1 INTEGRATION OF METABOLISM

Introduction to Metabolism

- It is a process that assimilate the food
- **2 types**
  - Catabolism
  - Anabolism

Catabolism

- Complex biomolecules → Simpler substances.
- Energy is liberated depending upon how it is trapped in the form of ATP.

Q. Why is energy stored as ATP?

Ans:

- If not stored as ATP will be released as heat, which leads to excess heat.
- Only ATP can be used by the anabolic pathways.
- Depending on how energy is trapped as ATP, Catabolism is 2 types:
  - **Substrate level phosphorylation**
    - → Energy is trapped as ATP at the substrate level only
    - → Substrates → Products
    - → ADP → ATP (characteristic of substrate level phosphorylation)
  - **Oxidative phosphorylation**
    - → Substrates → Products
    - → Energy is initially trapped in the form of reducing equivalence like NADH/ FADH2
    - → NADH/ FADH2 oxidized with the help of ETC, and if this ETC helps in phosphorylating ADP to form ATP (characteristic of oxidative phosphorylation)

Important Information

- NADPH can't enter ETC
- NADPH can't give energy

Q. Which of the reactions is an Oxidative phosphorylation?

Ans:

- Most probably the enzymes which end with kinase are involved in Substrate level phosphorylation
- In Oxidative phosphorylation, enzyme name ends with dehydrogenase

Important Information

- In humans metabolism oxidative phosphorylation is common (humans survives on Oxygen).

Examples of Substrate Level Phosphorylation

- **Glycolysis**
  - Phosphoglycerate kinase
  - Pyruvate kinase
- **TCA cycle**
  - Succinyl thiokinase
- **Muscle**
  - Creatine kinase

*Except these, all other comes under oxidative phosphorylation

Anabolism

00:06:27

- Simple substances → Complex forms
- Covalent linkages are formed (energy is used)
- **Examples**
  - Glycogen synthesis
  - Fatty Acid synthesis
  - Cholesterol synthesis
  - Protein synthesis
  - Gluconeogenesis

Important Information

- 11 high energy phosphates are required for Gluconeogenesis
- Only background is catabolic
- Pathway is anabolic

Amphibolic Pathway

- Runs in both anabolic and catabolic directions
- Depends on energy status of the cell
  - **Low energy** - Catabolic (ATP formed)
  - **High energy** - Anabolic (ATP utilized)
- **Only example is TCA cycle**
  - **Low energy** - Clockwise direction (10 ATP formed)
  - **High energy** - Anticlockwise direction (intermediates are accumulated and used in anabolism)

Integration of Metabolism

00:11:32

Low Energy

Glucose
↓
Enters cell
↓
Undergoes glycolysis
↓

Forms two molecules of Pyruvate (through aerobic glycolysis)

↓

7 ATPs formed

Absence of oxygen

Two molecules of Pyruvate

↓

lactate dehydrogenase

Two molecules of Lactate

↓

2 ATPs (anaerobic glycolysis)

Presence of oxygen

Two molecules of Pyruvate

↓

2 Acetyl CoA (Pyruvate dehydrogenase complex)

↓

2 NADH (Gives 5 ATPs in ETC)

↓

Acetyl-CoA Pushed to TCA (final oxidation and gives CO_2) this gives- 10 ATP (2 Acetyl-CoA- 2x10=20 ATPs)

Important Information

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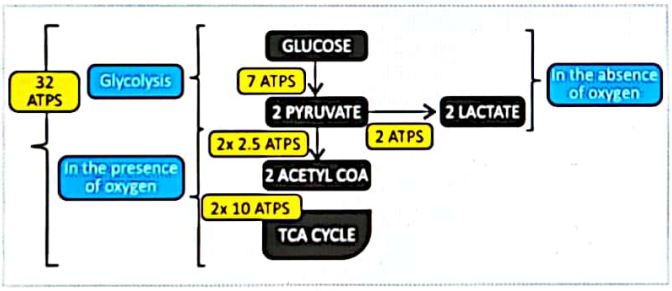
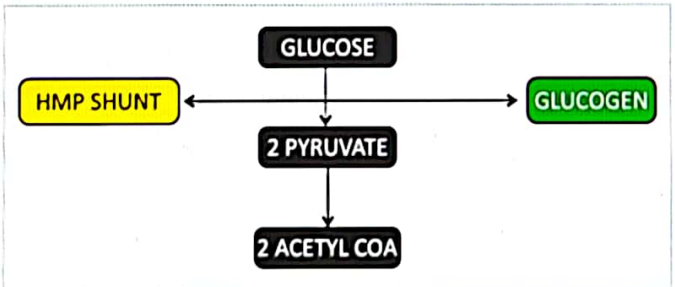
- When Glucose gets completely oxidized we get,
  - Glycolysis 7 + 5 = 12 ATPs
  - TCA gives 20 ATPs
  - Total = 32 ATPs



High Energy



- Glucose-6-Phosphate (G6P) is inhibited in high energy
- G6P enter into alternate pathways
  - Glycogen synthesis
  - HMP shunt

Important Information

- **G-6-phosphatase:** G6P → Glucose
- **G-6-phosphate dehydrogenase:** G6P pushed to HMP shunt
- **HMP**
  - Also called PPP (Pentose Phosphate Pathway)
  - It acts as a source for Ribose-5-Phosphate (R5P)
  - R5P foundation for nucleotides
  - Acts as a source of NADPH

Differences between NADH and NADPH

NADH	NADPH
• Enter into ETC • Each NADH gives 2.5 ATP	• Never enters ETC • Can't give ATPs • NADPH act as hydrogen source • **Significance** o Reductive biosynthesis of lipids (gives more energy) o Regeneration of glutathione (antioxidant mechanism of RBC) o Coenzyme of Ribonucleotide reductase (Nucleotides → Deoxynucleotide) o Necessary for Cyt P450 enzymes
Sources • Glycolysis • TCA • Fatty acid oxidation • Amino acid oxidation	**Sources** • HMP shunt • Cytoplasmic isocitrate dehydrogenase • Malic enzyme (Malate → Pyruvate)

Antioxidation of RBCs

- RBC acts as major source of oxidative stress in our body
- Thus need antioxidant mechanism
- RBC's uses glutathione (GSH)
- 2 GSH + H_2O_2 → 2 H_2O + GSSG (Enzyme: Glutathione peroxidase)

- Regeneration of Glutathione is necessary to get GSH back with the help of glutathione reductase.
- GSSG → GSH (NADPH acts as a hydrogen source)

Q. What happens in G6PD deficiency?

Ans:

HMP shunt is defective
↓
NADPH can't formed
↓
No Regeneration of Glutathione
↓
Increased H_2O_2
↓
RBC lysis
↓
Hemolytic anemia (following exposure to oxidative stress)

Usually seen in

- Fava beans intake
- Antimalarial drug -primaquine intake
- Acetyl-CoA is accumulated in high energy
- **Used for**
  - **Fatty acid synthesis:** Excess fatty acid stored as Triacylglycerol (↑)
  - **Cholesterol synthesis:** Excess cholesterol stored as Cholesterol esters (↑)

Important Information

- Acetyl-CoA is the shortest fatty acid
- 2 Acetic acids → Butyric acid
- 2 Butyric acid → Octanoyl-CoA
- 2 Octanoyl-CoA → Palmitic acid

Preferred Fuel for Cells

- Choice of fuel depends on anaerobic or aerobic nature of the cells
  - **Anaerobic Cells - Glucose** (Glycolysis)
    - → RBCs
    - → Retinal cells
    - → Corneal cells
    - → White muscle fibers (without myoglobin)
    - → Renal medulla (less blood supply)
  - **Aerobic Cells - Glucose and Fatty Acids** (Mostly use fatty acids as they give more ATP)
    - → Cardiac muscle fibers
    - → Red muscle fibers
    - → Neurons (Doesn't use Fatty Acids)

Important Information

- Neurons are surrounded by BBB, all fatty acid are non polar so they are attached to albumin within the circulation and it cannot cross the BBB thus neurons do not have access to fatty acids

RBCs	Neurons
Use glucose anaerobically	Use glucose aerobically
2 lactate and 2 ATPs	32 ATPs and becomes CO_2

Preferred Fuel for - Liver

- Amino acids are used (liver has glucokinase enzyme which has low affinity for glucose)
- Fatty acids can't be used because every dietary fat gets absorbed as chylomicron and it get into lymphatics and then to systemic circulation (not into portal circulation)

Blood Glucose Maintenance in the Fed State and Starvation

- **Dietary glucose** - 2 to 2.5 hours
- **Liver glycogenolysis**
  - Starts after 2 to 2.5 hours
  - Lasts for 12 to 18 hours
- **Gluconeogenesis**
  - Starts after 6 hours of previous meal
  - Overlaps with liver glycogenolysis

Gluconeogenesis 00:46:04

- In this process, two molecules of pyruvate are combined to form glucose (reversal of irreversible steps glycolysis).

