Lipoprotein 'x'

- Abnormal Lipoprotein found in two conditions:
  1. LCAT deficiency
  2. Cholestatic states e.g. I° Biliary cirrhosis and I° Sclerosing cholangitis
- Rich in amphipathic lipids (PL & cholesterol)
- Poor in neutral lipids (TG & cholesterol ester)
- Density lies between LDL and VLDL
- Onion like or vesicular appearance
- Protein present is albumin (Major) + variable apoproteins (small amount)

HYPER LIPOPROTEINEMIA (FREDRICKSON CLASSIFICATION)

00:28:13

Type	Defect	LP	TG	Ch	Common names
I	Lipoprotein Lipase Or Apo C-II defect	Chylo > VLDL	↑	Normal	Familial Hyperchylomicronemia
II a	LDL Receptor or Apo B100	↑ LDL	N	↑	Familial Hypercholesterolemia
II b	Unknown	↑ VLDL ↑ LDL	↑	↑	Familial combined hyperlipoproteinemia
III	apo E	↑ Chylo–remnant ↑ VLDL remnant	↑	↑	Broad β diseases / Remnant removal disease / Dys β lipoproteinemia

00:32:32

Important Information

Clinical Features recognition to tackle hyperlipoproteinemia related clinical questions

• Tendon xanthoma	↑ Cholesterol
• Eruptive xanthoma	↑ TG
• Palmar & Tubero eruptive xanthoma	↑ Chylo-remnant & ↑ VLDL remnant
• Milky plasma	↑ Chylomicrons
• Acute pain in abdomen [Acute pancreatitis]	↑ TG

Previous Year's Questions

Q. Type I hyperlipoproteinemia is characterized by? (NEET - Jan - 2019)

A. Elevated LDL
B. Elevated HDL
C. Elevated lipoprotein lipase
D. Elevated chylomicrons

Previous Year's Questions

Q. A patient has multiple tendon xanthomas. Serum cholesterol (398 mg/dl) & LDL (220 mg/dl) were found to be raised. Statins were given to this patient. What is the diagnosis? (NEET Sep 2021)

A. Lipoprotein lipase deficiency
B. Familial hypercholesterolemia
C. Tangier's disease
D. Huntington's disease

ABETALIPOPROTEINEMIA

00:34:13

- Absent or decreased beta lipoproteins (LDL, VLDL, IDL, chylomicrons)
- defect in absorption of lipids due to defective transport protein resulting in
  - defective absorption of fat-soluble vitamins
  - Vitamin K deficiency leading to bleeding manifestations
- Clinical features
  1. Acanthocytosis (crenated RBCs present in blood)
  2. Neurological problems
  3. Steatorrhea

FRIEDWALD'S EQUATION (FOR CALCULATING LDL)

00:35:47

- Total cholesterol = VLDL + HDL + LDL
- So, LDL = Total cholesterol – HDL – VLDL
- As VLDL = TG/5
- So, LDL Cholesterol (mg/dl) = Total cholesterol- HDL cholesterol – TG/5 **Or** LDL cholesterol (mmol/L) = Total cholesterol – HDL cholesterol – TG/2.2

Figure 69.1

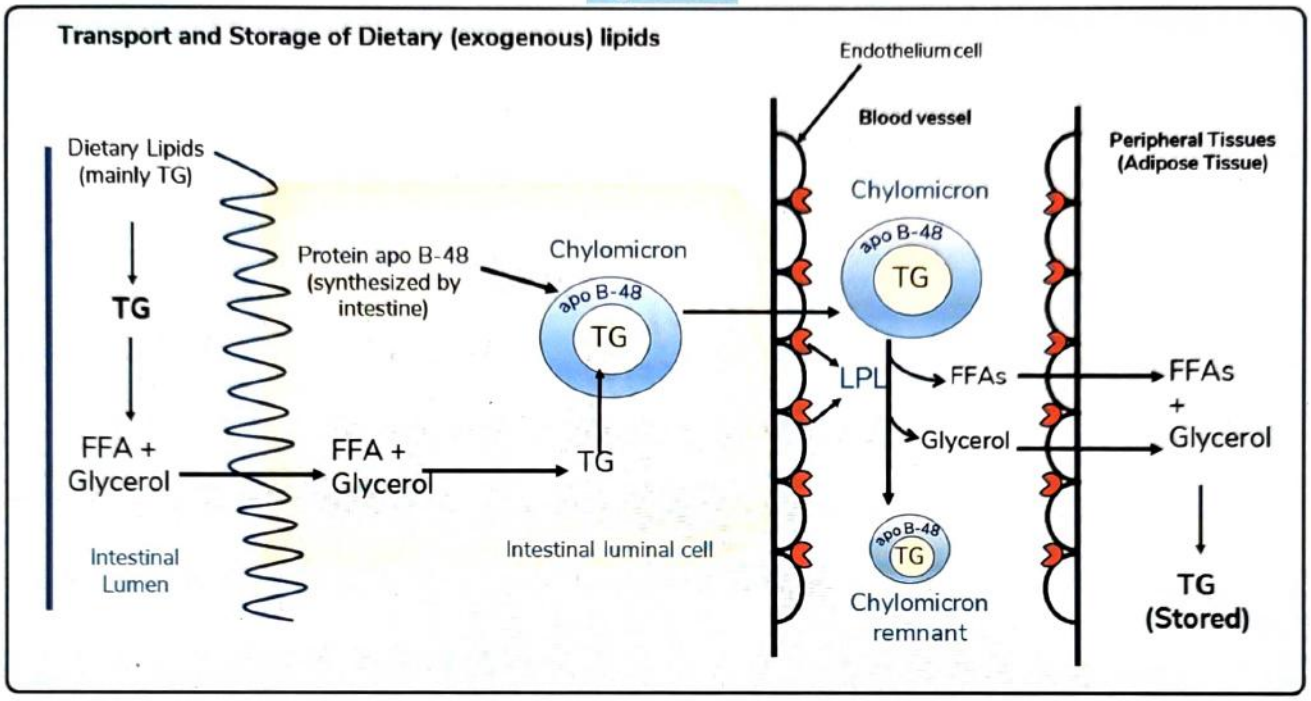
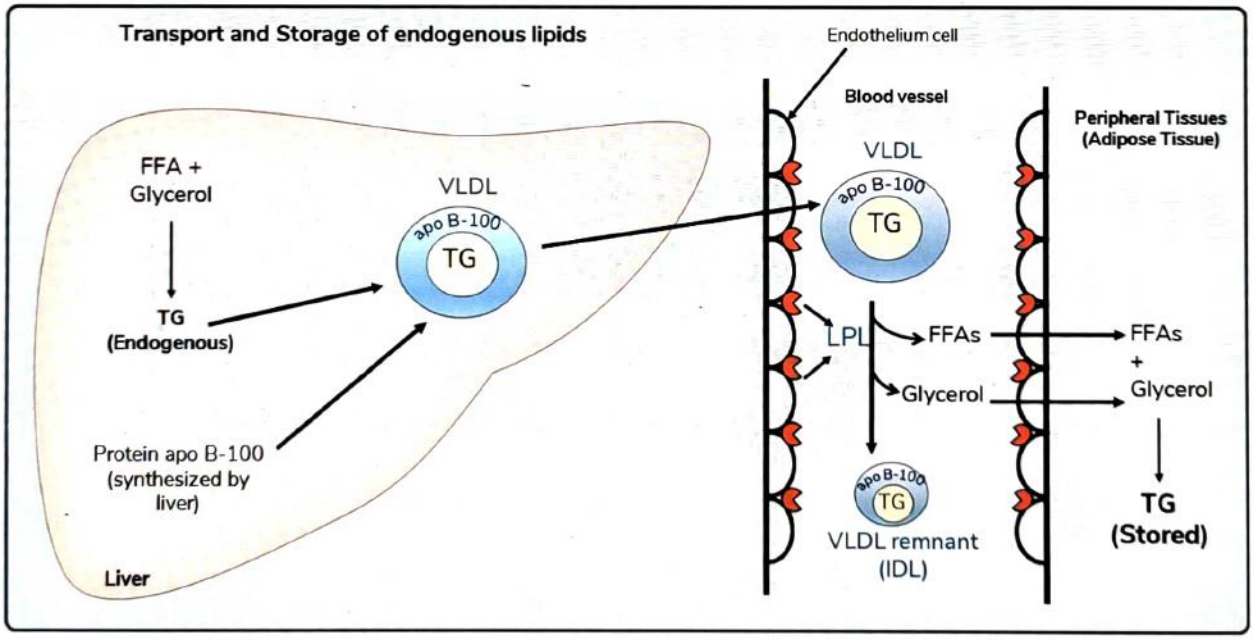


Figure 69.2



CLINICAL QUESTIONS

Q. A 28-year-old man was found to have elevated cholesterol levels of 325 mg/dL on a routine checkup. His father consistently had high levels of cholesterol throughout his life and eventually died of a heart attack at the age of 42. He was prescribed to take lovastatin, and his cholesterol levels dropped to 170 mg/dL. The most likely reason for the elevated cholesterol in this patient is due to a mutation in which one of the following proteins?

A. Microsomal Triglyceride transfer Protein (MTP)
B. LCAT
C. Lipoprotein lipase
D. LDL receptor

Answer: D

Solution

Patient condition suggests that it might be a case of Familial Hypercholesterolemia which is either due to defect in LDL receptor present on liver or defective Apo B100 protein present on LDL. It is genetically heritable disease passed in an autosomal dominant fashion in the family. The condition leads to high levels of cholesterol in the blood of the patient leading to atherosclerosis and other cardiovascular diseases at a very young age.

LDL receptor is required for liver uptake of LDL and further processing of the endogenous cholesterol while apo B100 protein present on LDL acts as legend for this receptor and help in engulfment of LDL in the liver. Defect in either of this results in inability to clear excess endogenous cholesterol and leading to its accumulation in the blood vessels hence increasing the chances of forming atherosclerotic plaques.

Regarding other options:

Deficiency/ Defect	Disease
MTP deficiency	Abetalipoproteinemia
LCAT deficiency	Fish-eye disease (partial deficiency) Norum Disease (complete deficiency)
Lipoprotein lipase deficiency	Familial hyperchylomicronemia

Reference: Harper's 30th ed/pg. 258

Q. An infant presented with diarrhea. Blood examination revealed acanthocytes, undetectable cholesterol and triglyceride. VLDL and LDL fractions were absent on lipoprotein electrophoresis. Therapeutic supplementation of daily doses of oral vitamin A, 25-hydroxy vitamin D, and vitamin K were prescribed with vitamin E added later on. The mutation analysis of which of the following could have been done to confirm the diagnosis?

A. Lipoprotein lipase
B. Microsomal triglyceride transfer protein
C. Cholesterol Ester transfer protein
D. Lecithin Cholesterol Acyl transferase

Answer: B

Solution

Absence of all beta-lipoproteins fractions VLDL and LDL in lipoprotein electrophoresis indicates abetalipoproteinemia or Bassen-Kornzweig syndrome. It is a rare autosomal recessive disorder due to defect in Microsomal Triglyceride Transfer Protein (MTP). This defect interferes with the normal absorption of fat and fat-soluble vitamins from food.

So, DNA sequencing of MTP protein gene would confirm this condition by revealing any mutations present in this gene which would ultimately affect the function of this protein.

Regarding other options:

Cholesterol ester transfer protein (CETP) transfers the cholesterol esters from HDL2 to VLDL in exchange for triacylglycerol from VLDL. Its deficiency results in increased HDL.

Lipoprotein lipase deficiency causes familial hyperchylomicronemia whereas LCAT deficiency causes Norum's disease with symptoms such as hemolytic anemia and end stage renal disease.

Reference: Harper's 30th ed/pg. 259

Q. A 10-yr old boy was admitted for surgery of cleft lip. Suddenly he had acute pain in abdomen. On examination, xanthomas were observed. Blood examination revealed milky plasma. Which lipoprotein is most likely increased in this patient?

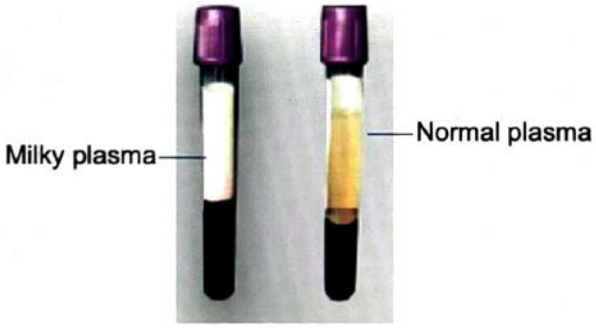
A. VLDL Remnant
B. Chylomicron
C. Triglycerides
D. Cholesterol

Answer: B

Solution

Milky plasma is a characteristic feature of increased chylomicrons in blood. This is a picture of Type I Hyperlipoproteinemia, which is due to the defect in Lipoprotein Lipase enzyme.

The patient plasma will show following features:



First test tube: Creamy layer on the top after centrifugation.
Second test tube: Normal serum on the top after centrifugation.
Chylomicrons mostly contains TGs, but TG is a simple lipid whereas chylomicron is a lipoprotein. The question is asking about lipoprotein so, the most accurate answer is b.

Reference: Harper's 30th ed/pg. 236

70 HDL

HIGH DENSITY LIPOPROTEINS

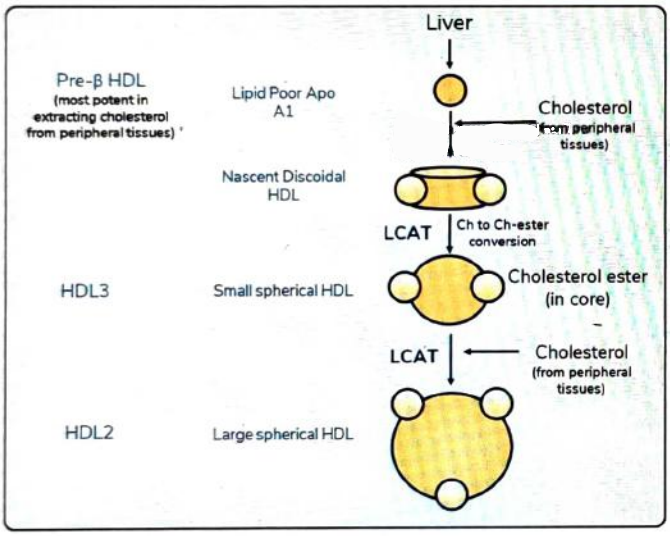
Synthesis and Structure

00:00:30

- Synthesized in Liver (mainly) & small intestine
- Has maximum phospholipids
- Proteins present are Apo A, C, E
  - Apo C & E are only synthesized in Liver and transferred to intestine when required.
- Contains LCAT enzyme
  - Converts cholesterol to cholesterol Ester (discussed earlier in lipoprotein notes)

Various forms of HDL and their synthesis

00:01:33



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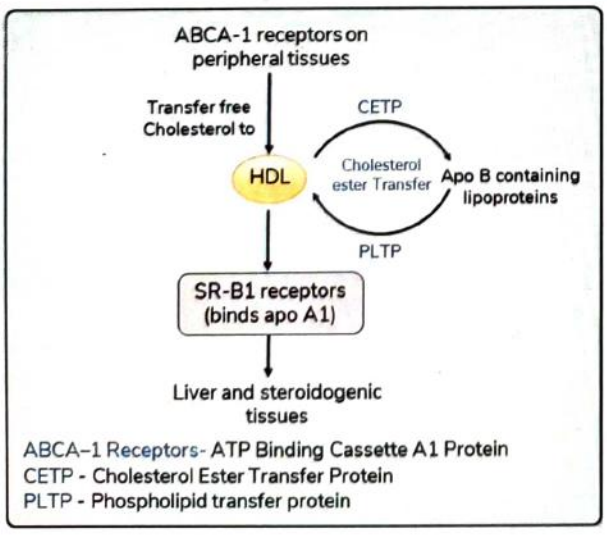

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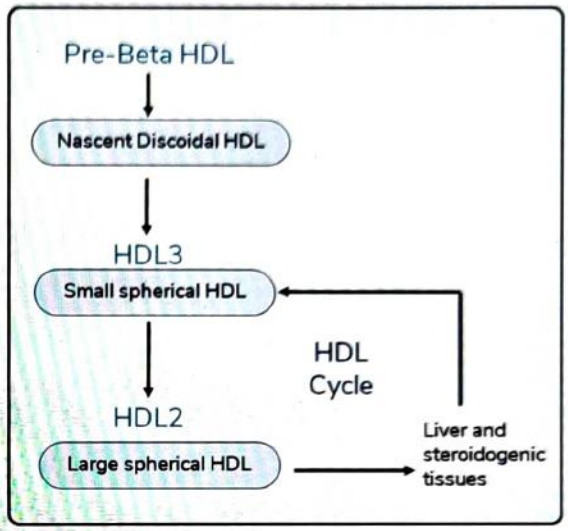
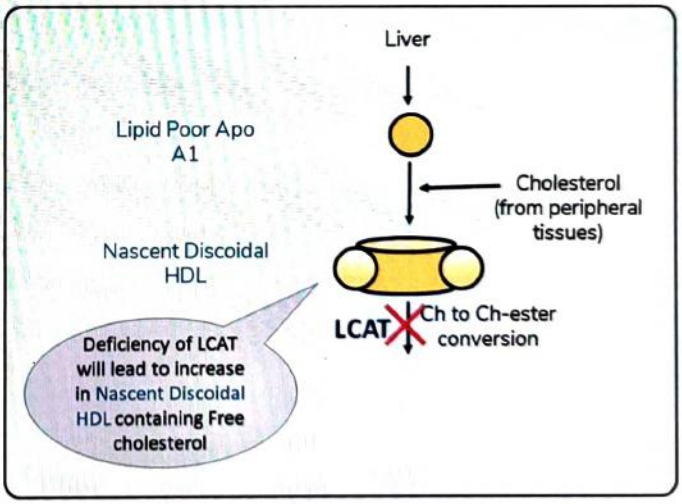




Previous Year's Questions

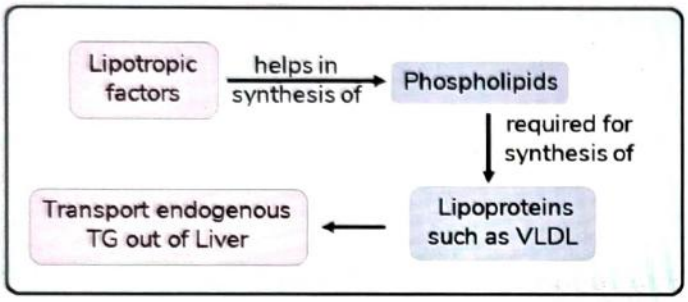
Q. Which of the following is cardio protective? (FMGE Dec 2019)

A. HDL
B. LDL
C. VLDL
D. Chylomicrons

71 LIPOTROPIC FACTORS

LIPOTROPIC FACTORS

- These are factors which are required for the synthesis of phospholipids
- They help in mobilization of TG out of liver cells.



- Deficiency leads to fatty liver (due to TG accumulation in liver)

- Lipotropic factors:
  - EFA/PUFAs
  - Vitamins- B_9 and B_{12}
  - Three amino acids
    1. Serine
    2. Glycine
    3. Methionine
  - Others: Choline, Betaine and Inositol

Previous Year's Questions

Q. Which of the following is a lipotropic factor? (JIPMER Nov 2017)

A. Sphingomyelin
B. Choline
C. Cardiolipin
D. Orotic acid

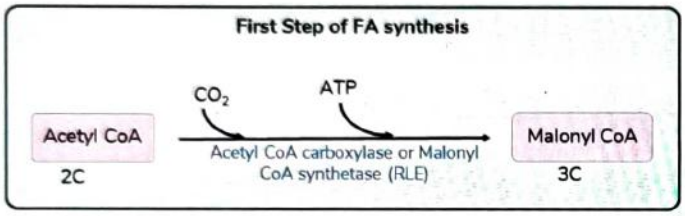
72 FATTY ACID SYNTHESIS

FATTY ACID SYNTHESIS

00:00:19

Characteristics

- Anabolic pathway
- Occurs in cytoplasm
- Activated by Insulin



Previous Year's Questions

Q. Active metabolite form in synthesis of fatty acid is? (AIIMS Nov 2017)

A. Acetyl CoA
B. Malonyl CoA
C. Stearate
D. Palmitate

FA synthase complex

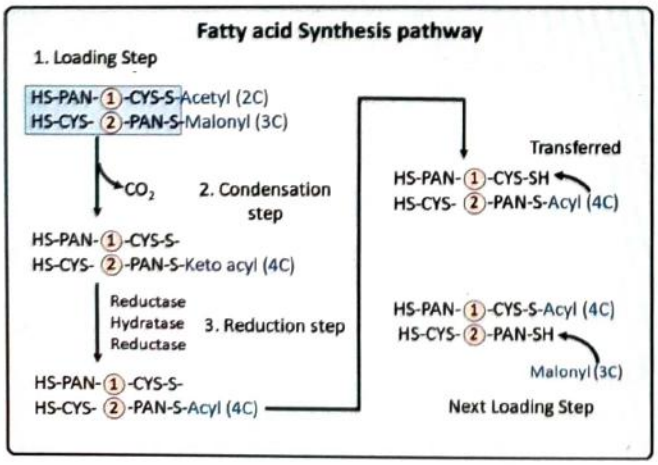
00:01:50

- Main enzyme
- Dimer (X- shaped as detected by X-Ray crystallography)
- Two monomers are joined by disulphide bonds

Refer Figure 72.1

Pathway

00:06:50



1. Loading step

- Acetyl always loaded at Cys end
- Malonyl always loaded at PAN end

2. Condensation step

- CO_2 is removed from Malonyl CoA
- After this step:
  - Upper Monomer is empty
  - Lower monomer has Keto Acyl [4C]
- Acetyl (2C) + Malonyl (3C) – CO_2 (1C) = 4C

3. Reduction step

- Removal of Keto group
- Requires 3 enzymes
  1. Reductase (NADPH is used)
  2. Hydratase
  3. Reductase (NADPH is used)
- 2 NADPH are used

After this step

- Upper monomer → empty
- Lower monomer → Acyl (4C)

The above three steps are repeated and again to make Long chain FA.

Next cycle

- Acyl (4C) from the Lower monomer is transferred to upper monomer
- Malonyl CoA (3C) comes at PAN end (next Loading Step)
- Cycle repeats again & again

Note

- In 1st cycle, 5 carbons are loaded & only 4 carbons are added [-1C at condensation]
- In all subsequent cycles, 3 carbons are loaded & only 2 carbons are added

Previous Year's Questions

Q. FA is synthesized from?

Ans: Acetyl CoA

Why not malonyl CoA?

- Because extra Carbon of Malonyl CoA is not getting added in newly synthesized FA
- 1st enzyme carboxylase added one CO_2 to form malonyl CoA but 2nd enzyme FA synthase removed the CO_2. So, only CO_2 of acetyl CoA are used.

Previous Year's Questions

Q.De- Novo synthesis of Fatty Acid requires which coenzyme? (FMGE June 2019)

A. NADPH
B. TPP
C. FAD
D. NAD

Previous Year's Questions

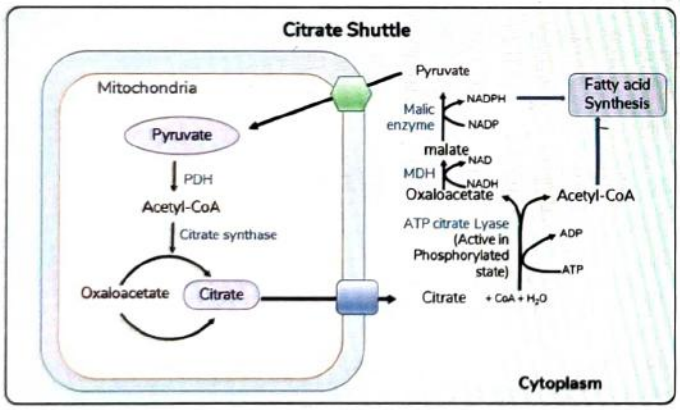
Q. Major product of fatty acid synthesis is? (AIIMS Nov 2017)

A. Acetyl CoA
B. ATP
C. Citrate
D. Palmitate

CITRATE SHUTLE

00:17:15

- Used for the transport of Acetyl CoA from mitochondria to cytoplasm for fatty acid synthesis



ATP Citrate Lyase

- Uses ATP (Generally, Lyases do not use ATP)
- Anabolic enzyme but active in phosphorylated state.
  - Generally anabolic enzymes are active in dephosphorylated state.

ELONGATION OF FATTY ACID

00:21:53

- Up to 10 carbon fatty acid is made in cytoplasm by normal de novo FA synthesis process
- Further elongation of FA chain (upto C-24) is done by fatty acyl elongase enzymes
- Occurs in ER
- Either NADH or NADPH can be used (NADPH is preferred)
- Includes loading, condensation and reduction steps
- C-22 and C-24 FAs are specially required in brain for the formation of sphingomyelin.

DESATURATION OF FATTY ACID

00:22:57

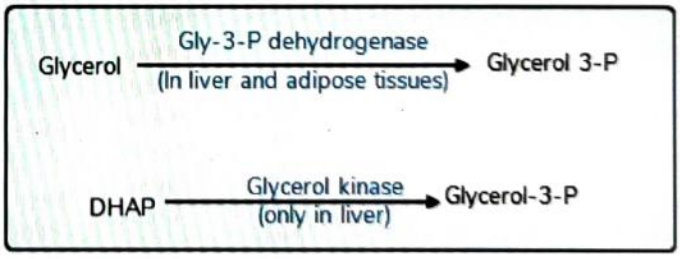
- Occur in liver ER
- Done by desaturases
- Enzymes doing desaturation are 4, 5, 6 and 9 desaturases
- Create double bond at 4, 5, 6 and 9 positions
- Humans do not have enzymes to create double bond beyond 9 position.
- So, the fatty acid containing double bond beyond 9 positions (PUFAs) need to be obtained in diet as essential fatty acids.

FORMATION OF TRIGLYCERIDES (TG)

00:24:40

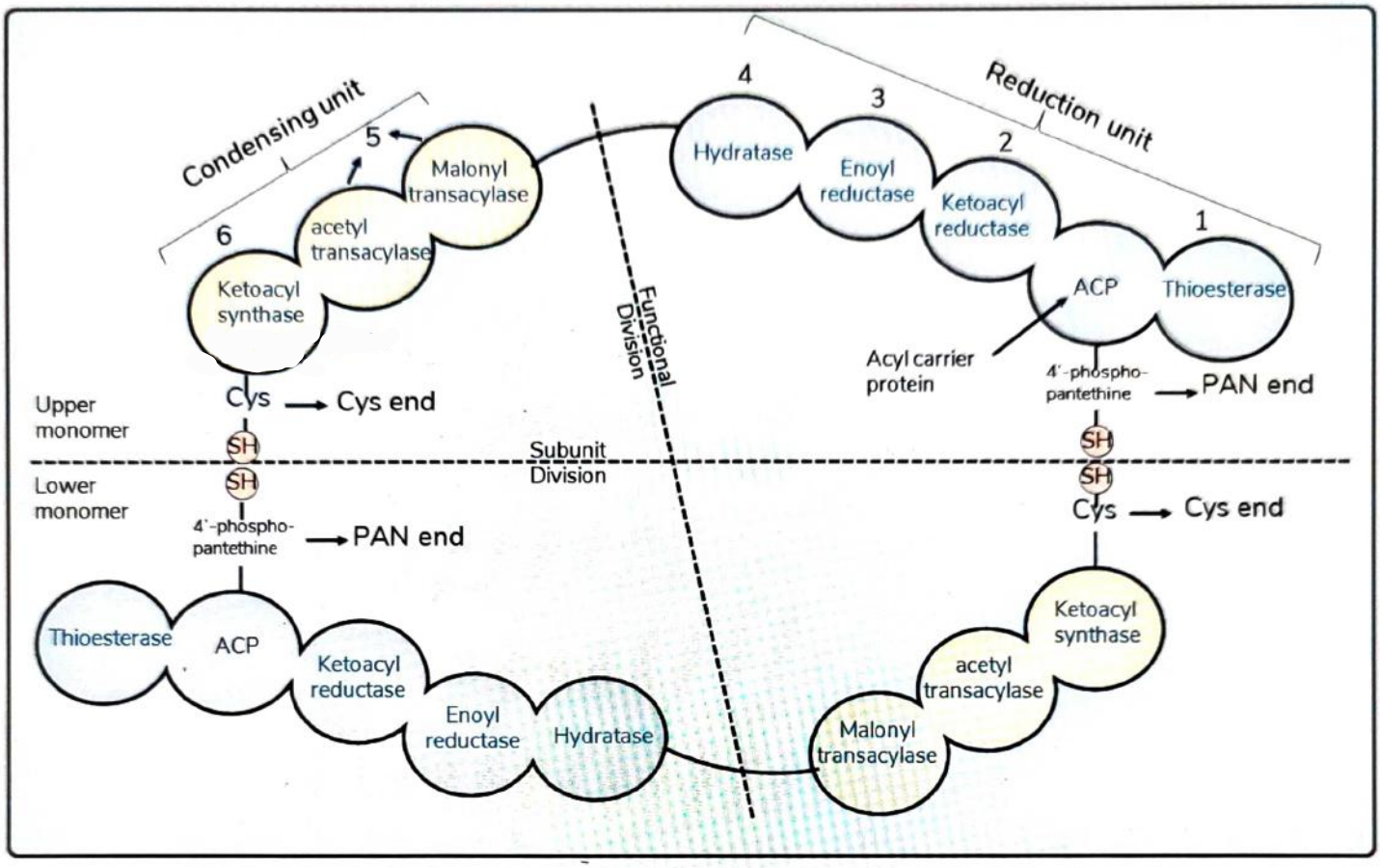
- TG = 3FAs + 1 Glycerol-3-P

Sources of Glycerol-3-P



- As, DHAP can only be obtained from glucose during glycolysis
- So, Adipose tissue strictly depend on glucose uptake for TG synthesis

Figure 72.1


Condensing unit
Reduction unit
6
5
4
3
2
1
Malonyl transacylase
acetyl transacylase
Ketoacyl synthase
Hydratase
Enoyl reductase
Ketoacyl reductase
ACP
Thioesterase
Functional Division
Acyl carrier protein
Upper monomer
Lower monomer
Subunit Division
Cys
Cys end
SH
4'-phospho-pantethine
PAN end
Thioesterase
ACP
Ketoacyl reductase
Enoyl reductase
Hydratase
Malonyl transacylase
acetyl transacylase
Ketoacyl synthase

CLINICAL QUESTIONS

Q. A newborn has severe respiratory problems and showed little development. In few days, he had severe muscle and neurological problems. His liver biopsy revealed normal enzyme levels of gluconeogenesis, glycolysis, the citric acid cycle, and the HMP shunt but a very low level of acetyl CoA carboxylase. The most likely reason for infant's respiratory problems is:

A. Glycogen depletion
B. Ketoacidosis
C. High levels of citrate
D. Low levels of phosphatidylcholine

Answer: D

Solution

Acetyl CoA carboxylase catalyze rate limiting step of formation of malonyl CoA in fatty acid synthesis pathway. So, the deficiency of acetyl CoA carboxylase reduces the ability of the patient to synthesize fatty acids.

The fetus utilizes fatty acids provided from mother. However, after birth the child must synthesize its own FAs and inability to do so can lead to growth retardation, muscle and neurological problems. In particular, the lungs require surfactant to function properly.

Surfactants are lipoproteinaceous substances synthesized in alveolar type II cells, which facilitate gas exchange by lowering alveolar surface tension. It contains significant amounts of dipalmitoyl phosphatidylcholine, the most common surfactant which requires palmitate formed mainly by de novo fatty acid synthesis.

Regarding other options:

As pyruvate carboxylase in gluconeogenesis is not affected so, there is no biotin deficiency
None of the other answers listed would result in all of the symptoms given.

Reference: Harper's 30th ed/pg. 250

73 KETONE BODY PATHWAYS

TWO PATHWAYS

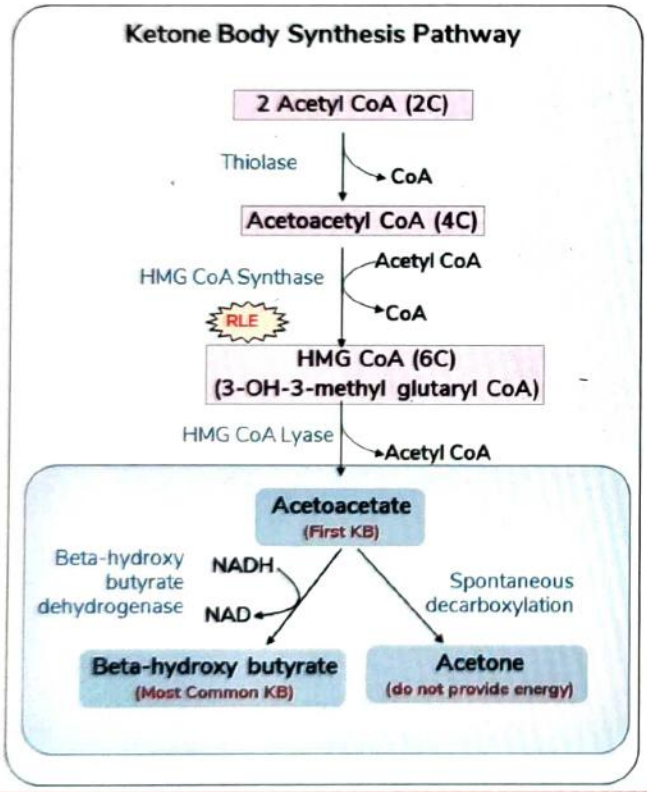
1. Ketone body synthesis
2. Ketone body utilization

Similarities 00:00:43

- Both are catabolic pathway
- Both occur in mitochondria
- Both are activated by glucagon and inhibited by insulin

Differences

- KB synthesis occurs only in Liver whereas KB utilization occurs in vital organs brain and heart & also in muscles but never occurs in liver. 00:02:13



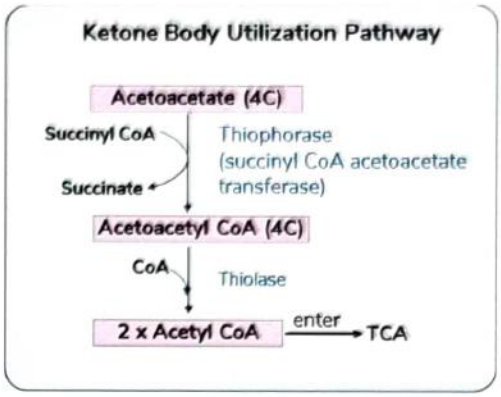
Important Information

Thiolase is a common enzyme for 4 lipid metabolic pathways:

1. Ketone body synthesis
2. Ketone body utilization
3. Cholesterol synthesis
4. β-oxidation of FA

- Acetoacetate is called as 1° KB whereas beta-hydroxybutyrate and acetone are 2° KBs.
- Acetone like smell or fruity odor in breath is diagnostic of ketoacidosis.

00:06:51



Important Information

- In first step of KB utilization, high energy CoA is added without using ATP. So, rule of business (i.e. activation using ATP) is not followed.
- Liver cannot use ketone bodies as it lacks thiophorase.

Previous Year's Questions

Q. Ketone bodies are not utilized by: (JIPMER May 2019)

A. Brain
B. RBC
C. Heart
D. Skeletal muscle

ENERGETICS OF KB UTILIZATION 00:10:29

From acetoacetate

- 2 acetyl CoA → TCA → 20 ATPs (each Acetyl CoA gives 10 ATPs in TCA cycle)
- But, 1 succinyl CoA is used which ~1 ATP used (succinyl CoA to succinate give 1 ATP)
- So, net yield of ATP = 20 - 1 = 19 ATPs

From beta-hydroxy butyrate

- Conversion of beta-hydroxy butyrate → acetoacetate produces = 1 NADH
- 1 NADH → ETC → 2.5 ATP
- So, net yield of ATP = 19.0 + 2.5 = 21.5 ATPs

TESTS FOR DETECTION OF KETONE BODIES

00:12:01

1. **Rothera's test**
- Purple colour ring at junction of 2 liquids = Positive test
- Given positive by Acetoacetate & Acetone

2. **Gerhard's test**
- Given positive by Acetoacetate only

3. **Test for β-Hydroxy Butyrate**: this is the main ketone body found in blood and urine of patients during ketoacidosis
- But no specific test is available for β-Hydroxy Butyrate
- So, in sample, β-Hydroxy Butyrate is first oxidized to acetoacetate first
- Then we can perform Rothera's or Gerhard's test.

CLINICAL QUESTIONS

Q. A 17-year-old girl with Type 1 diabetes contracted viral gastroenteritis which resulted in vomiting, nausea, difficulty taking fluids. She was not eating and forgot to take her insulin shots during her illness. She becomes weak and confused and is taken to the emergency room (ER). The doctor immediately notices a fruity odor to her breath which is due to:

A. Acetoacetate
B. Beta hydroxybutyrate
C. Acetyl-CoA
D. Acetone

Answer: D

Solution

Lack of food intake and missed insulin dose has caused diabetic ketoacidosis in the patient. The main fuel under such conditions is ketone bodies Acetoacetate, Beta hydroxybutyrate and acetone (See ketone body synthesis figure in text).

The fruity odor is due to acetone, which is being exhaled. The acetone is derived from the spontaneous decarboxylation of acetoacetate to acetone within the blood and tissues. Due to volatile nature of acetone, the breath of patient smells like acetone.

Reference: Harper's 30th ed/pg. 227

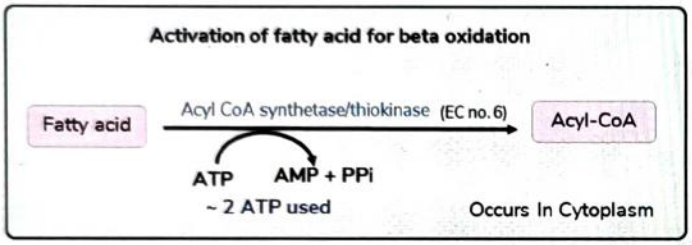
74 BETA-OXIDATION OF FATTY ACIDS

β-OXIDATION OF FATTY ACID

00:00:21

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

1. Rule of business / activation of FA

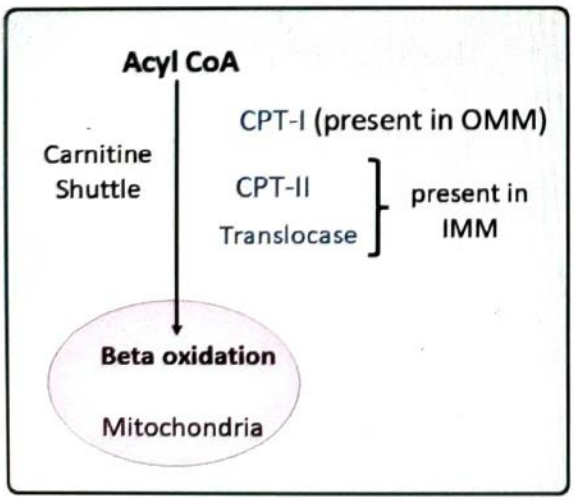


- This reaction serves as a control point for regulation of beta-oxidation in the body.
- Only at the time of necessity (starvation), β-oxidation will be initiated by body by allowing acyl CoA formed in cytoplasm to go into mitochondria.