Steps Involved in Gluconeogenesis

- There are four steps with three enzymes

Pyruvate kinase: It is a two step process

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

PFK-1: Step-3

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

Hexokinase/ Glucokinase: Step-4

Reversible step (Glycolysis)	Irreversible Step (Gluconeogenesis)
• Glucose to Glucose 6 phosphate by enzyme hexokinase	• Glucose-6-phosphate to glucose by enzyme glucose-6-phosphatase.

- **Other definition:** New glucose is formed from non carbohydrate precursors

Requirements for Gluconeogenesis

- **3 principle substrates**
  - Glycerol
  - Lactate
  - Alanine
- **Energy**
  - 11 high energy phosphates are needed

Recall ATPs

- **Aerobic** - 7 ATP
- **Anaerobic** - 2 ATP
- Complete oxidation
  - **Glucose** - 32 ATP
  - **Palmitic acid** - 106 ATP
  - **Stearic acid** - 120 ATP

Peripheral Lipolysis

- Peripheral lipolysis is important for Gluconeogenesis
- Adipose tissue triacylglycerol is cleaved with hormone sensitive lipase (HSL) and forms Glycerol + Fatty acid
- Levels of Glycerol and free fatty acid increased in blood
- Now insulin inhibits HSL
- Glucagon, GH, NE and cortisol stimulate HSL (thus the name HSL)
- This is the reason adipose tissue melt while starvation
- Glycerol + Fatty acid reach liver
- Glycerol is used as a substrate for gluconeogenesis
- Lactate is given by all anaerobic cells
- Alanine is brought from muscle protein breakdown (prolonged period of time)

Important Information

- Do not starve for long time as it leads to muscle breakdown
- In starvation there is an increase in free fatty acid in the circulation by lipolysis followed by fatty acid oxidation.
- Fatty acid oxidation defect leads to hypoglycemia
- Cardiac muscle fibers can be affected, leading to cardiomegaly and cardiomyopathies
- If red muscle fibers are affected, present as exercise intolerance
- In early neonatal period fatty acid defects will present with hypoglycemia

Enzyme Regulation - Covalent Modification 01:03:36

- Enzyme which increases plasma glucose is stimulated by Glucagon via phosphorylation
- Enzyme which decreases plasma glucose is stimulated by Insulin via dephosphorylation

Enzymes Activated by Phosphorylation

- **Liver glycogenolysis**
  - Glycogen phosphorylase
  - Debranching enzyme
- **Gluconeogenesis**
  - Pyruvate carboxylase
  - Phosphoenolpyruvate carboxykinase
  - Fructose-1,6-bisphosphatase
  - Glucose-6-phosphatase
  - Fructose 2,6-bisphosphatase
- HSL

MCQs

Q. Glucose on complete oxidation provides how many ATPs?

A. 7
B. 2
C. 32
D. 10

Q. In starvation which of the following is increased in the circulation?

A. Glucose
B. Insulin
C. Chylomicron
D. Free fatty acid

Q. Which of the following pathways is Amphibolic?

A. Glycolysis
B. Gluconeogenesis
C. Fatty acid oxidation
D. TCA cycle

Q. All the following are sources if NADPH except?

A. Malate dehydrogenase
B. Malic enzyme
C. Isocitrate dehydrogenase
D. HMP shunt

PREVIOUS YEAR QUESTIONS

Q. RBCs use which of the following in starvation? (INICET July 2021)

A. Glucose

B. Ketones

C. Amino Acids

D. Fatty acids

Q. Which cells are affected mostly in glycolytic enzyme defects?

Ans. RBCs

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

A. Hormone sensitive lipase

B. Lipoprotein lipase

C. Acid lipase

D. Alkaline lipase

2 CARBOHYDRATE CHEMISTRY & METABOLISM

Carbohydrate Chemistry and Metabolism

Q. The marker enzyme of microsomes is
A. Galactosyl transferase
B. Cathepsin
C. Lactate dehydrogenase
D. Glucose 6 phosphatase

- Microsome is nothing but Endoplasmic Reticulum.
- Galactosyl Transferase is important for glycoprotein Synthesis (Golgi Complex)
- Cathepsin are Proteases. Present in Lysosomes and enzyme markers of Lysosomes.
- Lactate Dehydrogenase is the enzyme of Glycolysis which happens in Cytoplasm.
- Glucose 6 phosphatase is an enzyme of gluconeogenesis (last step of gluconeogenesis occurs in ER), so Glucose 6 phosphatase is an enzyme marker for microsomes

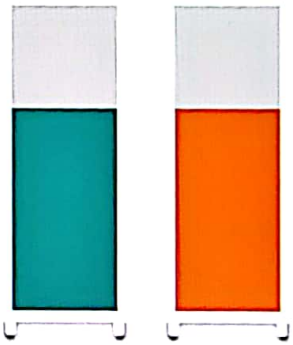
S.No.	Organelle	Marker Enzyme	
1.	Nucleus	DNA & RNA Polymerases	
2.	Endoplasmic Reticulum	Glucose 6 Phosphatase	
3.	Golgi Complex	Galactosyl Transferase	
4.	Mitochondria	Outer membrane	Monoamine Oxidase
		Inner membrane	ATP Synthase / Succinate Dehydrogenase
5.	Lysosome	Cathepsin	
6.	Cytoplasm	Lactate Dehydrogenase	
7.	Peroxisome	Catalase	

Q. Primaquine can precipitate hemolytic anemia in individuals with an enzyme deficiency. The enzyme is related to which pathway?
A. Gluconeogenesis
B. Glycolysis
C. HMP shunt
D. Glycogen metabolism

Q. On prolonged starvation, which of the following tests will be positive?

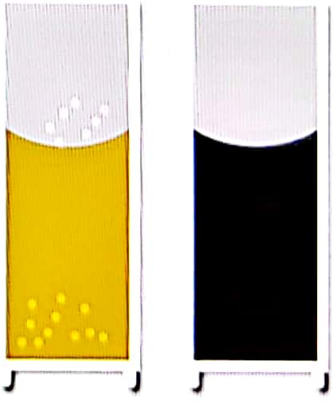
A.

B.



C.

D.



Ans: Rothera's test - Detect ketone body

Explanation:

A. Xanthoproteic acid – Aromatic amino acids (phenylalanine, tyrosine, tryptophan)
B. Benedict's test – reducing sugars
C. Rothera's test - Detect ketone body
D. Either Alkaptonuria or benzidine test

S.No	Abnormal Constituent	Color Reaction	Observation
1	Reducing sugar (glucose, galactose etc)	Benedicts test	Blue Green
2	Ketone bodies	Rothera's test	Purple color ring
3	Protein	Sulphosalicylic acid test	White ppt
4	Blood	Benzidine test	Dark green or black ppt
5	Bile salts	Hays test	Sulphur sinks to bottom
6	Bile pigments	Fouchet's test	Bluish or greenish ppt.

Q. Which of the following is not a glucogenic substrate?
A. Acetyl CoA
B. Glycerol
C. Lactate
D. Alanine

Explanation

- Gluconeogenesis is reversal of glycolysis
- **Substrates**
  - Glycerol
  - Lactate
  - Alanine

Criteria of gluconeogenesis substrate:

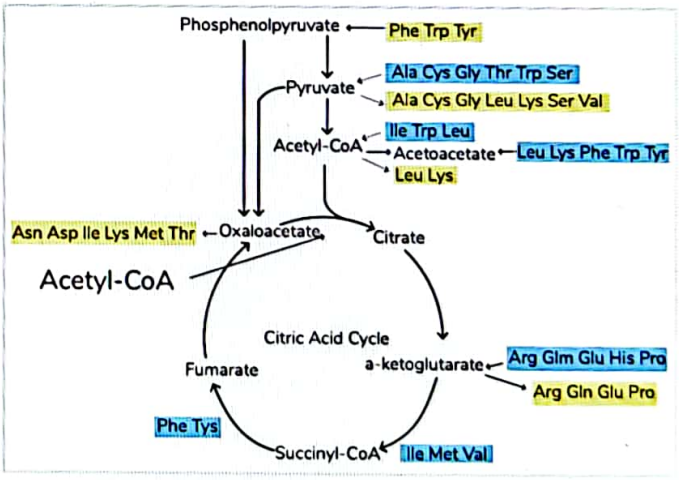
- Substrate of gluconeogenesis should be able to give rise to one of the glycolytic intermediates, or Citric acid cycle intermediates other than acetyl CoA.
- Those intermediates should be able to get into gluconeogenesis. Acetyl CoA can't.
- Conversion of pyruvate to Acetyl CoA by pyruvate Dehydrogenase is irreversible.

Citric Acid Intermediates

- By the end of the TCA cycle, OAA is regenerated.
- First enzyme of the TCA cycle is citrate synthesis; which condenses Acetyl CoA and OAA to form citrate
- OAA can get into gluconeogenesis.
- First enzyme of gluconeogenesis is pyruvate carboxylase.
  - It converts pyruvate to OAA.
- Then OAA is converted to PEP.
- Finally gets into gluconeogenesis.

Even Chain Fatty Acids - Not Gluconeogenic

- Even chain FA undergoes ß oxidation to form 8 Acetyl CoA (which cannot get into gluconeogenesis)



Odd Chain Fatty Acids - Gluconeogenic

- Undergoes oxidation to form propionyl CoA
- **Example:** 7C FA undergoes oxidation to form 5C FA and release 2C Acetyl CoA.
- Then 5C undergoes ß oxidation to form 3C FA (propionyl CoA) and release 2C Acetyl CoA.
- Majority of products come out as Acetyl CoA.
- But 1 propionyl CoA can get into citric acid cycle after getting converted into Succinyl CoA

Q. Thiamine deficiency causes lactic Acidosis because of inhibition of?

A. PDH
B. Pyruvate decarboxylase
C. PEPCK
D. Transketolase

Fates of Pyruvate

- Pyruvate is a central metabolite, can have multiple fates
- Choice of fate depends on the energy status of the cell

Low Energy

- Fate depends on whether the cell is aerobic or anaerobic
- **Anaerobic**
  - ATP generated by Anaerobic glycolysis
  - Converts pyruvate to Lactate by Lactate Dehydrogenase (LDH)
- **Aerobic:** Converts pyruvate to Acetyl CoA by Pyruvate Dehydrogenase (PDH)

High Energy

- **Depends on:** Person is well fed or in starvation
- **Starvation:** Aim is to Increase blood glucose by stimulating glycogenolysis and gluconeogenesis
- **For gluconeogenesis:** Gain energy by oxidizing fuels (fatty acids and amino acids)
- Pyruvate to OAA by pyruvate carboxylase then into gluconeogenesis
- **Well fed:** Starts using pyruvate for anabolism by converting pyruvate to alanine (enzyme -SGPT)

AST/SGOT

- Asp + αKG → OAA + Glu

ALT/SGPT

- Ala + αKG Glu + Pyr
- **Transaminase:** AST and ALT
- **AST/SGOT:** involved in breakdown of aspartate.
- Aspartate will react with keto acids (alpha ketoglutarate)
- Aspartate gives its amino acid to alpha ketoglutarate converting it into glutamate.
- That way aspartate turns to OAA (keto acid)
- **Enzymes**
  - Based on Forward reaction - AST
  - Based on Reverse reaction- SGOT
- **ALT:** Alanine react with alpha ketoglutarate.
- Converts alpha ketoglutarate to glutamate.
- Alanine and amino acid turns into pyruvate.
- **Enzymes**
  - Based on Forward reaction- ALT
  - Based on Reverse reaction -SGPT

How to remember: LP/Lumbar puncture

Important Information

All Fates

- Lactate by Lactate Dehydrogenase
- Acetyl CoA by Pyruvate Dehydrogenase
- Alanine by Alanine Transaminase /SGPT
- OAA by Pyruvate Carboxylase

Thiamine Dependent Enzymes

00:33:41

- **PDH** (pyruvate dehydrogenase)
- **Alpha Ketoglutarate Dehydrogenase**
  - Similarities
    - → PDH turns Pyruvate to Acetyl CoA
    - → AKD turns alpha ketoglutarate to Succinyl CoA
    - → Both in mitochondria
    - → Both catalyze oxidative carboxylation reactions.
    - → Both need Thiamine pyrophosphate.
    - → Both inhibited by arsenite.
    - → Both involve 3 subunits and 5 coenzymes
- **Branched chain keto acid dehydrogenase enzyme (defect causes maple syrup urine disease)**
- **Transketolase**

Thiamine Deficiency

PDH: When PDH is inactive pyruvate cannot turn to Acetyl CoA instead turns to Lactate causing Lactic Acidosis

Dry Beriberi

- Aerobic utilization of glucose is affected.
- Therefore neurons are affected.

BCKADH (Branched chain keto acid dehydrogenase enzyme): MSUD

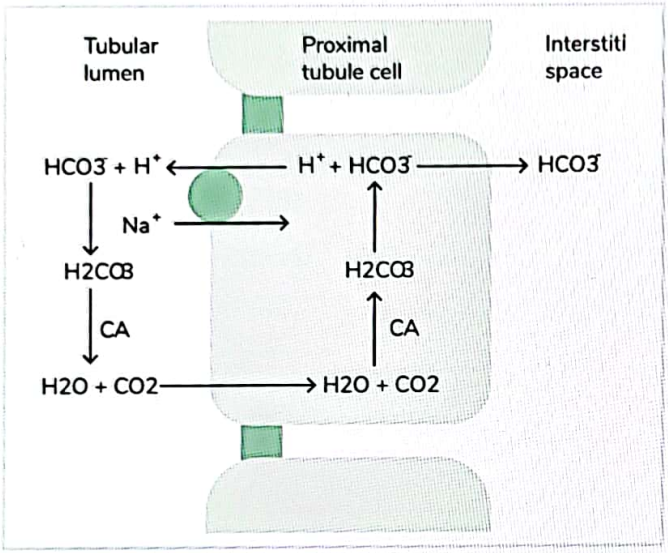
- Responds to Thiamine supplementation
- If Thiamine deficiency is suspected, estimate RBC transketolase activity
- B1 deficiency: transketolase activity should be measured in RBCs

Q. Drug of choice in Mountain sickness (FMGE Dec 2021)

A. Thiazides
B. Furosemide
C. Acetazolamide
D. Spironolactone

- When a person is climbing uphill quickly, not getting acclimatized to high altitude.
- Uphill above sea level, partial pressure is low, so hyperventilation - Wash away CO_2 which causes respiratory alkalosis

- Respiratory alkalosis
  - Detrimental.
  - Central chemoreceptors in medulla only respond to increase in H+ concentration in CSF
- Central chemoreceptors are not stimulated (H+ low)
- Respiratory center is depressed
- Acetazolamide causes normal anion gap metabolic acidosis
- Causes inhibition of enzyme carbonic anhydrase



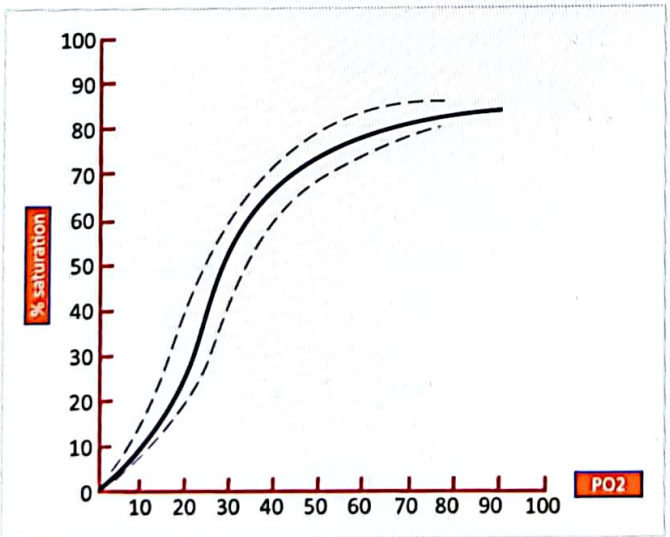
Q. Which of the following shifts the given curve to left?

A. Hypoxia

B. Acidosis

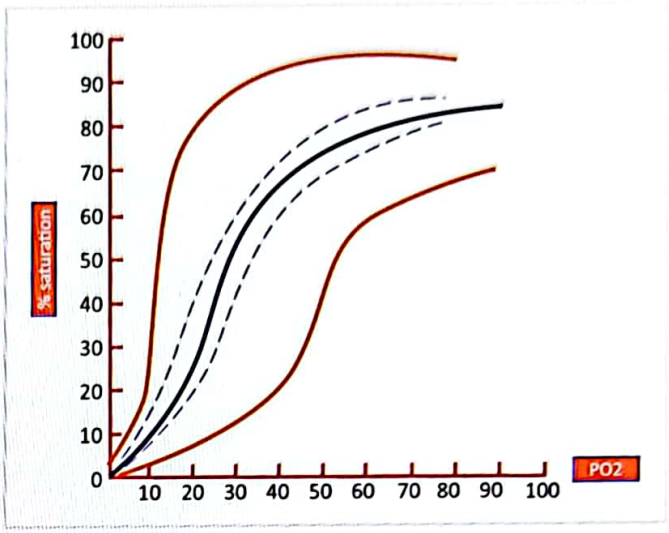
C. Anemia

D. Decrease in body temperature



Explanation:

- Sigmoidal curve is shown
- Oxyhemoglobin dissociation curve
- Along Y axis percentage saturation
- Along X axis Partial pressure of O2



- Shifting of the curve to the right or left tells us about P50
- P50 is the partial pressure of O2 at which Hb is 50% saturated (usually 28 mm Hg for normal adult Hb)
- Shifting of curve to the right – High P50 and low affinity
- Shifting of curve to the left – Low P50 and high affinity
- P50 and affinity are inversely related.
- Acidosis shift curve to right due to bohr effect (Reciprocal binding of oxygen and H+ i.e when oxygen binds H+ leave)
- Shifting of curve to the right (deoxygenation)
  - Hypoxia
  - Increase in body temperature
  - Anemia
  - Acidosis
- Increase in the rate of metabolism in hypoxia, increase in body temperature and anemia (causes increase in glycolysis)- leads to production of 2,3 BPG.