CPT -1 (Carnitine Palmitoyl Transferase I)

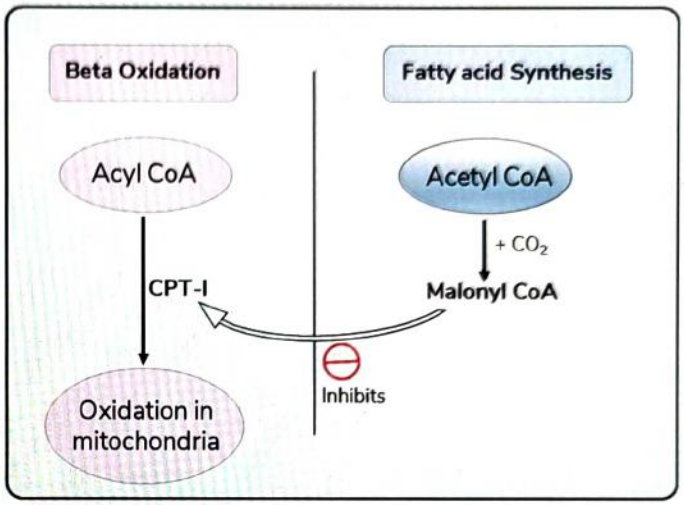
- Outermost enzyme which will decide the entry of Acyl CoA into mitochondria
- Only Long chain FA [14-20C] requires the carnitine system
- Medium chain (6-12C) and Short chain (2-4C) FA can directly enter into mitochondria
- CPT-I has a role in reciprocal regulation

00:03:12



RECIPROCAL REGULATION

00:05:59



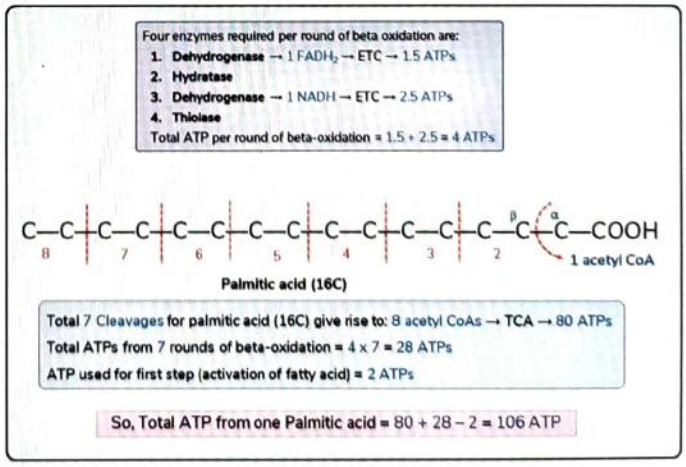
In fed state

- Malonyl CoA is formed from FA Synthesis
- Inhibits CPT-I
- That inhibits β-oxidation of FA

In starvation

- Malonyl CoA is not formed
- No inhibition on CPT-I
- β-oxidation of FA can occur

00:06:07

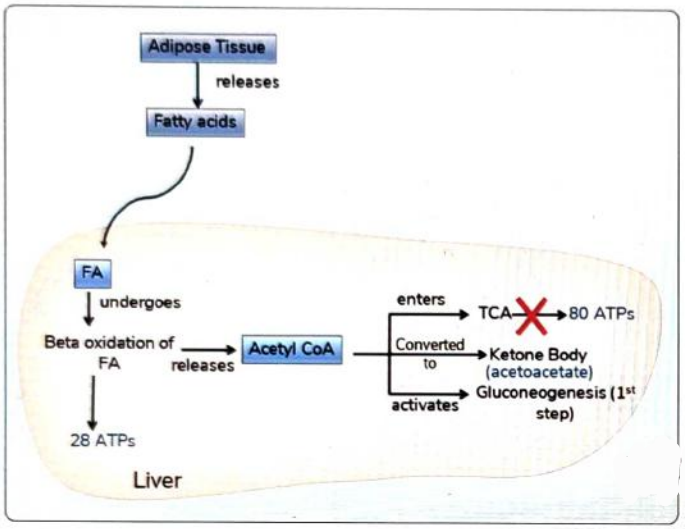


00:13:06

Q.In beta oxidation of palmitic acid if the final product is acetoacetate then net gain of ATP is

A.21

B. 26
C. 106
D. 129
Ans: B
Acetyl CoA will not enter TCA to produce 80 ATPs.



So, Total ATP from one Palmitic acid = 28 – 2 (used for activation) = 26 ATP

DEHYDROGENASES

00:14:50

1. LCAD (Long chain Acyl CoA Dehydrogenase) → Breaks up to 12C
2. MCAD (Medium chain Acyl CoA dehydrogenase)→ Breaks below 12C

MCAD Deficiency

- No symptoms in fed state (as β-oxidation is not required in fed state)
- Leads to non-ketotic hypoglycaemia during fasting/ starvation
  - Also, as the preferred route for acetyl CoA is TCA (refer to figure in question above)
  - So, Ketone body Synthesis and Gluconeogenesis will not occur (non-ketotic hypoglycaemia)
- Very Low in energy during fasting/starvation
  - Only LCAD is functional which can break up to only 12 C
  - So, very low amount of acetyl CoA will be formed in Liver
  - Low acetyl CoA will release very less energy from TCA cycle
- Dicarboxylic acidosis occurs chronically due to gradual accumulation of 12C FA in liver

TYPES OF HYPOGLYCEMIA

00:19:49

1. Non-ketotic hypoglycaemia

- Hypoglycemia without formation of Ketone body
- Causes
  1. Insulinoma
     - Insulin, an anabolic hormone, inhibits KB formation
     - So, high insulin will lead to low blood glucose but no KB
  2. Any defect in β-oxidation e.g.
     - Jamaican vomiting sickness
     - Carnitine deficiency
     - MCAD deficiency

2. Ketotic-hypoglycaemia

- Hypoglycemia along with formation of KB
  - Occurs in glycogen storage disease such as Von Gierke's Disease
  - Alcoholism
- Aka fasting hypoglycemia

JAMAICAN VOMITING SICKNESS

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

MINOR PATHWAYS FOR FATTY ACID OXIDATION

00:22:22

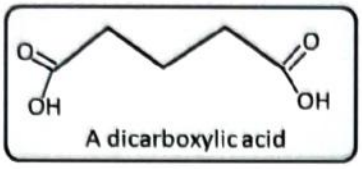
α - Oxidation

- Occurs in peroxisomes & ER
- For branched chain FA
- Removal of 1 carbon from α carbon atom
- NO ATPs produced
- **Refsum's disease**
  - Defect in α oxidation of peroxisomes
  - Phytanic acid not oxidised

  Rx: Restrict dairy products & green leafy vegetables (contain phytanic acids)

ω – Oxidation

- Dicarboxylic acids are formed
- 12 carbon FA accumulates in MCAD deficiency → ω oxidation → Dicarboxylic acids



A dicarboxylic acid

OXIDATION OF VLCFA (VERY LONG CHAIN FA) (>20C)

- VLCFAs are found in brain
- Oxidation is induced by high fat diet
- Oxidation occurs in peroxisomes up to octanoyl CoA & remaining occurs in mitochondria

Zellweger syndrome

- Defect in peroxisomes in all body cells causing defect in oxidation of VLCFA

Previous Year's Questions

Q. In Zellweger Syndrome, which of the following is absent? (NEET - Jan - 2019)

A. ER
B. Golgi apparatus
C. Mitochondria
D. Peroxisomes

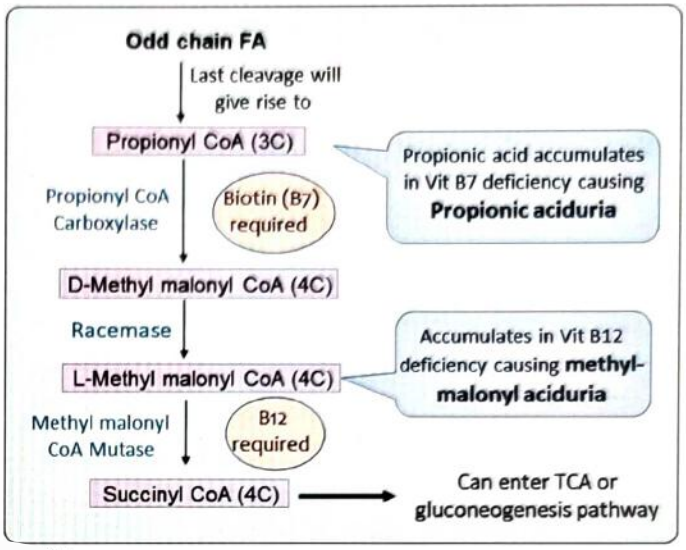
Previous Year's Questions

Q. In a cerebrohepatorenal syndrome, which of the following accumulate in brain? (AIIMS Nov 2018)

A. Pyruvate
B. Short-chain fatty acid
C. Very long-chain fatty acid
D. Acetyl CoA

OXIDATION OF ODD CHAIN FATTY ACID

00:26:08



CLINICAL QUESTIONS

Q. A 5-month-old boy was hospitalized following a seizure. He was not eating enough, prior to his hospital admission due to a stomach infection. The blood glucose reported at the time of admission was 28 mg/dl (normal= 60-100mg/dl). Urine analysis showed no ketone bodies, but presence of a variety of dicarboxylic acids. Finally, a diagnosis of medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency was made. In this patient, the best explanation for fasting hypoglycemia is:

A. Decreased ability to convert acetyl CoA to glucose
B. Increased production of ATP and NADH
C. Increased conversion of acetyl CoA to acetoacetate
D. Decreased acetyl-CoA production

Answer: D

Solution

MCAD deficiency results in decreased production of acetyl CoA, due to Impaired oxidation of medium chain fatty acids (<12 C in length). As acetyl-CoA is an allosteric activator of gluconeogenic enzyme pyruvate carboxylase, hence, glucose levels fall.

MCAD patients have non-ketotic hypoglycemia during starvation due to lack of availability of ketone body substrate acetyl-CoA. The dicarboxylic acid is produced as a result of omega oxidation of medium chain FA causing dicarboxylic acidosis.

Regarding other options:

Acetyl CoA can never be used for the synthesis of glucose due to irreversible nature of link reaction.
Acetoacetate is a ketone body, and with MCAD deficiency ketogenesis is decreased. Impaired fatty acid oxidation means that less ATP and NADH are made.

Reference: Harper's 30th ed/pg. 231

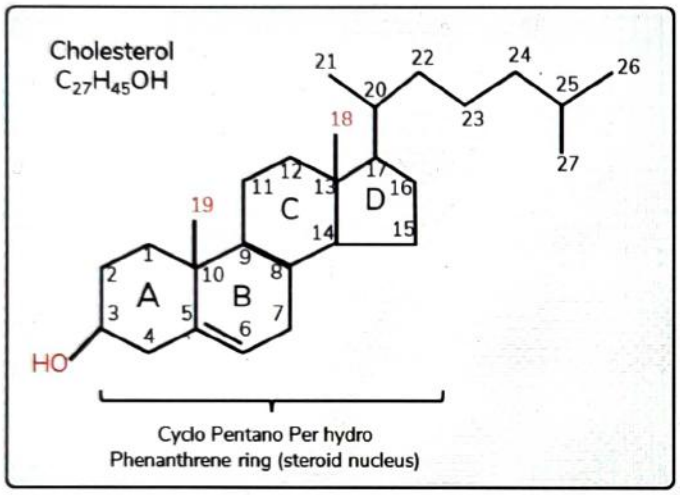
75 CHOLESTEROL SYNTHESIS

CHOLESTEROL

00:00:32

Structure

Contains Cyclo Pentane Per hydro Phenanthrene ring



- Steroid + ≥ 1 Alcohol = Sterol
- Sterols
  1. Cholesterol
     - 'Chole' means Bile
     - Exclusively present in animals
  2. Ergosterol
     - Ergus – fungus
     - Present in plants

Uses

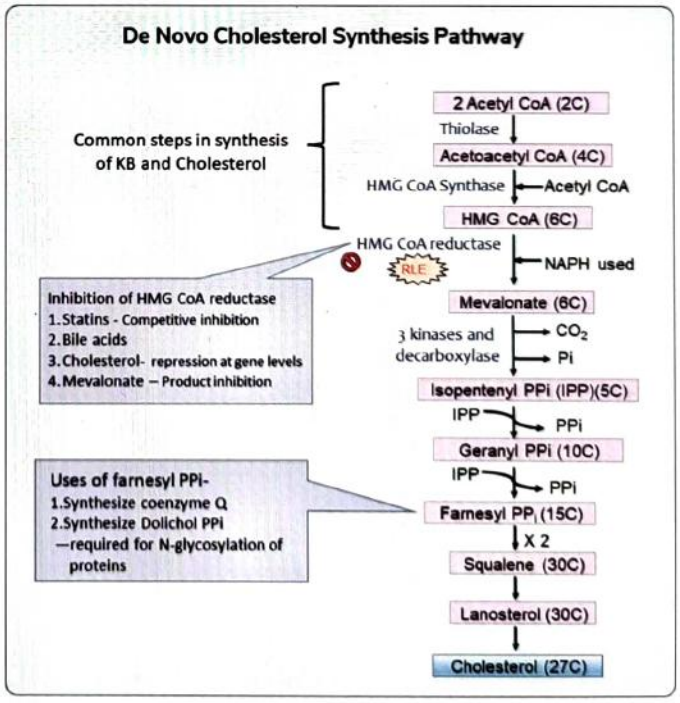
- maintains membrane Fluidity
- Required for the synthesis of
  - Vit D
  - Bile acids / Salts
  - Steroid hormones

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

Cell Compartments

- up to the formation of squalene → Cytoplasm
- Squalene to Cholesterol → Smooth ER

- Changes from Squalene to Cholesterol (In SER) → Total 19 reactions
  1. 30 C → 27 C
  2. 6 double bonds → 1 double bond
  3. Add OH to position 3
  4. Cyclization
  5. First Cyclic Compound formed → Lanosterol



DENOVO CHOLESTEROL SYNTHESIS

00:03:21

Nature of pathway

- Anabolic pathway
- activated by Insulin by Dephosphorylation of RLE HMG CoA reductase

- HMG CoA (β/3-OH-3-MethylGlutaryl CoA) is a ccommon metabolite for
  - Synthesis of KB and Cholesterol
  - Leucine catabolism

Important Information

Q. Statins treatment in few patients can cause Red colored urine?

- Statins will cause inhibition of synthesis of CoQ (required in ETC) → Low Muscle ATP formation → Lack of energy → Muscle cell damage → Myoglobin → Red colored urine

Previous Year's Questions

Q. Which of the following is not an enzyme in cholesterol biosynthesis? (AIIMS June 2020)

A. HMG CoA reductase
B. HMG CoA lyase
C. HMG CoA synthase
D. Thiolase

76 BILE ACIDS

BILE ACIDS

00:00:17

- In body, COOH group of bile acids is in ionized form (-COO)
- They combine with Na^+/K^+ to form salts known as Bile salts
- Bile acids are formed from cholesterol
- Changes in Cholesterol to convert to Bile acids
  1. α-Hydroxylation
  2. Reduction of B ring of cholesterol
  3. 27C reduced to 24C
  4. Oxidation of the terminal C to COOH group

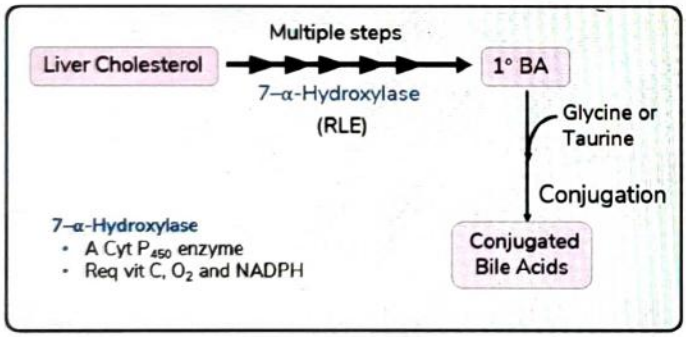
Uses

00:01:47

- Bile Acid formation is the only way of cholesterol excretion from body
- Digestion/ Emulsification of lipids and fat-soluble vitamins (due to their amphipathic nature)

Synthesis of bile acid from Cholesterol

00:02:26



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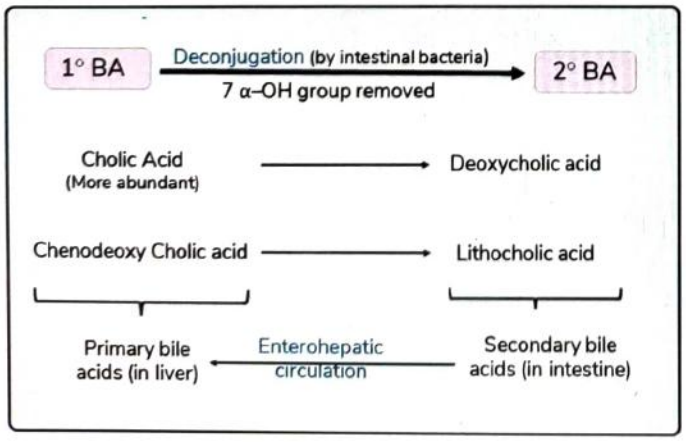
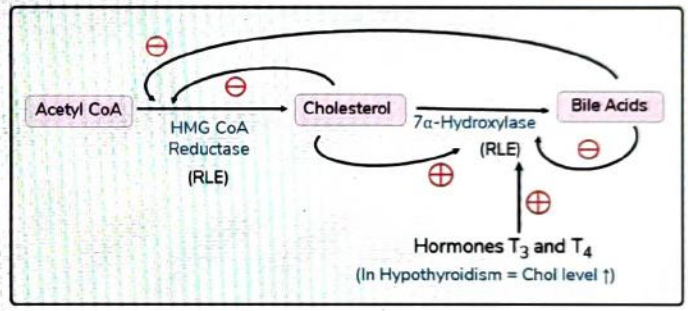


Important Information

- 98% - 99% of 2° BA undergoes Enterohepatic recirculation.
- Bile released from liver contains both 1° BA and 2° BA
- Bile acid with Least Enterohepatic recirculation → Lithocholic acid

Regulation of Bile acid synthesis

00:06:23



URSODEOXYCHOLIC ACIDS (2° BA)

00:07:39

- Ursus': means Bear (found in bear bile)
  - Very low concentration in human bile

Formation

Chenodeoxy Cholic acid (1°BA) → Ursodeoxycholic acid

Functions

- Hepato protective
- Modify BA pool
  - ↑ Hydrophilic BA pool, ↓ Hydrophobic BA pool
- Immunomodulatory
- Cytoprotective (Delay Gastroesophageal Varices)
- Can be used in treatment of
  - Primary Biliary Cirrhosis
  - Obstetric Cholestatis – To relieve itching
  - Cholesterol Gall Stones medically (non-surgically)
  - Cystic Fibrosis associated liver disease
  - Non-alcoholic fatty liver disease.

LEARNING OBJECTIVES

UNIT VII: MOLECULAR BIOLOGY

The unit on molecular biology starts with a chapter on nucleotide structure and metabolism with relevant details on purine and pyrimidine de novo and salvage pathways for biosynthesis and disorders associated due to enzyme deficiencies such as Lesch Nyhan syndrome, orotic aciduria and SCID. The 2nd chapter gives basic information on DNA structures and naming conventions for DNA and chromosomes. "Genetic disorders" chapter contain information on structural defects and single gene defects and along with important pointers on how to recognize various inheritance patterns. Mitochondrial DNA and associated diseases are discussed in chapter 80.

Chapters 81-83 deal with DNA replication and repair with focus on various enzymes involved in eukaryotes and prokaryotes. A short chapter klenow fragment provide details on method to synthesize it and it's uses in molecular biology techniques. DNA repair briefly discuss single and double strand break repair systems and disorders due to defective repair.

Chapters 84-88 discuss various types of RNA and mRNA synthesis by transcription process and post transcriptional modifications such as capping and tail addition and splicing in case of eukaryotic mRNA by RNA enzymes ribozymes. "Operon model" chapter discuss the regulation of transcription in E. coli using Lac operon as an example. Chapters 90-91 have information on protein synthesis process translation. Genetic techniques chapter briefly discuss important genetic techniques used for diagnosis of genetic disorders and analysis and modification of genome for practical purposes. Epigenetics and genome imprinting provide information on reversible regulation of gene expression by chemical modification and permanent inhibition of gene by imprinting. A new but widely popular technique on gene modification known as CRISPR is discussed in last chapter.

Major learning objectives

- To learn structure and properties of nitrogenous bases, nucleotides, DNA and RNA
- To understand processes and enzymes involved in DNA replication and repair
- To know various RNA types, introns, process of transcription and post transcriptional modification to synthesize mature RNA
- To understand the operon concept for regulation of gene transcription
- To study processes and enzymes involved in translation and protein synthesis
- To learn genetic techniques for study of genetic disorders and other molecular biology processes
- To know epigenetic and genome imprinting for regulation of gene expression

77 NUCLEOTIDES

Nucleic Acid (DNA/RNA)= Polymers of Nucleotides

Components of Nucleotides 00:01:00

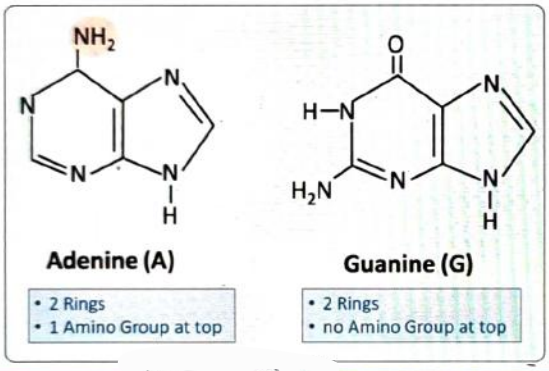
1. Nitrogenase base
2. Sugar
3. Phosphate

NuceloSide = Nitrogenase base + Sugar

So, Nucleotides = Nucleoside + Phosphate

NITROGENOUS BASES 00:02:38

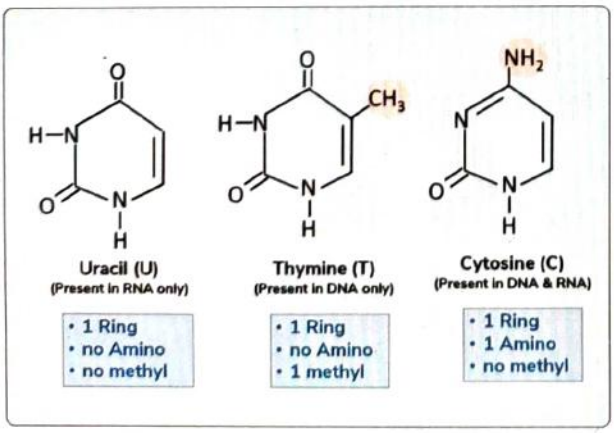
1. Purines



Important Information

An amino group is also present in Guanine. So, be aware that you need to look at the top position of hexacyclic ring for presence or absence of amino group

2. Pyrimidines 00:04:19



How to remember

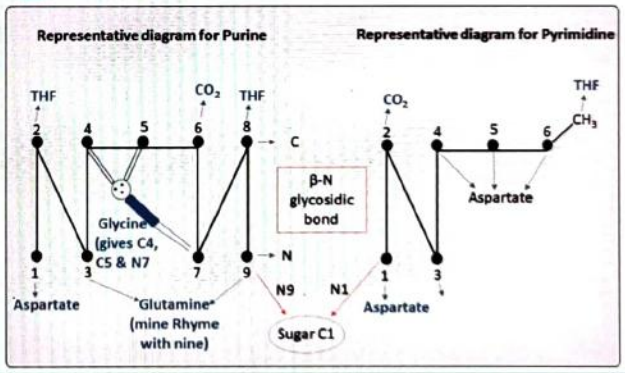
Thymine matching with Methyl

Important Information

Thymine (Pyrimidine) is different from Thiamine (vitamin B1)

Source of C & N atoms of Purine and Pyrimidines 00:07:01

How to remember



Summary 00:21:39

Purine		Pyrimidine	
N1	Aspartate	N1, C4, C5, C6	Aspartate
N3, N9	Glutamine	N3	Glutamine
C4, C5, N7	Glycine	C2	CO_2
C6	CO_2	Extra CH_3	THF
C2, C8	THF		

- beta-N-glycosidic bond
- b/w C1 of sugar & N9 of purine
- b/w C1 of sugar & N1 of pyrimidines

Important Information

Product of catabolism of N-bases

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

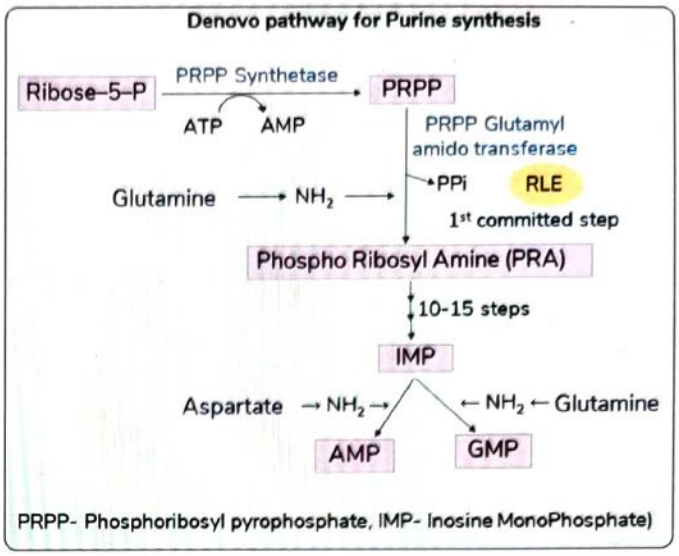
Nomenclature of Nucleotides 00:24:19

N–base	Nucleoside	Nucleotide form
Adenine	Adenosine	AMP, ADP, ATP, dAMP, dADP, dATP
Guanine	Guanosine	GMP, GDP, GTP, dGMP, dGDP, dGTP
Cytosine	Cytidine	CMP, CDP, CTP, dCMP, dCDP, dCTP
Uracil	Uridine	UMP, UDP, UTP (deoxy formsNA)
Thymine	Thymidine	dTMP, dTDP, dTTP (ribose forms NA)

PURINE SYNTHESIS 00:29:30

1. Denovo pathway

- 15-20 steps
- High energy consuming pathways
- Ribose–5–P
  - Act as primer
  - Only source is HMP



Important Information

PRPP is used in synthesis of

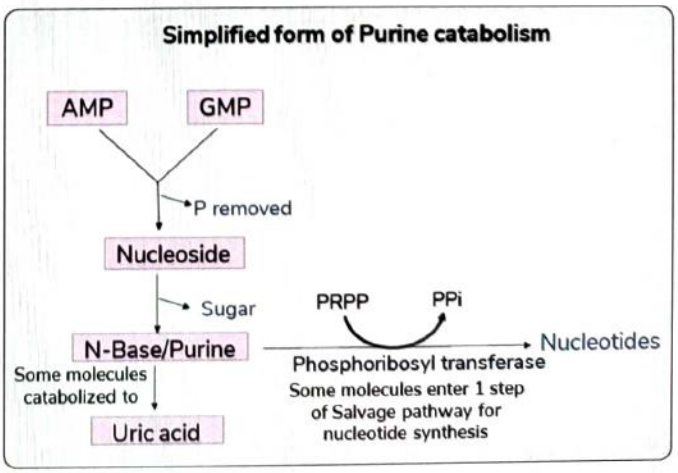
1. Purine (denovo/salvage)
2. Pyrimidines
3. Histidine
4. Niacin

IMP

- Ist nucleotide to be formed
- It is a parent nucleotide which further give rise to AMP & GMP
- Nitrogenous base in IMP is Hypoxanthine

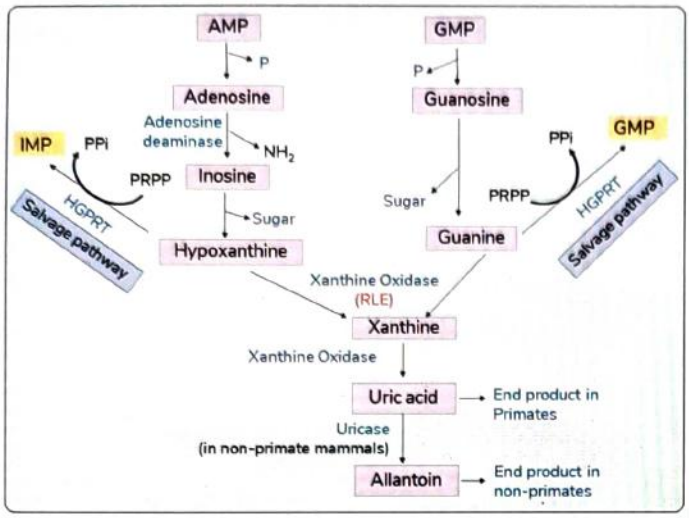
1. Salvage pathway 00:37:30

- Less energy consuming pathway (only 1 step)
- Salvage means"Saved from degradation" i.e. not allowed to go to catabolism
- Occurs in RBC, WBC, brain & bone marrow



PURINE CATABOLISM

00:41:07



Xanthine Oxidase

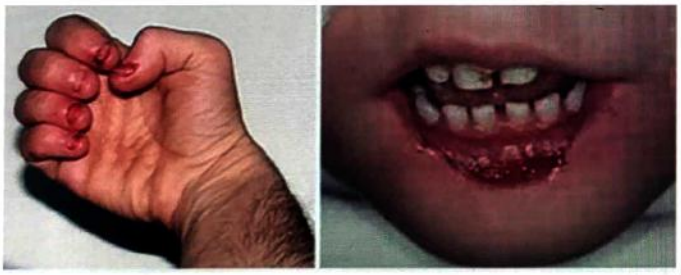
- RLE
- Inhibited by Allopurinol (Suicidal inhibitor of Xanthine Oxidase)
- Allopurinol used for Gout treatment

Purine Catabolism Disorders

00:44:58

Lesch Nyhan Syndrome

- Complete deficiency of HGPRT
- Gout d/t ↑↑ Nitrogenous bases ↑↑ Uric acid
- Self-mutilation d/t ↑ PRPP (neurotoxic)



Kelly Seegmiller Syndrome

- Partial deficiency of HGPRT
- Only gout present

Adenosine Deaminase (ADA)

00:47:26

- Used for conversion of adenosine to Inosine by removal of amino gp
- Important in B & T Lymphocytes
- Easily measured in any fluid of the body
  - if ↑ADA→ Suggestive of TB
  - if ↓ADA
    → Both B & T Lymphocytes affected
    → Leads to Severe Combined Immunodeficiency (SCID)

Previous Year's Questions

Q. A patient has swelling in MCP joints. Serum uric acid levels were raised. Doctor will prescribe medicine against which enzyme:

(FMGE Aug 2020)

A. Thymidylate synthase
B. Xanthine oxidase
C. ADA (Adenosine Deaminase)
D. HGPRT

Previous Year's Questions

Q. The enzyme deficient in Lesch Nyhan syndrome is:

(NEET May 2018)

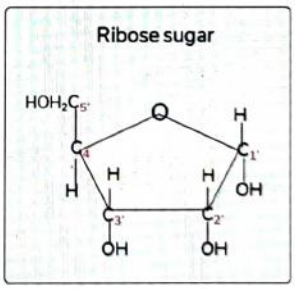
A. Adenosine Deaminase
B. PRPP synthetase
C. HGPRTase
D. Xanthine oxidase

SUGAR

00:49:34

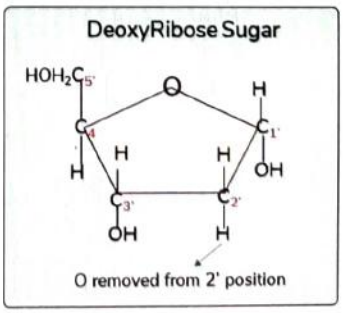
- Present in the form of pentose sugar
- 2 types: Ribose and Deoxyribose

1. Ribose

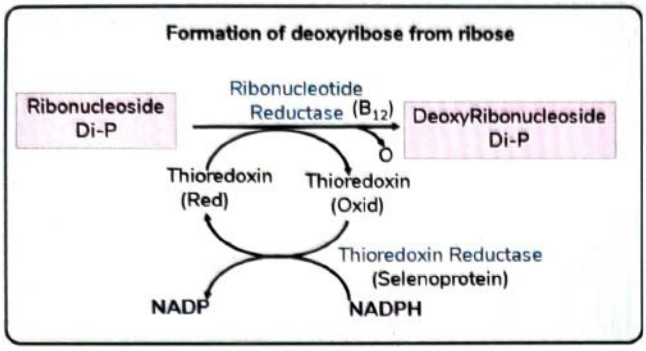


- 5C, 4OH, Furanose form and Aldehyde
- O is shared b/w functional & second last carbon

2. Deoxyribose



- One O atom is removed from OH of 2nd position carbon
- Rest of structure is same as ribose

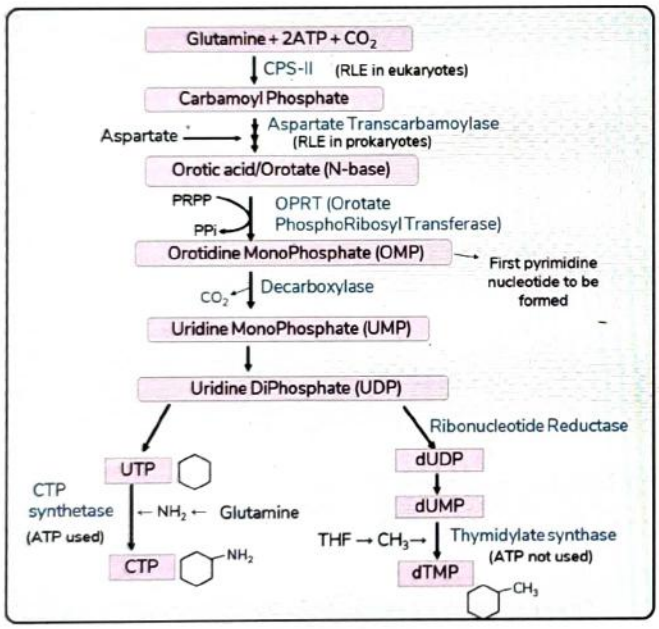


Q. Ultimate donor of hydrogens for the conversion of Ribonucleoside Di-P to Deoxyribonucleoside Di-P?

Ans: NADPH

PYRIMIDINE SYNTHESIS

00:56:43



OPRT + Decarboxylase

01:00:00

- Bi-functional enzyme (single protein with 2 enzymatic activities)
- Deficiency leads to Orotic Aciduria

Orotic aciduria

- Type I: both enzymes are deficient
- Type II: only one enzyme is deficient (mostly Decarboxylase)
- C/F
  - Growth Retardation and developmental delay
  - Neurological defects
  - Megaloblastic anaemia which is non-responsive to B_{12} or folic Acid R_x
- R_x: Give only Uridine as other nucleotides can be synthesized from this

Important Information

- **Conversion of U to C [UTP to CTP] occurs at the level of Triphosphate**
- **Conversion of U to T [UTP to TMP] occurs at the level of Monophosphate**

CLINICAL QUESTIONS

Q. A 17- month- old girl suffered from recurring respiratory infections. Injection of Diphtheria-Pertussis-Tetanus (DPT) and Typhoid vaccine produced only a minimal response. The lysate of girl's erythrocytes was found to lack detectable adenosine deaminase activity. Her mother and father both showed approximately 50% of the normal red cell adenosine deaminase activity. Diagnose the disease.

A. SCID
B. Crushing Muscular Trauma
C. Lesch –Nyhan Syndrome
D. Hypokalemia

Answer: A

Solution

Severe combined immunodeficiency (SCID) occurs due to deficiency of enzyme ADA which convert adenosine to inosine (and deoxyadenosine to deoxyinosine). In the absence of this enzyme an accumulation of deoxyadenosine occurs. Deoxyadenosine is toxic and will accumulate in the blood cells, eventually forming dATP through salvage reactions. The dATP will, in part, inhibit ribonucleotide reductase, and the cells with the high dATP levels will not be able to proliferate when signaled to do so due to the lack of deoxyribonucleotide precursors leading to lack of DNA synthesis in immune cell precursors. In SCID, the thymus is virtually absent, and there is virtually no T- cell and B-cell production.

Regarding other options:

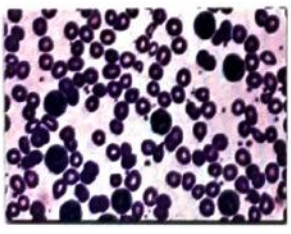
In Crushing Muscular Trauma, muscle cell damage results from pressure or crushing of skeletal muscles.

Lesch–Nyhan syndrome occurs due to deficiency of enzyme Hypoxanthine Guanine Phospho Ribosyl Transferase (HGPRT). Symptoms include hyperuricemia and self -mutilation.

Hypokalemia is a condition due to a reduction in normal potassium values. Owing to low serum potassium levels, potassium leaves the cells and is replaced by protons from the circulation. The loss of protons from the blood leads to the alkalosis.

Reference: Harper's 30th ed/pg. 341

Q. A 4-year-old girl presented to hospital with failure to thrive. Her peripheral blood smear is shown in figure. Vitamin B_{12} & folate were given but did not improve condition. Enzyme assay from the cultured PBMC showed deficiency of OPRT (Orotate Phospho Ribosyl Transferase). What could be the probable diagnosis?

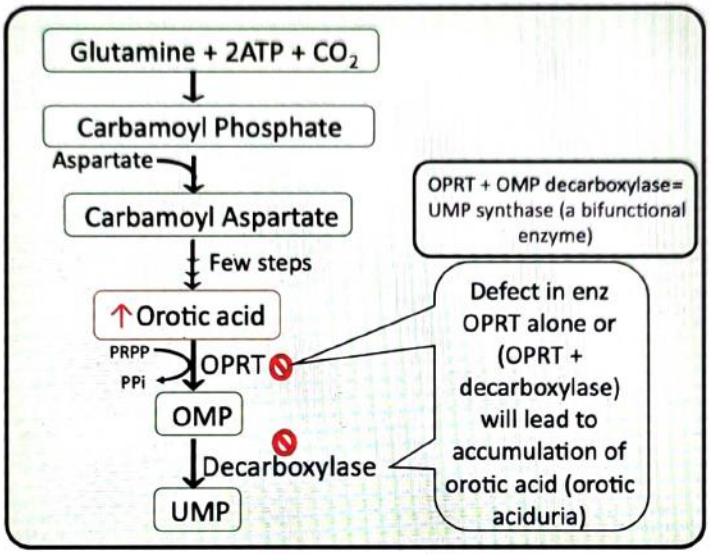


A. Orotate deficiency
B. Gout
C. Orotic aciduria
D. SCID

Answer: C

Solution

Orotic aciduria occurs due to deficiency of orotate phosphoribosyl transferase and OMP decarboxylase and leads to defect in DNA Synthesis. Clinical features include excretion of orotic acid in urine, severe anemia: Megaloblastic and not improved with folate/vitamin B_{12}/vitamin C supplementation and retarded growth



Rx: Feeding diet rich in uridine / cytidine

Reference: Harper's 30th ed/pg. 727

Q. A 3-year-old boy exhibiting developmental delay mental retardation, poor muscle control, gout, chronic renal failure and has started to bite his lips and fingers. The blood examination showed high levels of uric acid and he is diagnosed with Lesch-Nyhan syndrome. This child has an inability to catalyze which one of the following reactions?

A. Thymine + Deoxyribose 1-phosphate → Deoxythymidine + PPi
B. Adenine + 5'-PRPP → AMP + PPi
C. Uracil + Ribose 1-phosphate → Uridine + Phosphate
D. Guanine + 5' PRPP → GMP + PPi

Answer: D

Solution

Lesch–Nyhan syndrome occurs due to a deficiency of enzyme HGPRT that converts the free bases hypoxanthine, or guanine, plus PRPP (5'-phosphoribosyl 1'-pyrophosphate) to the nucleotides IMP, or GMP, plus pyrophosphate (see fig purine catabolism in text).

Regarding other options:

The reactions in answer choices a) and c) are part of the pyrimidine salvage pathways, using pyrimidine nucleoside phosphorylase, in which a nucleoside is formed from the free base and (deoxy)ribose 1-phosphate.

The reaction in the choice b) is catalyzed by adenine phosphoribosyltransferase, and is analogous to the HGPRT reaction, other than that adenine is the substrate, and not hypoxanthine or guanine.

Reference: Harper's 30th ed/pg. 820

78 BASICS OF GENETICS

NUCLEIC ACID

00:00:30

DNA (Deoxyribo Nucleic Acid)	RNA (Ribo Nucleic Acid)
• Double stranded (ds) • Has A T C G • Has Deoxyribose sugar	• Single stranded (ss) • Has A U C G • Has ribose sugar

Previous Year's Questions

Q. The bases which are present in human DNA? (FMGE Dec 2019)

A. Adenine-guanine-thiamine-uracil
B. Adenine-guanine-cytosine-uracil
C. Adenine-guanine-cytosine-thiamine
D. Adenine-inosine-cytosine-thiamine

00:01:48

Important Information

Q. Main difference b/w DNA & RNA?
A. Sugar
B. Nitrogenous base
Because most of the nitrogenous bases in both DNA and RNA are same. So, the main difference is sugar.

Absorption of UV-light

00:03:05

- Due to conjugated double bonds present in the ring.
- Nucleic acids absorb UV light at 260 nm due to Nitrogenous bases.
- This absorption is more for Purines as compared to pyrimidines.
- Amino acids and proteins also absorb UV light at 280 nm
  - It is due to aromatic amino acids
  - maximum for tryptophan
- NAD and NADP absorb light at 340 nm
- Porphyrin absorb at 400 nm (Sorett Band)

Previous Year's Questions

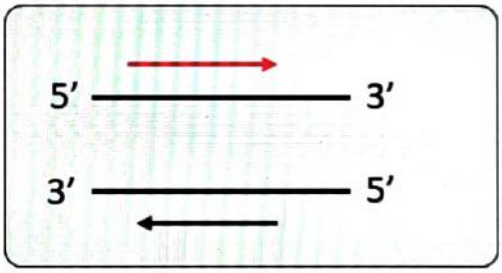
Q. Amino acid which absorbs UV light at 280 nm? (NEET 2020)

A. Tryptophan
B. Histidine
C. Aspartate
D. Ornithine

DNA

00:05:13

- Right-handed
- ds in both Prokaryotes and Eukaryotes
  - Prokaryotes DNA is circular (two ends are joined to form closed circle)
  - Eukaryotes DNA is linear (two ends are free)
- 2 strands are Anti-parallel



DNA Replication

00:07:02

- Occurs very fast due to bidirectional nature from Ori (Origin of replication)
  - In prokaryotes there is only 1 origin of replication
  - In eukaryotes there are multiples Origin of replications

00:08:45

Important Information

Chargaff's Rule
- No. of Purines = No. of Pyrimidines
  - A = T and G = C
  - A + G = T + C

Previous Year's Questions

Q. Adenosine is 28%, what is the % of cytosine?
(NEET 2020)

A. 22%
B. 26%
C. 44%
D. 56%

Types of DNA

00:09:52

B DNA	A DNA	Z DNA
MC type and Most stable from	Present in RNA-DNA duplex	Has zig-zag backbone
10 bp per turn	11 bp per turn	12 bp per turn
Right-handed	Right-handed	Left-handed
Found in Hydrated environment	Found in Dehydrated environment	Found In the area of Regulation of gene expression

Nucleosomes = DNA + Histone proteins

00:11:40

- **Histone proteins**
  - Rich in Basic AA
  - Carry positive charge
- **DNA**
  - Has PO_4^{2-} groups
  - So, carry negative charge
- DNA and Histones are tightly packed due to interaction between opposite charges
- **Heterochromatin**
  - When DNA is bound to histones it is not free for transcription
  - Due to tightly packed nature, genes on this DNA are inactive
  - It is darkly stained DNA

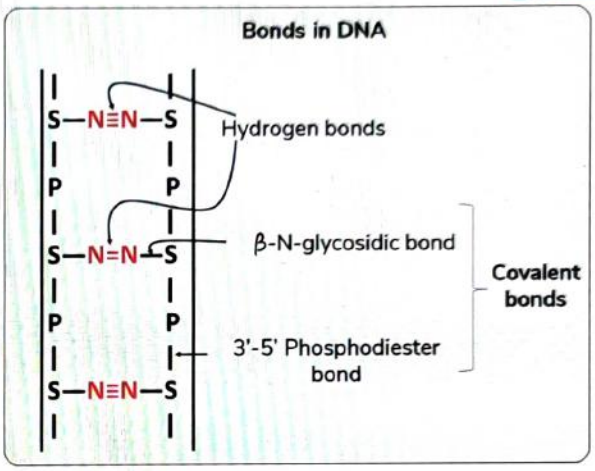
PTM's of Histones

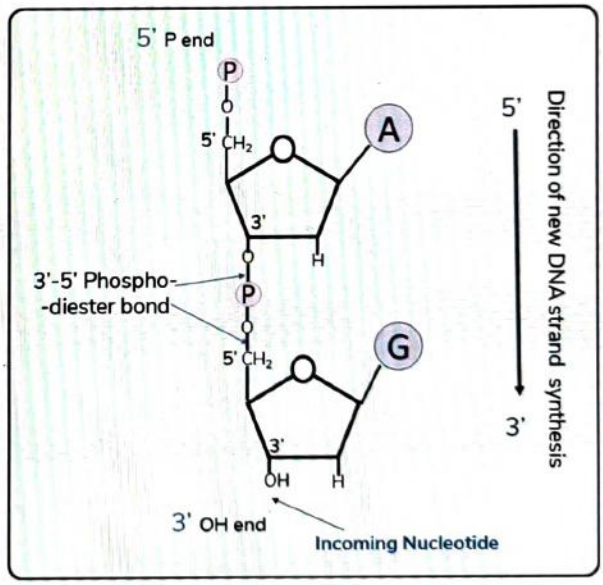
00:13:50

- PTM means Post Translational Modifications
- Helps in regulation of gene expression
- Various ways of histone modifications are:
  - Acetylation (addition of CH_3COO^-)
  - Phosphorylation (addition of PO_4^{2-})
  - Methylation
  - ADP Ribosylation
  - Sumoylation
  - Mono ubiquitylation
- Acetylation and Phosphorylation of histone adds negative charge
  - This -ve charge repels negative charge of DNA.
  - So, DNA become free from histones and available for transcription.
  - So, PTMs are one way of regulation of gene expression
  - Acetylation & Phosphorylation of Histones leads to Euchromatin formation
  - Euchromatin is loosely packed DNA and appear lightly stained
- Methylation of DNA leads to Genes inactivation

Bonds in DNA

00:18:39





00:25:34

Important Information

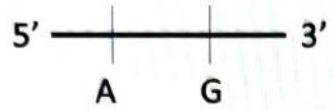
Di-deoxynucleotide

- If a di-deoxynucleotide is added in the growing nucleotide chain, it itself can be added but, it will not allow next incoming nucleotide to be added causing stop of DNA synthesis.