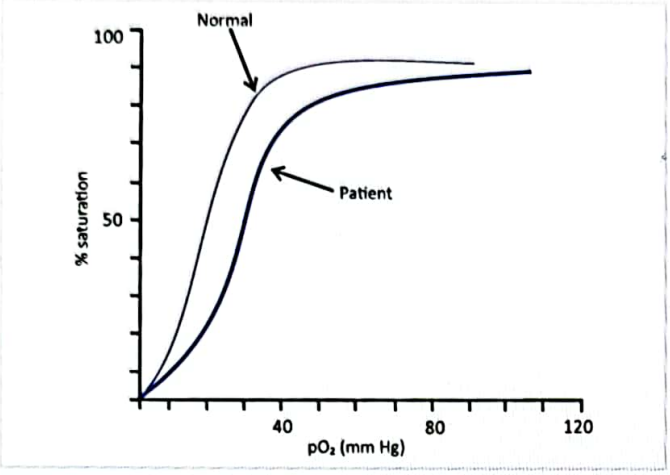
Effect of 2,3 BPG

- Increase in the rate in glycolysis
- For all of these there is a shunt pathway
- It is called rapaport leubering shunt
- Advantage is it gives rise to 2,3, BPG
- 2,3, BPG reduces the affinity of Hb for oxygen helps in unloading of oxygen to the tissues.

Shifts the given curve to left

- Conditions:
  - Alkalosis
  - Decrease in body temperature

Q. A 10 month old child is being evaluated for the underlying cause of hemolytic anemia. In the diagram, the oxygen dissociation curve for hemoglobin in his erythrocytes is compared with that of normal blood cells. Which of the following enzyme deficiencies is most likely to account for hemolytic anemia in this patient?



A. Phosphofructokinase
B. Glucose-6-phosphate dehydrogenase
C. Pyruvate carboxylase
D. Pyruvate kinase

Explanation

- A case hemolytic anemia
- High 2-3 BPG
- **Metabolic causes**
  - G6PD deficiency (most common)
  - Glycolytic enzyme defects
- Pyruvate carboxylase is excluded as it is an enzyme of gluconeogenesis (Pyruvate carboxylase defect presented as hypoglycemia)
- G-6-PD is also excluded as no oxidative stress here
- Phosphofructokinase is the rate limiting enzyme, if this is affected it will have low 2,3-BPG
- Here we have high 2,3-BPG
- Thus it is Pyruvate kinase defect

Important Information

- In G6PD deficiency the following are seen,
  - Oxidative stress
  - After intake of primaquine, fava beans, dapsone
  - Exercise intolerance
- **2,3-BPG is low:** Phosphofructokinase
- **2,3-BPG is high:** Pyruvate kinase

Q. Following an early morning run, a 29 year old man consumes a carbohydrate rich south Indian breakfast. Which of the following will most likely be activated in his liver after breakfast?

A. Cytoplasmic PEPCK
B. Membrane GLUT4 transporter
C. Cytoplasmic PFK2
D. Cytoplasmic glycogen phosphorylase

Explanation

- Blood glucose is high
- So no gluconeogenesis or glycogenolysis stimulation is needed in well fed state.
- **Thus**
  - No Cytoplasmic glycogen phosphorylase
  - No Cytoplasmic PEPCK
- Membrane GLUT4 transporter is not present in liver
- Thus, Cytoplasmic PFK2

Types of GLUT Transporters

GLUT Transporter	Location
GLUT 1	RBCs, Neurons, Placenta
GLUT 2	Enterocytes, Hepatocytes, Pancreatic beta cells, PCT
GLUT 3	RBCs, Neurons, Placenta
GLUT 4	Skeletal and cardiac muscle, adipose tissue
GLUT 5	Fructose absorption

- GLUT transporter belong to the facilitated passive diffusion mechanism.
- SGLT belong to secondary active transport mechanism.
- GLUT 2 are present along the basolateral side
- Sodium glucose cotransporter 1 is at apical side

Important Information

- Cytoplasmic PFK1: rate limiting enzyme of glycolysis
- Cytoplasmic PFK2: Tandem enzyme which regulates glycolysis and gluconeogenesis

Enzyme Regulation

- Enzyme which increases plasma glucose is stimulated by Glucagon via phosphorylation
- Enzyme which decreases plasma glucose is stimulated by Insulin by dephosphorylation
- **Ex:** Glycogen phosphorylase is an enzyme which is stimulated by phosphorylation (phosphoryl kinase enzyme)

Regulation of Glycolysis and Gluconeogenesis

- Both are reversible to each other
- Regulated in such a way that one pathway is activated and another one is inactivated (allosteric regulator)
- Otherwise they end up in a futile cycle

- **Example:** A starvation person used 11 ATP molecules (2 Pyruvate, 1 Fructose 6 Phosphate)
- If phosphofructokinase enzyme is failed then fructose-6-phosphate is converted to fructose 1,6 bisphosphate, then it enters into glycolysis.
- This process is called a futile cycle

Facts for the Regulation of Glycolysis and Gluconeogenesis

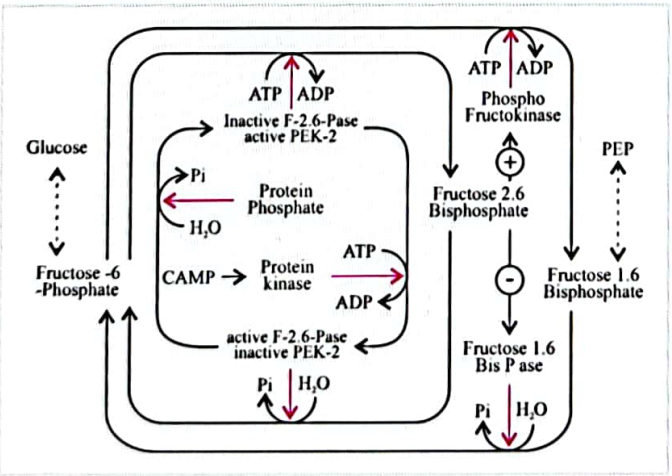
- Common allosteric regulator (fructose 2,6 bisphosphate)
- Fructose 2,6 bisphosphate is a stimulator of glycolysis (PFK-1).
- Fructose 2,6 bisphosphate is an inhibitor of gluconeogenesis (Fructose 1,6 bisphosphatase).
- Fructose 2,6 bisphosphate is the product of a tandem enzyme - PFK2.

Important Information

Tandem Enzyme

- Bi functional enzyme (one protein with opposite enzymatic activity)
- One enzymatic activity synthesizes the compound (protein is phosphorylated)
- Another one catalyzes the same compound (protein is dephosphorylated)
- PFK-2 has both PFK-2 and fructose 2,6 bisphosphatase activity
- Reversible conversion of fructose 6 phosphate to fructose 2,6 bisphosphate (PFK-2)

- When tandem enzyme gets phosphorylated it will act as Fructose 2,6 bisphosphatase and when the same enzyme gets dephosphorylated it will act as PFK2.



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

Rate Limiting Enzymes 01:21:55

Pathway	Rate Limiting Enzyme
Glycolysis	PFK-1
TCA Cycle	Isocitrate dehydrogenase
Gluconeogenesis	Fructose-1,6-bisphosphatase
Glycogen Synthesis	Glycogen synthase
Glycogenolysis	Glycogen phosphorylase
HMP shunt	Glucose-6-phosphate dehydrogenase

Energetics

Pathway	ATPs Generated/ Utilized	For Glycogenolysis Glucose
Aerobic glycolysis	7	8
Anaerobic glycolysis	2	3
TCA Cycle	10	
Complete oxidation of glucose	32	33
Glycogen Synthesis	- 3	
Gluconeogenesis	- 11	
HMP shunt	Glucose-6-phosphate dehydrogenase	

Important Information

- Free glucose is not the final product of glycogenolysis
- It is glucose-1-phosphate which becomes glucose-6-phosphate
- This enters into glycolysis.
- Bypassing the hexokinase step

Chemical Inhibitors of Enzymes

Enzyme	Inhibitor
Glyceraldehyde-3-phosphate dehydrogenase	Iodoacetate
Phosphoglycerate kinase	Arsenate
Enolase	Fluoride (Na_2F is given as anticoagulant is plasma glucose estimation)
Pyruvate dehydrogenase complex and alpha ketoglutarate dehydrogenase complex	Arsenite
Aconitase	Fluoroacetate (suicidal inhibition)
Succinate dehydrogenase	Malonate