00:27:45

Important Information

For a new DNA molecule



Q1. Which is correct?
A. 5' AG 3'
B. 3' GA 5'
C. Both

Q2. Which is correct?
A. AG
B. GA
C. Both

If the direction not given, then Left (A) is 5' and Right (G) is 3'

NUCLEASES

00:31:30

- Two types

1. Exonucleases	2. Endo/Exci nucleases
• Cutting from sides • 5' →3' Exonucleases • 3' → 5' Exonucleases	• Cut anywhere in between • Special type is Restriction endonuclease that cut at a specific site → Palindromes

00:33:24

Important Information

Q. Which of the following is a palindrome?
A. TAAT
B. GGCC

Palindrome definition: same sequence on both strands (when read in 5' to 3' direction)
5' TAAT 3'
3' ATTA 5'

- not same when read in 5' to 3' direction
- So, TAAT is not a palindrome

5' GGCC 3'
3' CCGG 5'

- both strands Read same (GGCC) in 5' to 3' direction
- So, it's a palindrome

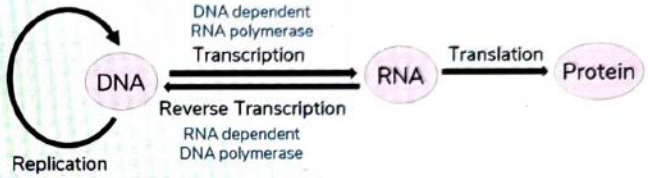
Previous Year's Questions

Q. Restriction endonuclease will act on which of the following: (AIIMS Nov 2019)

A. AAGCTT
B. AAGAAG
C. TACGAG
D. GAGAGG

00:36:32

Central Dogma of Molecular Biology



CHROMOSOMES

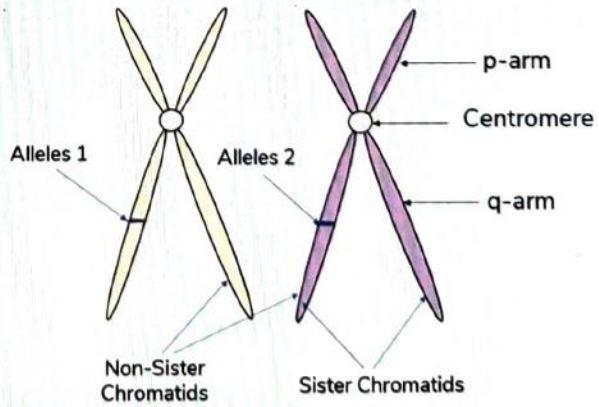
00:38:37

- 23 chromosomes = 22 Autosomes + 1 sex chromosome

Haploid state (n)	Diploid state (2n)
• 23 chromosomes • Present in Germ Cells	• 23 pairs of chromosomes • Present in somatic Cells

00:39:51

Homologous Chromosomes: A pair of chromosomes one derived from father side and other from mother side



Same Alleles: AA or aa (Homozygous condition)
Different alleles: Aa (Heterozygous condition)

Alleles	Genotype and Expression
A (Dominant allele)	Can express in both homozygous (AA) as well as heterozygous (Aa) condition
a (Recessive allele)	Can express only in homozygous (aa) but not in heterozygous (Aa) condition

Types of chromosome: Depending upon position of centromere 00:44:32

Centromere Position	Chromosome	Diagram	Example
Centre	Metacentric		Primitive, Chr 1,3
Near the centre	Submetacentric		most of human autosome & X chr
Close to end	Acrocentric		Y chr and some autosomes (13, 14, 15, 21, 22)
At the end	Telocentric		Not present in human

BARR BODIES 00:46:28

- Inactive condensed X chromosome
- Number of Barr Bodies = No. of X chromosomes - 1

	Genotype	No. of Barr Bodies	Phenotype
Normal Male	XY	0	Normal
Normal female	XX	1	Normal
Turner Syndrome	XO	0	Female with no Barr body
Klinefelter's Syndrome	XXY	1	Male with Barr body
Super Female	XXX	2	Female with two Barr bodies

ONE GENE ONE PROTEIN THEORY 00:48:18

- Says that one gene can give rise to one protein
- However, 20,000 gene in humans give rise to 2.5 lakh proteins that cannot be explained by this theory.
- So, there are two exception to one gene protein theory
  1. Alternate splicing
  2. RNA Editing

CLINICAL QUESTIONS

Q. Analysis of one strand of a double-stranded piece of DNA displayed 25 mol% A, 20 mol% T, 35 mol% G, and 20 mol% C. Which one of the following accurately represents the composition of the complementary strand?

A. [A] is 25 mol%, [T] is 20 mol%, [G] is 35 mol%, and [C] is 20 mol%.
B. [A] is 20 mol%, [T] is 25 mol%, [G] is 20 mol%, and [C] is 35 mol%.
C. [U] is 20 mol%, [T] is 25 mol%, [G] is 20 mol%, and [C] is 35 mol%.
D. The composition of the complementary strand cannot be determined from the data given.

Answer: B

Solution

In a double stranded DNA, Chargaff's rule is followed. That is the base pairs in double-stranded DNA require that [A] = [T], and [C] = [G].

Therefore, if the concentration of A in one strand is 25 mol%, the concentration of T in the complementary strand must also be 25 mol%.

Hence the base composition of complementary strand is:

In given strand	In complementary strand
A = 25	T = 25
T = 20	A = 20
G= 35	C= 35
C= 20	G = 20

Reference: Harper's 30th ed/pg. 360

Q. If one strand of DNA contains the sequence "ATCGCGTAACATGGATTCGG", what will be the sequence of the complementary strand using the standard convention?

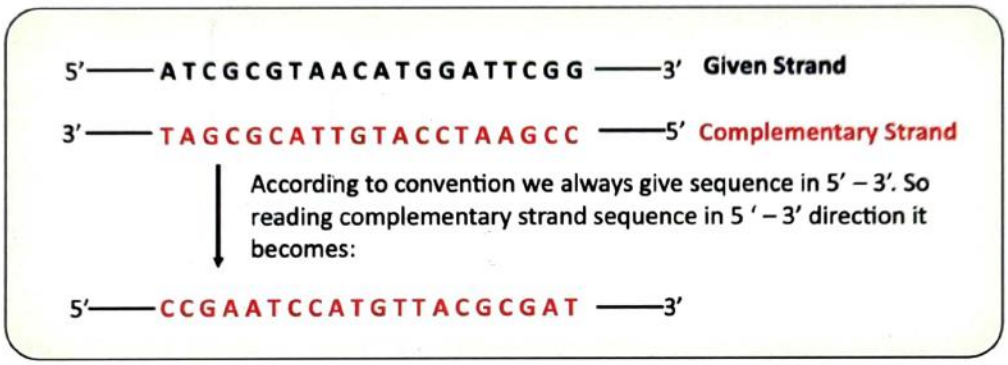
A. TAGCGCATTGTACCTAAGCC
B. CCGAATCCATGTTACGCGAT
C. ATCGCGTAACATGGATTCGG
D. None of the above

Answer: B

Solution

If one strand of DNA contains the sequence 5' ATCGCGTAACATGGATTCGG 3' the sequence of the complementary strand will be 5' CCGAATCCATGTTACGCGAT 3'

- DNA and RNA are directional molecules. They are always written in the sequence of 5' to 3'.
- Phosphodiester bonds link the 3' and 5' carbons of adjacent monomers. Each end of a nucleotide polymer thus is distinct.
- We therefore refer to the "5' end" or the "3' end" of a polynucleotide, the 5' end being that with a free or phosphorylated 5' hydroxyl group.
- DNA sequences are complementary and antiparallel to each other.
- So, the complementary sequence of ATCGCGTAACATGGATTCGG will be CCGAATCCATGTTACGCGAT.

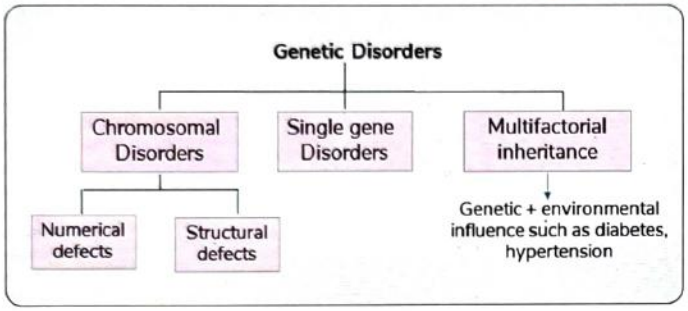


Note:In question or answer option if nothing is mentioned about the direction of the sequence, you have to automatically assume that they are asking in 5'-3' direction as it is internationally accepted convention.

Reference: Harper's 30th ed/pg. 345

79 GENETIC DISORDERS

00:00:20



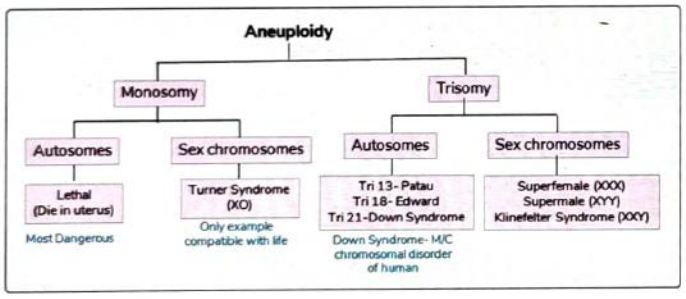
CHROMOSOMAL DISORDERS/ DEFECTS

00:01:18

1. **Numerical Defects**

- Normal number of chromosomes → Euploidy
  - Haploid = n = 1 set of 23 chromosomes
  - Diploid = 2n = 2 set of 23 chromosomes
- Abnormal (not in multiple of n) → Aneuploidy

00:03:06



00:06:37

2. Structural Defects

1) Insertion deletion of > 1 gene

2) Point mutation: only 1 base change
- Transition: Purine is replaced by Purine
- Transversion: Purine is replaced by Pyrimidine or vice versa
- Silent Mutation: Amino acid not changed
- Missense mutation: AA changed
- Non-sense Mutation: Insertion of stop codon

3) Frameshift Mutation: when insertion or deletion is not in multiple of 3
- Whole codon reading frame is shifted leading to misreading of entire gene

Important Information

Severity of Damage
Frameshift> Non-sense > Missense

4) Inversion: alteration in the position of genes within the same chromosome
5) Translocation: Exchange of gene material between two different chromosomes e.g. T (8, 14) Burkitt lymphoma.

Previous Year's Questions

Q. Type of mutation seen in sickle cell anaemia: (AIIMS May 2019)

A. Insertion
B. Deletion
C. Point mutation
D. Frameshift mutations

SINGLE GENE DISORDER

00:12:24

1. Classical /Mendelian inheritance disorders	2. Non-Mendelian inheritance diorders
• Autosomal dominant • Autosomal recessive • X-linked dominant and recessive • Y-linked	• Mitochondrial Disorders • Genomic imprinting • Trinucleotide repeat expansion disorders • Germline Mosaicism

00:14:16

AD (Autosomal Dominant)

- Familial hyper Cholesterolemia

AR (Autosomal Recessive)

- Most of biochemical enzyme defects
- All MPS disorder's except Hunter
- All glycogen storage disorders
- All urea cycle disorder's except OTC
- All sphingolipidosis except Fabry's disease
- All amino acid disorders e.g. PKU, Albinism, HCU etc
- Orotic aciduria
- ADA deficiency
- Wilson's disease
- Hemochromatosis

X-linked Recessive

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

Important pointers to determine inheritance patterns

00:17:37

If both male & female are affected with equal frequency	Autosomal disorder
If atleast one parent is affected	Dominant disorder
If neither of parent is affected (heterozygous (Aa) carrier parents)	Recessive disorder
If mother to all offspring	Mitochondrial Inheritance
If transmitted from father to all sons	Y-linked
If there is no male to male transmission	X-linked recessive
More males are affected, and affected son born to unaffected mother (carrier)	X-linked recessive

Previous Year's Questions

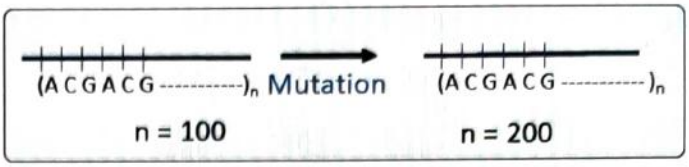
Q. Mother side uncle has disease... Her son has disease. Which type of inheritance is this?
(FMGE Aug 2020)

A. X-linked recessive
B. X-linked dominant
C. Autosomal dominant
D. Autosomal recessive

TRINUCLEOTIDE REPEAT EXPANSION

00:20:55

- Change in number of trinucleotides (e.g. ACG) sequence repeats.



- Causes severe neurological defects. e.g.
  - Huntington disease
  - Fragile X syndrome
  - Friedrich ataxia
  - Myotonic dystrophy

GERMLINE MOSAICISM

00:22:36

- Parents not affected i.e. no defective alleles present in parents
- Mutation occur after zygote is formed (postzygotic mutation in child)
- Child affected first will have normal somatic cells but germ cell will have the mutated gene
- This child has mosaic DNA i.e. different DNA in somatic and germ cells
- This child will pass disease to next progeny and next progeny will be affected

CLINICAL QUESTIONS

Q. A patient suffering from severe pain throughout his body was brought to hospital. He regularly has such episodes in last few years. The pain starts on vigorous exercising. Blood analysis showed a reduced blood cell count (anemia), elongated red blood cells rather than concave. He was diagnosed with Sickle cell anemia. The type of mutation seen in sickle cell anemia is:

A. Point
B. Insertion
C. Deletion
D. Frame shift

Answer: A

Solution

Sickle-cell anemia occur due to a single base alteration which is called point mutation. Due to this mutation in the gene the resulting protein which originally should have glutamate at the 6th position of β-chain of hemoglobin is now replaced by Valine by change of valine codon (GTG) for glutamate codon (GAG).

Point mutation: Alteration in a single base is point mutation. This is of two types depending on the type of base replaced.
a. Transition: Purine replaced by purine or pyrimidine replaced by pyrimidine.
For example: A ↔ G or C ↔ T.

b. Transversion: Purine replacing pyrimidine or pyrimidine replacing purine.
For example: A ↔ T or C ↔ G or A ↔ C or T ↔ G.

Reference: Lehninger's 6th ed/pg. 172

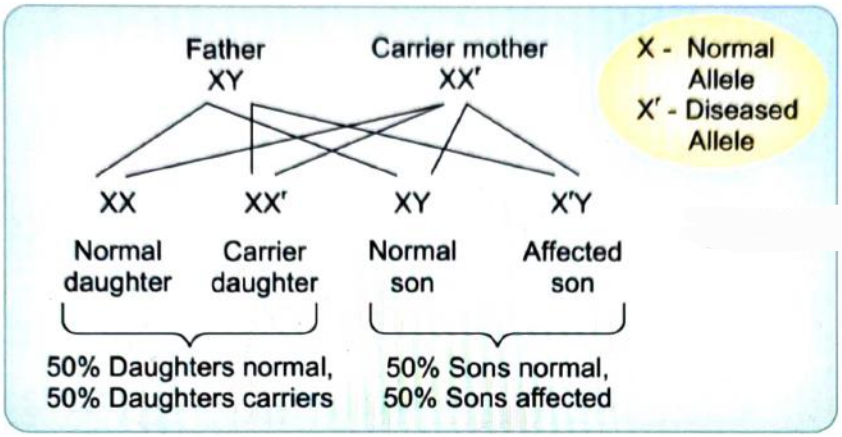
Q. A 5-year-old boy has short stature, coarse facies, stiffening of the joints, and mental retardation. His parents, 7-year-old brother and a 9-year-old sister, all are unaffected. Mother had a family history of a disease with similar progressive symptoms in one of her brothers who died at 16 years of age and in one of her nephews (her sister's son). The patient's mother is pregnant. The risk of fetus having the same disease is:

A. Virtually 0%
B. 25%
C. 50%
D. 100%

Answer: B

Solution

The patient's normal parents (unaffected father and carrier mother), one normal sister (normal daughter) and normal brother (normal son) indicates an X-linked recessive inheritance according to the chart below.



As pedigree chart of mother's family show no presence of disease in mother (normal daughter in fig) but in one brother (affected son in fig.) and in nephew from his sister (son of carrier daughter in fig), it again confirms an X-linked recessive inheritance pattern.

Coarse facial features is a typical feature of Mucopolysaccharidosis (MPS) and Hunter syndrome has X-linked recessive pattern.

Given X-linked recessive inheritance, the mother must have the abnormal allele on one of her X chromosomes (she is a carrier) in order for her son and brother to be affected. Her fetus has a 1/2 chance of being a boy and a 1/2 chance of being affected given male sex, resulting in a 1/4 (25%) overall risk of being affected.

Reference: Robbin's 9th/e pg. 142

80 MITOCHONDRIAL DNA

MITOCHONDRIAL DNA

00:00:16

- Only present in Eukaryotes
- Resembles prokaryotic DNA
  - Circular dsDNA
  - No Introns
  - Not attached to histone proteins
- mtDNA has high rate of mutation as compared to nuclear DNA due to
  - No introns (introns prevent mutation)
  - No DNA repair enzyme in mitochondria
  - High OFR production from ETC operation
- Contains around 16,000 bp, 37 genes (1% of total cellular DNA)
- No histones
- ETC requires 67 proteins. 13/67 = 19% of proteins are derived from mitochondrial DNA

Previous Year's Questions

Q. True regarding mitochondrial DNA is:

(NEET 2019)

A. Linear double stranded
B. Inherited from mother
C. Low mutation rate
D. All respiratory proteins are synthesized within mitochondria it self

00:04:22

Important Information

- Mitochondria have their own ribosomes to synthesize their proteins
- Mitochondria has a unique genetic code

Codon	Universal Codon	mtDNA
UGA	Stop codon	Tryptophan
AGG & AGA	Arginine	Stop Codons
AUA	Isoleucine	Methionine

MITOCHONDRIAL DISORDERS

00:05:58

General features

- Maternal inheritance i.e.
  - if mother has disease all offspring will be affected
  - if father has disease no offspring will be affected
- Mostly aerobic organelle affected → Lactic Acidosis
- CNS affected (CNS depends on aerobic respiration for energy)
- Most of patients have Retinitis Pigmentosa

Disease related to mutations in mitochondrial DNA

1. MELAS:Mitochondrial Encephalopathy Lactic Acidosis and Stroke like episode
2. Leigh Syndrome
3. Leber Hereditary Optic Neuropathy
4. NARP Syndrome: Neuropathy, Ataxia, Retinitis Pigmentosa
5. MERRF: Myoclonic Epilepsy and Ragged Red Fibres in muscles
6. CPEO: Chronic Progressive External Ophthalmoplegia
7. Kearns Sayre Syndrome
8. Pearson Syndrome: Lactic Acidosis, Pancreatic Insufficiency, Pancytopenia

Previous Year's Questions

Q. In which of the inheritance, if father is affected no offspring is affected, but if mother affected, all offspring affected?

(AIIMS May 2019)

A. Mitochondrial
B. X linked recessive
C. Autosomal dominant
D. Autosomal recessive

81 DNA REPLICATION

ENZYMES OF DNA REPLICATION

00:07:40

1. **Helicase**

- Causes strand separation
- Use ATP
- Create positive supercoils

2. **Topoisomerase** 00:08:21

- Relieve positive supercoils by cutting and re-joining
- Do not use ATP
- Have nuclease and ligase activity
- Two Types
  - Type 1- cuts one strand
  - Type II- cut both strands

- DNA gyrase
  - A special topoisomerase in prokaryotes
  - Uses ATP
  - Relives positive supercoils and creates negative supercoils

- Helicase & topoisomerase work in tandem

3. **Single Strand DNA Binding Proteins (SSBs)** 00:09:54

- Prevents reannealing by binding single DNA strands in prokaryotes.
- Replication Protein A (RPA) will do this function In Eukaryotes

Helicases, Topoisomerases & SSBs constitute 'Unwinding proteins'

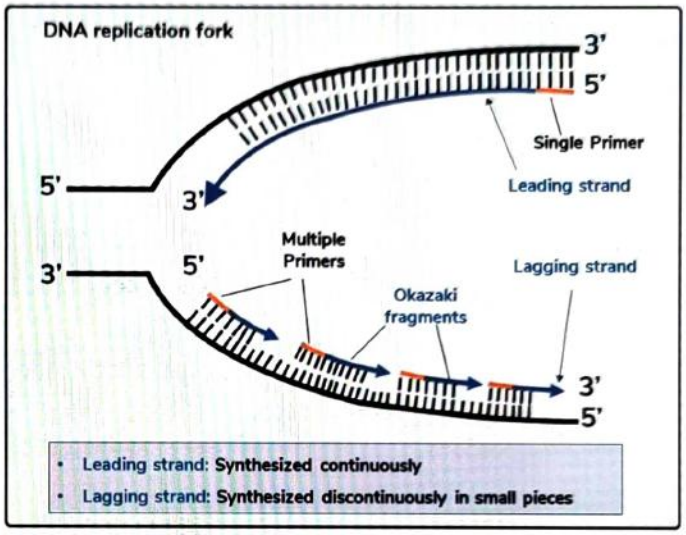
4. **Primases** 00:11:42

- Synthesizes the primers (RNA primer) using DNA as Template
- So, called as DNA dependent RNA Polymerase
  - In Eukaryotes: α–polymerase acts as primase
  - In Prokaryotes: DNA-G protein acts as primase

Important Information

- 1 primer is required for leading strand
- Multiple primers are required for lagging strand
- Length of a primer is ~11 nt and Okazaki fragment is 1000 nt long
- Primosome: Primase + Helicase

00:13:43



5. **DNA Polymerase III** 00:15:48

- Synthesizes both leading & lagging strands
- DNA Dependent DNA Polymerase
- Adds 600 to 1000 nt/sec
- Maximum rate of DNA synthesis out of all polymerase (I, II, III)

Previous Year's Questions

Q. True about DNA polymerase III:- (NEET May 2018)

A. Required for translation
B. Has DNA repair function
C. Forms okazaki fragments & needs RNA primer
D. Has no proof-reading activity

6. **DNA Polymerase I** 00:17:50

- Removes RNA primers from both leading & lagging strands
- Creates a single gap on leading strand and multiple gaps on lagging strand
- Fills gap only on lagging strand

Keep in mind that a gap is still left on leading strand (discussed later)

Previous Year's Questions

Q. Which is incorrect about DNA polymerase I?
(NEET May 2018)

A. Not required in bacteria
B. Role in primer removal
C. Fill gaps between okazaki fragments
D. Involved in DNA replication

7. **DNA Ligase** 00:19:30

- Creates 3'-5' Phosphodiester bond to join DNA with DNA
- Uses ATP
- Acts only on lagging strand

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

Somatic cells 00:21:36

- Have limited no. of divisions d/t gap present in leading strand
  - The gap left is called telomere shortening
  - With further divisions, telomere shortening increases & cell division stops after some divisions

TELOMERE 00:22:50

- Ends of chromosome
- Has $(TTAGGG)_n$ sequence repeated 'n' no. of times
  - Telomere Shortening occurs at this area
  - responsible for aging & death

Germ cells/Stem cells

- Have infinite no. of divisions
- Gap left in leading strand is filled by Telomerase

8. **Telomerase** 00:25:17

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

Previous Year's Questions

Q. All are true about telomerase EXCEPT:
(INI CET Nov 2020)

A. has reverse transcriptase activity
B. Maintains fidelity of DNA replication
C. Maintains length of DNA
D. Found only in eukaryotes

00:27:41

	Proof reading	Repair
Correction	• correction during synthesis	• correction after synthesis
Enzymatic activity	• 3' → 5' Exonuclease activity	• mostly endonuclease activity • Sometimes 5' → 3' Exonuclease activity
Enzyme in prokaryotes	• DNA Polymerase I, II, III	• DNA Polymerase I and II (Main)
Enzyme in eukaryotes	• All Polymerases except α & β polymerase	• β polymerase (main) • ε polymerases (minor role)
Phase of cell cycle	• occurs in S phase	• Most repairs occur in G_1 phase

PROKARYOTIC vs EUKARYOTIC DNA POLYMERASE 00:32:30

E. coli (Prok)	Eukaryotic		Function
	Nucleus	**Mitochondria**	
DNA Pol I	• RNase H • FEN-1 • δ-polymerase (minor role)	• RNase H • FEN-1 (Flap endonuclease)	Remove primer and fill the gap
DNA Pol II			DNA proof reading and repair β → DNA repair γ → Mitochondrial DNA synthesis
DNA Pol III			ε → Leading strand δ → Lagging strand
DNA-G		α	Primase

How to remember

Mnemonic for DNA polymerases
gaMMa (γ) → Mitochondrial
Lεading (γ) → ε-Polymerase
LAδδing → δ-polymerase

INHIBITORS OF DNA REPLICATION 00:37:28

Inhibitors interacting directly with DNA

- Bind b/w stacked bases, disrupting normal structure of DNA
- Acridine, Ethidium, Actinomycin

Inhibitors of deoxyribonucleotide synthesis

- 6-Mercaptopurine (Purine synthesis)
- 5-Fluorouracil (Thymidylate synthesis)
- Hydroxyureas, Sulfonamides (Both)

Inhibitors of replicative enzymes

- Arylhydrazinopyrimidines (DNA Polymerase III)
- Fluoroquinolones (DNA gyrase)

CLINICAL QUESTIONS

Q. A group of researchers while testing an experimental drug discovered a mutant of E. coli which was unable to replicate in the presence of drug. Later analysis of mutant strain revealed that there is defect in removing the RNA primers during the replication. Which of the following enzyme is most likely affected by this drug in this strain?

A. Helicase
B. DNA polymerase I
C. DNA polymerase III
D. Ligase
E. Primase

Answer: B

Solution

DNA polymerases are the main enzymes involved in DNA replication. In prokaryotes such as E coli, there are three DNA polymerases named as DNA polymerase I, II and III performing different functions. Other enzymes required for replication include helicase, topoisomerases, primase and ligase.

Before polymerization can occur, the two parental DNA strands are unwounded using helicase for cutting and topoisomerases by removing supercoiling. As DNA polymerase can only add nucleotides to exiting chain hence a short RNA primer is synthesized by primase enzyme using DNA as template. One primer is required for leading strand, but multiple primers are required for lagging strand. Leading strand is synthesized continuously in 5' → 3' direction (Fig. in text) and it needs only one RNA primer while lagging strand is synthesized in short stretches in 5' → 3' direction and requires many RNA primers.

After the proofreading DNA polymerase I removes RNA primers by 5'-3' exonuclease activity and fill the gap by synthesizing DNA using 5'-3' polymerase activity.

DNA ligase catalyzes the formation of phosphodiester bond between DNA synthesized by DNA polymerase III and that formed by DNA polymerase I using energy (ATP).

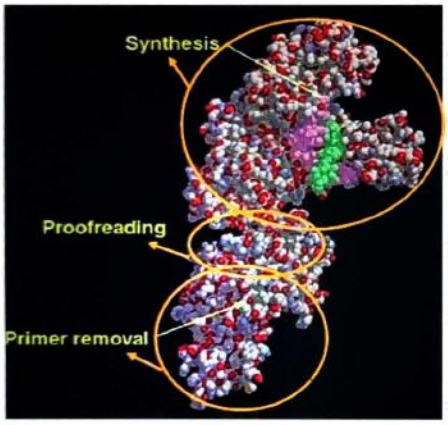
Reference: Harper's 30th ed/pg. 382-383

82 KLENOW FRAGMENT

DNA POLYMERASE

00:00:38

- In prokaryotes [I, II, III]
- In Eukaryotes [α, β, γ, δ]
- All the DNA polymerases (I, II, III) have 2 activities
  1. 5' → 3' polymerase activity → Synthesis
  2. 3' → 5' Exonuclease activity → Proof Reading
- DNA polymerase I has one extra activity i.e.
  - 5' → 3' exonuclease activity → RNA primer removal
- So, DNA Polymerase has
  1. 5' → 3' exonuclease (primer removal)
  2. 5' → 3' polymerase activity (Synthesis)
  3. 3' → 5' Exonuclease activity (Proof Reading)

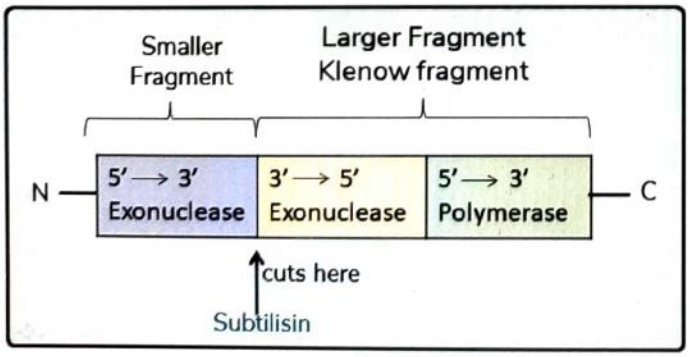


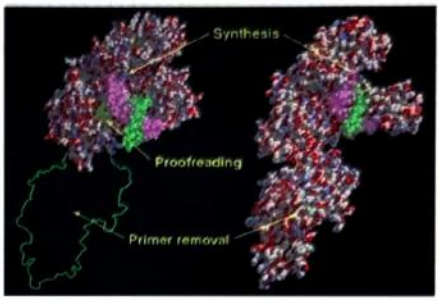
DNA Polymerase I

KLENOW FRAGMENT

00:02:58

- Subtilisin enzyme derived from *Bacillus subtilis* is an Endopeptidase enzyme
- It releases two fragments from DNA polymerase I
  1. Smaller fragment with 5' → 3' exonuclease activity
  2. Larger fragment towards the 'C' terminal known as Klenow fragment which is lacking 5' → 3' exonuclease activity





Previous Year's Questions

Q. Klenow fragment is formed by loss of fragment having which activity? (AIIMS May 2018)

A. 5'→3' polymerase
B. 3'→5' exonuclease
C. 5'→3' exonuclease
D. 3'→5' polymerase

Why to use Klenow Fragment?

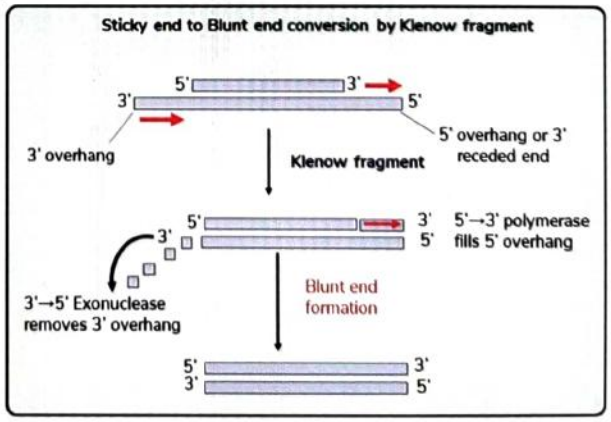
- 5' → 3' exonuclease activity of DNA polymerase I makes it unsuitable for many molecular biology applications. So klenow fragment is used for those applications instead.

Uses of Klenow fragment

00:05:23

1. Used to remove 3' overhang
2. Can fill 5' overhang (3' receded end)

- These two processes can convert sticky ends of DNA to blunt end



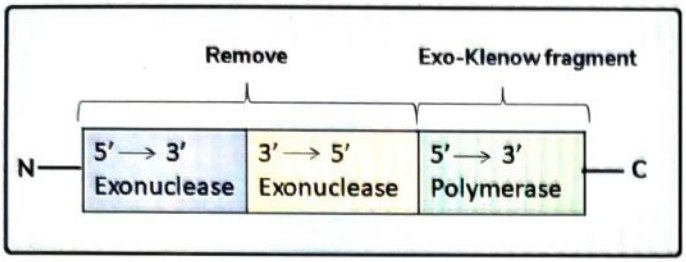
3. Convert ssDNA to dsDNA by polymerase activity

- Used earlier in PCR before the discovery of thermostable Taq polymerase

4. Can be used to produce Radioactive DNA probes

EXO-KLENOW FRAGMENT

00:08:18



Uses

- Used in Microarray for creating fluorescent probes
- Deoxy adenine (dA) & deoxy thymine (dT) tailing
- To prepare gene libraries for next generation sequencing

CLINICAL QUESTIONS

Q. A scientist while performing a DNA cloning experiment have amplified a gene that do not contain a restriction site for producing sticky ends. So, he must do a blunt end ligation. But the stock of plasmid in which he wants to insert this gene is already cut with a restriction enzyme which produces sticky ends. Which of the following enzyme he will use to flush the sticky ends of the plasmid?

A. Klenow fragment
B. Alkaline phosphatase
C. Primase
D. DNA ligase

Answer: A

Solutions:

Klenow Fragment is synthesized from DNA polymerase I by treating with Endopeptidase enzyme Subtilisin which releases two fragments from DNA polymerase I:

1. Smaller fragment with 5'→3' exonuclease activity
2. Larger fragment towards the 'C' terminal known as Klenow fragment which is lacking 5'→3' exonuclease activity.

The sticky ends have 3' overhangs which can be digested with Klenow fragment (has 3'→5' exonuclease activity) and 5' overhangs which can be filled with 5'→3' polymerase activity of klenow fragment to produce blunt-end DNA (see fig in text)

Extra Information:

Table: List of various enzymes used in molecular biology and their function

Tool	Role
Type II restriction enzymes	Cleaves DNA at specific sites
DNA ligase	Phosphodiester bond formation between 2 fragments
Polynucleotide kinase	Catalyzes the transfer of γ phosphate from ATP
Alkaline phosphatase	Removes the phosphate group from 5' end to prevent self-annealing of sticky ends of DNA
Reverse transcriptase	RNA dependent DNA polymerase for cDNA synthesis
RNAase H	Removes RNA from DNA-RNA hybrid
Exonuclease III	Removes nucleotide from 3' end
Phage γ exonuclease	Removes nucleotide from 5' end
Terminal nucleotidyl transferase	In homopolymer tailing to convert blind end to sticky end

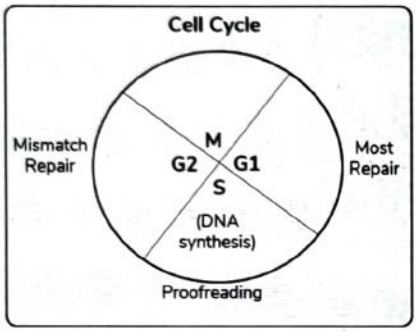
Reference: Lehninger's 6th ed/pg. 1017

83 DNA REPAIR

CELL CYCLE AND REPAIRS

00:00:51

- DNA synthesis and proofreading occur in S phase
- Only mismatch repair occur in G2 phase (to correct any shortcoming in proofreading)
- Most of repair occurs in G1 Phase



00:03:36

Type of repair	Cause	Damage	Disease
Nucleotide excision repair	UV radiation damage	T-T dimers	Xeroderma pigmentosa
Base excision repair	Spontaneous, IR rays, viral inf, chemical	C → U conversion	Rare, MUTYH associated polyposis
Mismatch repair	Proofreading error	Mismatched base	HNPCC (Hereditary Non-Polyposis Colorectal Cancer

Previous Year's Questions

Q. All are true regarding xeroderma pigmentosa except: (JIPMER Nov 2017)

A. Base excision repair defect
B. Skin manifestations are seen usually within 2 years
C. Usually patients die in 2nd or 3rd decade
D. Metastasis of malignant tumors commonly occur in these patients

Previous Year's Questions

Q. A patient who is a diagnosed case of HNPCC, which of the following is the most common repair associated with it? (NEET PG Sep 2021)

A. Mismatch repair
B. Nucleotide excision repair
C. Base excision repair
D. Homologous recombinant repair

REPAIR MECHANISMS

00:08:03

Single strand break repair

- All types of DNA damage mentioned in table have defect in one of the strands and so they can use other strand as a template and can repair the damaged DNA.

Double strand break repairs

00:08:37

1. Homologous repair/recombination (HR)
- homologous chromosome is used as template for repair
- Error free
- Defective HR can lead to
  - Bloom syndrome
  - Werner syndrome
  - Ataxia Telangiectasia-like disorders
  - Breast cancer (susceptibility 1 and 2)
  - Rothmund-Thomson syndrome
  - Nijmegen breakage syndrome

2. Non-homologous End-Joining (NHEJ) repair
- More error prone due to use of non-homologous chromosomes
- Defect in NHEJ will lead to
  - SCID (Severe Combined Immuno Deficiency)
  - Radiosensitive SCID

Previous Year's Questions

Q. In SCID, which type of DNA repair mechanism is defective? (AIIMS Nov 2017)

A. Double strand break repair
B. Nucleotide excision repair defect
C. End joining repair
D. Mismatch repair

CLINICAL QUESTIONS

Q. A 10-year old girl is brought to the dermatologist by her parents. She has many freckles on her face, necks, arms and hands and parents report that she is highly sensitive to sunlight. On examination, two basal cell carcinomas are observed on her face. The process which is most likely defective in this patient is?

A. Repair of double stranded breaks
B. Removal of mismatched bases from the 3' end of Okazaki fragments
C. Removal of pyrimidine dimers from DNA
D. Removal of uracil from DNA.

Answer: C

Solution

The patient is showing characteristic of condition known as Xeroderma pigmentosum. These individuals have sensitivity to sunlight with extensive freckling on parts of the body exposed to the sun. They are also prone to developing skin cancer at a young age.

It is caused by defect in DNA repair process known as nucleotide excision repair which corrects for pyrimidine dimers formed by ultraviolet light damaged of DNA.

For other options refer to the table in text.

Reference: Dinesh Puri 3rd ed/pg. 465

Q. An experiment was conducted with rats in which they are fed nitrate rich food for several days. The DNA analysis of intestinal cells showed a lot of deaminated cytosine. The most probable reason for this finding is defect in which of the following?

A. Base Excision Repair
B. Nucleotide excision repair
C. Mismatch Repair
D. Homologous repair

Answer: A

Solution

Presence of deaminated bases is due to defective base excision repair which corrects for abnormal bases such as uracil, hypoxanthine and xanthine formed by spontaneous deamination of original bases cytosine, adenine and guanine respectively. This defect causes MUTYH associated polyposis in humans, which is a rare disease.

Enzyme involved in base excision repair are as:

1. DNA glycosylases
2. AP endonucleases
3. DNA polymerase I
4. DNA ligase

Reference: Lehninger's 7th ed/pg. 1028

Q. On colonoscopy examination of a 40-year-old man, he was found to have a right-sided, mucinous colon cancer, with no other lesions or polyps seen. He has a family history of colon cancers and ovarian cancers. He most probably has defect in which of the following processes?

A. Defective transcription-coupled repair
B. Nucleotide excision repair
C. Mismatch repair
D. Base Excision repair

Answer: C

Solution

The patient has Hereditary Nonpolyposis Colorectal Cancer (HNPCC), which is due to specific mutations in proteins involved in mismatch repair and is primarily right-sided with absence of any polyps.

It occurs due to mutations in any of seven different genes involved in mismatch repair (which is distinct from the generalized DNA repair system, mutations in which lead to xeroderma pigmentosum).

These genes are all tumor suppressor genes.