Few Facts to Remember

- **Malate shuttle**
  - **Glycolysis**
    - → Passage of NADH from cytoplasm to mitochondria
    - → Occurs in cytoplasm
    - → In this, 2 NADH is received per glucose entering into glycolysis
  - **Gluconeogenesis**
    - → Passage of oxaloacetic acid from mitochondria to cytoplasm

Important Information

- In white muscle fibers and neurons we have glycerol phosphate shuttle instead of malate shuttle

Malate shuttle	Glycerol Phosphate Shuttle
All cells	White muscle fibers and neurons
NADH cytoplasmic → NADH mitochondrial	NADH cytoplasmic → $FADH_2$ Mitochondrial

*If cells follows glycerol phosphate shuttle minus 2 from the total ATPs

Pathway	ATPs Generated/ Utilized	Glycerol Phosphate Shuttle
Aerobic glycolysis	7	5
Complete oxidation of glucose	32	30

Enzyme Defects

- **Essential fructosuria**
  - Fructose is found in urine (Benedict's test is +ve)
  - Caused by a defect of fructokinase
- **Hereditary fructose intolerance**
  - Intolerance to fructose
  - After intake of table sugar consumption
  - May show
    - → Hypoglycemia
    - → Lactic acidosis
    - → Hyperuricemia
    - → Jaundice
    - → Hepatomegaly
  - Caused by defect of Aldolase B
- **Classical Galactosemia**
  - Caused by defect of GALPUT (Galactose-1-Phosphate Uridyl Transferase)
  - May show
    - → Hypoglycemia
    - → Lactic acidosis
    - → Hyperuricemia
    - → Hepatomegaly

Important Information

- **Classical galactosemia**
  - Presentations are observed even when the child is mother fed
  - +ve Benedict's test
  - Oil drop cataract (characteristic)
- **Hereditary fructose intolerance**
  - Presentations are absent when the child is mother fed
  - +ve Benedict's test
  - Cataract changes are not that common

3 LIPID CHEMISTRY & METABOLISM/HEME SYNTHESIS

Fatty Acid Synthesis 00:01:01

- **Location:** Cytoplasm
- The building block of fatty acid and cholesterol is acetyl CoA.
- **Rate limiting enzyme:** Acetyl CoA carboxylase

Fatty Acid Synthase Complex

- Present in cytoplasm
- Two units, and every unit has 8 subunits but only 7 enzymatic activities
- One of the subunit has acyl carrier protein activity (carries fatty acid getting synthesized)
- Every unit has a cysteine end, and 4-phosphopantetheine ends.
- Simultaneously 2 fatty acids are synthesized

One Liners

- Most commonly synthesized fatty acid by fatty acid synthase complex is palmitic acid (16C)
- Cycles involved in giving 16C fatty acid: (n/2)-1 = (16/2)-1 = 8-7 = 7 cycles
- Number of NADPH required for synthesizing palmitic acid - 14 (2 per cycle)
- Number of ATPs required to synthesize palmitic acid - 7 (1 per cycle)

Acetyl CoA Carboxylase

- Anabolic enzyme
- **Stimulated** by high energy status indicators
  - NADH
  - $FADH_2$
  - ATP
  - Citrate
  - Insulin
- **Inhibited** by low energy status indicators
  - ADP
  - NAD
  - FAD
  - Glucagon
  - Acyl CoA (product)

Fatty Acid Oxidation 00:07:55

- **Location:** Mitochondria and peroxisomes

To Remember: The only oxidation that can happen in cytoplasm is glycolysis (anaerobic)

- **Functions of peroxisome**
  - Very long chain fatty acid oxidation
  - Ether lipid (plasmalogen) synthesis
  - Branched-chain fatty acids

Mitochondrial vs Peroxisomal Oxidation

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

- **When oxidation happens in Mitochondria**
  - Hydrogen atom is removed from β carbon atom and goes to NAD and FAD, which further forms NADH and $FADH_2$.
  - When NADH and $FADH_2$ go through an electron transfer chain, give rise to ATP.
- **When oxidation happens in the peroxisome**
  - Hydrogen atom from β carbon atom is removed and goes to the oxygen molecule
  - This then forms hydrogen peroxide (H_2O_2)
  - To detoxify hydrogen peroxide, the peroxisome is equipped with catalase enzyme

Phases of Fatty Acid Oxidation

- **1st phase –** n carbon containing Fatty acids undergoes β oxidation to form n/2 acetyl CoA
- **2nd phase -** Every acetyl CoA enters into the citric acid cycle and comes out as CO_2, and every citric acid cycle will gives 10 ATPs

Energetics of Complete Oxidation of Fatty Acids

Formula for Total ATP,

$$[(\frac{n}{2} - 1) \times 4] + [\frac{n}{2} \times 10]$$

Formula for Net ATP,

$$[(\frac{n}{2} - 1) \times 4] + [\frac{n}{2} \times 10] - 2$$

Q. Why -2 in Net ATP?

Ans. To convert the fatty acid into acyl CoA we use two high energy phosphates

Examples

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

Total ATP

$$[(\frac{n}{2} - 1) \times 4] + [\frac{n}{2} \times 10]$$

= 7 x 4 + 8 x 10
= 108 ATPs

Net ATP,

$$[(\frac{n}{2} - 1) \times 4] + [\frac{n}{2} \times 10] - 2$$

= {7 x 4 + 8 x 10} - 2
= 106 ATPs

Ans: Palmitic acid on complete oxidation provides 106 ATPs

2. Net ATPs in stearic acid (18c)

Net ATP

$$[(\frac{n}{2} - 1) \times 4] + [\frac{n}{2} \times 10] - 2$$

= {8 x 4 + 9 x 10} - 2
= 120 ATPs

Ans: Stearic acid on complete oxidation provides 120 ATPs

Regulation of Fatty Acid oxidation

- **Rate limiting enzyme:** Carnitine Acyl Transferase-I
- **Stimulated** by low energy status indicators
  - ADP
  - NAD
  - FAD
  - Glucagon
  - Acyl CoA (product)
- **Inhibited** by high energy status indicators
  - NADH
  - $FADH_2$
  - ATP
  - Insulin
  - Malonyl CoA

To Remember

- Malonyl CoA is an intermediate of fatty acid synthesis
- If Malonyl CoA is present in the cell, then this means that the cell is in anabolic state
- Means it is rich in energy, thus less oxidation is needed
- So it inhibits the fatty acid oxidation

MCQ

Q. A 25 year old male presents with an episode of pancreatitis. His cholesterol is 190 mg/dL and Triglyceride level is 1180 mg/dL. His post heparinized lipoprotein lipase activity is low. On mixing study, the LPL activity normalizes. Which of the following is true about his condition?

a. He will respond to fresh frozen plasma administration
b. It is a defect of lipoprotein lipase
c. It is a defect of Apo CIII
d. It is an autosomal dominant condition

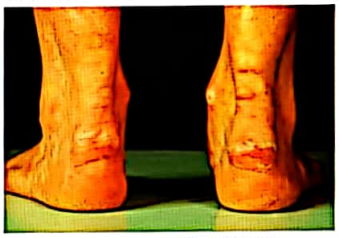
Ans. a. He will respond to fresh frozen plasma administration

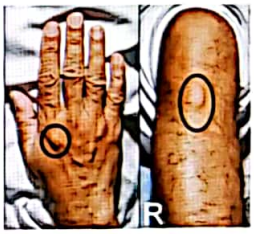
Explanation

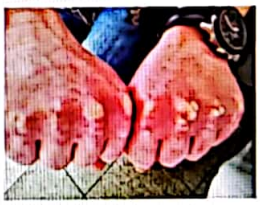
- All the clinical findings are suggestive of type 1 lipoproteinemia (familial chylomicronemia syndrome)
- Familial chylomicronemia syndrome is caused due to defect of Apo C-II/ Lipoprotein lipase

Hyperlipoproteinemias 00:26:36

- **Friedrickson's classification** (6 types)
  - Type I
  - Type IIA
  - Type IIB
  - Type III
  - Type IV
  - Type V
- **3 categories**
  - **Hypercholesterolemia** (clinical features)
    → Tendon xanthomas

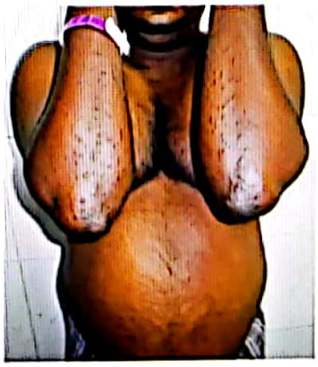






→ Accelerated atherosclerosis

  - **Hypertriglyceridemia** (clinical features)
    → Eruptive xanthomas



→ Recurrent pancreatitis
→ Lipemia retinalis
- **Both**

Phrynoderma vs Eruptive xanthomas

- Phrynoderma – Eruptive lesion with keratin plug is present (vit A deficiency)
- Eruptive xanthomas - Keratin plug is absent

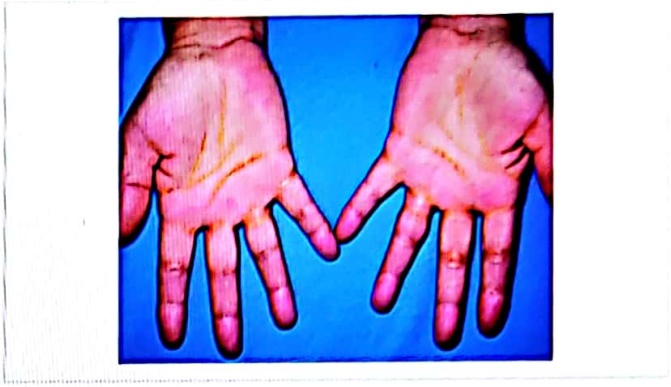
Q. What are the autosomal dominant hyperlipoproteinemias?
Ans. Type 2a, 4, and 5.