Reference: Harper's 30th ed/pg. 390

84 TYPES OF RNA

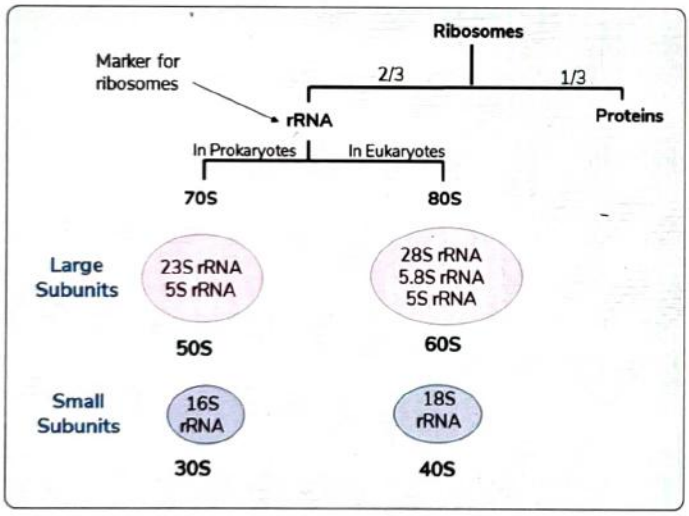
TYPES OF RNA

00:00:26

RNA Type	Abundance	Feature	Synthesis
rRNA (ribosomal)	80%	Most abundant	Nucleolus
tRNA (transfer)	15%	Smallest and has maximum modified bases	Nucleus
mRNA (Messenger)	5%	Most heterogenous	Nucleus

rRNA [Ribosomal RNA]

00:03:05



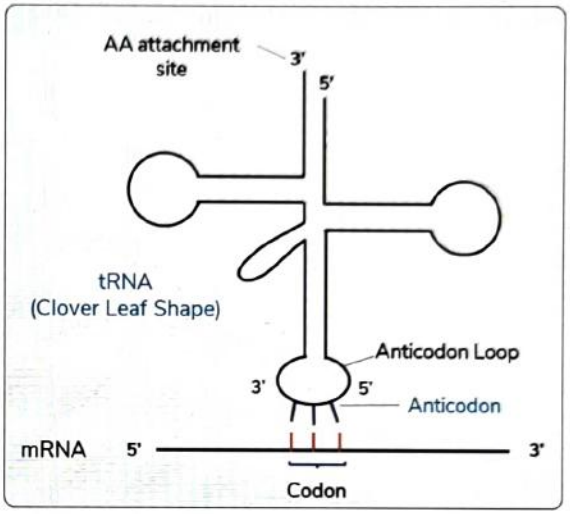
Important Information

- 16S rRNA sequence of prokaryotes is complimentary to Shine Dalgarno Sequence
- 23S rRNA of Prokaryotes and 28S rRNA of Eukaryotes are Ribozyme (RNA having enzyme activity)

tRNA [Transfer RNA]

00:06:44

- Has clover leaf shape



Codon & Anticodon

- Codon is present on mRNA whereas Anticodon present on tRNA
- They have complimentary base pairing & their binding help in translation
  - During translation, codon can see anticodon but it can't see which amino acid is attached.
  - Ex: codon is for cysteine, then anticodon is also for cysteine.
  - so, ideally a cysteine should be attached at 3' end of this tRNA
  - Suppose, by chance, if this tRNA brings a wrong AA (say alanine), it will be added & cause a mutation (more details in translation)

CODING AND NONCODING RNAs

00:09:30

Coding RNA: RNA with codons e.g. mRNA

Non-coding RNA (nc RNA): All RNAs without codons e.g.

- tRNA (variable size)
- rRNA (70-90 nucleotides)
- Other nc RNA

Large nc RNA (> 200 nt)	Small nc RNA (< 200 nt)
1. lnc RNA (Long nc RNA) 2. Linc RNA (Long intervening nc RNA)	1. pi RNA (piwi interacting) o (Mc small nc RNA) 2. mi RNA (micro RNA) 3. si RNA (Small interfering) 4. sn RNA (Small nuclear) 5. sno RNA (Small Nucleolar)

Previous Year's Questions

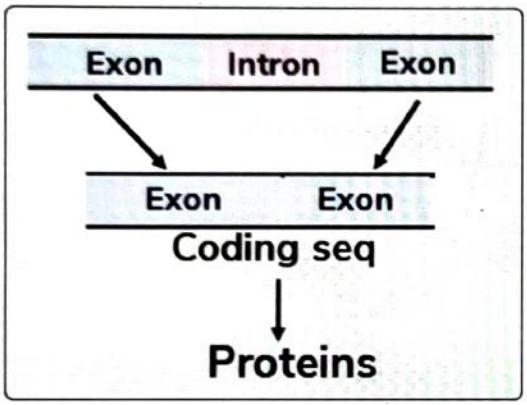
Q. Binding site of miRNA on mRNA? (INI CET July 2021)

A. 5' UTR
B. 3' UTR
C. Gene promotor
D. Gene body

85 INTRONS

INTRONS

- Introns: Intervening sequences between Exons



- ≥ 98% of coding genome
- Only present in eukaryotes
- Can be transcribed, but not translated
- Presence of introns prevent mutations

00:02:44

Eukaryotic nuclear DNA	Eukaryotic mitochondrial DNA
• Have introns	• Do not have introns (same as prokaryotes)
• Less chance of mutations (as introns prevent mutations)	• High rate of mutations occurs as compared to nuclear DNA

86 TRANSCRIPTION

TRANSCRIPTION

- Formation of RNA from DNA in nucleus
- Enzyme of transcription: DNA dependent RNA polymerase

RNA polymerase 00:00:51

- do not require primer
- cannot do proofreading
- **Types**
  1. In prokaryotes, only single type present
  2. In Eukaryotes

Eukaryotic RNA polymerase types	RNA synthesized
Type I	All rRNA except 5S rRNA
Type II	mRNA, miRNA, lncRNA few snRNA & snoRNA
Type III	tRNA, 5S rRNA, few snRNA & snoRNA
Mitochondrial RNA Polymerase	Mitochondrial RNA

RNA polymerase Holoenzyme (complete enzyme) 00:03:44

- **5 subunits: 2α, β, β', ω, σ**

Subunits	Function
α, ω	Enzyme assembly
β	main catalytic subunit
β'	Template (DNA) binding
σ	Initiation of transcription (binds promoter)

TATA Box and Shine Dalgarno Sequence 00:05:25

Refer Figure 86.1

Tata Box	Shine Dalgarno Sequence
• Sequence Present at -10 position upstream to TSS on DNA	• Present at -10 position upstream to AUG codon on mRNA
• Helps in initiation of transcription	• Helps in initiation of translation
• Present in both Prokaryotes & Eukaryotes o Prok → Pribnow Box o Euk → Hogness Box	• Only present in prokaryotes
• Rich in T & A	• Rich in A & G

Template / Anti Sense / Non-Coding / Minus Strand 00:11:25

- Template strand: as new RNA is getting synthesized using this strand as template

Non-Template / Sense / Coding / Plus Strand

- Coding strand: as it has the same codons as new RNA (see fig)
- Sense strand: as it has same sense of direction as new RNA (see fig)

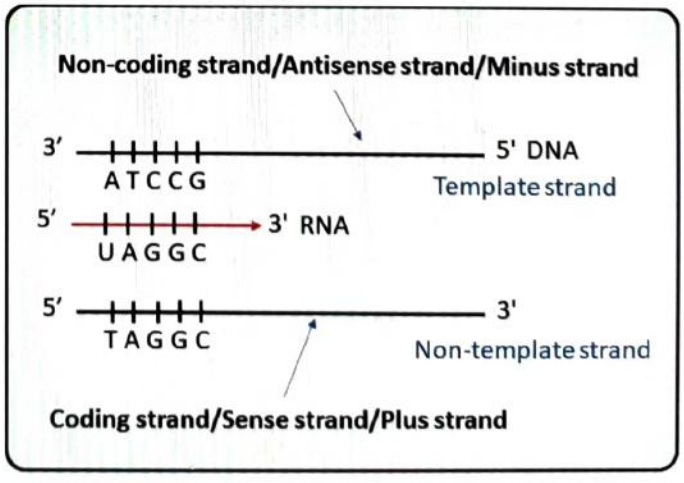
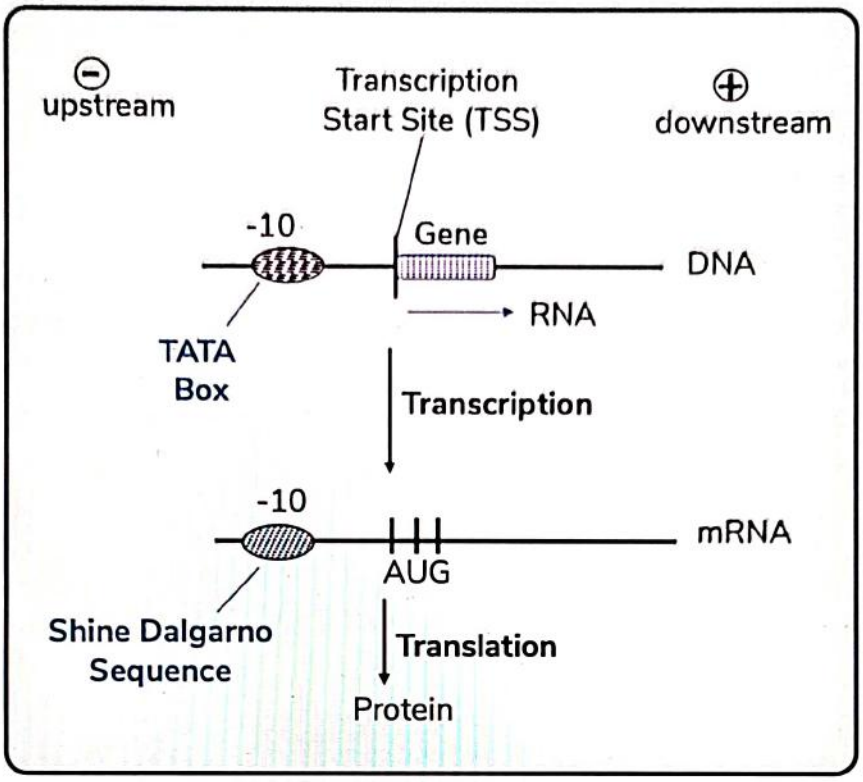
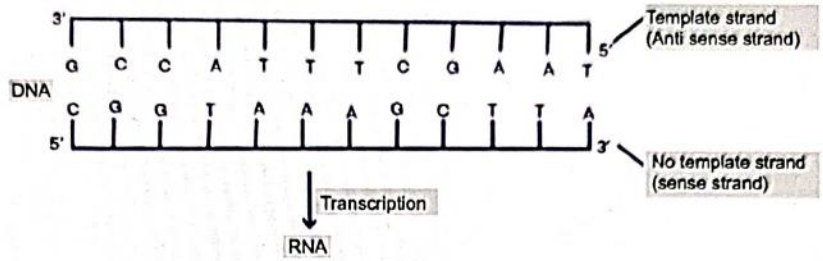


Figure 86.1


⊖
upstream
Transcription
Start Site (TSS)
⊕
downstream
-10
Gene
DNA
RNA
TATA
Box
Transcription
-10
mRNA
AUG
Shine Dalgarno
Sequence
Translation
Protein

CLINICAL QUESTIONS

Q. The figure below shows the base sequence on template & non-template strand of DNA. What is the base sequence of RNA?

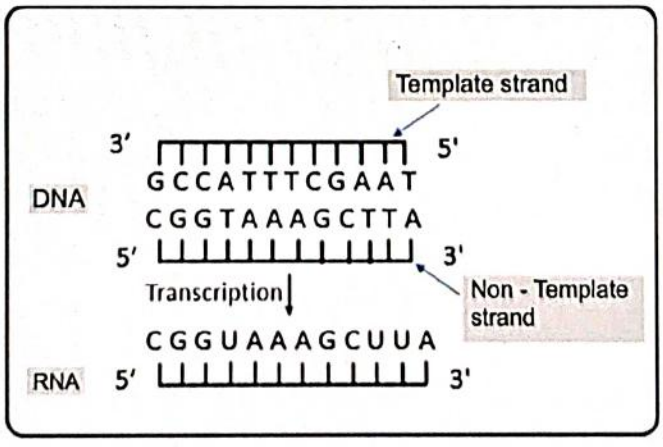


A. CGGUAAAGCUUA
B. AUUCGAAAUGGC
C. GCCATTTCGAAT
D. UAAGCUUUACCG

Answer: A

Solution

- Base sequence and direction of new RNA is always same as the non-template strand.
- But there is uracil in place of thymine.
- Whenever sequence is written without mentioning the direction, then it is always 5' on left side & 3' on right side.



Reference: Harper's 30th ed/pg. 362

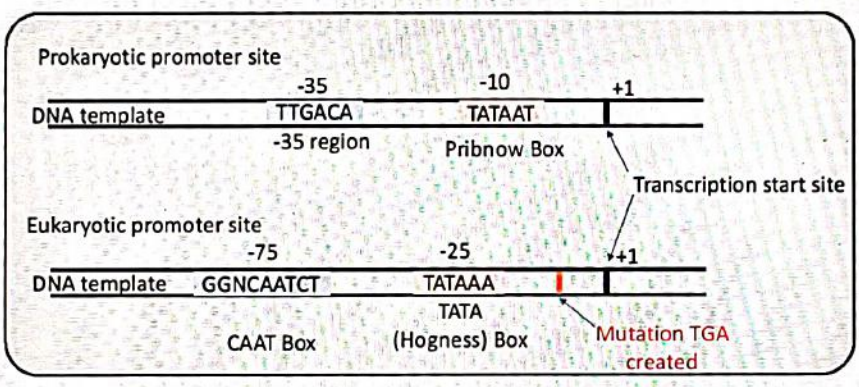
Q. The mutation of normal codon to a stop codon in DNA often leads to harmful conditions. In an experiment, an error prone PCR to amplify a gene introduced a mutation that created a stop codon TAG between the TATA box and the transcription initiation site. The most likely outcome of this mutation will be?

A. No effect
B. Loss of transcription
C. A shorter protein
D. A mistake in splicing

Answer: A

Solution

There is no coding sequence between the TATA box (the promoter region where RNA polymerase binds) and the transcription initiation site, so the presence of a TAG in the DNA will not affect protein synthesis.



In fact, the RNA containing UAG in its sequence will not even be synthesized because the transcription initiation site occurs after this sequence in the DNA.

The introns and exons are not affected by this mutation, and splicing will occur normally.

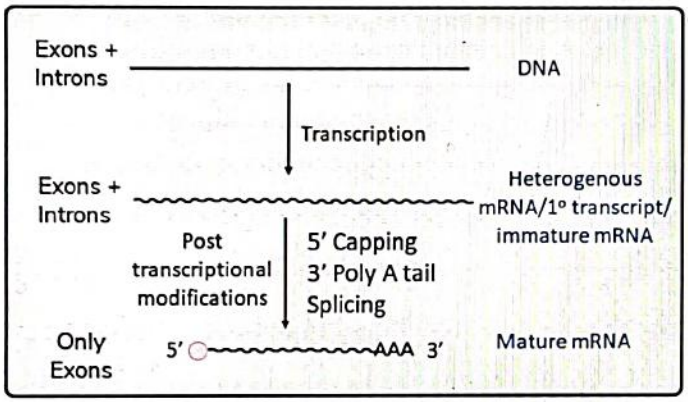
Thus, the mRNA produced would be of normal size and would produce a normal sized protein.

Reference: Harper's 30th ed/pg. 400

87 POST TRANSCRIPTIONAL MODIFICATIONS

POST TRANSCRIPTIONAL MODIFICATIONS

00:00:20



Introns prevents mutations

- Introns are transcrined but not translated
- Whenever mutation occurs, it most probably occurs in introns (larger in number)
- Introns are excised in post translational modification
- So, Incidence of mutation is diminished

Processess in post transcriptional modifications

00:03:10

1. 5' cap addition

- 7 methyl guanosine cap
- Methyl group is donated by SAM (in cytoplasm)
- Prevents the attack from 5' exonuclease

2. 3' Poly A tail addition 00:04:31

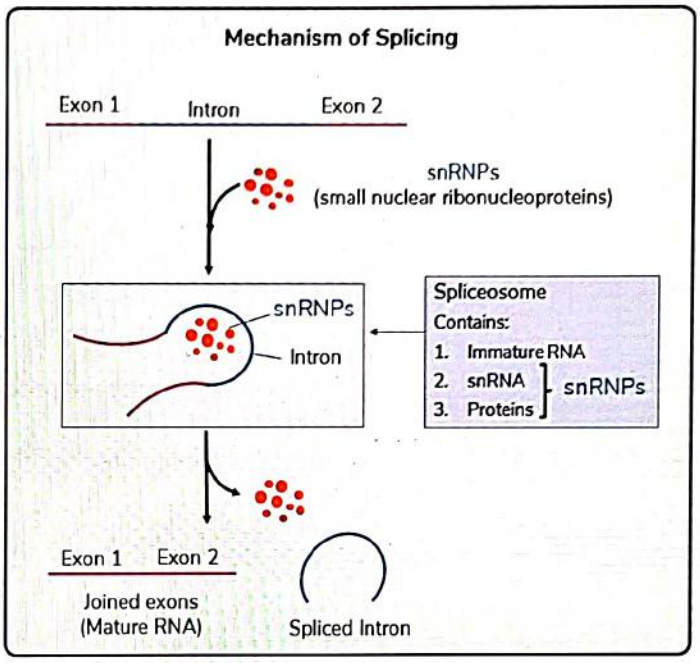
- Functions
  1. Prevents attack from 3' exonucleases
  2. Helps the RNA to exit from nucleus to cytoplasm

- added by Poly Adenylate Polymerase
  - Uses ATP as subsatrate (AAAA---)
  - No. of AAAAAs added =40–200

- poly A tail is not translated
- Added in all mRNA Except in mRNA for histone proteins

3. Splicing 00:07:08

- Involves removal of introns
- Done by snRNA (small nuclear ribozyme)



These 3 modifications occur in all cells & called as RNA processing

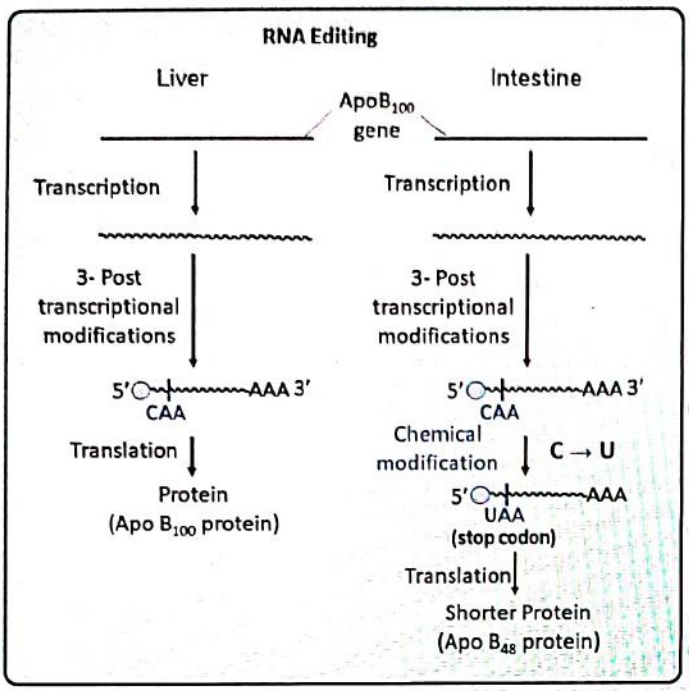
Previous Year's Questions

Q. Which of the following does not require 5' capping? (AIIMS June 2020)

A. tRNA of alanine
B. U6 snRNA
C. mRNA for histone
D. siRNA

4. Differential RNA processing/ RNA editing/Chemical modifications of RNA 00:10:51

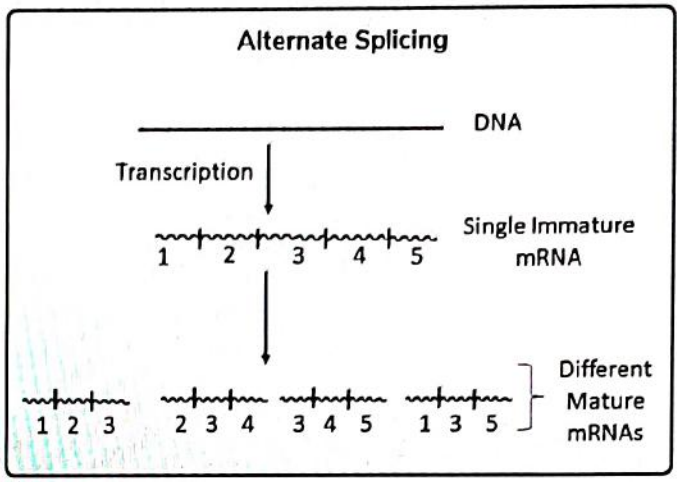
- Occurs in few cells
- Exception to one gene one protein theory i.e. one gene gives more than 1 protein. e.g
- Single $ApoB_{100}$ gene give rise to
  1. Apo B_{100} in Liver
  2. $ApoB_{48}$ in Intestine
  - A chemical modification, C to U conversion, gives rise to stop codon in intestinal mRNA which will cause shorter protein Apo B48 synthesis



Alternate splicing

00:16:00

- Also an exception to one gene one protein theory
- Gives rise different mature mRNAs from single type of transcript



Previous Year's Questions

Q. Apolipoprotein B_{48} is a made by? (NEET Jan 2020)

A. RNA editing
B. RNA interference
C. RNA splicing
D. DNA translocation

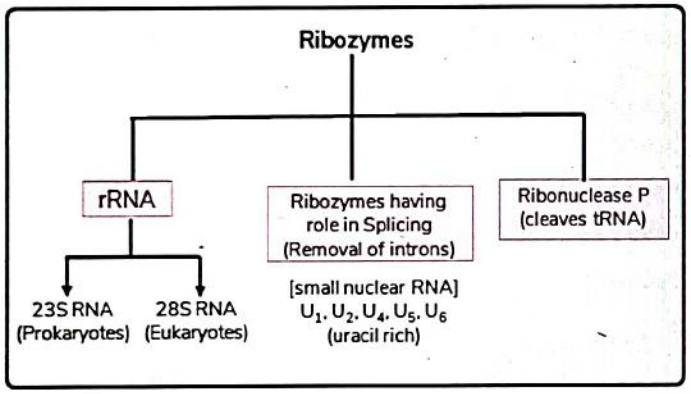
88 RIBOZYMES

RIBOZYMES

- Ribozyme means RNA acting as enzymes (commonly proteins acts as enzymes)
- Substrates of Ribozymes is mostly RNA
- They mostly catalyse the synthesis and breakage of phosphodiester bond.
- No ATP is used in their reaction

Similarity of Protein enzymes & Ribozymes 00:01:34

- Specificity
- Accelerate rate of reaction
- Kinetic behavior
- Can be competitively Inhibited 00:02:52



rRNA acting as ribozyme

- 23S rRNA in Prokaryotes and 28S rRNA in Eukaryotes
- They have Peptidyl Transferase activity required for elongation and termination of Translation

Ribozymes having Role in Splicing

Ribonuclease P

- an endonuclease which cleaves the tRNA
- Structurally a Ribonucleoprotein with RNA portion acting as enzyme.
- Create a mature 5' end of tRNA
- Ubiquitous (present in all cells of the body)

Important Information

Telomerase is not a Ribozyme: RNAase H is not a Ribozyme

RNAase H

- An endonuclease
- Non-sequence specific
- Does not act on free or ssRNA
- Cleaves RNA in RNA-DNA duplex producing ss DNA
- Used in Synthesis of cDNA by reverse transcriptase

Telomerase: Refer to DNA replication notes

Previous Year's Questions

Q. Which of the following is not a Ribozyme? (NEET Jan 2019)

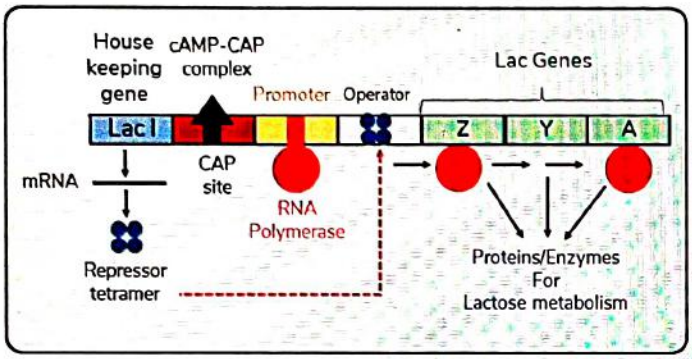
A. Transpeptidase
B. Ribonuclease
C. Peptidyl transferase
D. Poly A polymerase

89 OPERON MODEL

LACTOSE OPERON IN E. COLI

00:00:17

- Operon model: Operating unit
- Function to switch on or switch off the genes



In Normal State:

- Glucose is mostly present
- No need to use lactose
- No need to make lactose utilizing proteins
- So, lac Z, Y, A genes of operon model are kept in switch off state to save energy used in protein synthesis

Mechanism of switching off Z, Y, A genes

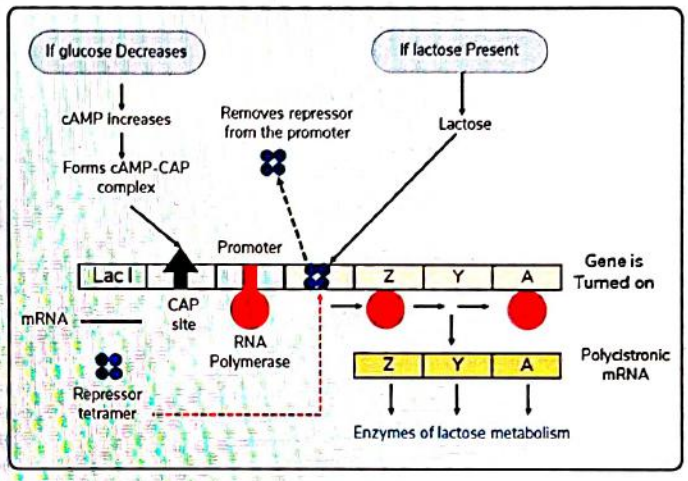
00:02:21

- **Lac I Gene** (I stands for Inhibitory)
  - Housekeeping / constitutive gene (always active)
  - Forms repressor tetramer (protein) all the time
- Repressor tetramer binds to Operator site
- Promoter is present upstream to operator
  - RNA Polymerase is present binding to promoter
- Repressor Tetramer by binding to operator inhibits RNA Polymerase movement and thus inhibits transcription

Mechanism of switching on Z, Y, A Genes

00:05:08

- Occurs in Two situations:
  1. If ↓ Glucose in environment of E. coli
  2. Presence of Lactose in environment of E. coli



CLINICAL QUESTIONS

Q. An E. coli culture expressing a recombinant protein was grown in lactose containing broth media. After 10 hours of growth, glucose is added to the media which suppressed the fermentation of lactose. Which of the following is the best explanation for this suppression?

A. Repressor protein is present and bound to operator
B. Repressor protein is present and bound to promoter
C. Glucose is bound to the promoter
D. cAMP is low in the cell

Answer: D

Solution

Culturing E. coli in lactose containing media results in binding of lactose to the repressor protein and causing a conformation change and removal of repressor from the operator site. This will allow RNA polymerase to go ahead and transcribe the gene. cAMP is a positive regulator for this operon & in combination with CAP protein activate the transcription of Lac genes by binding to CAP site. But in the presence of glucose cAMP levels are low due to inhibition of adenyl cyclase hence transcription is inhibited (See fig in text).

Regarding other options:

The main mechanism of lac operon is effect of cAMP (option d) and not binding of repressor protein to the operator. Repressor protein binds to operator region and not promoter (option a). Glucose itself does not act by binding to repressor or promoter in contrast to lactose which bind to repressor protein or promoter region (option c).

Reference: Harper's 30th ed/pg. 431-432

90 CODON

CODON

00:00:23

- Nucleotide Triplets e.g. ACG 3 bases make 1 codon
- 4 bases are used to make codons: A U C G
- $4^3 = 64$ codons combinations are possible

Q. If 4 bases make 1 codon, the total codons? 00:01:38

Ans: Total $4^4 = 256$ codons are possible

- Out of 64, 3 are stop codons i.e. do not code for amino acids
  1. UAA
  2. UAG
  3. UGA
- So, for 20 AA, 61 codons are present
  - or on an average, for each amino acid, 3 codons are present
  - Each amino acid has more than 1 codon, known as degeneracy/redundancy of codon
- AA that do not show degeneracy (only 1 codon present):
  1. Methionine - AUG
  2. Tryptophan - UGG

00:04:06

Important Information

- Few amino acids have 6 codons (max number possible) e.g. Mnemonic - SALE
  - S - Serine
  - A - Arginine
  - LE - Leucine

Codon	Amino acid
AAA	Lysine
UUU	Ph-alanine
CCC	Proline
GGG	Glycine
AUA	Isoleucine

CODON PROPERTIES

00:07:02

1. Degeneracy

- Each amino acid has more than 1 codon

2. Unambiguous

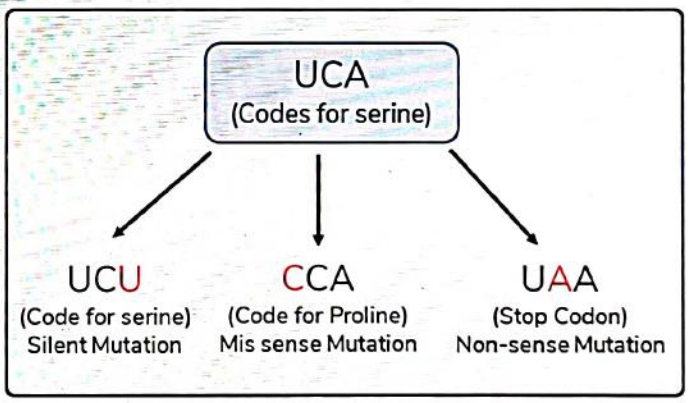
- Each codon is specific for its amino acid e.g. AUG will always code for methionine

3. Non overlapping and Commaless

- Means that the codon is read from a fixed starting point as a continuous sequence of bases, taking three at a time, without any punctuation between codons.
- E.g. AUCGGGACUGCA will be read as AUC GGG ACU GCA

4. Universal

- Genetic code has been well conserved during evolution with only slight differences
- Degeneracy Prevents Mutation 00:08:26



All these are point mutations

Previous Year's Questions

Q. Degeneracy of codon means? (JIPMER Nov 2018)

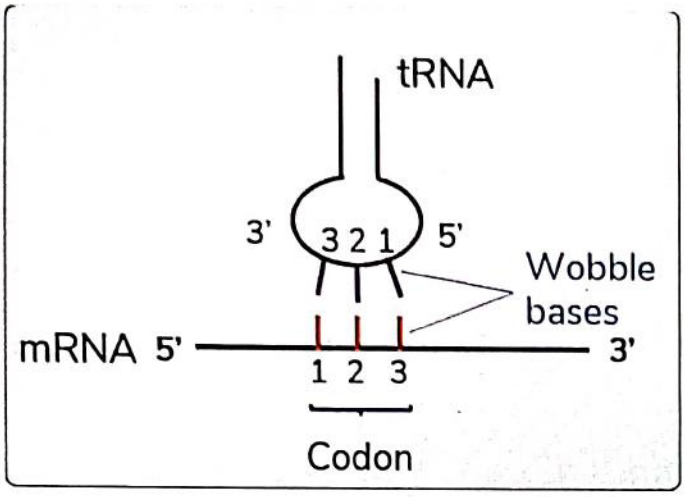
A. Comma less
B. Unambiguity
C. One amino acid having more than one codon
D. One codon coding for more than one amino acid

WOBBLE HYPOTHESIS

00:11:16

Wobble bases

- 3rd base of the codon on mRNA and 1st base of anticodon on tRNA in 5' to 3' direction
- These positions are wobble positions



Wobble hypothesis says that

- For binding between bases present in these positions, some imprecision is allowed. i.e. one base in anticodon can bind with various bases of codon
- Wobble bases can base pair via non-Watson Crick base pairing
  - Watson Crick base pairing is: A only pair with T and G only with C
- Occur in RNA only
- Does not follow general Watson-Crick base pairing rule

Wobble base pair combinations

1st base of anticodon	3rd base of codon
I (hypoxanthine)	A, U, C (Mn- Area Under Curve)
U	A, G
G	C, U
A	U
C	G

How to remember

- Hypoxanthine wobble base pairing
  I - A, U, C
  Mn- Area Under Curve

The advantage is that due to this pairing, a single tRNA can recognize more than one codon for a specific AA.

SYNONYMOUS CODONS

00:15:27

- All similar codons for 1 AA. e.g. GGG, GGC, GGU, GGA are synonymous codons code for glycine only

CLINICAL QUESTIONS

Q. An E. coli culture expressing a recombinant protein was grown in lactose containing broth media. After 10 hours of growth, glucose is added to the media which suppressed the fermentation of lactose. Which of the following is the best explanation for this suppression?

A. Repressor protein is present and bound to operator
B. Repressor protein is present and bound to promoter
C. Glucose is bound to the promoter
D. cAMP is low in the cell

Answer: D

Solution

Culturing E. coli in lactose containing media results in binding of lactose to the repressor protein and causing a conformation change and removal of repressor from the operator site. This will allow RNA polymerase to go ahead and transcribe the gene. cAMP is a positive regulator for this operon in combination with CAP protein activate the transcription of Lac genes by binding to CAP site. But in the presence of glucose cAMP levels are low due to inhibition of adenyl cyclase hence transcription is inhibited (See fig in text).

Regarding other options:

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Reference: Harper's 30th ed/pg. 431-432

91 TRANSLATION

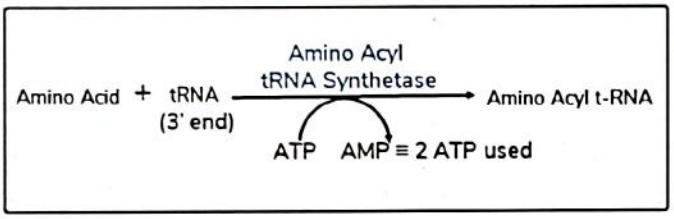
STEPS OF TRANSLATION

00:00:34

1. Activation of AA/charging of tRNA
2. Initiation
3. Elongation
4. Termination

1. Activation of AA/charging of tRNA

00:01:31



Amino Acyl tRNA Synthetase

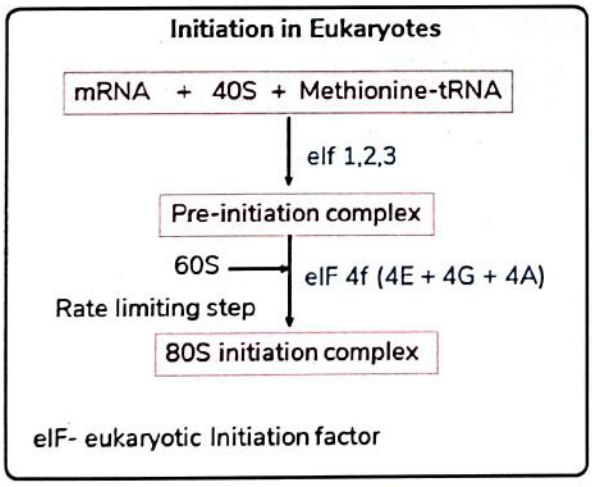
00:02:37

- Have 20 isoenzymes (one for each AA)
- Is the only point of proofreading during translation
- Responsible for fidelity/accuracy of protein synthesis

Initiation

00:05:55

- First AA
  - In Eukaryotes: Methionine
  - In Prokaryotes: formyl Methionine
- Initiation codon: AUG (codes for methionine)



Important Information

Factors required for formation of 80S initiation complex: Mnemonic- FEGA
4F - 4E - 4G - 4A

Translation in Prokaryotes

00:09:11

Refer figure 91.1

Initiation

- A sequence in 16S RNA (part of ribosome) is complementary to Shine-Dalgarno sequence on mRNA and help in their binding for formation of 30S initiation complex.

Elongation

00:11:57

- 'P' site (Polypeptide site): Polypeptide is released from this site at the end of translation
- 'A' site (Acceptor Site): All AA except first Methionine are accepted here
- Codon UUU: Codes for phenylalanine and recognize anticodon AAA on tRNA.
- However, In the figure, lysine is attached to the phe-tRNA which will get inserted and will cause mutation as codon on mRNA cannot correct for the wrong amino acid attached to the tRNA.

- Peptidyl transferase
  - Is 23S RNA ribozyme
  - Works at P-site,
  - Cuts the amino acid from P site and bring it to A site
  - Joins two amino acids by a peptide bond formation without using any ATP or GTP

- Translocation
  - Movement of ribosome on mRNA
  - Require GTP and elongation factor EF-G

Termination

00:18:56

- **Releasing factor (RF)**
  - Misnomer i.e. name indicates that it helps in release. However, it does not help in release. It only recognizes the stop codon

- Peptidyl transferase releases the polypeptide from P site.

Factors in translation in prokaryotes & eukaryotes

00:21:47

	Prokaryotes	Eukaryotes
Initiation	IF 1, 2, 3	eIF 1, 2, 3, 4F
Elongation	EF– Tu, Ts, G	eEF 1α, 1β, 1γ, eEF2
Termination	RF 1, 2, 3	eRF

- Factors used in translocation: EF-G and eEF-2

Previous Year's Questions

Q. Which of the following binds mRNA with ribosome in eukaryotes? (AIIMS Nov 2019)

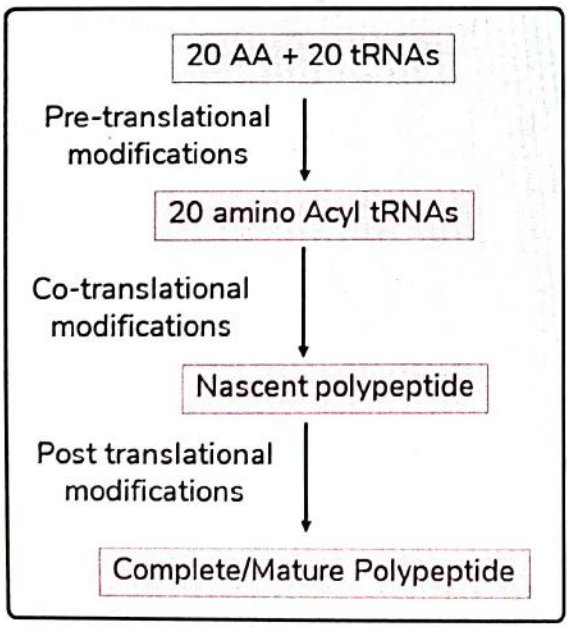
A. Poly A tail
B. tRNA
C. 7-methylguanosine cap
D. Shine dalgrino sequence

Important Information

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

Ans:
2 ATPs → for the activation of AA
2 GTPs → 1 GTP used for entry at A site
→ 1 GTP used for translocation
So, 4 high energy phosphates are used to add one AA in the growing polypeptide chain

MODIFICATIONS IN TRANSLATION 00:27:58



Co-translational Modifications

- Occurs in case of Selenocysteine and Pyrrolysine (21st and 22nd amino acids)

Post Translational Modifications (PTMs)

- Glycosylation (MC PTM)
- Phosphorylation
- Methylation
- Ubiquitylation
- Hydroxylation
- Biotin attachment to carboxylases
- Vitamin K dependent Carboxylation of Glutamate
- Proteolysis of protein (Cleavage of a sequence in protein)
- N-Acetylation
- Acylation
- S ~ S bond formation

Previous Year's Questions

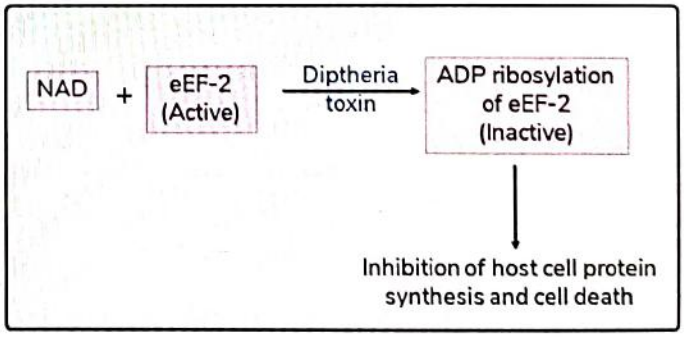
Q. Which amino acid does not include post translational modification? (AIIMS Nov 2017)

A. Selenocystiene
B. Triiodothyronine
C. Hydroxy-proline
D. Hydroxy-lysine

BACTERIAL TOXINS 00:34:26

- Examples are Cholera, Diphtheria and Pertussis toxins
- Inhibit host cell protein synthesis
- Inhibits EF by their ADP ribosylation
- Source of ADP ribosylation is NAD

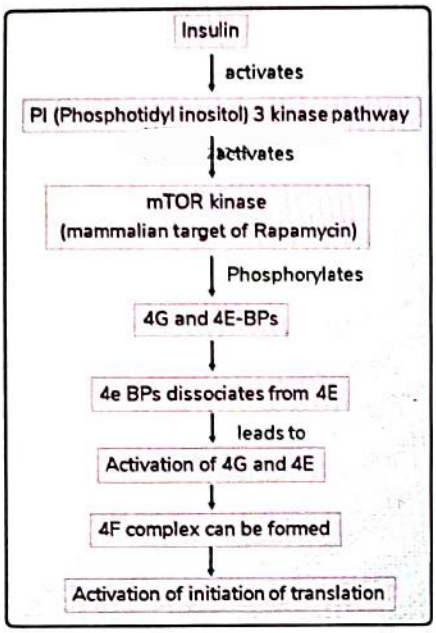
Mechanism of Action



INSULIN 00:38:28

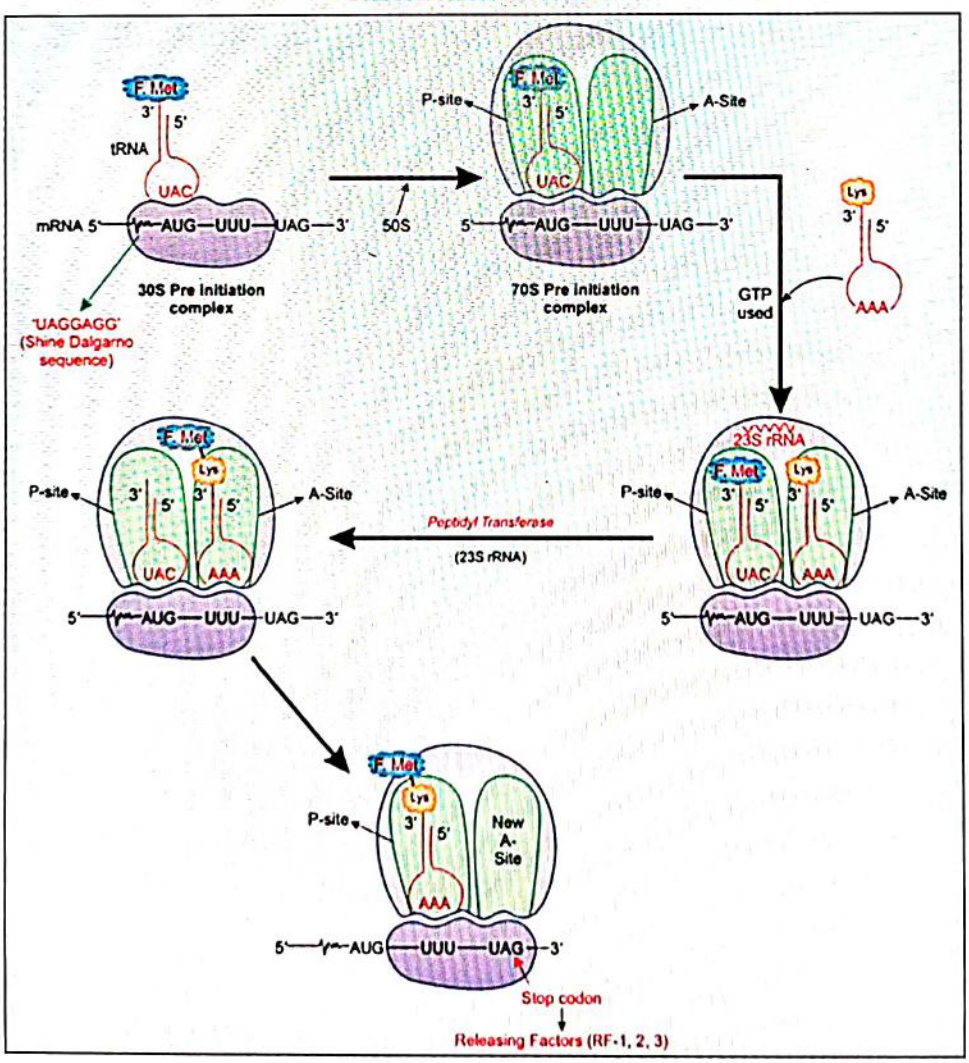
- Anabolic hormone and activate protein synthesis
- Does so by phosphorylation of eIF 4G and eIF 4E-BPs

Mechanism of activation of protein synthesis by Insulin



This same effect is also exerted by growth factors and mitogenic growth polypeptides

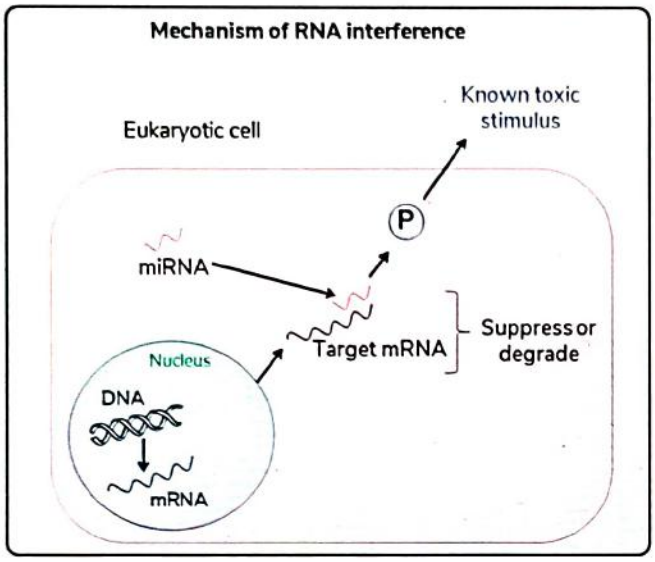
Figure 91.1



92 GENETIC TECHINQUES

RNA INTERFERENCE/ GENE SILENCING/GENE KNOCKDOWN

00:00:33



- Known toxic stimulus: Cell already has genetic machinery (DNA and protein) to detoxify the toxin.
- But the detoxification gene is not always active and induced only when required
- When stimulus come, gene will be transcribed and translated to make protein against the toxin e.g. antibody produced against as antigen
- When toxic stimulus is no longer present, cell must stop production of protein to save energy of cell.
- RNA interference using miRNA is one of the mechanisms to stop this production.
- This operates at the level of stopping translation by suppressing or degrading target mRNA
- In other words, one RNA is interfering in the function of other RNA
- Aka as Silencing technique: Gene is present, but function is silenced
- Aka Gene knockdown: gene is present, but its function is down

00:06:33

Important Information

- Gene knock out: Gene deleted
- Gene knock in: Gene inserted
- Gene knockdown: Gene is present, but its function is suppressed

Significance of gene knockdown

- To inhibit function of a harmful gene without causing the harmful effect associated with gene knockout.

Mechanism of miRNA silencing

- miRNA + proteins→RISC complex (RNA Induced Silencing Complex) → Binds to 3' end of target mRNA to be degraded

Enzyme used in the formation of miRNA

- RNA pol II (synthesis in nucleus)
- DROSHA
- PASHA
- Dicer endonucleases

2 Types of micro RNA

Synthesized from DNA	Synthesized from cytoplasmic RNA
• E.g. mi RNA	• e.g. tRNA, Viral RNA Aka si RNA (silencing or small interfering RNA)
• bind to 3' end of target mRNA	• Can bind anywhere on target mRNA

Advantages of gene knockdown

- Rapid
- Inexpensive
- Highly specific (selective silencing of gene)
- Can inactivate a diseased gene

Important Information

- RNA interference: gene inhibition at the level of translation
- Genomic imprinting: gene inhibition at the level of transcription

Previous Year's Questions

Q. RNAi acts through? (INI CET July 2021)

A. Knock out
B. Knock down
C. Knock in
D. Knock up

PCR (POLYMERASE CHAIN REACTION)

00:13:57

- used for amplification of DNA

Components of PCR

1. Heat is used for denaturation and 2 strands separation
2. DNA to be amplified
3. 2 primers (1 for each strand)
4. Enzyme: Taq polymerase [derived from *Thermus aquaticus* bacteria]
5. Substrates: Deoxyribonucleotides
6. Mg^{2+} and Buffer

Remember that Dideoxyribonucleotide is never the component of PCR

Steps of PCR in sequence

1. Denaturation → Two strands get separated
2. Annealing → Primers get annealed (attached) to DNA
3. Extension → Polymerization of DNA

Previous Year's Questions

Q. PCR steps are? (INI CET Nov 2020)

A. Denaturation, annealing, elongation
B. Annealing, denaturation, ligation
C. Ligation, denaturation, annealing
D. Denaturation, annealing, elongation, hybridization

REAL TIME PCR / QUANTITATIVE PCR

00:17:52

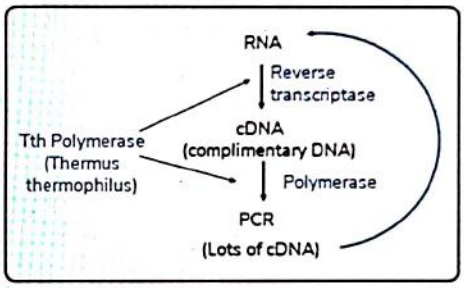
- Normal PCR is End-PCR i.e. only at the end of whole PCR process, we get the products and do the analysis.
- If there is a problem in amplification in middle of process, we cannot know about it
- Real time PCR can circumvent this problem by analysing DNA as it is getting synthesized
- Aka quantitative PCR as we can determine the quantity of DNA synthesized

Mechanism

- 5 components of a normal PCR + SYBR Green dye
- SYBR Green dye
  - Fluoresces when bound to ds DNA
  - Monitors the amplification of target DNA as it gets amplified by the monitoring the amount of fluorescence and back calculating the amount of DNA from this value
  - Amount of amplification can be known and quantified in real time

RT-PCR (REVERSE TRANSCRIPTASE PCR)

00:21:41



Advantages of PCR

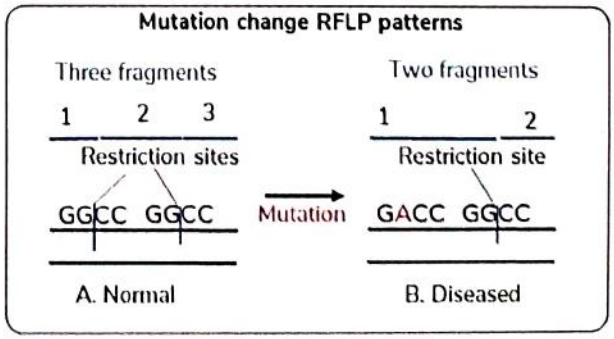
- Quick
- Automated
- Highly Sensitive
- Can be used to study RNA as well as DNA
- Can detect mutations

RFLP [RESTRICTION FRAGMENT LENGTH POLYMORPHISM]

00:23:58

Restriction fragments: The fragments obtained after digestion with restriction endonuclease enzyme.

RFLP: Polymorphism (variability) in the length of fragments formed by restriction enzyme

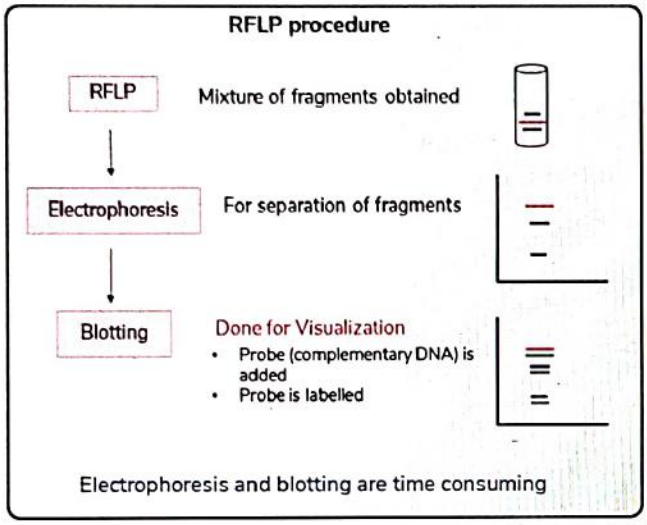


Uses of RFLP

- Detects mutations (Single)
- Genome mapping
- Paternity testing
- Localization of genes for genetic disorders
- Determination of risk of diseases

Limitations

- Can detect only single mutation at a time
- Can detect only those mutation which affect palindrome
- Lengthy procedure



BLOTTING / HYBRIDIZATION

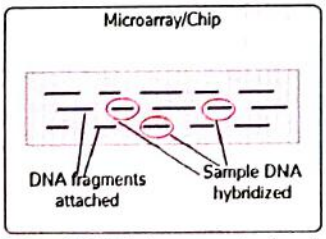
00:32:39

Blotting technique	Sample	Probe
Southern blotting (DNA-DNA hybridization)	DNA detected	DNA
Northern blotting (RNA-DNA hybridization)	RNA detected	DNA
Western blotting (Antigen-antibody reaction) Immunoblotting	Protein (antigen) detected	Antibody

Single gene expression analysis can be done by Northern or Western blotting or both

MICRO ARRAY/CHIP

00:35:02



Device used: a lot of DNA fragments is placed on a small device looking like a chip.

Important Information

Chip is different from ChIP which stands for Chromatin Imuuno Precipitation used for analysis of PTMs of histone proteins

Uses of chip

- Can detect multiple mutations
- Can do multiple gene expression analysis
- Can do comparative genomic hybridization
- Can detect SNPs (single nucleotide polymorphisms)
- Can do global pattern of gene expression (different mRNAs coming from different genomes)
- Can detect genetic transfer of the disease

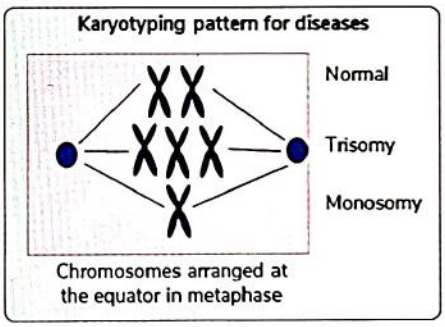
Limitation

- Can't detect monosomy & Trisomy

KARYOTYPING

00:39:14

- Best technique for detecting monosomy & trisomy
- Cells are arrested in metaphase stage using colchicine chemical
- In Metaphase, all chromosomes lie at equator
- So, they can be eailsy analyzed by seeing under microscope for any change in their number



- Chromosomes are stained with Giemsa stain to observe a G-banding

Limitations

- Lengthy (culture of cell required)
- Cannot be done in any phase of cell cycle
- Cannot detect micro deletions, amplifications and complex translocations

FLUORESCENT INSITU HYBRIDIZATION (FISH)

00:41:43

- In Situ: done in morphologically intact cell, tissue or organ
- Rapid technique (< 24hrs): as culturing of cells is not required
- Can be done in any phase of cell cycle
- Can detect microdeletions, amplifications, monosomy & trisomy
- Can detect gene location on a chromosome

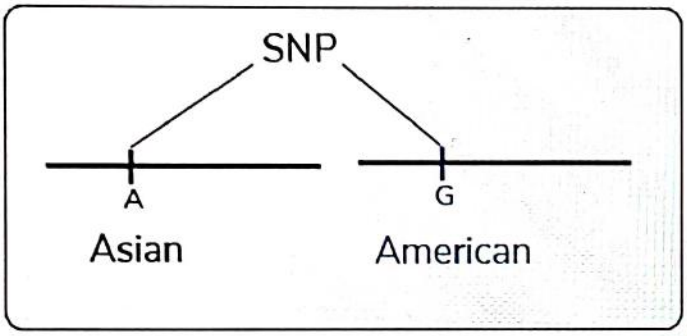
FRAP (FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING) 00:43:45

- Used to detect movement of proteins from one compartment of cell to another
- Fluid mosaic model of cell membrane was detected using this technique.

DNA MARKERS 00:45:03

- Relate to various Polymorphisms in DNA

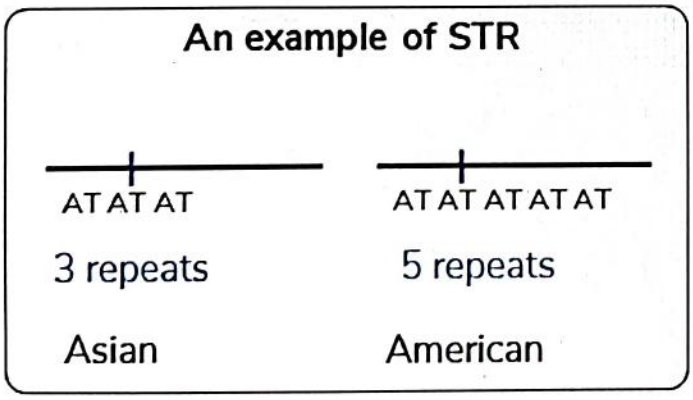
1. SNPs (Single Nucleotide Polymorphisms)



- Single nucleotide variations in the genomes
- MC polymorphism
- ~ 10 million SNPs in human genome
- SNPs occur once in every 300 bases

2. Repeat length polymorphisms

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



3. RFLP

Previous Year's Questions

Q. False about microsatellite is: (JIPMER May 2018)

A. Repeat size is 12-17 base pairs
B. Found in carcinoma colon
C. More prone to variation
D. DNA repeats

DNA SEQUENCING TECHNIQUES 00:51:47

1. **Sanger's method:** Dideoxy nucleotide method
   - Sanger's reagent: 1-fluoro 2,4 dinitro Benzene (FDNB)
   - Edman Reagent: Phenyl Isothiocynate (PITC) used for aa sequencing
2. **Maxam & Gillbert technique:** Chemical cleavage method
3. **NGS (Next Generation Sequencing):** Large scale and automated

Advantages of NGS

- High accuracy
- Fast turnround time for high sample volumes
- Can detect unknown/new mutation
- Can detect mosaicism
- High sensitivity
- Can do gene expression profiling
- Can discover ncRNA and protein binding site on DNA

Important Information

Techniques which can detect mutations

- PCR
- RFLP
- Microarray
- Sequencing
- Karyotyping
- FISH

Previous Year's Questions

Q. Which of the following methods uses RNA? (INI CET July 2021)

A. Western blot
B. RT-PCR
C. Sangers method
D. G-banding

CLINICAL QUESTIONS

Q. A patient presented with complains of fever, cough, backpain, runny nose and breathing difficulties to the outpatient department. He was a suspected case of COVID-19. Which technique will be best to confirm the diagnosis:

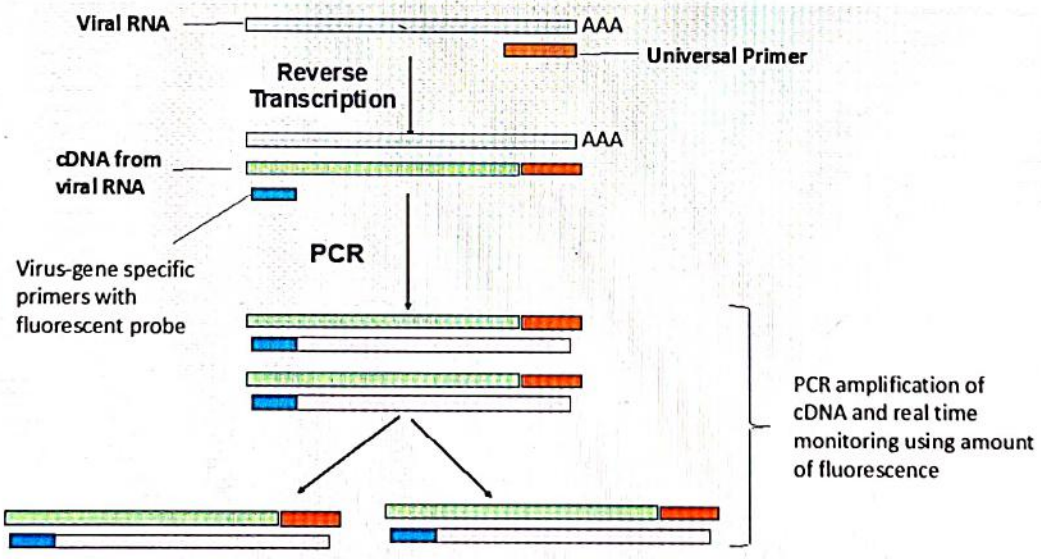
A. Antigen-antibody test
B. PCR
C. Real-Time Reverse Transcriptase PCR
D. Reverse Transcriptase PCR

Answer: C

Solution

The best test to detect Covid-19 viral infection is real time Reverse Transcriptase PCR (rRT-PCR) assay. In this assay the swab sample from nose is used. The amount of viral nucleic acid present in the sample gives an estimation extent of infection.

The viral RNA in the sample is first converted to cDNA using reverse transcriptase enzyme and then cDNA is detected and quantified using viral specific primers in a PCR reaction performed and monitored in real time using fluorescent probes or dye (See fig).



When the fluorescence reaches a threshold, the sample is said to be positive for virus infection. By monitoring how many cycles it takes to reach this level, one can determine the severity of the infection: the fewer the cycles, the more severe the viral infection is.

Reference: - Lehninger's 7th ed/pg. 331

Q. A tall 23-year-old male has feminine characters such as sparse facial hair, small testes, and gynecomastia. He also had poor coordination and language and reading difficulties. The physician suspecting of Klinefelter syndrome will order which of the following test to confirm his diagnosis?

A. USG abdomen
B. Echocardiography
C. Triple test
D. Karyotyping

Answer: D

Solution

Klinefelter's syndrome (XXY) can be detected by karyotyping. This test will directly observe the presence of extra X chromosome in his chromosome preparations.

Karyotyping is used for study of chromosome and is considered best technique to detect Monosomy and Trisomy. Sample used is blood, bone marrow, amniotic fluid and placental tissue. Most common stain used is Geimsa/G-banding.

Reference: Nelson's 20th ed/pg. 622

93 EPIGENETICS AND GENOMIC IMPRINTING

EPIGENETICS

00:01:37

- "Epi" genetics means "above" genetics
- In other words, this is change in DNA but not change in DNA code
- Chemical modification of DNA e.g. DNA methylation
- Changes are reversible and can be transmitted to next generation
- Can lead to gene activation or gene inhibition

GENOMIC IMPRINTING

00:02:43

- An Epigenetic phenomenon
  - Imprinting means inhibited; Genomic means related to genes
  - A method of Gene regulation
- Two alleles are present for any gene (one from each parent)
- In most cases, both alleles are expressed
- < 1% of cases, only one allele is expressed & other is imprinted

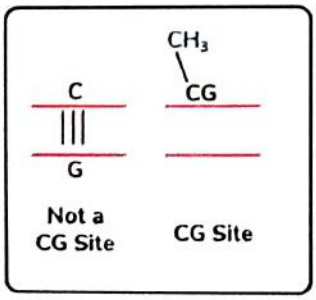
00:04:42

Paternal Imprinting	Maternal Imprinting
• Allele from father is imprinted/inhibited • Allele from mother is working	• allele from mother is imprinted/inhibited • Allele from father is working

Mechanisms of Genomic Imprinting 00:05:13

1. **DNA methylation**

- Most common mechanism
- Occurs at CG site/CG Island/CpG sites (C & G together present on same strands)
- Cytosine is usually methylated in CG site that causes inactivation of the gene



- DNA methylation can be detected by Na bisulfite method

2. **PTM of Histones:**

- Histone Deacetylation
- Histone Methylation
- Methods to detect PTMs: Chromatin Immuno Precipitation (ChIP)
  - CH → CHromatin; IP → ImmunoPrecipitation

Previous Year's Questions

Q. Which of the following does not favor permissive euchromatin due to changes occurring at cytosine residues at CpG islands in DNA?
(AIIMS May 2018)

A. Methylation
B. Phosphorylation
C. Alkylation
D. Sumoylation

Disorders caused due to genomic imprinting 00:08:28

1. **Prader Willi Syndrome (PWS)**

- Related to imprinting of a particular gene e.g. ABC gene on Chr 15
- Under normal situation: Maternal allele is imprinted/ inhibited, and only paternal allele is working
- In PWS: paternal allele is also deleted resulting in both copies (father and mother) of gene not working giving rise to sign and symptoms

2. **Angel Man syndrome**

- Related to another gene e.g. XYZ on chromosome 15
- Under normal situation: Paternal allele is imprinted/ inhibited, and only Maternal allele is working
- In Angelman Syndrome: Maternal copy of allele is also deleted leading to various sign and symptoms

Important Information

- Prader – Paternal allele deleted
- Angel – Maternal allele deleted

CLINICAL QUESTIONS

Q. A patient presented with voracious appetite, obesity, short stature, hypogonadism, and mental disability. Karyotypic analysis showed a small deletion on the proximal long arm of chromosome 15. The physician suspected it to be a case of genomic imprinting. Which out of the following is a disease due to genomic imprinting?

A. Prader Willi syndrome
B. Marfan syndrome
C. EDS
D. Osteogenesis imperfecta

Answer: A

Solution

Genomic imprinting is seen in Prader Willi syndrome and Angelman Syndrome.

Prader Willi Syndrome (PWS): Gene on chromosome 15, for which maternal allele is normally imprinted and paternal copy is active. But, if paternal allele is deleted, then it leads to PWS. Other causes of PWS is maternal uniparental disomy i.e. both copies of maternal chromosomes are present. As maternal alleles are already imprinted in this case, so not expressed.

Regarding other options:

Marfan syndrome is due to mutation in fibrilin gene with clinical features such as tall stature, long limbs, lens dislocation, arachnodactyly and media of large arteries is weak (defective elastin).

Ehler-Danlos Syndrome (EDS) is a heterogeneous group of disorders characterized by stretchy skin and loose joints. Disease exist in many types depending on collagen affected. Collagen types affected are Type I, III, V.

Osteogenesis imperfecta also known as (brittle bone disease) is a subtype of EDS causing ostetoporosis due to mutation in gene COL1A1 or COL1A2 affecting collagen type I.

Reference: - Robbin's 9th ed/pg. 172-173

94 CRISPR

CRISPR – CAS 9 SYSTEM

00:00:26

- Causes double strand break.
- CRISPR – Clustered Regularly Interspersed Short Palindromic Repeats
- Cas-9: CRISPR associated endonuclease
  - This endonuclease is guided by CRISPR for cutting site
- It is an immune system in bacteria against bacteriophages
  - Memory of bacteriophage infection and immunity is transferred to future generation also
  - So, protect many future generations from viruses

Uses

00:02:27

- Can be adopted to be used in eukaryotes for purposes like
  - Gene deletion
  - Exogenous gene insertion
  - Multigene editing possible
  - Altering gene transcription and regulation

Advantages over older technique of DNA breaks

00:03:01

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

CRSIPR COVID-19 TEST

00:03:47

- Simple paper Strip test
- Detects the genomic sequence of SARS-CoV-2 virus
- A specially adapted Cas9 protein is used for detection

How it is better than RT-PCR test?

- More Accurate
- Quicker turnaround time
- Less expensive equipment
- Better ease of use

Name Given: FELUDA
FELUDA- FN cas9 Editor Linked Uniform Detection Assay

Previous Year's Questions

Q. In CRISPR-Cas 9 system. which repair mechanism is used for genome editing? (AIIMS Nov 2019)

A. Homologous repair
B. Non homologous end repair
C. Mismatch repair
D. Nucleotide excision repair

LEARNING OBJECTIVES

UNIT VIII: MISCELLANEOUS

- This unit is mainly focused on vitamin and minerals along with other important miscellaneous topics in biochemistry which are high yielding. Chapter 95 gives details of alcohol metabolism and biochemical changes occurring in the body due to excessive alcohol intake. Vitamin general chapter tells about the classification and general characteristic of water-soluble and fat-soluble vitamins. Chapters 97-100 contain details on fat-soluble vitamins A, D, E and K their sources, main biochemical functions and disease associated with their deficiencies and overdoses. Chapters 101-109 provide details on important functions and diseases due to deficiency of water-soluble vitamins.

- Chapter 110 to 113 provide classification of minerals as macro and micro minerals and discussion on important macro-mineral calcium and micro-minerals iron, copper, zinc, chromium selenium and fluorine. The individual chapter on these minerals include knowledge regarding their absorption, transport, their function and various diseases due to their deficiency or excess.

- Free radical chapter teaches about the mechanism of generation of oxygen free radicals (OFR) by incomplete reduction of O_2, uses of OFR, and then enzymatic and non-enzymatic antioxidants. Xenobiotics chapter talks about reactions and enzyme involved in detoxification of foreign substances from the body. Muscle energy systems chapter provide details on different energy providing pathways under aerobic and anaerobic conditions.

- Meister cycle is a mechanism of absorption of neutral amino acids. A chapter on buffer and titration curve touch in the basics of acid-base buffers, acid-base disorders and body's buffer mechanisms to compensate for these disorders. Polyamine pathway talks about general function and generation of important amines such as putrescine, spermine and spermidine. HBA1c value is widely used diagnostic tool for diabetes and discussion is given in chapter 123 whereas chapter 124 talks about the one carbon donors and acceptors and their functions.

- **Major learning objectives**
  - To know pathways and enzymes involved in Alcohol metabolism and consequences of alcoholism
  - To understand roles of fat-soluble and water-soluble vitamins in human metabolism
  - To know the absorption, transport and functions of different minerals
  - To learn diseases associated with vitamin and mineral deficiencies and excess
  - To understand free radical generation and their uses and types and roles of antioxidants
  - To know the processes involved in detoxification of xenobiotics
  - To learn about acid-base disorders and body buffering mechanism against it

95 ALCOHOL METABOLISM

ALCOHOL METABOLISM

Types of alcohols 00:00:25

- Ethanol (Least Toxic)
- Methanol
- Ethylene glycol
- Isopropyl alcohol (Most Toxic)

Alcohol Metabolism 00:01:15

- Major organ involved: Liver
- Organelle: Cytoplasm & Mitochondria
- Minor pathways occur in:
  - ER Microsomal Ethanol Oxidising pathway (MEOS)
  - Peroxisomes
- Follows zero order kinetics
  - Alcohol elimination occur irrespective of its concentration in body
- Site of alcohol absorption: Mainly small Intestine (80%) and also Stomach (20%)
- Energy produced: 7 kcal/gm (empty calories i.e. no vitamin or minerals supplied)

ALCOHOL PROPERTIES 00:03:39

- Water soluble
- Produced by fermentation of sugar (fruity odour)
- CNS depressant

3 stages of alcohol consumption effects

1. Excitement/Euphoria
- Due to increased dopamine
- Pupils are dilated

2. Incoordination
- Pupils dilated

3. Coma
- Pupils constricted

ENZYME INVOLVED IN ALCOHOL METABOLISM 00:05:11

Alcohol	Alcohol dehydrogenase →	Aldehyde	Aldehyde dehydrogenase →	Acid
Ethanol		Acetaldehyde		Acetic Acid
Methanol		Formaldehyde		Formic Acid

Alcohol Dehydrogenase (ADH)

- Many isoenzymes
- Most Abundant form: ADH 1A
- Present in Liver and Adrenal Glands
- Has NAD containing domain known as Rossman fold

Aldehyde Dehydrogenase (ALDH)

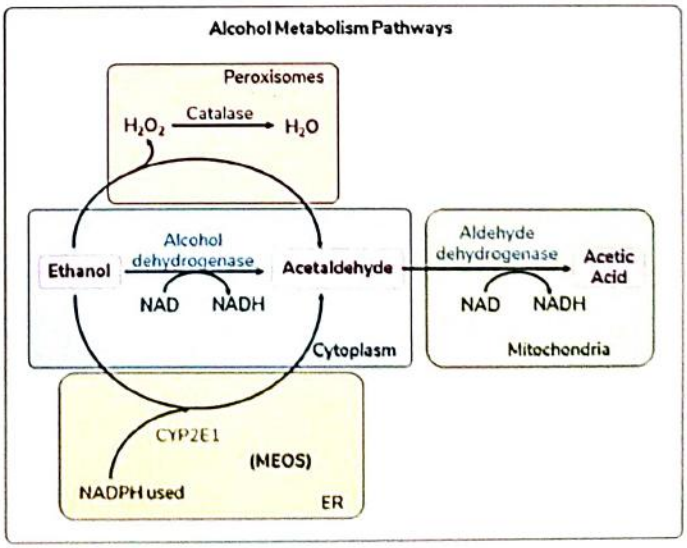
- Converts aldehyde to acid
- Fast reaction, so, do not allow aldehyde to accumulate
  - Acetaldehyde if accumulated give unpleasant feelings such as Vomiting, Diarrhoea, Flushing and Sweating.
- Activity varies with genetic variation
- Decreased activity in Asian population
  - So, acetaldehyde ↑↑ causing unpleasant symptoms- called as Asian Flush Syndrome
  - Symptoms are Nausea, Diarrhoea, Tachycardia, Vomiting, Hyperventilation, Flushing, Sweating

Important Information

How Acetaldehyde is toxic:

- Binds with glutathione
- Forms adducts with proteins and amino acids
- Damages mitochondria
- Inhibits microtubules

00:10:04

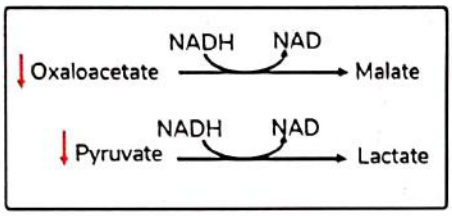


Microsomal Ethanol Oxidising System [MEOS]

- Induced only after high amount of alcohol is ingested as CYP2E1 has High Km so, requires a lot of substrate
- Protective in chronic Alcoholics as NADPH instead of NADH is involved, so, no NADH accumulation
- But too much use will produce ROS (Reactive oxygen species) which will damage DNA, proteins & Lipids

CONSEQUENCES OF INCREASED NADH IN ALCOHOLISM

00:14:08



↑NADH in liver occurs d/t alcohol metabolism which causes ↓oxaloacetate & ↓pyruvate which further leads to

1. **Lactic Acidosis**
   - d/t increased Pyruvate to Lactate conversion

2. **Hyperuricemia**
   - As Alcohol increases the breakdown of purine nucleotides causing increased uric acid production
   - Both over production & under excretion of uric acid occurs

3. **Hypoglycemia** due to
   - Decreased Gluconeogenesis [no pyruvate & OAA]
   - Poor diet due to Comatose state

4. **TCA inhibition**
   - due to NAD & OAA shortage
   - Acetyl CoA diverted to KB synthesis causing alcoholic ketosis

5. **β-Oxidation inhibition**
6. **Increased TG synthesis leading to fatty liver**

Important Information

- In alcoholics, fatty liver is due to
  1. increased endogenous synthesis of TG in Liver and not because of FA derived from adipose tissues
  2. β - Oxidation inhibition
  3. Impaired formation or release of VLDL

Note: There is no negative feedback control for alcohol metabolism. So, alcohol oxidation is preferred over other macromolecules when alcohol is in excess

Previous Year's Questions

Q. In fatty liver, which is not given: (FMGE Aug 2020)

A. Choline
B. Ethanol
C. Folic acid
D. Methionine

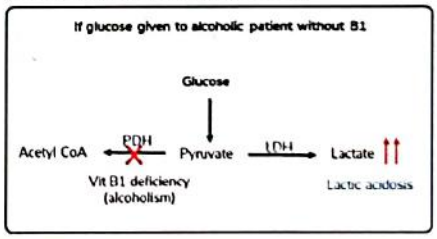
VITAMIN DEFICIENCY ASSOCIATED WITH ALCOHOLISM

00:20:01

- Vitamin B_1, B_3, B_6, B_9 and Vitamin A and D
- Alcohol interferes with the absorption of B_1 leading to Thiamine deficiency
- Severe thiamine deficiency Leads to Wernicke-Korsakoff Syndrome
  - Wernicke is Peripheral Neuropathy component with symptoms as Ataxia, Nystagmus and Ophthalmoplegia
  - Korsakoff is Psychosis Component with symptoms such as Memory toss, Confabulations and Cerebral hemorrhage

Treatment for alcoholic patients reaching hospital with hypoglycemia

- Give thiamine before giving glucose for hypoglycaemia to metabolize glucose via aerobic respiration and to avoid lactate formation.
- If glucose given without B_1 administration, it can worsen the symptoms of lactic acidosis as:



Previous Year's Questions

Q. A 20-year old alcoholic malnourished patient presented to hospital with respiratory distress. His pulse was 112/minute. Patient had edema, hypertension, systolic murmur along the left sternal edge and bilateral murmur along the left sternal edge. Bilateral crepitations were felt in the lungs. A diagnosis of congestive high output cardiac failure was made. Which vitamin is deficient? (NEET Jan 2019)

A. Vitamin B1
B. Vitamin C
C. Vitamin B2
D. Vitamin B6

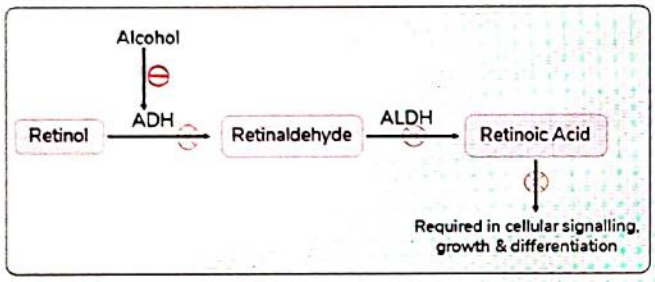
ALCOHOL DEADDICTION 00:23:28

Alcohol aversion therapy

- Disulfiram is given
  - It inhibits ALDH causing acetaldehyde accumulation if a person consumes alcohol
  - acetaldehyde ↑↑ will cause unpleasant symptoms
  - So, patient will fear of symptoms and avoid taking alcohol.

FETAL ALCOHOL SYNDROME 00:24:56

- If mother is taking alcohol during pregnancy it can cause various harmful effects on fetus.
- There is no safe defined amount of alcohol in pregnancy and is strictly C/I
- In fetus, ADH is inhibited in mother taking lots of alcohol



Sign & Symptoms

- Low IQ
- low birth weight
- Microcephaly
- Short Stature

Note: Alcohol can cross placenta and enter fetal brain by passing through blood brain barrier affecting brain and causing low IQ.

MARKERS FOR ALCOHOLISM 00:27:26

1. **Liver**

- Alcoholic Hepatitis occurs in chronic alcoholism
- Normally LFT marker is ALT
- But In alcoholism, liver maker enzyme is AST
- Also, there is raised Bilirubin in chronic hepatitis

2. **GGT (Gamma Glutamyl Transpeptidase)**

- Non-specific marker i.e. Increase in GGT in any form of fatty liver
- Can be used to detect binge drinking
- It takes 2-6 weeks of abstinence from alcohol for normal levels of GGT

3. **CDT (Carbohydrate Deficient Transferrin)**

- Transferrin: glycosylated Plasma glycoprotein used in iron transport
- Alcohol decreases glycosylation in chronic alcoholic patients

Detection of blood alcohol concentration 00:30:56

- If blood alcohol conc is = 1 mg%
- In urine = 1.3 %
- Alveolar air = 0.002%

Principle used in alcohol breath analyzers

- Subject's exhaled air goes in mouthpiece of analyzer from where it goes in to testing chamber
- Then blood alcohol conc is determined by back calculation using the above relation.
- blood alcohol > 30mg%, is punishable by Law in some cases.

ALCOHOL POISONING 00:32:57

Methanol Poisoning

- Methanol is aka Wood alcohol
- Methanol forms toxic Formaldehyde which can cause blindness

Ethylene Glycol poisoning

- Ethylene glycol is present in anti-freeze compounds
- Forms glycolic acid which can be converted to oxalic acid causing 2° hyperoxaluria
- Other symptoms: PCT necrosis and renal failure
- Diagnostic feature: fluorescent dye in urine + 2° hyperoxaluria
- Both also cause metabolic acidosis with increased anion gap
- Both use ADH and ALDH for their metabolism

Treatment

1. Using Ethanol that works on competitive enzyme inhibitor principle of alcohol metabolic enzymes
2. Using Fomepizole which inhibits enzyme ADH

CLINICAL QUESTIONS

Q. A 4-year-old baby boy landed in emergency with rapid breathing. He was cold & clammy, confused, and lethargic. His mother gave a history of accidental ingestion of automobile antifreeze. Ethanol administration is used as treatment in this poisoning because:

A. It conjugates with ethylene glycol
B. Inhibit enzyme alcohol dehydrogenase
C. Inhibit binding of ethylene glycol to alcohol dehydrogenase
D. Stimulate the excretion of ethylene glycol

Answer: C

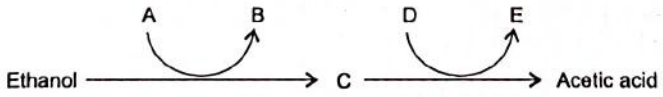
Solution

As metabolism of ethylene glycol is similar to ethanol and uses the same alcohol dehydrogenase, so, for the treatment, ethanol is used as antidote in ethylene glycol poisoning. Ethanol has low K_m for alcohol dehydrogenase; thus, it inhibits the binding of ethylene glycol with the enzyme by competitive inhibition causing the slowing down of glycolic acid production.

The most common source of ethylene glycol is automotive antifreeze or radiator coolant, where concentrations are high. Other sources of ethylene glycol include windshield deicing agents, brake fluid, motor oil, developing solutions for hobby photographers, wood stains, solvents, and paints.

Reference: Harper's 30th ed/pg. 261

Q. Disulfiram is used in alcohol deaddiction programs as an Antabuse for the treatment of alcoholism as patient on disulfiram therapy fear symptoms of flushing, tachycardia, nausea, vomiting, headache, vertigo, and anxiety within 30 minutes of alcohol ingestion. Below is a diagram depicting alcohol metabolism.



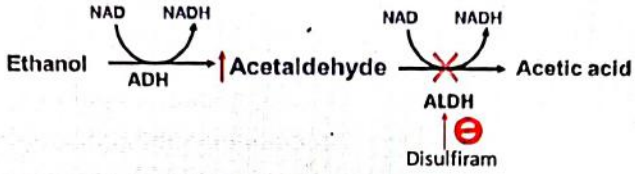
Elevated levels of which substance in the diagram may lead to the adverse effects described above?

A. A
B. B
C. C
D. D
E. E

Answer: C

Solution

Ethanol (CH_3CH_2OH) is converted to acetaldehyde (CH_3CHO) (substance C) by alcohol dehydrogenase (ADH, enzyme 1). Acetaldehyde is converted to acetate (CH_3CO_2) by acetaldehyde dehydrogenase (ALDH, enzyme 2). Both ADH and ALDH require the cofactor NAD^+ (substances A and D), which is reduced to produce NADH (substances B and E). Disulfiram irreversibly binds to NAD^+ binding sites on the ALDH resulting in the build-up of acetaldehyde (substance C). The accumulation of this toxic intermediate is thought to cause the adverse effects suffered by patients who ingest alcohol while also taking disulfiram.



Regarding other options:

NAD^+, which is represented by both A and D, is the rate-limiting step in EtOH metabolism. NAD^+ is consumed by ethanol metabolism, so it is not elevated in this situation and hence does not cause disulfiram-like symptoms.

Ethanol metabolism converts NAD^+ to NADH. NADH overproduction causes high NADH/NAD^+ ratio which in turn result in increased triglyceride synthesis and inhibition of lipolysis, ultimately resulting in hepatosteatosis but cannot cause disulfiram-like symptoms.

Reference: Devlin's 7th ed/pg. 412

96 VITAMINS GENERAL

VITAMINS

- Vitamins are organic compounds which are required in minute quantities but cannot be synthesized in body, so, they are essential in diet.