Category	Friedrickson's Type
Hypercholesterolemia	2a
Hypertriglyceridemia	1, 4, and 5
Both	2b and 3

Type-I Hyperlipoproteinemia

- **Other name:** Familial chylomicronemia syndrome
- **Defect:** Apo C-II or LPL
- Chylomicron metabolism will not be initiated
- Chylomicron stays in circulation
- Chylomicron carries dietary triacylglycerols from intestine to extra hepatic tissues
- This presents as massive elevation of TGL
- Leads to milky plasma
- **Features**
  - Presents as eruptive xanthomas or recurrent pancreatitis
  - Lipemia retinalis is also seen on examination
- Post heparinized blood sample is to be collected to know the enzyme activity (Low enzyme activity is diagnostic)
- Specific diagnosis
  - Mixing study is done
  - Equal vol of patient's post heparinized blood + pooled normal plasma (source of Apo C-II)
  - If LPL activity does not normalizes after mixing – LPL defect
  - If normalized - Apo C-II defect
- **Treatment**
  - **Apo C-II defect:** Fresh frozen plasma is administered
  - **LPL defect:** Dialysis to be performed

Q. 38 year old male presents with yellowish discoloration of palmar creases as shown in the image. His cholesterol is 300 mg/dL and Triglyceride is 280 mg/dL. What is expected in his lipoprotein electrophoresis?



a. A band at the site of application
b. Broad alpha band
c. Broad beta band
d. Absent alpha band
Ans. Broad beta band

Explanation

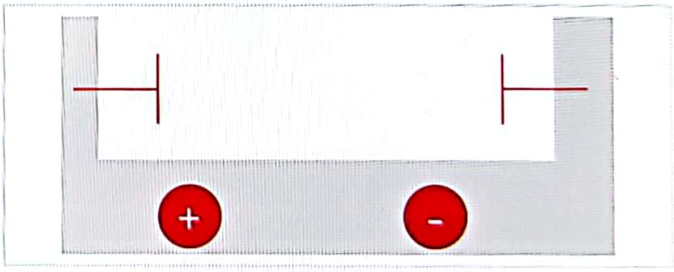
- Xanthoma Palmaris striae – pathognomic of Type III hyperlipoproteinemia
- Other name: Familial dysbetalipoproteinemia (broad beta band is seen)

Type-III Hyperlipoproteinemia

- Type-III is a defect of Apo E
- **Other name:** Remnant disease/ Familial dysbetalipoproteinemia
- Both Cholesterol and TGL increased
- Broad beta disease (broad beta band is seen)

Lipoprotein Electrophoresis

- Done on a glass slide
- **Support medium:** Agarose
- **Procedure**



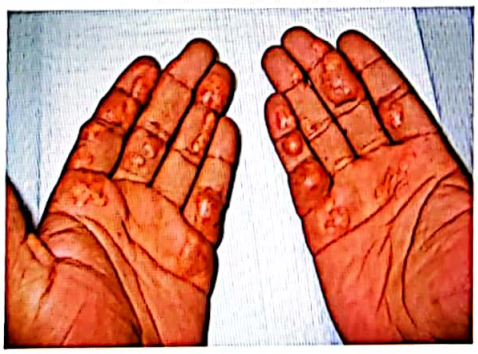
  - Plasma/ Serum is applied using micropipette to one side of the slide
  - Glass slide placed in electrophoresis tank
  - Connect close to the point of application to -ve electrode
  - Opposite side to +ve electrode
  - **Buffer:** Alkaline buffer of pH 8.3 is added (lipoproteins try to neutralize the alkaline pH by giving off their H+)
  - Lipoproteins are negatively charged and when we switch on the electric current lipoproteins start moving towards +ve electrode

- **Factors affecting migration**
  - **Number of -ve charges:** More charges, more migration
  - **Particle size:** Large size, less moment

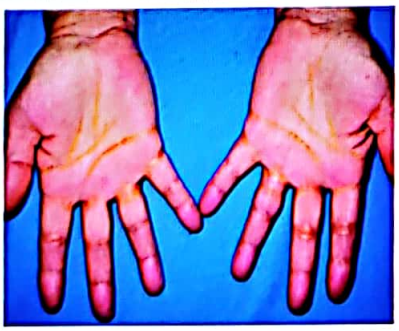
To Remember
- Alpha band (has HDL) moves the fastest
  - **Reason:** HDL has highest phospholipid or protein content
- Chylomicron is so huge, so it can't move at all (if present)
  - Fasting plasma of normal person doesn't show chylomicron
  - Chylomicron is seen **(at the point of application)**
    → Familial chylomicronemia syndrome (Apo C-II or LPL defect)
    → Lipoprotein X (LCAT deficiency)
    a. LCAT converts discoidal HDL to Spheroidal HDL
    b. Also associated with Obstructive jaundice
- Beta band (LDL)
- Pre beta band (VLDL) - ahead of beta band
- **In normal person** we can see 3 bands
  - Alpha band
  - Pre beta band
  - Beta band
- **If remnant disease**
  - Forms band in beta region
  - Making beta band broader

Note: Type-III hyperlipoproteinemia is caused by homozygous E2/E2 mutation

Dermal Pathognomic Features of Type-III hyperlipoproteinemia
- Palmar eruptive xanthomas



- Palmaris xanthoma striae



MCQ
Q. Most essential fatty acid is
a. Oleic acid
b. Linoleic acid
c. Arachidonic acid
d. Stearic acid
Ans. Linoleic acid

Things to Know
- **Linoleic acid**
  - 18C
  - Omega 6 type
  - Essential fatty acid
- **Alpha linolenic acid**
  - 18C
  - Omega 3 type
  - Essential fatty acid
- **Arachidonic acid**
  - 20C
  - Omega 6 type
  - Semi essential fatty acid
  - If diet has Linoleic acid
    → Need not take Arachidonic acid
    → Linoleic acid will get converted into Arachidonic acid
  - If diet do not have Linoleic acid, must take Arachidonic acid via diet

Q. Surfactant is
a. Lecithin
b. Cephalin
c. Plasmalogen
d. Cholesterol
Ans. Lecithin

Facts to Know
- Dipalmitoylphosphatidyl choline
- Surfactant reduces surface tension of the lungs
- Released by type 2 pneumocytes

Chemical and Common Names of Lipids 01:01:20

Chemical Name	Common Name
Phosphatidylcholine	Lecithin
Phosphatidylethanolamine	Cephalin
Diphosphatidylglycerol	Cardiolipins
Glycerophospholipids with ether linkage	Plasmalogen

Q. The rate limiting enzyme of fatty acid synthesis is
a. Acetyl CoA carboxylase
b. Carnitine Acyl Transferase I
c. Carnitine Acyl Transferase II
d. 7 alpha hydroxylase
Ans. Acetyl CoA carboxylase

Rate Limiting Enzymes of Lipid Metabolism

Pathway	Rate Limiting Enzyme
Fatty acid synthesis	Acetyl CoA carboxylase
Fatty acid oxidation	Carnitine Acyl Transferase I
Cholesterol synthesis	HMG CoA reductase
Ketone body synthesis	HMG CoA lyase or synthase
Bile acid synthesis	7 alpha hydroxylase

Q. A 34 year old male is diagnosed with papillary carcinoma of thyroid. He is treated with thyroidectomy. His wife learns online that the ketogenic diet can avoid neoplasia. Which of the following regulatory effects best explain the antineoplastic effect of ketogenic diet?

a. Acetyl CoA stimulates Pyruvate carboxylase
b. Malonyl CoA inhibits Carnitine Acyl Transferase
c. Citrate Inhibits PFKI
d. Acyl CoA stimulates Carnitine Acyl Transferase

Ans. Citrate Inhibits PFKI

Warburg Effect

- Neoplastic cell metabolic machinery is reprogrammed to use glucose through aerobic glycolysis.
- **Reasons**
  - ATP production is direct, thus rate of onset of ATP production is faster
  - Intermediates of glycolysis can be used as building blocks by rapidly dividing cells