Atypical vitamins: can be synthesized in our body by human enzymes 00:01:21

1. **Vitamin D:** Synthesized in skin from cholesterol
2. **Niacin (B_3):** Synthesized from amino acid Tryptophan

Be aware that Vitamins synthesized by bacterial flora in human body are not considered as atypical vitamins

CLASSIFICATION OF VITAMINS 00:02:45

Fat Soluble	Water Soluble
• A, D, E, K	• B complex, C
• All have a common structure	• No common structure
• They do not have common function	• Common function - act as Coenzymes

Important Information

Exception: Fat soluble Vitamin which act as coenzyme - Vit K

Q. Water Soluble form available for which fat-soluble vitamin?

Ans. Vit K - Synthetic form - K_3 /menadione is water soluble

GENERAL FEATURES OF FAT-SOLUBLE VITAMINS 00:05:51

1. Non-polar so, cannot be excreted via kidneys and tend to accumulate
   - Get stored in liver
   - If excess: toxicity of fat-soluble vitamin can occur
2. Common Isoprene unit (5C unsaturated, branched chain structure) as precursor
3. All are absorbed from intestine and are assembled into chylomicrons along with dietary lipids. Pancreatic enzymes and bile salts have a role in absorption of these vitamins.
   - Fat soluble vitamin as they are stored in our body so their deficiency is Rare

Important Information

- Fat-soluble vitamin as they cane be stored in our body so, their deficiency is Rare.
- Fat-soluble vitamin with least toxic effect - Vitamin E
- Water-soluble vitamins are polar and are not stored in body as they are excreted via kidneys so, their toxicity is rare.
- One water-soluble vit which get stored in body - Vit B_{12}

CAUSES OF DEFICIENCY OF FAT-SOLUBLE VITAMINS 00:09:09

1. Steatorrhea & Malabsorption e.g. Cystic Fibrosis, Celiac diseases
   - Vitamin K is the first fat soluble vitamin to be excreted in acute malabsorption.
2. Mineral oil intake e.g. Paraffin Oil
   - This oil is not absorbed in body and get excreted out and take fat-soluble vitamin with it.

IMPORTANT QUESTIONS ON FAT-SOLUBLE VITAMINS 00:10:54

Fish Liver Oils: Good sources of vitamin A and D but not vitamin E

Richest source of vitamin A: Halibut fish liver oil >> Cod fish liver oil >> Shark fish liver oil

Which fruit rich in vitamin A: Ripe Mango

Which vegetable (plant source) rich in vitamin A: Carrot

Richest source of vitamin D: Halibut fish liver oil

Which fruit or vegetable has vitamin D: None

Vitamins with No plant source: Vitamin D & vitamin B_{12}

Strict vegetarians will be deficient in which Vitamin: Vitamin B_{12} >> Vitamin D (Vit D can also be obtained from sunlight)

IMPORTANT QUESTIONS ON SOURCE OF ESSENTIAL FATTY ACIDS (EFA) 00:14:40

Richest sources of Essential Fatty acid?
Safflower oil > Sunflower oil > Corn oil, Soybean oil

Other sources of EFA: Olive oil, Ground nut oil, Coconut oil, Flaxseed oil, Fish oil

Richest source of Linoleic acid: Safflower oil

Richest source of Arachidonic acid: Safflower oil

Richest source of Linolenic acid: Flaxseed oil > Soybean oil

Richest source of Eicosapentaenoic acid or Timnodonic: Fish oils

Richest source of PUFAs: Safflower oil

Richest source of Saturated Fatty acids (SFAs): Coconut oil

Richest source of Mono-unsaturated fatty acids (MUFAs): Olive oil

00:17:37

Food	Poor source of
Egg	Carbohydrates and vitamin C
Milk	Fe and Vit C
Meat	Ca
Fish	Carbohydrates & Iodine

Limiting Amino Acids - amino acids lacking in particular foods 00:18:41

Food	Limiting amino acid
Maize lacks	Tryptophan + Lysine
Wheat lacks	Threonine + Lysine
Pulses lacks	Methionine + Cysteine

Previous Year's Questions

Q. A 35-year old Farmer on maize diet with diarrhoea, dementia and dermatitis. Which Vitamin is deficient in this patient? (NEET PG 2021)

A. Thiamine
B. Riboflavin
C. Niacin
D. Pyridoxine

IMPORTANT QUESTIONS ON WATER SOLUBLE VITAMINS AND MINERALS 00:19:30

Richest source of Vitamin C: Amla or Indian Gooseberry > Guava > Cabbage > Other citrus fruits

Non-Citrus fruit having vitamin C: Guava

Vegetable which is rich in vitamin C: Cabbage

Richest source of iron: Dried pumpkin seed > nuts and oil seeds e.g. pistachio nuts > cashew nuts

What is Golden Rice: Genetically modified crop rich in iron and vitamin A

ONE LINERS 00:21:27

B-complex vitamins involved in energy release: B_1, B_2 and B_3, B_5 (link reaction and TCA), B_7 (required in gluconeogenesis)

Vitamin Synthesized by bacterial flora: Vitamins B_2, B_5, B_7, B_9, B_{12} and Vit K,

- Bacterial flora also synthesizes short chain fatty acids

Vitamin def with neurological symptoms
B_1 - Dry Beri beri (affects CNS)
B_3 – Dementia, Delirium, Depression
B_6- Infants having seizures
B_{12} and Vit E -Progressive Peripheral Neuropathy

Dementia in which vit Def: B_1, B_3, B_{12} and E

S-containing Vitamins: Vit B_1 and B_7

3 vitamins playing role in metabolism of S-containing amino acid: B_6, B_9, B_{12}

Homocystinuria occurs in def of: Vitamin B_6, B_9 and B_{12}

Glossitis, cheilosis in def of: Vitamin B_2 and B_6

Alopecia: Vit B_6 and B_7 def or Vit A toxicity

Antioxidants vitamins: Vit A, C and E and Vit D (recent addition)

Bleeding occurs due to def of: Vit C and Vit K

Hemolysis occurs due to def of: Vit E

VITAMIN DEFICIENCY ANEMIAS

00:28:19

Microcytic hypochromic anemia	Megaloblastic anemia
• Vit C: Helps in absorption of Fe • Vitamin B_6: Coenzyme of heme synthesis enz ALA synthase	• Vit B_9: No neurological symptoms • Vit B_{12}: Progressive peripheral neuropathy

Important Information

Vit E def causes neuropathy but No megaloblastic anaemia

00:30:06

Vitamin which take part in one carbon transfer reaction: Vitamin B_9 and B_{12}

Amino acid which take part in 1C transfer reaction: glycine, serine threonine and methionine

Nutritional causes of cardiomyopathy
- Excess of iron (hemochromatosis)
- Deficiency of vitamin B_1, Selenium, Ca and Mg

Royal Bee Jelly: Rich in vitamin B_5, B_6 and B_7

Site of absorption
- Vitamin B_9: Jejunum and duodenum
- Vitamin B_{12}: Ileum

97 VITAMIN A

VITAMIN A

3 biologically active forms 00:00:21

1. Retinol (having Alcohol (-OH) gp)
- Role in reproduction

2. Retinal (having Aldehyde (-CHO) gp)
- Role in vision

3. Retinoic acid (having Carboxyl (-COOH) gp)
- Role in cell growth and differentiation

- Main form is Retinol: can be converted to other forms also.
- All 3 forms are known as Retinoid compounds.
  - Obtained from animal sources (such as eggs, fish, liver, milk, cheese) as Retinol ester 00:02:47

Important Information

- From plant source (ripe mango, carrot etc.) the form of Vit A obtained is β-carotene (have 2 retinals joined end to end)
- Most abundant form of vitamin A: β-carotene

Previous Year's Questions

Q. Most abundant form of pro-vitamin A is:- (NEET PG 2018)

A. Beta carotene
B. Alpha-carotene
C. Retinol
D. Retinaldehyde

Absorption of Vitamin A 00:03:30

Vit A is absorbed in Intestine in the form of Retinol
↓
In Liver
(can be stored as Retinol esters in Peri-sinusoidal cells/Ito cells as 1 year supply)
↓
In liver, form Retinol – RBP complex in 1:1 ratio
↓
In Circulation, form Retinol – RBP – Transthyretin to avoid retinol-RBP loss in urine

- RBP (Retinol Binding Protein) is a low MW protein formed in liver
- Retinol – RBP – Transthyretin (Ternary complex)
- Transthyretin (older name prealbumin)
  - Trans - Transport protein
  - Thy - Thyroxine
  - Retin - Retinol binding protein

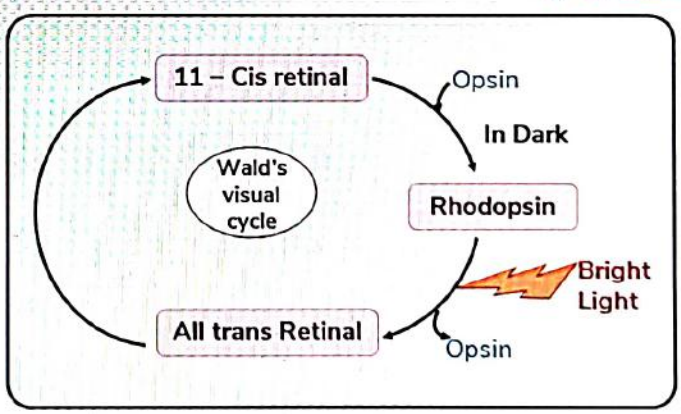
So, transthyretin helps in transport of thyroxine and RBP

Previous Year's Questions

Q. Vitamin A is stored in:- (NEET PG 2019)

A. Kupfer cells
B. Hepatocytes
C. Ito cells
D. Endothelial cells of liver

ROLE OF VITAMIN A IN VISION 00:06:20



00:08:00

Important Information

Rhodopsin

- Protein responsible for night vision
- In Bright Light, it gets bleached and becomes non-functional
- On moving into Dark, rhodopsin gets resynthesized
- This Time taken for rhodopsin re-synthesis is called as DARK Adaptation time
- This time is increased in Vit A deficiency

Other uses 00:09:34

- Antioxidant
- Photoprotective
- Maintenance of stability of lysosomes
- Glycoprotein synthesis
- Maintenance of epithelium
- Therapeutically, high doses of 13-cis Retinoic acid are used for treatment of severe cystic acne

Keep in mind that Retinoic acid is Teratogenic, so, used only when pregnancy test is negative

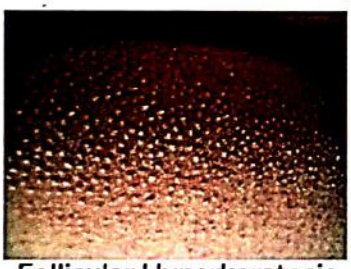
DEFICIENCY OF VIT A 00:11:24

- Earliest sign: Loss of sensitivity to green light
- Earliest symptom/ manifestation: Night blindness
- Most specific manifestation: Bitot's spots

Bitot's Spots

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

Bitot's Spot



Follicular Hyperkeratosis

- Dry scaly or tod like skin (at elbow or knees)
  - Due to non-maintenance of epithelium as vitamin has role in skin epithelium maintenance
  - Also occurs in essential fatty acid deficiency

Other Clinical features 00:14:32

- Xerophthalmia
- Keratomalacia
- Immunosuppression
- Skin ulceration
- Corneal ulceration, scarring
- Atrophy of urinary & respiratory tract epithelium leading to infection

Vitamin A deficiency is most common cause of preventable blindness

Previous Year's Questions

Q. Night blindness history, corneal drying and patient has Bitot's spots. Which of the following is found in this patient? (NEET PG 2021)

A. Keratosis pilaris rubra
B. Eczema
C. Phrynoderma
D. Icthyosis

Vitamin A toxicity 00:16:02

- Organelle affected: Lysosomes
- As vitamin A is stored in Liver, so, it occurs in people who consume bear's liver
- Clinical features are
  - raised Intracranial tension resembling Brain Tumour (Known as Pseudo Tumour Cerebri)
  - Hepatomegaly
  - Liver damage
  - Hyperlipidemia
  - Blurred Vision
  - Alopecia
  - Dry pruritic skin

Carotenemia 00:18:06

- Occurs due to excess intake of beta-carotenes
- Yellow staining of skin (but Not Sclera)

In Hyperbilirubinemia Jaundice – Yellow staining of skin + sclera.

CLINICAL QUESTIONS

Q. A 3-year old child showing growth failure, chronic cough and bronchitis. The child develops chronic diarrhea with light-colored, foul-smelling stools. Treatment with which of the following vitamin should be considered?

A. Vitamin A
B. Vitamin C
C. Vitamin B_1
D. Vitamin B_2

Answer: A

Solution

The symptoms of patient indicate respiratory and intestinal disease. In intestinal disorders such as chronic diarrhea or malabsorption due to deficient digestive enzymes, fat-soluble vitamins are poorly absorbed and can become deficient.

Hence, Supplementation of fat-soluble vitamins A, D, E, and K should be routinely done in these disorders. These vitamins must be administered intramuscularly or as oral emulsions (mixtures of oil and water).

The other vitamins listed are water-soluble which can be efficiently administered orally, and rapidly absorbed from the intestine.

Reference: Harper's 30th ed/pg. 456

98 VITAMIN D

VITAMIN D/SUNSHINE VITAMIN

- An atypical vitamin: can be synthesized in the body from cholesterol
- Sunshine vitamin: as it is synthesized in the presence of sunlight
- Active form: Calcitriol or 1,25 dihydroxy cholecalciferol

00:01:14

  - Three -OH groups present at positions 1 (added in Kidney), 25 (added in liver) and at 3 (present in cholesterol)

2 forms

1. D_2 - Ergocalciferol (obtained from plants, fungi and yeast)
2. D_3 - Cholecalciferol (obtained from Cholesterol)
   Both forms are converted to 25-OH D_3 in Liver

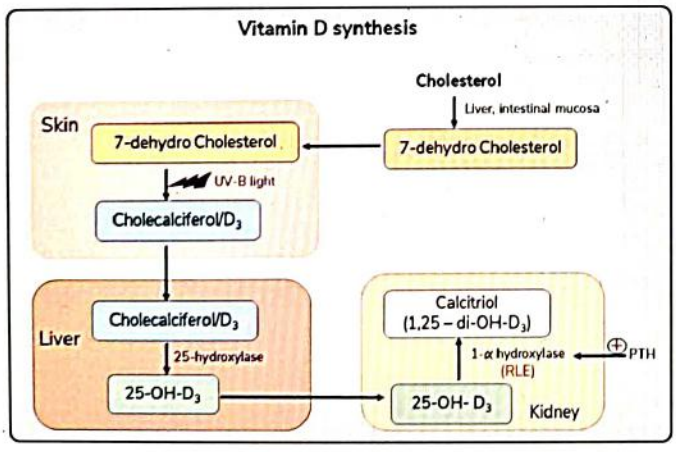
Calcitriol: considered as Hormone due to following reasons: 00:03:23

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

Note: But Calcitriol is not a proper hormone because it is not produced by some gland

SYNTHESIS OF VITAMIN D

00:05:18



Previous Year's Questions

Q. Vitamin D synthesis sequence is? (AIIMS 2020)

A. Skin → Liver → Kidney
B. Kidney → Liver → Skin
C. Skin → Kidney → Liver
D. Liver → Skin → Kidney

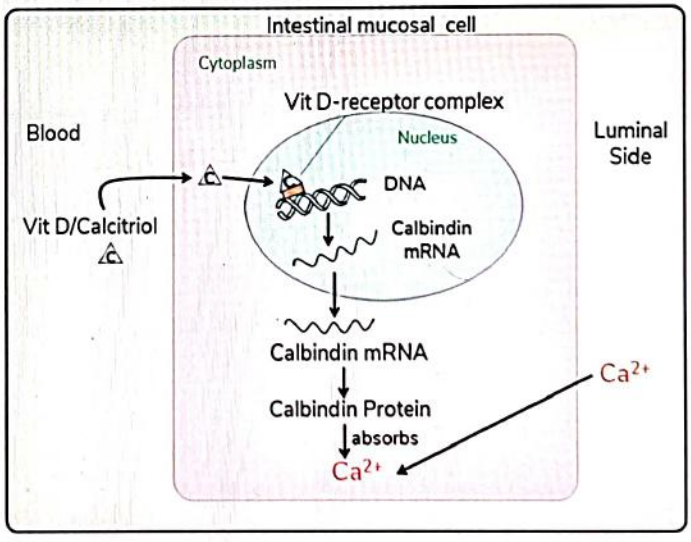
Ca Homeostasis: Involve Calcitriol & PTH 00:07:45

Vit D	PTH	Calcitonin
↑ Ca^{2+} in Blood ↑ Ⓟ in Blood	↑ Ca^{2+} in Blood ↓ Ⓟ in Blood (by excretion via kidneys)	↓ Ca^{2+} in Blood

ROLES OF VIT D

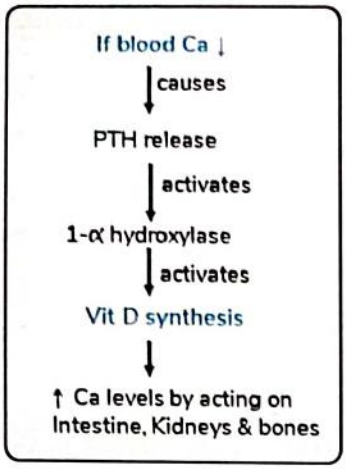
00:08:57

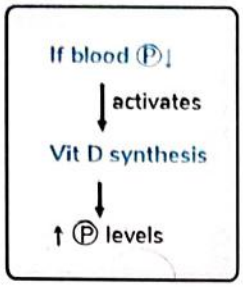
1. In intestine: ↑ Ca absorption with the help of protein Calbindin and ↑ Ⓟ absorption



2. In Kidneys: ↑ reabsorption of both Ca and Ⓟ
3. Bone: ↑ Bone mineralisation during bone growth and development. 00:11:42

- But if there is ↓Ca levels in blood, then Vitamin D will increase osteoclastic activity of bone resulting in removal of bone calcium and its release into blood.





00:13:55

Important Information

Production of Vitamin D α exposure to sunlight α I/Pigmentation of Skin

- **So, a dark skin coloured person will produce less vit D.**
- **Highest levels of Vit D are synthesized at the end of Summers**
- **Lowest levels of Vit D are synthesized at the end of Winters**

Deficiency of Vit D 00:15:05

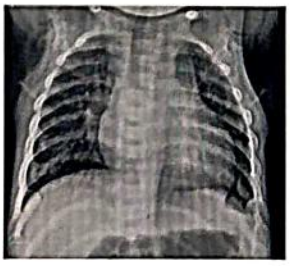
In Vitamin D deficiency

- ↓Ⓟ Levels in blood but blood Ca levels may be ↓ or normal.
  - Normal level is due to 2° to release of PTH which will ↑ Ca levels
- Also, there is ↑Alkaline phosphatase (ALP)

Deficiency in Children: Rickets 00:16:29

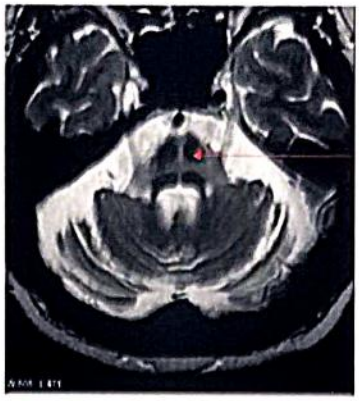
- Pigeon like chest
- Beaded appearance of RIBs aka Rachitic Rosary

Beaded appearance of Ribs



Rachitic Rosary

- Weight bearing bones – Bending
  - Bowlegs and Knock Knees
- Hot cross bun appearance of head due to non-closure of fontanelle



Hot cross Bun

Deficiency in adults: Osteomalacia 00:17:38

1. Bone pain
2. Prone to fracture
3. Muscle weakness
4. Soft bones like Pelvic bones affected (Waddling gait)

Renal Rickets

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

Causes of deficiency 00:16:43

1. Inadequate Sunlight exposure:
   - Common in women following Purdah/Burka system
   - Hospitalised bed ridden patients
   - Climate where Sunlight is not enough
2. Chronic kidney disease
3. Premature Infants
4. In Pregnancy + Lactation as requirements are high

Previous Year's Questions

Q. A 4-year old boy presented with developmental delay, recurrent chest infections and worsening bony pain. His serum PTH was normal. Calcium was normal and phosphate was low. There is no response to Vitamin D. The probable diagnosis is: (NEET PG 2021)

A. Nutritional rickets
B. X-linked hypophosphatemic rickets
C. Vitamin D dependent rickets type I
D. Vitamin D dependent rickets type II

Hypervitaminoses D 00:19:50

Symptoms

- Hypercalcemia
- Hypercalciuria causing Renal stones
- Loss of appetite
- Calcification of Soft tissues: calcium deposited in blood vessels causing hypertension

CLINICAL QUESTIONS

Q. A 70-year-old man presents to his physician with complaint of tenderness and aching of bone along with generalized muscle weakness. On consultation, it is evident that he has a very sedentary lifestyle with most of his time spent watching television in his room. He also reports a poor diet mostly devoid of fresh fruits and vegetables. A carpal spasm is noted after the cuff is inflated while taking the patient's blood pressure. Radiographs showed reduced bone density. Which of the following laboratory abnormalities would also be expected in this patient?

A. Elevated serum calcium
B. Decreased 1α-hydoxylase activity
C. Elevated 1,25-dihydroxycholecalciferol
D. Elevated parathyroid hormone

Answer: D

Solution

This patient is most probably suffering from vitamin D deficiency and osteomalacia looking at the symptoms of his poor diet and indoor lifestyle. Osteomalacia can manifest with bone pain and muscle weakness with reduced bone density on radiographic exam.

Vitamin D can be consumed from plants (ergocalciferol) or formed in sun-exposed skin (cholecalciferol). The active form of vitamin D is 1,25-dihydroxycholecalciferol (calcitriol), which is produced in the kidney by the enzyme 1α-hydroxylase (See vitamin D biosynthesis figure in text).

Calcium homeostasis in the body is maintained by interplay of calcitonin and parathyroid (PTH) hormones (refer to text).

Regarding other options:

This patient is suffering from hypocalcemia as indicated from reduced bone density. Vitamin D increases calcium reabsorption in the intestine as well as from both the distal convoluted tubule and collecting ducts of the kidney. So, Vitamin D deficiency will cause hypocalcemia (low serum calcium).

1a-Hydoxylase catalyzes the formation of 1,25 dihydroxycholecalciferol (calcitriol), the active form of vitamin D. In response to hypocalcemia, the body increases parathyroid hormone (PTH) secretion. PTH stimulates 1α-hydoxylase in order to maintain serum calcium. This patient has signs of hypocalcemia and would be expected to have increased activity of 1a-hydoxylase, not decreased.

This patient has vitamin D deficiency and osteomalacia. As 1,25 dihydroxycholecalciferol is synthesized from ergocalciferol (vitamin D_2), so, its levels would be decreased.

Reference: Lehninger's 6th ed/pg. 935

99 VITAMIN E

VITAMIN E/ α–TOCOPHEROL

- Most abundant & potent form of Vitamin E: α–Tocopherol
- Earlier also known as anti-sterility vitamin but this term is not used anymore as this effect was observed in animals only and not in humans.

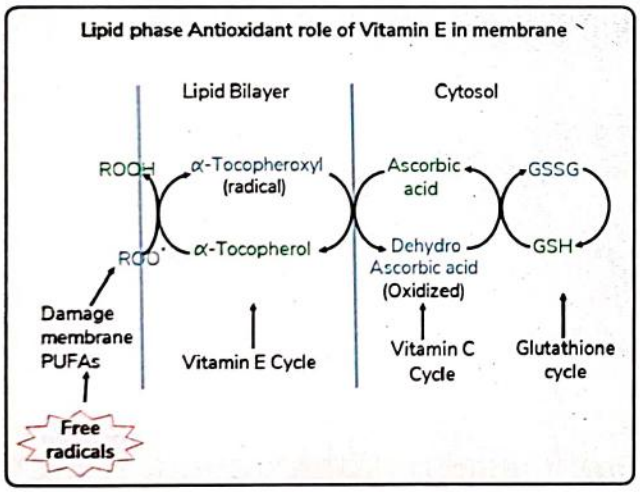
Sources 00:01:20

1. Richest source: Vegetable oils e.g. wheat germ oil, cotton seed oil, sunflower oil
2. Nuts

Excretion: Mainly in faeces via hepatobiliary route

Biochemical role 00:02:24

1. **Antioxidant:** Most potent lipid phase antioxidant (chain breaking antioxidant)
   - Protects cell membranes especially RBC membrane from free radical damage thus maintains stability of RBC membrane
   - **Anti-atherogenic:** Delays the formation of atherosclerotic plaque by converting oxidized LDL back to normal LDL 00:04:14



00:07:25

Important Information

Inter-relationship of vitamin E and Selenium

- Selenium is required for enzyme Glutathione peroxidase which converts GSH → GSSG
  - So, Se supplementation decreases the symptoms of Vit E deficiency
  - Vit E also decreases the requirement of Se

Previous Year's Questions

Q. Most potent lipid phase antioxidant: (FMGE 2019)

A. Vitamin A
B. Vitamin E
C. Vitamin C
D. Vitamin K

DEFICIENCY OF VIT E 00:08:34

- Hemolysis (d/t lack of protection of RBCs from free radical damage)
- CNS affected with Neurological presentation similar to B_{12} deficiency i.e.
  - Progressive peripheral neuropathy,
  - Axonal degeneration,
  - Spinocerebellar ataxia
- No megaloblastic anaemia
- Progressive external Opthalmoplegia

100 VITAMIN K

VITAMIN K

- K – 'Koagulation' (earlier name for Coagulation)
- Only fat-soluble vitamin with coenzyme function
- First vitamin to be excreted in acute malabsorption

Forms of Vitamin K 00:01:05

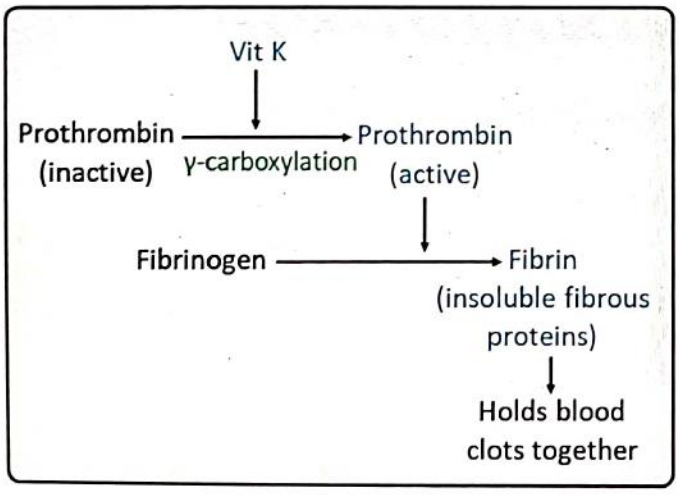
- 3 forms

1. K1 - Natural form - Phyloquinone - Plant source
2. K2 - Natural form - Menaquinone - Animal source
   - Synthesized by bacterial flora in intestine
   - Stored in Liver
3. K3 - Synthetic form – Menadione - Water soluble
   - Used for therapeutic purposes

Biochemical role: Blood clotting 00:02:33

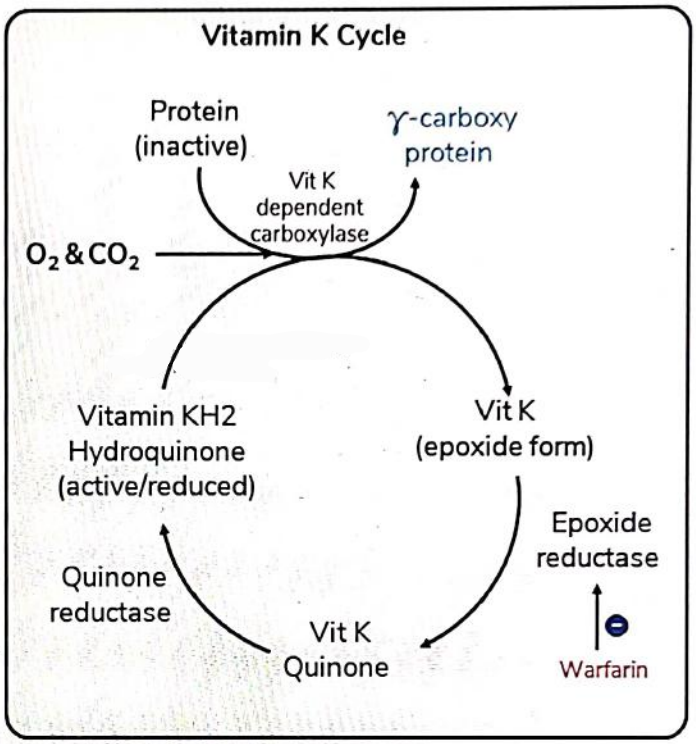
- By its coenzyme role in carboxylation (addition of COO^- group) helps in activation of:
  - Clotting proteins 2 (II), 7 (VII), 9 (IX), and 10 (X),
  - Protein C and S,
  - Nephrocalcin and osteocalcin,
- Addition of COO^- group increases the negative charge on these proteins that helps their interaction with more Ca^{2+} ion resulting in coagulation ($COO^- + Ca^{2+} \rightarrow$ Coagulation)

00:04:00



VITAMIN K CYCLE 00:05:01

- Occurs in hepatocyte microsomes.



Previous Year's Questions

Q. Which factor in warfarin therapy, have decreased gamma carboxylation of glutamate residue (NEET PG 2020)

A. Factor II
B. Factor V
C. Factor VIII
D. Factor III

Previous Year's Questions

Q. Vitamin K in its coenzyme form is regenerated by which enzyme? (AIIMS 2018)

A. Glutathione reductase
B. Pyruvate carboxylase
C. Dihydrofolate reductase
D. Epoxide reductase

VIT K DEFICIENCY

00:07:29

- Bleeding
- Easy bruising
- Ecchymotic patches
- ↑ Prothrombin time
- Foetal Haemorrhagic disease of newborn
  - Fatal and occurs quite often, so, all newborns are given vit K injection

Important Information

Reasons of Haemorrhagic disease of newborn

- Poor placental transfer
- Low Vit K content in early breast milk (colostrum)
- Hepatic Immaturity - Inadequate synthesis of coagulation proteins
- Vitamin K regeneration cycle is not fully developed
- Intestine of newborns is sterile (i.e. intestinal bacteria for Vit K synthesis not present)

Difference between Vit K and Vit C deficiency 00:09:36

Vit K deficiency	Vitamin C deficiency
• ↑ Prothrombin time • Haemorrhagic disease with no connective tissue problem	• ↑ Bleeding time • Gum hyperplasia, inflammation, loss of teeth, skeletal deformities in children (as vit C is required for collagen synthesis) • Anaemia (as vit C is required for Fe absorption • Poor wound healing
• Associated with o Fat malabsorption o Long term antibiotic therapy o Infants whose mothers were taking anticonvulsant therapy during pregnancy e.g. phenylhydantoins	• Associated with dietary deficiency of citrus fruits and green vegetables

CLINICAL QUESTIONS

Q. A full-term infant which is born at home and was doing well with breast-feeding starts bleeding from the umbilical cord and nostrils in next two days. The most likely cause of this condition is

A. Deficiency of vitamin K
B. Deficiency of vitamin C
C. Deficiency of vitamin E
D. Hypervitaminosis A

Answer: A

Solution

The diagnosis is hemorrhagic disease of the newborn due to vitamin K deficiency reasons for which are listed in the text. Because of these factors, vitamin K is routinely administered to newborns.

Regarding other options:

Hypervitaminosis A can cause liver toxicity but not bleeding,

Deficiencies of vitamin E (neonatal anemia) or vitamin C (extremely rare in newborns) have other symptoms besides bleeding

Reference: Harper's 30th ed/pg. 457

101 VITAMIN B_1

VITAMIN B_1/THIAMINE

- Careful to distinguish between Thiamine (a vitamin) from Thymine (a Pyrimidine)
  - Thiamine has a pyrimidine like ring in its structure
  - There is Sulphur in its structure also

Active form: TPP (Thiamine PyroPhosPhate) 00:01:35

> **Important Information**
>
> Vitamin B_1 is mainly involved in carbohydrate metabolism
>
> Q. Vitamin for which RDA depends on carbohydrate intake: Vitamin B_1

Richest source: Rice Polishing (outer Aleurone layer of grains is rich in Vit B_1)

Biochemical Role 00:03:15

- **Oxidative Decarboxylation reactions**
  - Coenzyme for PDH of link reaction, α-KG DH & Isocitrate DH of TCA cycle, BCAA-DH etc.
  - Hence, vitamin B_1 has a role in energy release in the cells.
  - B_1 deficiency will affect highly aerobic tissues such as brain and heart

- **Role in HMP**
  - B_1 is a coenzyme for enzyme Transketolase of HMP pathway, Hence, RBC Transketolase activity is used as a marker for B_1 deficiency
    - → Transketolase activity is tested not quantity for assessing B_1 deficiency
    - → A decrease in activity of this enzyme indicates B_1 deficiency

DEFICIENCY OF VIT B_1 - BERI-BERI 00:06:22

Dry Beriberi

- Affect CNS - as B_1 activates Cl^- channels in membrane of nervous system
- No oedema

Wet Beriberi

- Affect CVS
- Oedema present

Usually mixed Beriberi occurs i.e. overlap of wet and dry Beriberi

00:07:50

- Most common cause of B_1 deficiency is – alcoholism
  - Because alcohol interferes with the absorption of B_1
  - Because of poor diet and eating habits of alcoholics

- In chronic alcoholics severe vitamin B_1 deficiency occurs causing Wernicke-Korsakoff Psychosis syndrome
  - Wernicke component is encephalopathy- ataxia, nystagmus, ophthalmoplegia
  - Korsakoff component is psychosis- confabulations and psychosis

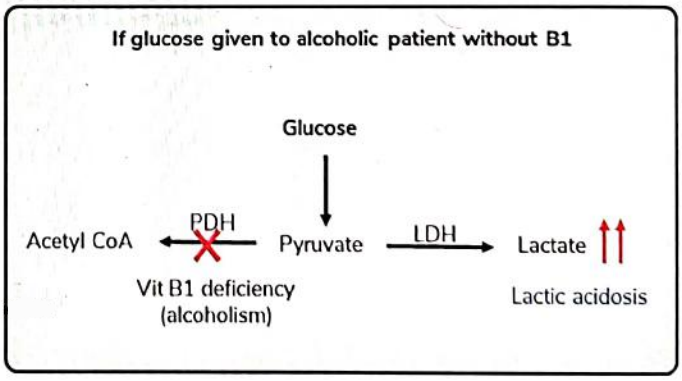
- Patient using diuretic have increase excretion of vitamin B_1 in urine

Lactic Acidosis in Vit B_1 deficiency

- As link reaction is inhibited due to PDH inactivity, excess pyruvate gets accumulated which gets converted to lactate causing lactic acidosis.

Treatment for alcoholic patients reaching hospital with hypoglycemia 00:10:28

- Give thiamine before giving glucose for hypoglycaemia to metabolize glucose via aerobic respiration and to avoid lactate formation.
- If glucose given without B_1 administration, it can worsen the symptoms of lactic acidosis as:



Previous Year's Questions

Q. Patient presents with inability to convert pyruvate to Acetyl CoA. Which of the following can be given in treatment? (JIPMER 2018)

A. Vitamin B_3
B. Vitamin B_1
C. Free fatty acids
D. Biotin

Previous Year's Questions

Q. A 20-year old alcoholic malnourished patient presented to hospital with respiratory distress. His pulse was 112/minute. Patient had edema, hypertension, systolic murmur along the left sternal edge. Bilateral murmur along the left sternal edge. Bilateral crepitations were felt in the lungs. A diagnosis of congestive high output cardiac failure was made. Which vitamin is deficient? (NEET PG 2019)

A. Vitamin B_1
B. Vitamin C
C. Vitamin B_2
D. Vitamin B_6

CLINICAL QUESTIONS

Q. A 20-year old alcoholic malnourished patient presented to hospital with respiratory distress. His pulse was 112/minute. Patient had edema, hypertension, systolic murmur along the left sternal edge. Bilateral crepitations were felt in the lungs. A diagnosis of congestive high output cardiac failure was made. Physician suspected a vitamin deficiency. Which of the following test will most likely support the diagnosis?

A. Erythrocyte transketolase activity
B. RBC Glutathione reductase
C. RBC thiamine levels
D. Serum thiamine level

Answer: A

Solution

Chronic alcoholics are at risk for thiamine deficiency. This is most likely a case of wet/cardiac Beri-Beri i.e. Vitamin B_1 deficiency. As Vitamin B_1 is mainly involved in oxidative decarboxylation reactions, so in B_1 deficiency, highly aerobic tissues e.g. heart and brain are affected the most.

Thiamine (vitamin B_1) deficiency is assessed by Erythrocyte transketolase activity as thiamine (B_1) is the cofactor for transketolase enzyme in HMP pathway in RBCs. Activity of Transketolase is measured (not the quantity)

Regarding other options:

There is no importance of checking serum or RBCs thiamine levels

RBC glutathione reductase is the marker for vitamin B_2 deficiency

Reference: Harper's 30th ed/pg. 555-556

102 VITAMIN B_2

VITAMIN B_2/ RIBOFLAVIN

Active form: FMN (Flavin Mononucleotide) & FAD (Flavin Adenine Dinucleotide)

Biochemical role: Coenzyme in Oxidation-Reduction reactions 00:01:08

- FAD present in - Complex II ETC
- FMN present in - Complex I ETC
- Enzymes which require FAD
  - PDH complex (Link Reaction),
  - Succinate Dehydrogenase and α-ketoglutarate DH (TCA),
  - Branched Chain Amino Acid DH (BCAA-DH),
  - L-amino acid oxidase and Monoamine oxidase

Important Information

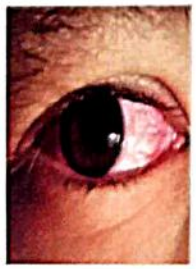
- B_2 and B_6 are required for synthesis of vitamin B_3 (niacin) from precursor tryptophan. So, pellagra like symptoms will occur in B2 and B6 deficiency also.

Marker enzyme: RBC Glutathione Reductase Activity

DEFICIENCY OF VIT B_2/ RIBOFLAVINOSIS

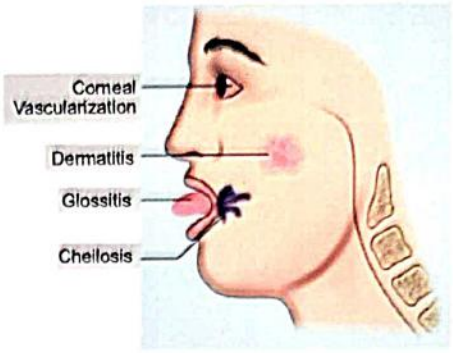
00:03:14

- **Characterized by 2Cs**
  - C - Corneal Vascularization
    → Aka pink eye due to increase vascularization of cornea
  - C - Cheilosis
    → Aka angular stomatitis due to cracks at the corner of the mouth
- **Other manifestations**
  - Seborrheic dermatitis
  - Glossitis/Magenta tongue/Geographical tongue



Corneal vascularization/ pink eye

Glossitis/ magenta tongue



Previous Year's Questions

Q. A middle-aged woman presents with fissures in mouth, tingling sensation and peripheral neuropathy. Investigations showed reduced glutathione reductase activity. Which vitamin deficiency is the likely cause of this? (AIIMS 2018)

A. Vitamin B_1
B. Vitamin B_2
C. Vitamin B_6
D. Vitamin B_{12}

Previous Year's Questions

Q. Corneal vascularization is seen in deficiency of which vitamin? (JIPMER 2017)

A. Vitamin B_1
B. Vitamin B_2
C. Vitamin B_3
D. Vitamin B_9

CLINICAL QUESTIONS

Q. A postoperative patient on IV fluids develops lesions in the mouth. An abnormally low excretion of 15µg riboflavin/mg creatinine was found on analysis of urine. Which of the following TCA cycle enzymes is most likely to be affected?

A. Succinate dehydrogenase
B. Malate dehydrogenase
C. Isocitrate dehydrogenase
D. Citrate synthase

Answer: A

Solution

The patient is showing a deficiency in riboflavin (urinary excretion of less than 30 µg/mg creatinine is considered clinically deficient).

Riboflavin is a component of the cofactor FAD (flavin adenine dinucleotide), which is required for the conversion of succinate to fumarate by succinate dehydrogenase (see table).

Table: Vitamins needed for TCA cycle

Coenzyme	Vitamin	TCA cycle enzymes
Thiamine pyrophosphate (TPP)	B_1 (Thiamine)	• α-ketoglutarate dehydrogenase (also requires other 4 cofactors like PDH)
Nicotinamide adenine dinucleotide (NAD^+)	B_3 (Niacin)	• Isocitrate dehydrogenase, • α-ketoglutarate dehydrogenase • Malate dehydrogenase
Flavin adenine dinucleotide (FAD)	B_2 (Riboflavin)	• Succinate dehydrogenase

Reference: Harper's 30th ed/pg. 121, 163, 164

Q. A patient presented in the OPD with complaints of tingling sensations in the hand and was feeling weak for past few days. He was a vegetarian but was not eating a proper diet for quite some time. A thorough clinical examination showed that the patient also had a red and softened tongue and roughed lips. The physician suspected a vitamin deficiency and ordered which of the following enzyme assay to detect this vitamin deficiency in this patient?

A. Glutathione reductase
B. Transketolase
C. Histidine load test
D. PDH

Answer: A

Solution

These are the typical presentation of the riboflavin deficiency and marker enzyme for riboflavin deficiency is glutathione reductase activity test as this enzyme need FAD as prosthetic group for its activity.

Symptoms of B_2 deficiency:

- Corneal vascularization- earliest sign of deficiency
- Glossitis: inflammation of tongue (magenta tongue)
- Cheilosis: redness and shiny appearance of lips.
- Angular stomatitis: lesions at mucocutaneous junction at corners of mouth, leading to painful fissures.
- Seborrheic dermatitis: rough scaly skin because of desquamation.

Extra Information:

Marker Enzymes/test for various vitamin deficiencies

B_1: Transketolase activity

B_6: Transaminase activity

B_9: Histidine load test

B_{12}: Serum methyl malonate

Reference: Harper's 30th ed/pg. 285

103 VITAMIN B_3

VITAMIN B_3/ NIACIN

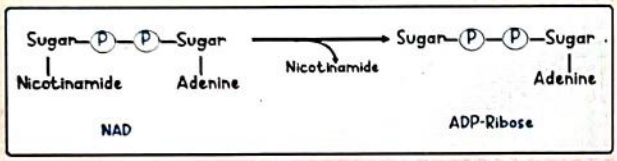
Active forms: NAD (Nicotinamide Adenine Dinucleotide) & NADP (Nicotinamide Adenine Dinucleotide Phosphate)

Biochemical role: Coenzyme in Oxidation–Reduction reactions 00:01:13

Important Information

Q. Which vitamin is required in DNA repair as a source of ADP-ribose?

A. Niacin in the form of NAD can donate ADP-ribose as:



- Niacin is an atypical vitamin i.e. it can be synthesized in body from tryptophan as:

Tryptophan (60mg) —(Vit B_2 and B_6; QPRTase (RLE))→ **Niacin (1mg)**

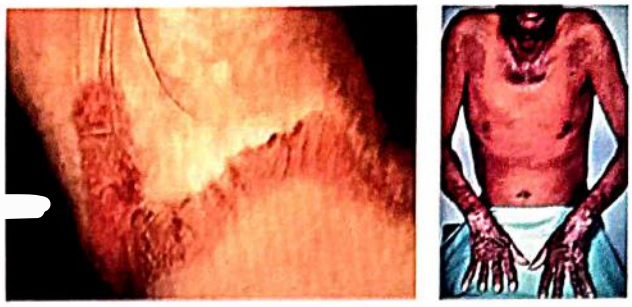
DEFICIENCY OF B_3 – PELLAGRA 00:03:39

Causes

- In people taking high corn diet/maize as Staple diet
  - Maize protein zein lacks tryptophan
- Due to anti-TB drug Isoniazid
  - Inhibits formation of active form of PLP (B6)
- Carcinoid syndrome
  - Too much tryptophan used to make serotonin, so, less is available for niacin synthesis
- Hartnup's disease (loss of tryptophan from body due to no absorption)

Symptoms 00:05:17

- Dermatitis (Photosensitive) → Casal's necklace appearance
- Dementia
- Delirium
- Depression
- Diarrhoea
- Death



Casal's Necklace

Photosensitive Dermatitis

THERAPEUTIC USE OF NIACIN 00:06:41

- Niacin in high doses can be used as Anti-Hyperlipidaemic agent
  - In high doses Niacin Inhibits hormone sensitive lipase (HSL).
  - HSL releases FAs from breakdown of TG. so, on its inhibition, FA will not be released in blood.
  - Hence, there will be ↓ TG and ↓ LDL and ↑ HDL in patients
- High doses of niacin can cause Niacin flush
  - Expansion of capillary occur, so, increase blood flow to skin surface.

Previous Year's Questions

Q. Which vitamin deficiency causes this?
(NEET PG 2020, 2021)

A. Thiamine
B. Niacin
C. Zinc
D. Pyridoxine

CLINICAL QUESTIONS

Q. A baby is hypotonic and shows that pyruvate cannot form Acetyl CoA in fibroblasts. Also, lactic acidosis is found. Administration of which of the following can revert this situation.

A. Biotin
B. Pyridoxal phosphate
C. Thiamine
D. Pyruvate

Answer: C

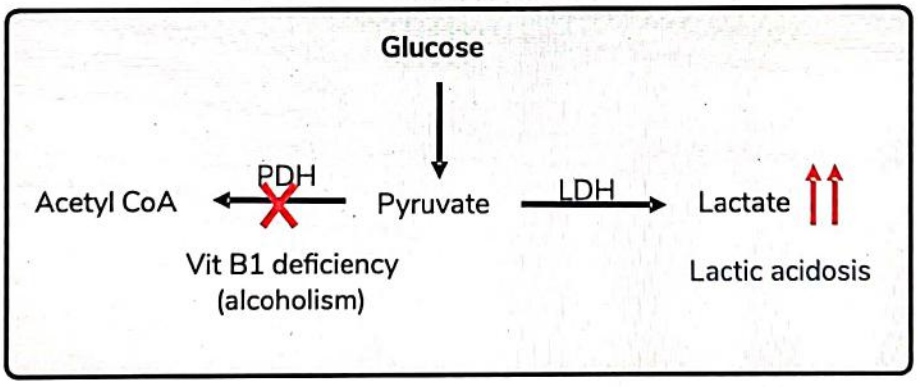
Solution

Congenital Lactic Acidosis may occur due to deficiency of thiamine (B_1) which is required for Pyruvate dehydrogenase (PDH) that converts pyruvate to Acetyl CoA.

PDH requires 5 coenzymes:

1. Lipoic acid
2. Thiamine (B_1)
3. Riboflavin (B_2) or FAD
4. Niacin (B_3) or NAD
5. Pantothenic acid (B_5)

In case of Thiamine deficiency, PDH reaction will be affected, and pyruvate will accumulate leading to the formation of lactate by LDH leading to Lactic Acidosis (see fig).



Reference: Harper's 30th ed/pg. 163

104 VITAMIN B_5

VITAMIN B_5/ PANTOTHENIC ACID

Active form: Coenzyme A e.g. Acyl CoA, Acetyl CoA

Biochemical role: coenzyme in acetylation and fatty acyl transfer reactions

- β-alanine is a component of pantothenic acid

DEFICIENCY OF B_5

- Def is rare causing Burning foot syndrome
- Hallervorden-Spatz syndrome aka Pantothenate kinase associated neurodegeneration
  - Associated with vit B5
  - Accumulation of iron in brain causing Free radical damage in brain (as Fe generates free radicals)
  - Autosomal recessive condition
  - **Clinical features**
    - → Dementia and Mental retardation
    - → Parkinson like features

CLINICAL QUESTIONS

Q. A 4-year old was suffering from anemia and significantly underweight for his age. He complaints of sensation of pain in lower extremities. The physician suspected burning feet syndrome and advised pantothenic acid supplementation. Which of the following conversions requires pantothenate as essential cofactor?

A. Glucose to ribose 6-phosphate
B. Alanine to glucose
C. Glucose to pyruvate
D. Oxaloacetate to citrate
E. Glutamate to a-ketoglutarate

Answer: D

Solution

Pantothenate is an essential part of coenzyme A which is required for reaction involving transfer of acyl group involving those associated with tricarboxylic acid cycle. In the very first step of TCA, acetyl CoA combines with oxaloacetate to form citrate involving transfer of acetyl group to oxaloacetate. Also, succinyl CoA synthesis from alpha-ketoglutarate requires CoA as coenzyme.

There are variety of other biochemical reactions such as fatty acid synthesis, beta oxidation, protein synthesis, vitamin A and vitamin D synthesis which require CoA mainly for acyl group transfer which activates the substrate for next reaction.

The synthesis of CoA from pantothenate in the cell involve phosphorylation reaction. Deficiency of pantothenic acid is rare, though it has been observed in severely malnourished populations where complains of paresthesia and dysesthesias (burning feet syndrome) are of common occurrence.

Options a, b, c and e do not require coenzyme A.

Reference: Harper's 30th ed/pg. 561

105 VITAMIN B_6

VITAMIN B_6/ PYRIDOXINE

Active form: PLP (Pyridoxal Phosphate)

Mechanism of action: formation of schiff base b/w Enz and Substrate

Biochemical role: coenzyme mainly in amino acid and protein metabolism reactions 00:01:06

Important Information

Q. Vitamin for which RDA is based on protein intake – Vitamin B_6

Only place in carbohydrate metabolism where vit B_6 is required is – glycogen phosphorylase in glycogenolysis

Previous Year's Questions

Q. Which vitamin is required for glycogen Phosphorylase? (AIIMS 2017)

A. PLP
B. TPP
C. Riboflavin
D. Lipoic acid

Role in amino acid and protein metabolism

- Transamination
- Deamination
- Trans sulfuration
- Cysteine synthesis
- Haem synthesis
- Simple decarboxylation of amino acids

DEFICIENCY OF VIT B_6 00:03:19

Def leads to

- Increased homocysteine in blood
- Increased homocystine in urine
- Excretion of xanthurenic acid in urine

Clinical features

- Anemia + Kidney oxalate stone + Neurological symptoms
- Alopecia
- Glossitis
- Cheilosis
- Convulsions
- Mental confusion
- Depression
- Carpel tunnel syndrome
- Neuropathy

00:05:08

Important Information

- Most common cause of vitamin B_6 deficiency is isoniazid therapy

Q. Seizures in Infants is caused by which Vitamin deficiency?

Ans: B_6

- Because B_6 is required for simple decarboxylation of glutamate for the formation of inhibitory neurotransmitter GABA
- Deficiency of GABA cause continuous excitation in brain.
- These seizures are non-responsive to anticonvulsive therapy

Previous Year's Questions

Q. Vitamin deficiency causing neonatal seizures? (AIIMS June 2020)

A. Thiamine
B. Pyridoxine
C. Riboflavin
D. Pantothenic Acid

Previous Year's Questions

Q. Sideroblastic anemia can be treated by? (INICET 2021)

A. PLP
B. TPP
C. Riboflavin
D. Lipoic acid

CLINICAL QUESTIONS

Q. A family member in TB positive patient's house was exposed and was found to give a positive tuberculin test (PPD) with a normal chest X-ray. He was placed on precautionary 6-month isoniazid therapy and developed peripheral neuropathies. Which of the following vitamins can be prescribed for treatment of neuropathies?

A. Riboflavin
B. Pyridoxine
C. Thiamine
D. Folate

Answer: B

Solution

- Isoniazid inhibits pyridoxal phosphokinase which converts pyridoxine to its biologically active form pyridoxal phosphate. Thus, Isoniazid therapy inhibits the formation of the active form of B_6.
- So, vitamin B_6 (pyridoxine) supplementation during isoniazid therapy is necessary in some patients to prevent the development of peripheral neuropathy.
- As Vit. B_6 (PLP) is required for Niacin synthesis from tryptophan, hence Isoniazid therapy also causes niacin deficiency and pellagra.

Reference: Harrison's 20th ed/pg. 2687

106 VITAMIN B_7

VITAMIN B_7/ VIT H/ BIOTIN

Biochemical Role: coenzyme in carboxylation reaction

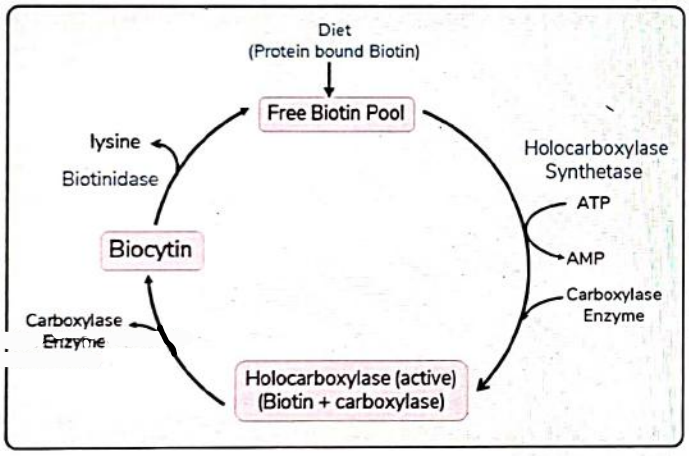
How to remember

All carboxylases require "ABC" – Mnemonic
A - ATP.
B - Biotin
C - CO_2
and also Mg

Important carboxylases 00:01:30

- Pyruvate carboxylase (gluconeogenesis)
- Acetyl CoA carboxylase (FA synthesis)
- Propionyl CoA carboxylase (Odd chain FA metabolism)

BIOTIN CYCLE 00:02:16



MULTIPLE CARBOXYLASE DEFICIENCY 00:04:45

- Deficiency of Holocarboxylase Synthetase and/or Biotinidase.
- Deficiency of Holocarboxylase Synthetase: Early onset (Infantile form)
- Deficiency of Biotinidase: Late onset (Juvenile form)

Clinical features

- Autosomal recessive
- An organic acidemia
  - NH_3 normal or ↑
  - Metabolic acidosis with ↑ anion gap
  - Ketosis
  - Odour - Tom Cat urine

Previous Year's Questions

Q. Tomcat urine odor is seen in: (JIPMER 2017)

A. Multiple carboxylase deficiency
B. Phenylketonuria
C. Hawkinuria
D. Maple syrup disease

Special C/F 00:06:45

- CNS: Encephalopathy, Seizures, Developmental delay
- Skin: Eczema
- Hair: Alopecia

Diagnosis: Enzyme assay in Lymphocytes
Treatment: Biotin supplementation

00:07:58

Important Information

Few reactions where CO_2 added but Biotin is not used

- CPS-I
- CPS-II
- Malic enzymes [converts Pyruvate (3C) to Malate (4C)]
- Gamma carboxylation of clotting factor done by Vit K dependent carboxylase
- Addition of Carbon number 6 in Purines

STREPTAVIDIN 00:09:26

- Protein obtained from bacteria Streptomyces avidinii
- Has high affinity for biotin (ligand)
- Strong ligand-protein interaction between biotin and streptavidin can be used for applications such as:
  - Protein purification
  - Detection of proteins, lipids and nucleic acids

DEFICIENCY OF VITAMIN B_7 00:10:45

- Most common cause of deficiency of Biotin- excess consumption of raw eggs

BIOTIN AS ANTI-EGG WHITE INJURY FACTOR

Egg white (in raw egg) contains protein avidin

↓

Avidin binds tightly with biotin

↓

Causes deficiency of biotin

↓

Gluconeogenesis enzyme Pyruvate Carboxylase requires B_7

↓

so, deficiency of B_7 will lead to Hypoglycaemia

Deficiency signs and symptoms 00:12:18

- Alopecia
- Muscle pain
- Bowel inflammation
- Hypoglycaemia

Previous Year's Questions

Q. A bodybuilder starts eating raw eggs for protein. He developed fatigue on moderate exercise. The doctor prescribes a vitamin. Which enzyme is deficient in him? (AIIMS 2019)

A. Glucose 6 Phosphatase
B. Pyruvate Carboxylase
C. PEPCK
D. Glycogen Phosphorylase

CLINICAL QUESTIONS

Q. A 20-month-old male infant presented with developmental delay, seizures, and eczema. His urine has a characteristic tom cat urine smell and blood analysis indicated organic acidemia. The physician discussed the disease with parents and advised them to start biotin supplementation. The defect in which of the following conversion is cause of acidemia in this patient.

A. Pyruvate to oxaloacetate
B. Acetyl CoA to citrate
C. Pyruvate to acetyl CoA
D. Succinate to oxaloacetate

Answer: A

Solution

- Tom cat urine disorder is a characteristic symptom of multiple carboxylase/biotinidase deficiency which is an autosomal recessive disorder due to defect in either holocarboxylase synthase and/or biotinidase deficiency. These enzymes are required to generate active form of biotin required for carboxylation reactions (see 1st fig in text).
- Biotin is a prosthetic group for all carboxylases and is involved in a wide range of metabolic processes, primarily related to the utilization of fats, carbohydrates, and amino acids. It is required in carboxylation reactions such as:
  - Acetyl CoA to Malonyl CoA done by enzyme Acetyl CoA carboxylase.
  - Pyruvate to Oxaloacetate done by enzyme Pyruvate carboxylase.
  - Propionyl CoA to Methyl Malonyl CoA done by enzyme Propionyl CoA carboxylase.

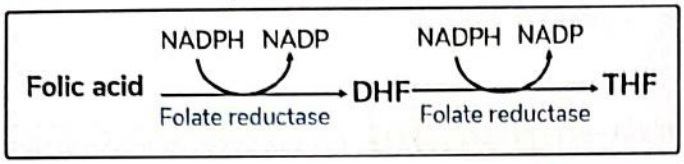
As the pyruvate to oxaloacetate conversion is inhibited in this patient, the excess pyruvate will form lactic acid causing acidosis.

Reference: Harper's 30th ed/pg. 561

107 VITAMIN B_9

VITAMIN B_9/FOLATE/FOLIC ACID

Active form: TetraHydro Folate (THF)



Biochemical Role 00:01:30

- Involved in 1-Carbon metabolism
- Purine and pyrimidine synthesis
- S-containing amino acid metabolism

DEFICIENCY OF FOLATE 00:02:00

Most common cause of def is Alcoholism, pregnancy

- **In pregnant mothers**
  - Folate def leads to neural tube defects in child
  - So, Folate supplementation is essential in pregnancy and even before pregnancy
- **In adults**
  - Megaloblastic Anemia due to
    → problem in the formation of purine and pyrimidines.
    → Cells keep growing in size, but nucleus is unable to divide.
  - No neurological sign and symptoms

Important Information

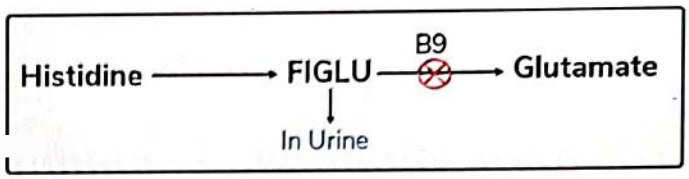
- In B_{12} deficiency there is Megaloblastic anaemia + Neurological symptoms

Clinical features 00:03:43

- ↑ blood homocysteine
- ↑ blood homocysteine
- FIGLU (Form Imino Glutamate) in urine

Diagnosis: Histidine loading test 00:04:16

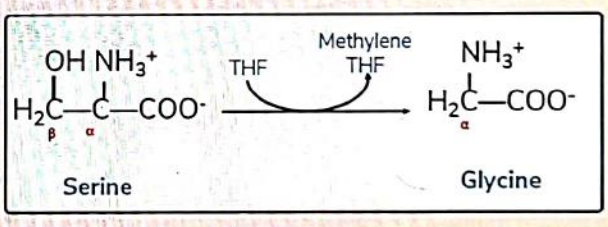
- FIGLU is an intermediate in Histidine metabolism and converted to glutamate in the presence of B_9
- So, Histidine Loading test is used to detect B_9 deficiency
- Excess histidine given to patient and checking for FIGLU in urine
- Presence of FIGLU indicates B_9 deficiency.



00:05:10

Important Information

- Form of folate used in treatment – N^5 formyl THF (due to high stability)
- Main form of folate in circulation – Methyl THF
- Form of folate for IC transfer reaction – methylene THF
- In I-C metabolism when serine gets converted to glycine, β carbon is added to THF as:



CLINICAL QUESTIONS

Q. A patient is presented with symptoms of peripheral nephropathy which are getting worsened gradually. Blood examination revealed larger than normal RBCs. What of the following is best treatment that can be given to help alleviate his symptoms?

A. Iron
B. Folic Acid alone
C. Folic acid along with Hydroxycobalamin
D. Vitamin B1 alone

Answer: C

Solution

This reasons behind for this patient's clinical manifestations are:

- Haematological: Megaloblastic anemia, Hypersegmentation of neutrophils due to secondary deficiency of folate (folate trap in B_{12} deficiency)
- Neurological: Demyelination in peripheral nerves and spinal cord due to accumulation of L-methyl malonic acid.

Vit def	Manifestation
Folate	Megaloblastic anemia
B_{12}	Megaloblastic anemia + neurological manifestations

Though Folic acid alone can treat anemia, but it cannot reduce symptoms of neuropathy as it is specifically due to B_{12} deficiency. So, administration of both is best treatment option in this case.

Reference: Harper's 30th ed/pg. 560

108 VITAMIN B_{12}

VITAMIN B_{12}/COBALAMIN

- Cobalamin contains cobalt in its structure

Two forms of Vit B_{12} 00:00:41

Methyl cobalamin	Deoxyadenosyl cobalamin
• major circulatory form • Involved in conversion of Homocysteine to Methionine	• Storage form (stored in liver) • required by enzyme methyl-malonyl CoA Mutase

Biochemical Roles

- Conversion of Homocysteine to Methionine
- Coenzyme for methyl-malonyl CoA Mutase
- Coenzyme for Ribonucleotide Reductase (required for ribose to deoxyribose conversion)

Previous Year's Questions

Q. Which B12 dependent enzyme is involved in amino acid metabolism? (INICET Nov 2020)

A. Methyl malonyl CoA isomerase
B. Homocysteine synthase
C. Folate reductase
D. Methionine synthase
E. Beta hydroxy butyrate synthase

Absorption of Vitamin B_{12} 00:02:27

Parietal cell in stomach
↓ secretes
Glycoprotein know as Intrinsic factor (IF)
↓
forms IF–B_{12} complex
↓
IF-B_{12} absorbed in ileum

VITAMIN B_{12} DEFICIENCY 00:03:18

Important Information

- In atrophic gastritis, IF not released, so vit B_{12} deficiency
- In Pernicious Anaemia (an auto Immune disease) - Antibodies against Intrinsic factor, so Vit B_{12} is not absorbed.

Most common causes of B_{12} def

1. Pernicious anemia
2. Ageing
3. in vegetarian (B_{12} not present in vegetarian foods)

Deficiency signs and symptoms 00:04:38

- Megaloblastic anemia + Neurological symptoms (progressive peripheral neuropathy)
- Subacute combined degeneration of spinal cord and demyelination → unique to B_{12} def
- Homocysteine is raised (refer to S-containing AA metabolism for details)
- L-methyl malonic is increased (refer to odd chain fatty acid metabolism)

00:06:22

Folate trap

↑↑ Methyl THF (Inactive form) —Vit B_{12}→ THF (active form)

↓ Cannot do any function

- If Vit B_{12} is deficient in body, active form of folate i.e. THF is not formed & Folate is trapped as methyl THF.
- It's also called as functional deficiency of Folate.

Diagnosis of B_{12} def 00:07:57

- **Schilling test**
  - Labelled B_{12} given orally
  - Unlabelled B_{12} is given intramuscularly
  - 24 hours urine B_{12} excretion measured
  - Monitor B_{12} excretion and absorption to know about B_{12} deficiency.

Previous Year's Questions

Q. Methyl tetrahydrofolate accumulation and functional deficiency of folate is seen in the deficiency of: (INICET July 2021)

A. Folic acid
B. Vit B_{12}
C. Vit B_1
D. Vit B_6

CLINICAL QUESTIONS

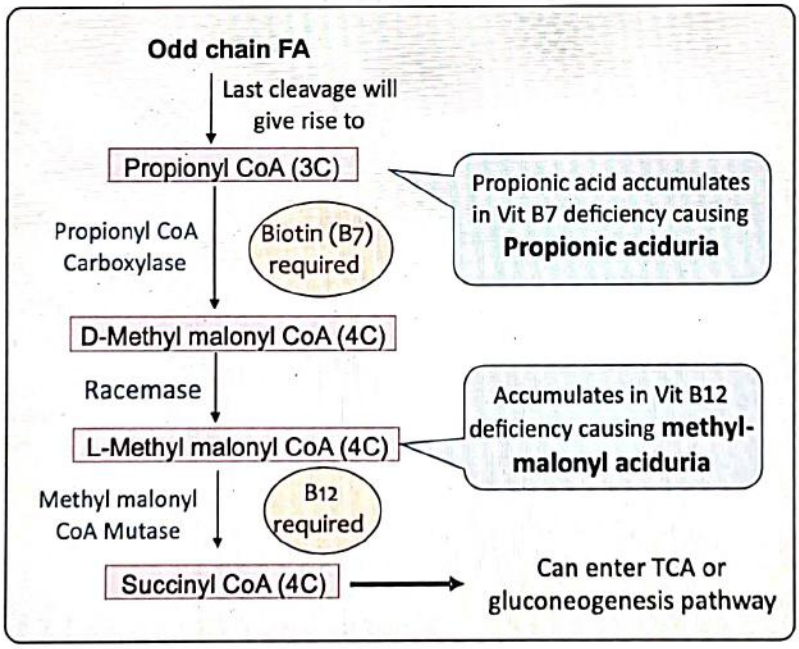
Q. A 60-year-old woman was severely malnourished and developed macrocytic anemia. Her blood and urine analysis further revealed elevated levels of homocysteine and methylmalonate (MMA). The leucocyte transketolase levels were also below normal levels. What symptoms out of following confirms that the anemia in this woman was due to a primary deficiency of cyanocobalamin (B_{12})?

A. Macrocytic anemia
B. Elevated methylmalonate
C. Severe malnutrition
D. Elevated homocysteine

Answer: B

Solution

- This person is showing macrocytic anemia and elevated homocysteine, both of which can be caused by either B_{12} or folate deficiency.
- However, enzyme Methylmalonyl CoA mutase specifically requires B_{12} and not folate for its activity.
- The coenzyme form of B_{12} i.e. adenosyl cobalamin is required for the activity of enzyme methylmalonyl CoA mutase, which covert methylmalonyl CoA, into succinyl CoA. Due to deficiency of B12, methyl malonyl may accumulate causing malonyl aciduria (See fig below).
- MMA is elevated in 90–98% of patients with B_{12} deficiency



Reference: Harper's 30th ed/pg. 558

109 VITAMIN C

VITAMIN C/ ASCORBIC ACID

- A Sugar acid

Active moiety: Ascorbic Acid

- Essential in human diet

00:00:53

Important Information

Synthesis

- Non-primates can synthesize vitamin C due to the presence of enzyme L-Gulono lactone oxidase but humans cannot synthesize it due to this enzyme's absence.

Source: Fresh fruits (vit C gets destroyed on heating)
Richest source: Amla (Indian Gooseberry)

Previous Year's Questions

Q. Due to which of the following enzyme deficiency, vitamin C cannot be synthesized in humans? (AIIMS 2018)

A. L-Glucuronic acid oxidase
B. L-Gulonic acid reductase
C. L-Gulonolactone oxidase
D. L-Gulonolactone reductase

Biochemical Roles 00:02:47

1. Hydroxylation reaction
- Collagen synthesis (helps in wound repair and healing)
- Bile acid synthesis (cofactor for RLE 7α-Hydroxylase)
- Tryptophan Hydroxylase (synthesis of serotonin and melatonin)
- Tyrosine hydroxylase (synthesis of catecholamines)
- Dopamine hydroxylase (for dopamine → NE conversion)

2. Vit C helps in absorption of Iron by converting Fe^{3+} to Fe^{2+} form
- Deficiency will cause micocytic, hypochromic anemia

3. Water phase anti-oxidant
4. converts Met-Hb → Hb (so, it prevents hemolysis)

Previous Year's Questions

Q. Which of the following vitamin increases the absorption of iron? (AIIMS 2018)

A. Vitamin A
B. Vitamin C
C. Thiamin
D. Riboflavin

DEFICIENCY OF VIT C

00:05:51

Scurvy: Collagen formation defective

C/F

- Bleeding gums
- Inflammation of gums
- Hyperplasia
- Tooth loss
- Bruises
- Petechiae
- Poor wound Healing
- Anaemia
- Increased bleeding time
- Glossitis

Infantile scurvy

C/F

- Pallor
- Diarrhoea
- Subperiosteal haemorrhage
- Skeletal deformities
- No bleeding gums (as teeth not erupted)

TOXICITY OF VIT C

00:07:29

- Rare condition
- Clinical features are
  - Oxalate kidney stone formation (ascorbic acid on oxidation → dehydroascorbic acid → oxalic acid)
  - Iron overload (due to excess iron absorption)

CLINICAL QUESTIONS

Q. A 30-year-old male visited his dentist to consult his loosening of teeth. A more detailed check-up reveals that his gums are swollen, purple, and spongy. Physical examination revealed multiple splinter hemorrhages near the distal ends of the nail on patient's fingers and wound healing is not proper in this patient. The patient is showing deficiency symptoms of:

A. Vitamin A
B. Folic Acid
C. Vitamin C
D. Vitamin B1

Answer: C

Solution

Vitamin C acts as a cofactor for the enzyme lysyl and prolyl hydroxylase which is necessary for post translation modification of collagen by hydroxylating the proline and lysine residue.

Hydroxylation of collagen residues

Proline $\xrightarrow[\text{(Require vit C \& } Fe^{2+})]{\text{Prolyl hydroxylase}}$ Hydroxyproline

Lysine $\xrightarrow[\text{(Require vit C \& Fe)}]{\text{Lysyl hydroxylase}}$ Hydroxylysine

These hydroxylated residues are necessary for hydrogen bonding between different collagen chains and hence provide strength to collagen fibers.

Vitamin C deficiency leads to defective post-translational modification of collagen and loss of tensile strength of blood vessels leading to:

- Bleeding gums
- Ecchymoses
- Easy bruisability
- Poor wound healing (Features of scurvy).

Reference: Harper's 30th ed/pg. 562

110 MINERALS: BASICS

MINERALS

Definition: Inorganic material mainly required as cofactor for many enzymes. So, they are essential in diet.

Classification of Minerals (Based on daily requirements)

00:01:03

Major elements or Macro-minerals	Trace elements or Micro-minerals
> 100 mg/day • Ca, P • Na, K, Cl • Mg, S	< 100 mg/day • Fe, Fl • Cu, Co, Cr • Mn, Mo • Zn, I, Se

Important topics and diseases in minerals

- Calcium homeostasis
- Fe - Mucosal Block Theory and Hemochromatosis
- Cu - Menke and Wilson Disease
- Zn - Acrodermatitis enteropathica
- Se - Keshan's Cardiomyopathy

Important Information

- Minerals with no urinary excretion – Fe and Cu
- Minerals having role in glucose tolerance – Zn, Cr and Mg

111 CALCIUM

CALCIUM

Major source: Milk
Other sources: egg, fish, meat etc.

ABSORPTION OF CALCIUM

00:00:47

Mechanism of Ca absorption: Refer to Vitamin D notes

Factors that favour Ca absorption

- Vit D
- PTH
- Acidity
- Basic amino acids Lysine and Arginine

Factors that decrease Ca absorption

- Phytate (in cereals)
- Oxalates (in Green leafy veg, tea, beetroot)
- Phosphates

Ca Homeostasis (for details refer to Vit D)

- **Three components involved** 00:02:37

1. Vit D (↑ blood Ca)
2. PTH C (↑ blood Ca)
3. Calcitonin (↓ blood Ca)

Causes of Hypercalcaemia 00:03:29

- Hyperparathyroidism
- Vitamin D toxicity
- Multiple Myeloma
- Thiazide diuretics
- Metastatic bone carcinoma

Cause of Hypocalcaemia 00:04:20

- Hypoparathyroidism
- Vitamin D def
- Dietary def of Ca
- Malabsorption syndrome
- Chronic renal failure (as kidneys form active vit D)
- Acute pancreatitis

112 IRON

IRON

Sources

00:00:38

- green leafy veg, jaggery, pulses
- In 3rd trimester of pregnancy there is good placental iron transport
- Milk is a poor source of Fe, Cu and vitamin C
- So, during weaning iron supplementation in diet of babies is very important.

Note: Food has Fe^{+3} (ferric) but absorption occurs in Fe^{+2} (ferrous) form

Factors that ↑ Fe absorption

00:02:28

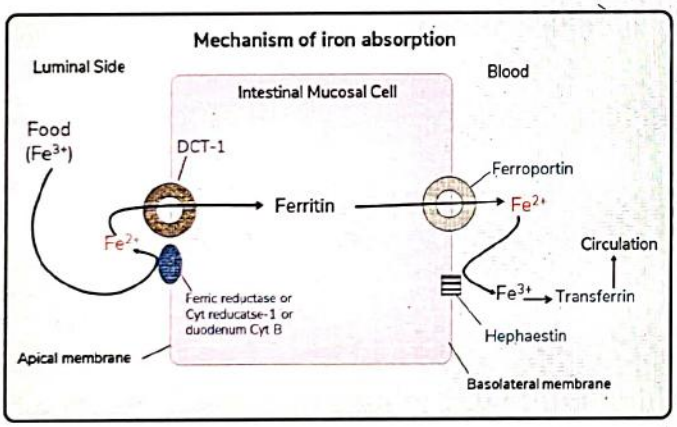
- Vit C
- Cysteine
- Acidic pH (HCl)
- All helps in Fe^{3+} to Fe^{2+} conversion

Factors that ↓ Fe absorption

- Phytates, Phosphates, oxalates
- Tannates (tea)

IRON ABSORPTION

00:03:46



Previous Year's Questions

Q. Which of the following vitamin increases the absorption of iron? (AIIMS May 2018)

A. Vitamin A
B. Vitamin C
C. Thiamin
D. Riboflavin

DMT-1/DCT-1 (Divalent Metal/Cation Transporter-1)

00:07:02

- Transports Fe^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}
- Coupled with H^+ transport
- Highly conserved during evolution
- Expression (synthesis) of this protein is regulated by body iron stores

Hephaestin

00:08:11

- Has homology with ceruloplasmin
- Both hephaestin and ceruloplasmin has ferroxidase activity

Hephaestin	Ceruloplasmin
• Mainly for Fe metabolism • A transmembrane protein	• Mainly for Cu metabolism • A plasma protein

Hepcidin

00:09:16

- **Acute phase protein** synthesized by liver
- Inhibits ferroportin and ↓ iron absorption
- In chronic inflammation → increase hepcidin synthesis → ↓ iron absorption → anemia (aka anemia of chronic inflammation.

Previous Year's Questions

Q. Hepcidin decreases iron absorption by inhibiting? (AIIMS May 2019)

A. Divalent metal transporter
B. Hephaestin
C. Ferroportin
D. Transferrin

00:10:16

Haptoglobin	Hemopexin
• Binds free Hb and clear free Hb from circulation	• Binds free Haem and protects body from its oxidative damage effects

Mucosal block theory of iron 00:11:12

- It explains method of iron regulation
- Iron is a one-way element i.e. Iron homeostasis is regulated at the level of absorption, not excretion
- It's because iron is toxic so is absorbed only whatever is required
  - If body iron stores depleted → Abs ↑
  - If body iron stores sufficient → Abs ↓
- Proteins taking part in this are: DCT-1, Ferritin and Hepcidin

Fe as Haem	Fe as Fe-S centre
• Hb, Mb, cytochromes, myeloperoxidase	• Xanthine oxidase, ribonucleotide reductase, ferredoxin, aconitase

IRON TRANSPORT AND STORAGE PROTEIN

00:14:03

Transferrin

- Beta globulin present in liver
- High affinity for Fe^{3+}
- Each transferrin has two Fe binding sites
- Only 1/3rd sites are occupied at any time

Important Information

TIBC: Total Iron Binding capacity of transferrin

- TIBC↑ indicates that there is Fe def anemia as there is an increase in transferrin to bind more iron for increasing Fe supply

Iron storage proteins 00:15:29

1. Ferritin

- Loosely bind to iron So, readily mobilized form of stored iron
- First marker to be ↓ in Fe def anemia, so it a more sensitive marker than transferrin

2. Hemosiderin

- Aggregates of several ferritin
- Has higher iron content
- Tightly bind to iron So, release iron more slowly

Fe DEFICIENCY ANEMIA

00:16:59

- Most common nutritional problem in India
- Hb ↓, Fe ↓, Ferritin ↓
- TIBC ↑ and Transferrin ↑
- Microcytic hypochromic

Rx: oral Iron + Vit C (increase abs) + Vit E (for tackling unabsorbed iron generated free radical)

Previous Year's Questions

Q. Which of the following dyads of plasma protein-function is/are correct: (PGI May 2019)

A. Transferrin- Transports iron

B. Thyroxin binding globulin- thyroxin binding and transport

C. Ceruloplasmin- Assisting in iron absorption by transferrin

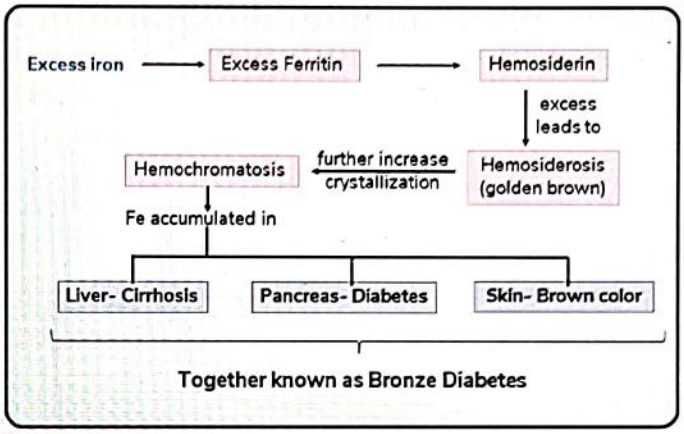
D. Haptoglobin- Heme binding and destruction

E. Hemopexin-Heme binding

IRON OVERLOAD

00:18:27

Hemochormatosis and Bronze Diabetes



CDT- CARBOHYDRATE DEFICIENT TRANSFERRIN

00:20:07

- Marker for chronic alcoholism
  - As alcohol decreases glycosylation of glycoprotein transferrin
  - So, CDT will be low in chronic alcoholics

113 COPPER

COPPER (Cu)

Sources

- Nuts, cereals, green leafy vegetables
- Meat, liver

Functions 00:00:47

1. cofactor for all oxidases
2. It is involved in iron metabolism as a part of protein Ceruloplasmin
- So, Cu def. will cause anemia which is also microcytic hypochromic like Fe Def anemia

Ceruloplasmin (Cp) 00:01:36

- Acute phase protein
- An alpha-2 globulin
- Synthesized by liver in the form of Apo–Cp
  - Apo-Cp + 6-8 atoms of Cu → Ceruloplasmin (active)
- Has ferroxidase activity (converts Fe^{2+} to Fe^{3+})
- ↓ Cp → Brain affected and Iron Overload causing pancreatic Diabetes

Excretion of copper 00:03:16

- Not excreted via kidneys
- Excreted via biliary route and not reabsorbed from GIT
- Any disease which hampers biliary excretion increase Cu in body
  - 1° biliary cirrhosis
  - Cholestatic hepatitis
  - 1° sclerosing cholangitis

Plasma Cu transport 00:04:35

1. 80% Ceruloplasmin } Tightly bound
2. 10% transcuperin }
3. 10% Albumin → loosely bound to Cu → so albumin is better Cu Transporter

DISORDERS OF COPPER METABOLISM

00:05:33

1. Menke's Kinky hair Syndrome
2. Wilson's Hepatolenticular degeneration
3. MEDNIK Syndrome

MEDNIK

- Inherited Autosomal Recessive disorder
- Mutation in AP1Si gene
  M - mental retardation
  E - Enteropathy
  D - Deafness
  N - Neuropathy
  I - Ichthyosis
  K - Keratoderma
- Increase Cu in plasma membrane of cell of organs affected
- In Mednik syndrome all Cu ATPases are affected
- So, has combined features of Menke and Wilson disease

CU TOXICITY 00:08:50

Cause: Excess use of brass utensils

C/F

- Hemolysis and renal damage
- Blue green stools and saliva

114 MENKE AND WILSON DISEASE

Difference between Menke's and Wilson's Disease 00:00:29

Menke'e Disease	Wilson's disease
• Cu Deficiency	• Cu Excess
• ATP-7A protein defective	• ATP-7B protein defective
• X-linked recessive	• Autosomal recessive
• ATP-7A is present in intestine for absorption of Cu. Its deficiency causes: o ↓ Cu (Cu stays in intestinal cell, unable to enter blood) o ↓ Ceruloplasmin (cp) (due to insufficient Cu to form cp)	• ATP-7B is present in liver to throw Cu in bile and also incorporates Cu in cp. Its deficiency causes: o ↑ Cu in liver (due to non-excretion from body) o ↓ Ceruloplasmin (cp) (due to non-incorporation of Cu in cp)

↓ ceruloplasmin (cp) is common between Menke and Wilson Disease

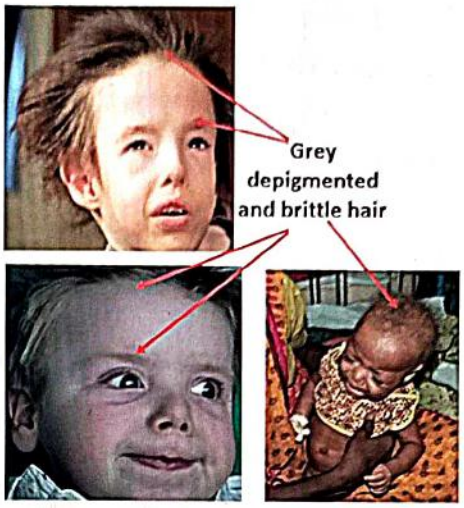
How to remember

- As Alphabetically Letter "M" in Menke comes before letter "W" in Wilson
- In 7A "A" comes before "B" in 7B.
- Cu deficiency "Less" comes before Cu excess "More"

MENKE'S KINKY HAIR SYNDROME 00:03:20

Clinical features

- Premature birth
- Hypotonia
- Growth retardation
- Mental retardation
- Grey depigmented hair:
  - Tyrosinase (an oxidase) is affected as oxidases require Cu
  - Tyrosinase is required for synthesis of Melanin.
  - So, melanin synthesis is decreased causing grey hair
- Brittle kinky hair
  - As lysyl oxidase affected, so defective and weak collagen synthesis
- Decreased Cu in blood and urine



Previous Year's Questions

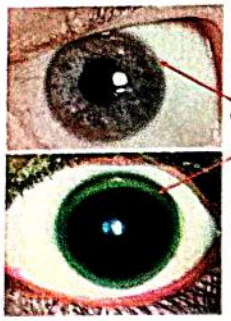
Q. Defect in Menke disease: (NEET 2019)

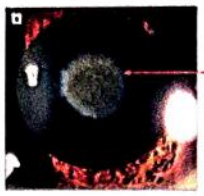
A. Lysyl hydroxylase
B. Lysyl oxidase
C. Prolyl hydroxylase
D. Prolyl oxidase

WILSON'S HEPATOLENTICULAR DEGENERATION 00:05:30

Clinical features

- Cu accumulates in liver causing liver damage
- When excess in liver, ↑ Cu can go to other extrahepatic tissues and cause following changes:
  - In Brain: Neurological degeneration
  - In Kidneys: Renal damage, urolithiasis
  - In Bone marrow & RBC: Hemolytic anemia
  - In Eyes
    - → sunflower cataract
    - → Kayser-Fleisher (KF) rings: green/golden ring in Descemet's membrane of cornea due to Cu deposition





Treatment: Give penicillamine as it will chelate Cu

Previous Year's Questions

Q. In Wilson disease there is less urinary excretion of: (NEET 2019)

A. Phosphorous
B. Methyl-Histidine
C. Phosphotyrosine
D. Serine

115 SELENIUM AND FLUORINE

SELENIUM (Se)

- Part of 21st amino acid selenocysteine and enzyme glutathione peroxidase
- Works in association with vit E as an antioxidant mineral
- Has a role in spermatogenesis

Se DEFICIENCY

00:01:12

Keshan's Disease

- Named because first reported in Chinese Province – Keshan and endemic to this region
- mainly affects children and women of child-bearing age due to their increased Se requirements
- **Clinical Features**
  - Cardiomyopathy
  - Muscular weakness and muscular dystrophy
  - Inability to gain weight
  - Hypothyroidism
  - Hypertension
  - Eczema
  - Arthritis
  - Selenium poor soil corelates with high incidence of cancer
  - Increased risk of stroke

Previous Year's Questions

Q. Keshan's cardiomyopathy due to deficiency of: (JIPMER Nov 2017)

A. Chromium
B. Selenium
C. Magnesium
D. Copper

Se TOXICITY

00:02:52

Aka known as Selenosis

- Occur due to accidental ingestion of anti-rust chemicals and metal polishings
- **Clinical Features**
  - Diarrhoea
  - Hair loss, weight loss
  - Falling of nails
  - Garlic breath odour

FLUORINE

00:03:53

Most common source of Fl: Drinking water

Role

- Growth of bones and teeth
- Provide resistance to enamel to prevent acid attack

Note: Double edged sword- both deficiency and excess are harmful

FLUORINE DEFICIENCY

00:04:50

- If levels are < 0.5 ppm → dental caries (Acidic destruction of enamel and dentine)

FLUORINE EXCESS

00:05:20

Aka Fluorosis

- If levels are > 5 ppm → dental fluorosis
  - Discoloration of teeth and mottling of dental enamel
  - First tooth to be affected is upper central incisors and 1st molar
- If levels are > 20 ppm → skeletal fluorosis
  - Osteoporosis, brittle bones and calcification of tendons and ligaments

Note: Fluoride toothpastes prescriptions has >300 ppm Fl so, should never be used for longer duration.