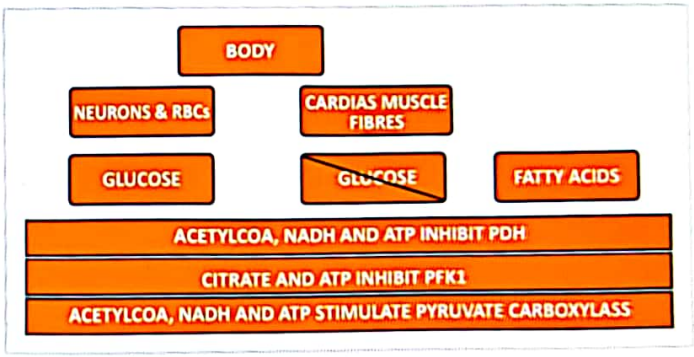
To Remember

- In fatty acid oxidation no direct ATP
- No intermediates - No building blocks
- Rate of onset of ATP production from fatty acid oxidation is a slower process
  - Some glucose will get into HMP shunt
    → Thus acts as a source of NADPH
    → Helps in regenerating glutathione which is an antioxidant mechanism (protects from oxidative stress by chemotherapy or radiotherapy)
    → Thus if glucose is used no glucose is left to enter HMP shunt

Glucose Sparing Effect

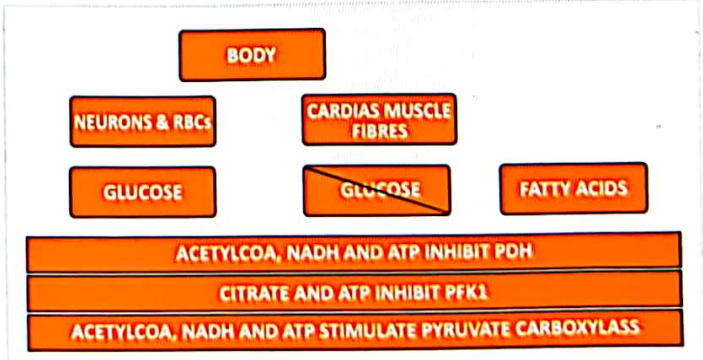
- Neurons and RBCs use only glucose
- Red and cardiac muscle fibers can use both glucose or fatty acid
- Thus cells which have more fatty acid oxidation, glucose should not be used
- This glucose is spared for Neurons and RBCs

- **Measuring the fatty acid oxidation** (products are measured)
  - **Signal-01:** nC fatty acid gives n/2 Acetyl CoA - Acetyl CoA is measured
  - **Signal-02:** Acetyl CoA reacts with oxaloacetate to form Citrate - Citrate is measured
  - **Signal-03:** H atoms bind with NAD and FADH to give NADH and $FADH_2$ - NADH and $FADH_2$ are measured
  - **Signal-04:** These give ATP - ATP is measured
- To conclude: Inhibiting the oxidation of glucose by fatty acid oxidation product.
- **Step-01: Acetyl CoA, NADH, and ATP inhibit PDH**
  - These inhibit pyruvate dehydrogenase complex
  - This accumulate pyruvate in the cells
  - Thus glycolysis is inhibited and glucose accumulated in cells
- **Step-02: Citrate and ATP inhibit PFK1** (better)
  - These inhibit glucose oxidation by inhibiting Phosphofructokinase-1
- **Step-03: Acetyl CoA, NADH, and ATP stimulate Pyruvate Carboxylase**
  - Where there is fatty acid oxidation gluconeogenesis is stimulated and inhibition of glucose oxidation
  - New glucose is produced and spared for Neurons and RBCs



Pasteur Effect

- Modified glucose sparing effect
- Inhibition of anaerobic oxidation by aerobic oxidation products
- NADH is never allowed to be accumulated
- Thus step 1 and 3 are excluded
- Only step 2 is involved – citrate and ATP inhibit phosphofructokinase



Keto Diet

- Expect to thrive on a zero carbohydrate and high fat diet.

Zero carbohydrate diet
↓
No glucose
↓
No aerobic glycolysis
↓
Difficult for neoplastic cells to thrive
↓
Additionally, patient is on high fatty diet
↓
All these fats oxidize
↓
Acetylcholine CoA and citrate are formed
↓
Therefore there will be high citrate and ATP
↓
Inhibit phosphofructokinase-1 levels

- High fatty diet
- High Acetyl CoA
- High citrate and ATP
- Inhibit phosphofructokinase 1

Q. Which of the following is not associated with ketosis in type 1 diabetes?

a. Decreased beta oxidation of fatty acids
b. Decreased Acetyl CoA entering TCA cycle
c. Increased beta hydroxybutyrate dehydrogenase
d. Increased mobilization of fatty acid to liver

Ans. Decreased beta oxidation of fatty acids

Explanation

- Low insulin
- Stimulation of catabolic pathway Like fatty acid oxidation.
- Therefore, in type 1 diabetes there is an increase in fatty acid oxidation.

Type 1 Diabetes 01:23:00

Low insulin
↓
Stimulates hormone sensitive lipase - present in adipose tissue
↓
Acts on triacylglycerol (TAG)
↓
Cleaves into glycerol and fatty acid
↓
Both reaches the liver
↓
Here insulin Inhibits HSL - normal function

In type 1 diabetes - there is low insulin
↓
Therefore activation of HSL occurs - leads to weight loss
↓
Fatty acids - oxidized to acetyl CoA in liver
↓
Not able to enter TCA cycle due to less OAA (In diabetes gluconeogenesis increases that decreases OAA)
↓
Acetyl CoA accumulates and forms ketone bodies

Steps of Ketone Bodies Formation

2 Acetyl CoA - with the help of thiolase
↓

Converts to aceto Acetyl CoA
↓ + other Acetyl CoA

Convert to HMG CoA in the presence of HMG CoA synthase
↓
In the presence of HMG CoA lyase - releases one acetyl CoA
↓
Acetoacetate is formed - 1st ketone body
↓
Undergo spontaneous decarboxylation
↓
Forms acetone - 2nd ketone body - responsible for fruity odor of breathing diabetic ketoacidosis
↓
Acetoacetate also converts to beta hydroxybutyrate in the presence of beta hydroxybutyrate dehydrogenase, needs NADH - 3rd ketone body

- In acute diabetic ketoacidosis - NADH is high.
  - More beta hydroxybutyrate is formed.

Q. Is ketosis observed in diabetes because of :

a. Low availability of OAA
b. Excess OAA
c. Low energy
d. Low fatty acid oxidation

Ans. Low availability of OAA

Q. Liver cannot utilize ketone bodies because of lack of :
a. Thiolase
b. Thioesterase
c. Thiophorase
d. Aconitase
Ans. Thiophorase

Explanation
- Ketone body synthesis occurs in the liver.
- Can't be utilized by the liver due to lack of thiophorase.

Q. Fatty acid oxidation is present with all except?
a. Hypoglycemia
b. Ketosis
c. Hyperammonemia
d. Dicarboxylic aciduria
Ans. Ketosis

Fatty Acid Oxidation Defects
- Non ketotic hypoglycemia.
- Medium chain acylCoA dehydrogenase deficiency - sudden infant death syndrome.
  - Enzyme of fatty acid oxidation.
  - Gluconeogenesis - affected indirectly.
  - Leads to hypoglycemia.
- Less insulin in liver - activates HSL.
  - Excess peripheral lipolysis in adipose tissue.
  - More fatty acids enter the liver.
  - Fatty acid oxidize to give Acetyl CoA - Do not enter TCA.
  - Therefore, it forms ketone bodies.
- Fatty acid oxidation defect - decreases gluconeogenesis.
- To increase gluconeogenesis - amino acid oxidation occurs.
  - Ammonia is released - Hyperammonemia.
- Dicarboxylic aciduria
  - When mitochondria cannot oxidize fatty acids,
  - They enter peroxisomes.
  - Oxidizes all fatty acids into small chains which cannot be oxidized further.
  - Peroxisomes start meditating omega oxidation - another carboxylic group is added.
  - Hence called dicarboxylic aciduria.

GLYCOSPHINGOLIPIDOSIS 01:39:16

Enzyme defect	Disorder	Clinical Features
Beta galactosidase	GM1 gangliosidosis	• Neurological • systemic manifestations - hepatosplenomegaly, skeletal dysplasia. • Accumulation of GM1 ganglioside.
Alpha galactosidase	Fabry's disease	• Reddish purple spots - angiokeratomas. • Early atherosclerotic changes and CKD • X linked recessively inherited. • Accumulation of globoside
Hexosaminidase A	Tay Sachs disease	• Cherry red spots • Neurological manifestations • No organomegaly , no skeleton displasia • Accumulation of GM2 ganglioside.
Hexosaminidase A and B	Sandhoff's disease	• Cherry red spots • Neurological manifestations • No organomegaly • Accumulation of GM2 ganglioside + globoside.
Neuraminidase	Sialidosis	• Gingival hypertrophy • Generalized swelling , coarse facial features , macroglossia • Neurological manifestations • Organomegaly • Accumulation of sialyl oligosaccharides.
Beta glucosidase / beta glucosyl cerebrosidase	Gaucher's disease	• Crumpled tissue paper appearance • Erlenmeyer flask deformity • Anemia • Thrombocytopenia • Hepatomegaly
Ceramidase	Farber's disease	• Granulomatous disorder. • Painful subcutaneous nodules. • CKD

- Tay Sachs disease + Sandhoff = GM2 gangliosidosis.
- Alpha glucosidase or acid Maltase defect causes Pompe's disease.