CLINICAL QUESTIONS

Q. A 14-month old infant is presented with dilated cardiomyopathy, hypothyroidism, inability to gain weight and failure to thrive and diagnosed with Keshan's cardiomyopathy. Which of the following mineral supplements can help improve this child condition?

A. Chromium
B. Selenium
C. Magnesium
D. Copper

Answer: B

Solution

Keshan's disease is due to deficiency of selenium in the body. Selenium as selenocysteine is an essential component of the enzyme glutathione peroxidase and selenium containing enzyme 5'-deiodinase converts thyroxine (T4) to triiodothyronine in the thyroid gland.

For Clinical features of Keshan's disease refer to text.

Reference: Harper's 30th ed/pg. 249

116 ZINC AND CHROMIUM

ZINC

Uses 00:00:29

- Cofactor for 300+ enzymes e.g. Carbonic anhydrase, alcohol DH, ALP, DNA and RNA polymerase etc
- Enhances Immunity
- Stabilizes Insulin, so, deficiency leads to impaired glucose tolerance
- Prevents diarrhoea (decrease risk, severity and duration of Diarrhoeal episodes)
  - Regulates intestinal movement of water and electrolytes
  - WHO recommends Zinc along with low osmolarity ORS solution for preventing and treating Diarrhoea in children.

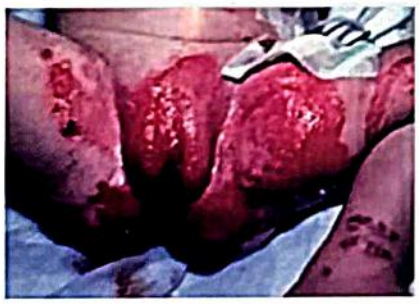
Zn DEFICIENCY 00:02:51

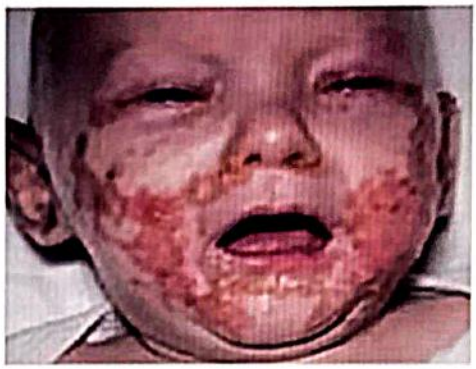
Symptoms

- Delayed wound healing
- ↓ Immunity
- Diarrhoea
- Hypogonadism

Acrodermatitis Enteropathica 00:03:20

- Rare, Autosomal Recessive genetic disorder
- Defect in zinc absorption from intestine so, Zn deficiency
- Simultaneous diarrhoea + dermatitis
- Inflammation around nose, mouth, anus, cheeks, elbow etc.
- Also occurs in some infants after weaning from breast milk as breast milk has picolinic acid (piconilic acid increases Zn absorption from intestine)





Previous Year's Questions

Q. Use of Zinc in diarrhoea is because it: (FMGE Nov 2018)
A. Reduces the risk, duration and severity of diarrheal episodes
B. Enhances immune response
C. Regulates intestinal transport & absorption of water and electrolytes
D. All

CHROMIUM 00:04:56

GTF (Glucose Tolerance Factor)

- This factor is synthesized in vivo from dietary chromium.
- It enhances the action of insulin 3 folds. So, chromium deficiency leads to impaired glucose intolerance.

CLINICAL QUESTIONS

Q. A baby girl is brought to a hospital for severe dialarhoea and dermatitis. She is also low in weight for her age. Physical examination shows the rash is not typical of yeast infection and it is distributed beyond the genital area and is prevalent on knees and tissue around mouth and anus. She also has swelling of the hands and feet (edema) and has lost her hair. Which one of the following minerals supplements most likely help in relieving her symptoms?

A. Lead
B. Arsenic
C. Zinc
D. Chromium

Answer: C

Solution

She is most probably suffering from Acrodermatitis enteropathica that is a rare autosomal recessive disorder characterized by abnormalities in zinc absorption.

It is characterized by simultaneous occurrence of skin inflammation (Dermatitis) and Diarrhea. Skin on cheeks, elbows and knees and tissue around mouth and anus are inflamed.

Administration of zinc along with new low osmolarity oral rehydration solutions/salts (ORS), can reduce the risk, duration and severity of diarrheal episodes for up to three months.

References: Devlin's 7th ed/pg. 1091-1092

117 FREE RADICALS

FREE RADICALS

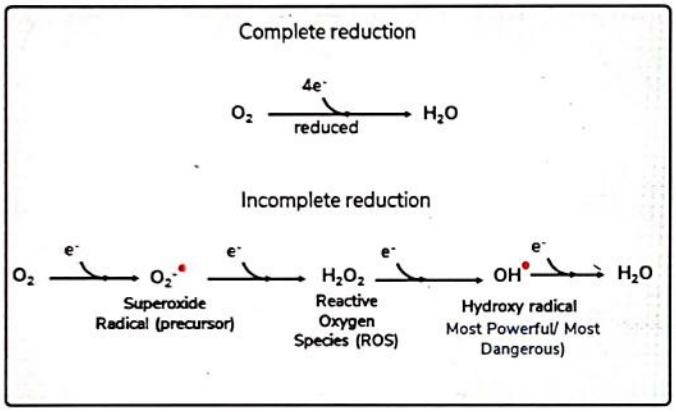
Def: Any molecule/molecular fragments having 1 or more than 1 unpaired electron in its outer orbit.

Properties

- It has an independent existence
- Short lived
- Gains e- from surrounding compounds & converting them to more dangerous free radicals so, can start chain of reactions

Formation of FR 00:01:56

- Incomplete reduction give rise to O_2 FRs



Oxygen free radicals 00:04:27

- Superoxide radical ($O_2^{-\bullet}$)
- Hydroxyl radical ($OH^{\bullet}$)
- Hydroperoxyl radical ($Ho_2^{\bullet}$)

Other free radicals

- Malon Dialdehyde (MDA)
- Oxidised LDL
- Prostanoids (formed from prostaglandins)
- Oxysterol (formed from cholesterol)
- Nitric oxide (EDRF)

$$NO \longrightarrow \text{Peroxy nitrite} \longrightarrow OH^{\bullet}$$

How oxygen free radicals generated in body 00:06:18

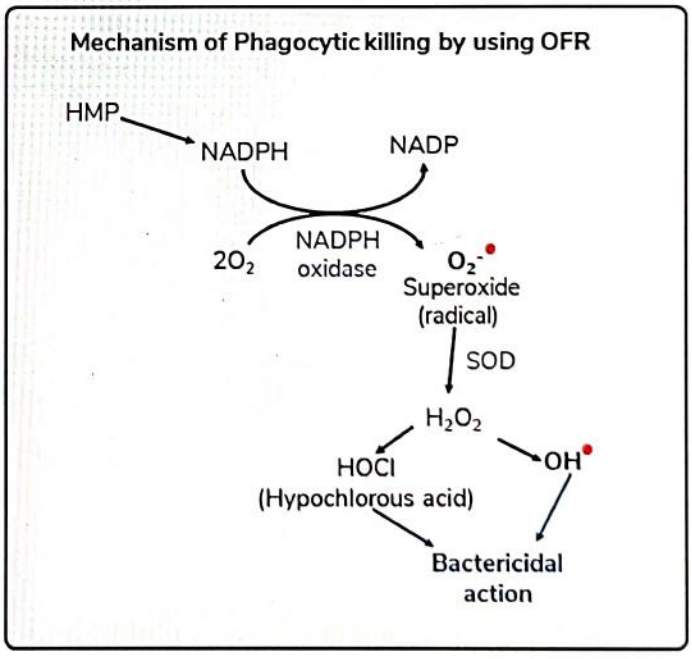
- There is increased chance of formation of FR whenever there is excess free O_2 used in some pathway or rxn such as
  1. ETC
  2. Oxidation–reduction reactions
  3. Exogenous agents
     - Ionizing radiations
     - Cigarette smoke
     - Carbon tetrachloride
  4. Transition metals
     - Fe, Cu (Cuprous & Ferrous are more reactive than cupric & Ferric)
     - Fenton reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet}$$

     - Other transition metals are Co and Ni

Uses of OFR 00:09:19

1. Enzymes → at active site, OFR helps in catalysis
2. Phagocytosis
   - Done by neutrophils and monocytes
   - Use OFR to Kill bacteria



- Increase in oxygen consumption during this mechanism is called as Respiratory Burst

Damage by OFR 00:13:47

- Mainly Macromolecules are damaged

1. Most susceptible is PUFAs by lipid peroxidation
   - ALEs – Advanced Lipid Peroxidation End products are formed
   - Chain of reactions started
2. Nucleic acids (DNA) damage leads to
   - Chain breaks
   - Mutations
   - Cell death
   - Cancer
3. Hb → met Hb
4. In Proteins: conformational changes occur, and SH group is oxidized
5. In Carbohydrates: AGEs (Advanced Glycation End Products) formed

Diseases due to OFR 00:16:13

1. Parkinsonism
2. Alzheimer's disease
3. Cancer
4. Rheumatoid arthritis
5. Ageing
6. Autoimmune Diseases
7. Mutations
8. Infertility
9. Atherosclerosis
10. DM
11. Cataract and Retinopathy
12. Respiratory Distress Syndrome

ANTIOXIDANTS 00:17:28

1. **Enzymatic**
2. **Non-Enzymatic**

Enzymatic

1. SOD (Super Oxide Dismutase)

$2O_2^{\bullet -}$ (Superoxide Radical (Substrate)) —SOD, Dismutation reaction→ H_2O_2 (ROS) (Relatively Harmless)

- 3 isoenzyme forms of SOD
  1. Cytoplasmic: requires Cu
  2. Mitochondrial: requires Mn
  3. Extra cellular: requires Cu + Zn

2. Glutathione Peroxidase

Glutathione (Reduced) —Glutathione peroxidase→ Glutathione (Oxidized)

Glutathione: Refer to HMP notes for details.

3. Catalase

$2H_2O_2$ (Hydrogen peroxide) —Catalase→ $2H_2O + O_2$

Previous Year's Questions

Q. Cofactor for Mitochondrial SOD (FMGE Aug 2020)

A. Mn
B. Cu
C. Zn
D. Mb

Non-Enzymatic Antioxidant 00:21:27

1. Vitamins: A, E(most potent), C, D
2. Thiol Antioxidants: Glutathione, Thioredoxin, Lipoic acid
3. Flavonoids
4. Selenium
5. Melatonin
6. Transferrin and Ceruloplasmin
7. Coenzyme Q
8. Uric Acid

Food rich in Antioxidants

- Turmeric
- Spinach
- Coffee beans

Two Classes of Antioxidants 00:23:19

1. Chain breaking antioxidants	2. Preventive antioxidants
• Interfere with chain propagation 1. Vitamins 2. SOD 3. Uric Acid	• Reduce the rate of chain initiation 1. Glutathione peroxidase 2. Catalase

Artificial Antioxidants

1. Propyl gallate
2. Butylated hydroxyl toluene (BHT)
3. Butylated hydroxyl anisole (BHA)

Measurement of Free Radicals 00:25:14

1. FOX assay (Ferrous Oxidase in Xylenol)
2. Estimation of MDA (Lipid peroxidation marker)
3. Pentane & Methane measurement

Previous Year's Questions

Q. Most potent lipid phase antioxidant
(FMGE June 2019)

A. Vitamin A
B. Vitamin E
C. Vitamin C
D. Vitamin K

118 XENOBIOTICS

XENOBIOTIC COMPOUNDS

Definition: Foreign substances to which human body is exposed to & they get metabolized in body & excreted out of body safely.

Detoxification 00:01:30

- Making the xenobiotics substance more soluble to be excreted out of the body safely
- Major organ for detoxification: Liver
- Mainly these substances are thrown out of the body by kidneys

Xenobiotic reactions: 2 phases 00:02:35

Phase I rxn	Phase II rxn
• Makes the compounds Hydrophilic/ Polar	• Makes the compounds more soluble to be thrown out of the body via Kidneys

Phase I reactions 00:04:22

1. Hydroxylation (mc)
2. Hydrolysis
3. Oxidation
4. Reduction

CytP450 00:05:03

- Enzymes used for Phase I hydroxylation
- Most versatile biocatalyst i.e. can use variety of substrates
- Membrane bound enzymes
- present in microsomes & IMM
- Absorb light at 450 nm
- Highly inducible by their own substrates (by ↑ the rate of transcription of genes of these enz)
  - Advantage of inducible nature is high efficiency
  - Disadvantage is rapid development of tolerance against the drugs. So, dose of drugs needs to be increased e.g.
    → Epileptic drug Phenobarbital
    → Dose have to be increased to 3-4 times within the 1st week of starting of this drug.

Important Information

CYP3A4

- most active isoenzyme form of CytP450
- It uses NADPH & O_2
- It is a haem containing enzyme
- Most important enzyme of Xenobiotic metabolism
- perform hydroxylation/mono-oxygenation reactions.

Isoenzymes of cytochromes 00:10:15

- Comes from different genes (closely related)
- Broadly divided into 2 categories

1. Lipid metabolizing enz	2. Drug metabolizing enz
• Has tight substrate specificity	• Has broad specificity so variety of drugs can be metabolized
• Responsible for o ω- oxidation of FA o Denaturation of FA o Synthesis of Steroids	• Responsible for metabolizing the drugs

Phase II reactions 00:12:24

Conjugation

- MC of all xenobiotic reactions
- Conjugating agents

1. Glutathione
2. PAPS (Phospho Adenosyl Phospho Sulphate)
   - Responsible for Sulfation reactions
3. SAM (S-Adenosyl Methionine) for Methylation
4. Glycine: responsible for conjugating
   - Bile acids
   - Benzoic acid → Hippuric acid (Soluble form)
5. Glucuronic acid: for conjugation of Bilirubin
6. Acetyl CoA for Acetylation

119 MUSCLE ENERGY SYSTEMS

In muscles different energy systems gets activated depending on the energy requirements.
The three systems that are used sequentially in an exercising muscle are as:

1. Phosphagen system 00:00:43

- Uses creatine phosphate as fuel
- Used in first 8-10 seconds of muscle contraction
- Is quickest source of energy
- Does not uses O_2 and also does not produce lactate
- Is the most direct form of energy production
- Is depleted quickly as phosphocreatine is of limited supply in muscles

2. Anaerobic/Lactate system 00:02:18

- Used between first 1-3 minutes of muscle contraction
- Produces ATP using glucose derived from muscle glycogen as fuel
- Produces lactate by anaerobic glycolysis and produce 2 ATPs/glucose

3. Aerobic system or Mitochondrial Respiration 00:03:06

- Used after 3 minutes of exercise
- Uses aerobic pathway including TCA & ETC so, also called as mitochondrial system
- Is most efficient as it provides more amount of energy (32 ATP from 1 glucose)
- Can also use variety of fuels such as blood glucose, muscle glycogen, fats, lipids and proteins.
- Is the slowest pathway (as ETC and TCA will take time to occur)

Previous Year's Questions

Q. During exercise, most rapid way to synthesize ATP is: (AIIMS 2018)

A. Glycogenolysis
B. Glycolysis
C. Phosphocreatine
D. TCA cycle

Summary of Muscle energy systems 00:04:44

	Muscle energy systems	Power (Rate of ATP production	Capacity (Total ATP produced)	Fuel uses
In Sequence ↓	**Phosphagen system**	Very high	Very low	Creatine-Phosphate or Phosphocreatine
	Anaerobic/ Lactate system	High	Low	Muscle Glycogen
	Aerobic system/ Mitochondrial respiration	Low	Very High	Muscle glycogen, blood glucose, adipose tissue and intramuscular fat

120 MEISTER CYCLE

MEISTER CYCLE/γ-GLUTAMYL CYCLE

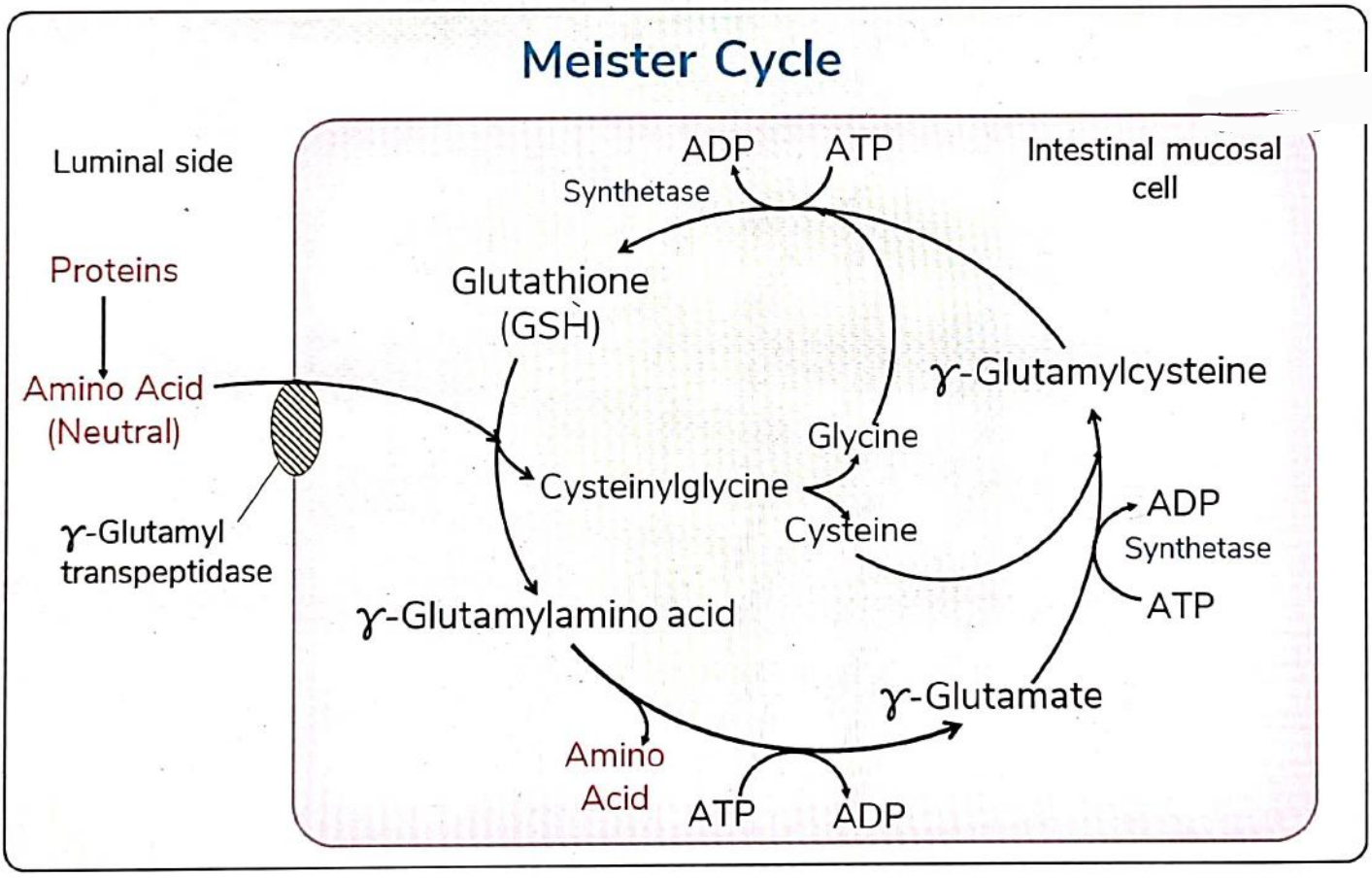
It is

- Used for transport of amino acids especially neutral amino acids into the cells.
- Occurs at three sites in body - in Intestine, Kidneys and Liver.
- Cannot be used for transfer of proline and hydroxy-proline.
- Makes use of tripeptide Glutathione (made of Glutamate + Cysteine + Glycine).

How to remember

GLUTA-THI-ONE
GLUTA – Glutamate
THI - Cysteine THIOL (Sulfur group (SH) containing AA)
ONE - Glycine (simplest smallest AA)

The cost of transfer of one amino acid via this cycle is 3ATPs which is high.



121 BUFFERS AND TITRATION CURVE

BUFFERS AND TITRATION CURVE

Basics

00:01:00

- Normal pH range of blood is – 7.35 to 7.45 (slightly alkaline)
- Acids are produced during metabolism
  - Blood resist change in pH by buffering action
- Normal pH range of Urine is 6.5 -7.0 (slightly acidic)
- Main acid forming reaction in body is:

$$H_2O + CO_2 \longleftrightarrow \underset{\text{Carbonic acid}}{H_2CO_3} \xrightarrow{\text{Carbonic anhydrase}} H^+ + HCO_3^-$$

- Carbonic anhydrase
  - Zn-requiring enzyme
  - Highest conc. is in RBC and Renal tubules

BASICS CHEMISTRY OF ACIDS AND BASES

00:03:45

Acid

- Proton Donor

$$\underset{\text{Acid}}{HA} \longrightarrow \underset{\text{Proton}}{H^+} + \underset{\text{Salt}}{A^-}$$

- Strong acid: dissociate completely
- Weak acid: give H^+ less rapidly
- The acids formed in the body are generally weak acids

Base

- Proton Acceptor or donates OH^-

$$NaOH \longrightarrow Na^+ + OH^-$$

Amphoteric substances

- Acts as both acid and base e.g. Amino acids and proteins.
- Contain both acidic and basic groups:

$$\text{Base} \quad NH_2 \xrightarrow{+H^+} NH_3^+$$

$$\text{Acid} \quad COOH \xrightarrow{\text{Donates } H^+} COO^- + H^+$$

pH

- $pH = -\log [H^+]$
- pH = 7 (Neutral/Normal); pH < 7 (acidic); pH > 7 (basic)

Buffers

00:07:41

- **Def-** Solutions in which pH change does not occur on addition of strong acid or base
- Usually a combination of weak acid with its conjugate base
- Weak acid when dissolved in aqueous solution, creates an equilibrium as:

$$\underset{\text{Weak Acid}}{HA} \rightleftharpoons H^+ + \underset{\text{Conjugate base}}{A^-}$$

Handerson-Hasselbalch equation

00:09:41

$$pH = pK_a + \log\frac{[Salt]}{[Acid]} \; or \; \log\frac{[Base]}{[Acid]}$$

Where pH = pH of medium in which acid is dispersed
pK_a = dissociation constant of acid

- Buffer is most effective when:
  - pH = pK_a or [Salt] = [Acid] or 50% of acid is ionized
  - Maximum Buffering capacity range = pKa ± 1
  - Minimum buffering capacity occur at pH = pI (isoelectric point)
    → At this pH precipitation occurs due to insolubilization and neutralization of charges

BUFFERING MECHANISM IN BODY

00:13:40

Three tier defence mechanism for resisting change of pH

1. Lungs
2. Kidneys
3. Chemical buffers

1. Buffering action of Lungs

00:14:42

- Also called as Neural respiratory system/control = CNS + Respiratory

If blood becomes acidic	If blood becomes alkaline
↓	↓
Hyperventilation occurs	Hypoventilation occurs
↓	↓
More CO_2 thrown out	Acid remains in body
↓	↓
Acid decreased	Neutralizes alkali

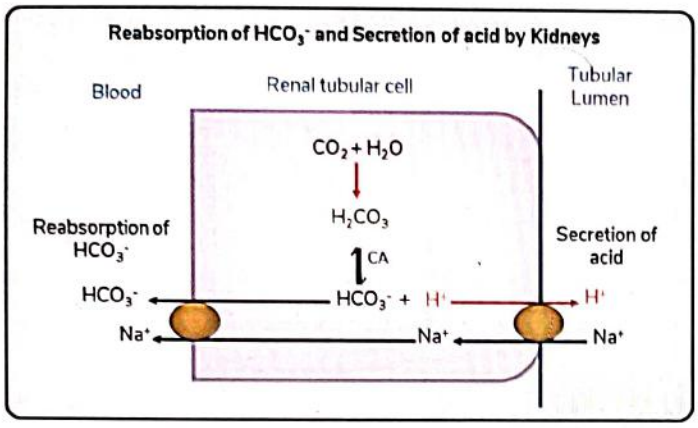
2. Buffering action of Kidney

00:17:01

- Kidneys work by regulating plasma bicarbonate using

1) Reabsorption of HCO_3^-
2) Formation of titratable acid-secreted out

3) Formation of NH_4^+
   - source of ammonia is Glutamine
   - Traps H^+ ions in NH_4^+ thus decreasing acidosis by throwing NH_4^+ in urine



3. Chemical Buffers in Intracellular and extracellular Fluids

00:20:09

1) Bicarbonate buffer (major extracellular buffer)
- pKa ~ 6.1
- ideal buffer- both components of this buffer can be altered for blood pH maintenance
- Two components
  (1) HCO_3^- (maintained by kidneys)
  (2) CO_2/H_2CO_3 (maintained by respiratory system)

2) Phosphate buffer (major Intracellular buffer/major urinary buffer)

3) Protein Buffer (largest amount)
- Protein albumins and globulins in blood acts as buffer e.g.
  - Haemoglobin in RBCs
  - presence of Histidine amino acids (38 histidine in 1 HB molecule)
  - Imidazole group of Histidine provide buffering action due to pKa = 6.5 to 7.4 very near to physiological pH of blood

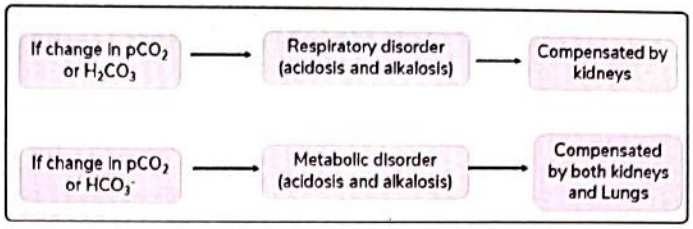
Previous Year's Questions

Q. Which of the following is the most effective buffer for pH of 7.4? (JIPMER Nov 2018)

A. Carbonic acid buffer with pKa of 6.1
B. Phosphate buffer with pKa of 6.9
C. Glutamate buffer with pKa of 8.7
D. Acetate buffer with pKa of 4.5

ACID BASE DISORDERS

00:24:30



Important Information

↓ pCO_2 → Respiratory Alkalosis
↑ pCO_2 → Respiratory Acidosis
↑ HCO_3^- → Metabolic Alkalosis
↓ HCO_3^- → Metabolic Acidosis

Anion Gap
- Def: Difference of sum of all major cations and anions in the blood
- Anion Gap = $(Na^+ + K^+) - (Cl^- + HCO_3^-)$
  - K^+ is very less so it can be ignored
- Normal Anion gap value is = 12 ± 2 meq/L
- Used for differential diagnosis of metabolic acidosis
- Metabolic acidosis is of two types:

1. **With Increased anion gap**
- Ketoacidosis (Diabetic or starvation)
- MSUD
- Biochemical organic acidemia
- Lactic acidosis
- Renal failure
- Urimea
- Toxins or poisoning of Salicylates, Methanol or Ethylene glycol

2. **With Normal Anion gap**
- Renal tubular acidosis
- ACE inhibitors
- GI loss of bicarbonate

Previous Year's Questions

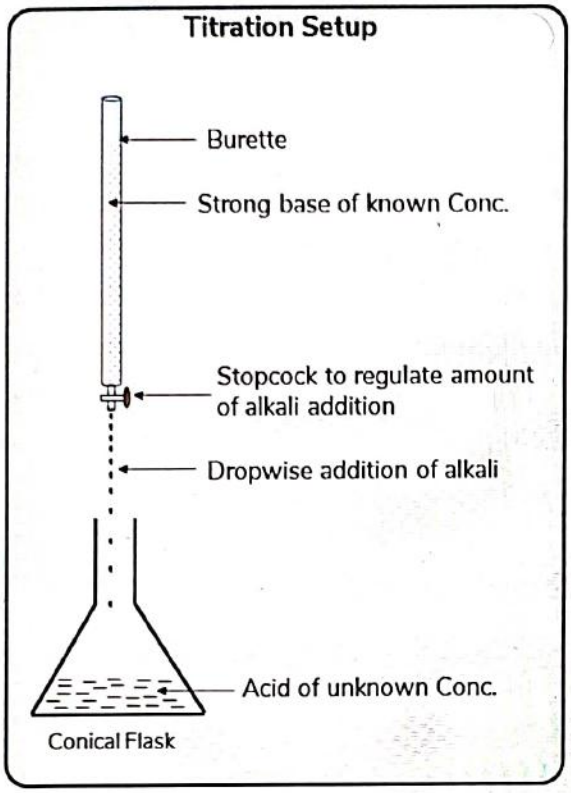
Q. High anion gap acidosis occurs in: (INICET Nov 2020)

A. Renal tubular acidosis
B. Diabetic ketoacidosis
C. Lactic acidosis
D. Salicylate poisoning

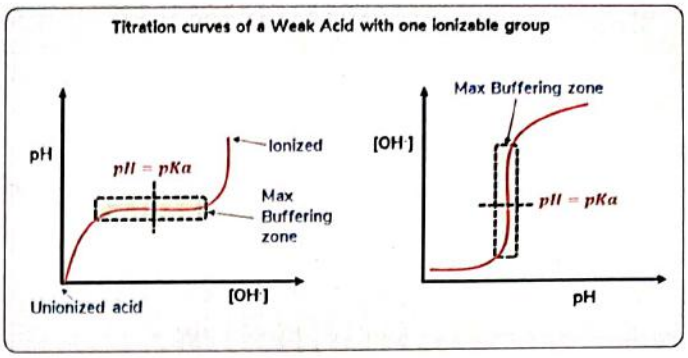
TITRATION CURVE

00:29:41

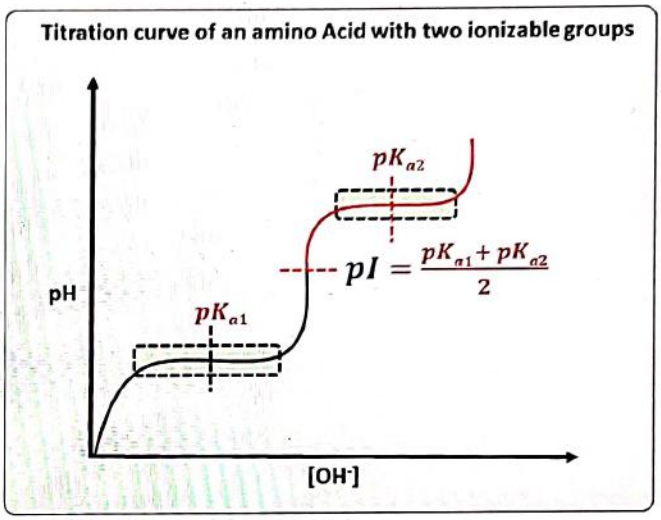
- **Def:** Graphical plot of pH of buffer against amount of alkali added.
- Titration is done to find amount of acid in a solution
- End point is to reach pH = 7



00:32:00

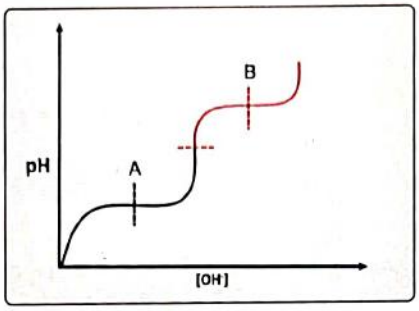


00:34:52



CLINICAL QUESTIONS

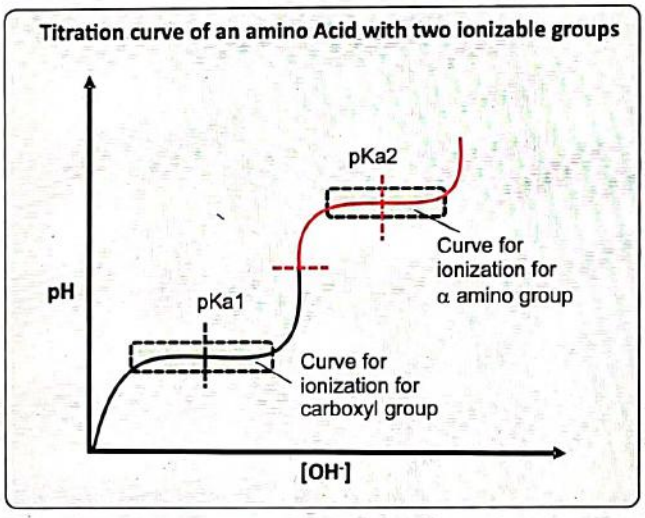
Q. The graph below shows a titration curve of a common biochemical compound. Which of the following statements about the graph is true?



A. The compound has only one ionizable function
B. The compound has at least two ionizable functions
C. Points A and B on the graph represent the minimum buffering capacity region
D. Point A could represent the range of ionization of an amino function

Answer: B

Solution



The figure in the question shows the titration curve of glycine, an amino acid with two dissociable protons—one from the α-carboxyl group (COOH) and the other from the α-amino group (NH_2).

The curve clearly demonstrates two ionizable functions, one that ionizes at low pH is of α-carboxyl group and other at high pH would be of α-amino group.

Regarding other options:

The maximum buffering capacity of any ionizable function is at the pH equivalent to the pKa of the dissociation, as represented by points A and B on the graph.

Point A is due to the ionization of a relatively strong acid like a carboxyl group while point B represents ionization of a base like the α-amino group.

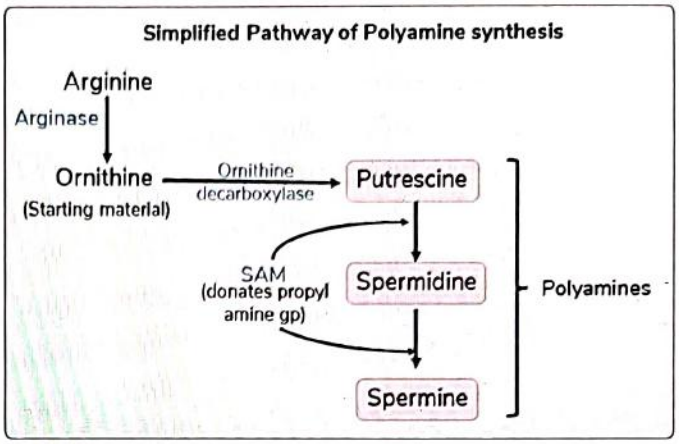
Reference: Harper's 30th ed/pg. 12,20-21

122 POLYAMINE PATHWAY

POLYAMINE PATHWAY

00:04:17

- 'Poly' means 'Many' amine (NH_2) group
- NH_2 group → NH_3^+ gives positive charge, so,
  - Polyamines are highly positively charged
  - Polyamines interact with negatively charged cellular structures e.g.
    - → Nucleic acid (DNA and RNA)
    - → Proteins
    - → Membrane phospholipids



Roles of Polyamines

00:01:57

- DNA stability
- Chromatin modelling
- Increase rate of cellular proliferation, so, used as growth factors in cell cultures
- Inhibits enzymes e.g. protein kinase
- Help in membrane fusion required during endo or exocytosis
- Opening of few channels and help in transport of substances across the cells
- Changes in polyamine is associated with many diseases and ageing

123 HbA1C

HbA1C: Glycated/glycosylated Hemoglobin

Characteristics 00:00:39

- Glycosylation occurs by formation of N-glycosidic linkage between a hexose sugar (Glu/ Gal/Fru) and N-terminal amino acid of hemoglobin
- It is a normal and continuous process
- In a person with normal blood glucose, approximately 5% of Hb is glycated
- HbA1C is reported as ratio of glycosylated Hb/total Hb and given as '%' with no units.
- It is non-enzymatic and irreversible reaction and persists throughout the RBC lifespan (120 days).
- HbA1C value are of diagnostic value in a diabetic patient

00:02:58

HbA1C levels	Significance
<6.0 % (5.7%)	Normal person
6.0–6.5%	Pre-diabetic
≥ 6.5%	Diabetic

- HbA1C determination is a frequently used test to assess diabetic condition these days.

Advantages of HbA1C over direct blood glucose measurement 00:03:37

1. It does not require a fasting sample.
2. It gives an estimate of average blood glucose concentration for past 6-8 weeks because lifespan of RBCs is 120 days and glycation is irreversible.

Uses

1. To check the status of DM
2. To check the response to treatment in a diabetic patient

Methods to determine HbA1C 00:05:23

1. HPLC based methods:These methods are gold standards for measurement of HbA1C and mainly use Ion-exchange chromatography.
2. Affinity chromatography: mainly used in point of care devices
3. Other less common methods
   - Immunoassay
   - Enzymatic assay
   - Capillary Electrophoresis.

Previous Year's Questions

Q. Glycosylated Hb (HbAIC) is best detected by (INICET July 2021)

A. Ion exchange
B. Affinity chromatography
C. Isoelectric focussing
D. Electrophoresis

124 1C METABOLISM

1C METABOLISM

Definition: One-carbon (1C) metabolism consist of series of interlinking metabolic pathways mainly including folate cycle and methionine cycle

> **Important Information**
>
> CO_2 and HCO_3^- are not considered member of IC pool.

Significance 00:01:33

- 1C metabolism serve to activate and transfer 1C units for biosynthetic processes such as:
  - Purine and Pyrimidine synthesis
  - Synthesis of polyamines
  - Amino acid synthesis (Glycine serine and cysteine)
  - Synthesis of creatine
  - Synthesis of phospholipids
- Any block in these processes will lead to inhibition of cellular proliferation and developmental delay

Components of 1C metabolism 00:03:17

- Vitamins: B_7 (Biotin) for carboxylation rxn, B_9 and B_{12}
- AA: Methionine for SAM (S-Adenosyl Methionine)

00:05:02

Source of THF forms	Donors of 1C	Acceptors of 1C
• Tryptophan	• N^{10} formyl THF (most Oxidized)	• C2 of purine • Formyl-methionine • CO_2
• Histidine	• N^5, N^{10} methenylTHF	• C8 of purine
• Glycine, serine, choline and betaine	• N^5, N^{10} methylene THF	• Glycine • dUMP to dTMP conversion
	• N^5 methyl THF (Most reduced)	• B_{12} (Methyl Cobalamin)

PREP NUGGETS

Prep Nuggets

Amino acid disorders	Enzyme deficient
Phenylketonuria	.....
.....	Homogentisate dioxygenase
Maple syrup urine	.....
Albinism	.....

Prep Nuggets

Glut	Function
Glut 1	.....
Glut 2	.....
Glut 3	.....
Glut 4	.....
Glut 5	.....

Prep Nuggets

Enzyme inhibitors

	Km	Vmax
Competitive	.....	.....
Non competitive	.....	.....
Un-competitive	.....	.....

Prep Nuggets

Lipoprotein

	Lipid	Protein
Chylomicron	.....	.....
VLDL	.....	.....
LDL	.....	.....
HDL	.....	.....

Prep Nuggets

Lysosomal storage disease	Enzyme deficient
Mucopolysaccharidosis	.....
I – cell disease	.....
.....	acid maltase
Sphingo lipidoses	.....
Wolman's disease	.....

Prep Nuggets

Clinical features	Disease
CNS + Skin + hair	.....
.....	PKU
Hypopigmentation	.....
CNS + burst sugar urine	.....