To Remember
- **No cherry red spots in**
  - Krabbe's
  - Gaucher's
  - Fabry's
  - Mnemonic: KGF
- **No mental retardation in**
  - Gaucher's
  - Fabry's
  - Mnemonic: GF

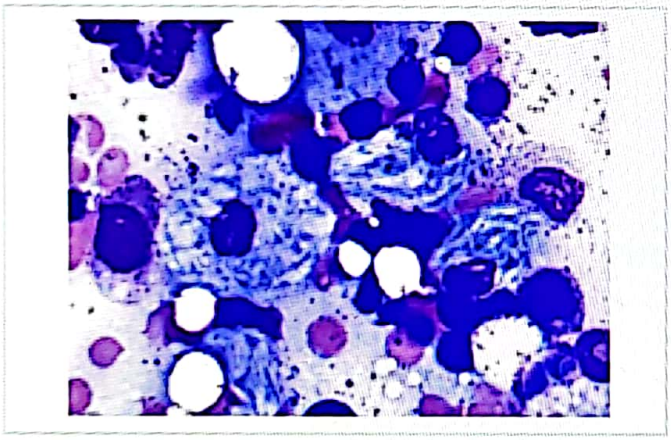
- **No hepatosplenomegaly in**
  - GM2 gangliosidosis
  - Krabbe's disease
- **Beta galactosidase defect can cause 3 diseases**
  - **Krabbe's disease** - Beta galactosidase present in myelin. → Primarily presents with blindness and deafness.
  - **GM1 gangliosidosis** - Beta galactosidase present in GM1 ganglioside.
  - **Morquio B disease** - Beta galactosidase present in keratin sulfate.

Lipid Accumulation

- Cerebrocytes - accumulates in Gaucher's disease.
- Globocytes - accumulates in Fabry's disease and Sandhoff's disease.
- Gangliocytes -
  - GM1 - GM1 gangliosidosis.
  - GM2 - Tay Sachs disease and Sandhoff's disease.

NEET PG 2023

Q. Child presented with refractory anemia. Below is provided the smear of bone marrow biopsy of the child. The probable enzyme deficiency is?



a. Beta glucocerebrosidase
b. 1,4 alpha glucosidase
c. Hexosaminidase A
d. Hexosaminidase B

Ans. Beta glucocerebrosidase

Explanation

- Image shows crumpled tissue paper appearance.
- Seen in Gaucher's disease.
- Beta glucocerebrosidase deficiency.

NEET PG 2023

Q. A child presented with difficulty in vision on examination cherry red spots were seen on the macula. There was no organomegaly. Identify the disease

a. Gaucher's disease
b. Hunter's disease
c. Tay Sachs disease
d. Niemann pick disease

Ans. Tay Sachs disease

Explanation: Neurological manifestations + Cherry red spot + no organomegaly - Tay Sachs disease.

Q For the first few months and 8 months old child's growth and development were normal, then symptoms such as deafness, blindness, atrophied muscle inability to swallow, and convulsions begin to appear. During the fundus inspection a cherry red macula was also seen in both eyes. Suspecting sphingolipidoses, 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 Sachs disease
c. Gaucher's disease
d. Fabry's disease

Ans. Sandhoff's disease

Q. True regarding competitive inhibition of an enzyme is

a. Km is increased
b. Km is unaltered
c. Km is decreased
d. Vmax is decreased

Ans. Km is increased

Explanation

- In competitive inhibitor there is a competition between inhibitor and substrate to bind to the enzyme
- So if Substrate concentration is more, then substrate wins
- As overcoming is possible Vmax is achieved (so Vmax is normal)
- Km is the substrate concentration at ½ Vmax
- So Km is increased to achieve the Vmax
- Thus Km increases in competitive inhibition

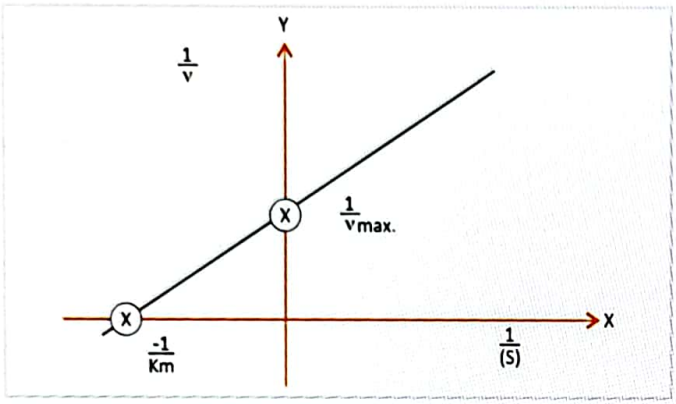
Effect of Inhibitors on Km and Vmax 02:02:53

Type of inhibition	Vmax	Km
Competitive inhibition	Normal	Increased (Substrate concentration)
Uncompetitive inhibition	Low	Low
Mixed inhibition	Low	high
Non competitive inhibition (type of mixed inhibition)	Low	Normal

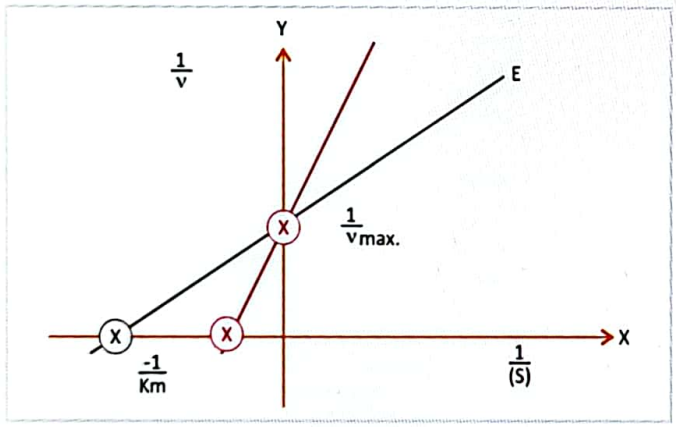
Lineweaver Berke Plot

In the absence of the inhibitor

- Y axis = 1/V
- X axis = 1/[S]
- This gives a straight line
- If it is extrapolated it cut the X axis at one point and y axis at another point
- The point at which it touches the y axis is 1/Vmax
- The point at which it touches the x axis is -1/Km
- This graph is obtained in the absence of the inhibitor

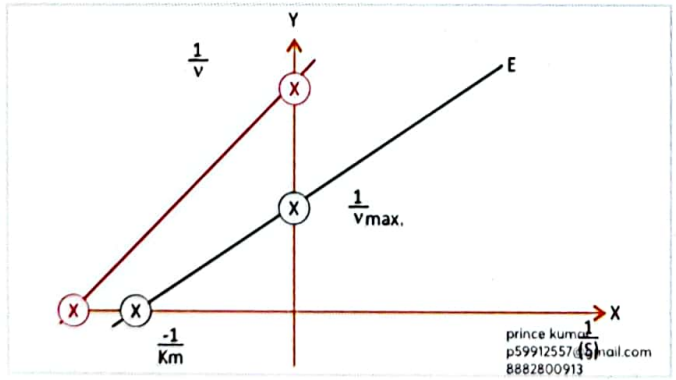


Presence of a competitive inhibitor Graph



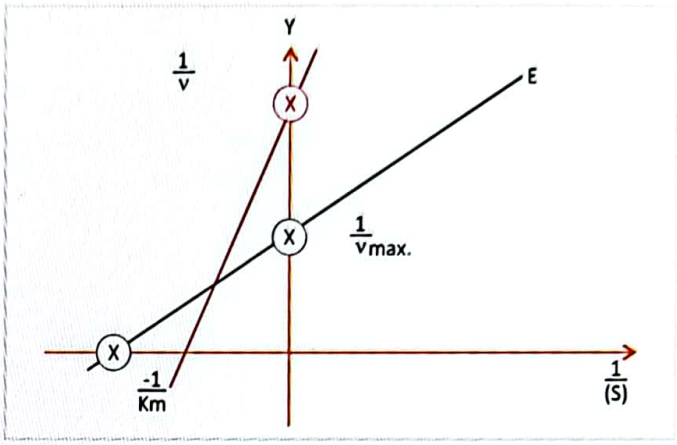
- Vmax is normal, 1/Vmax is normal
- Km is elevated, 1/Km is low
- If the line cuts the X axis close to zero
- Hence it is a competitive inhibitor

Presence of a uncompetitive inhibitor Graph



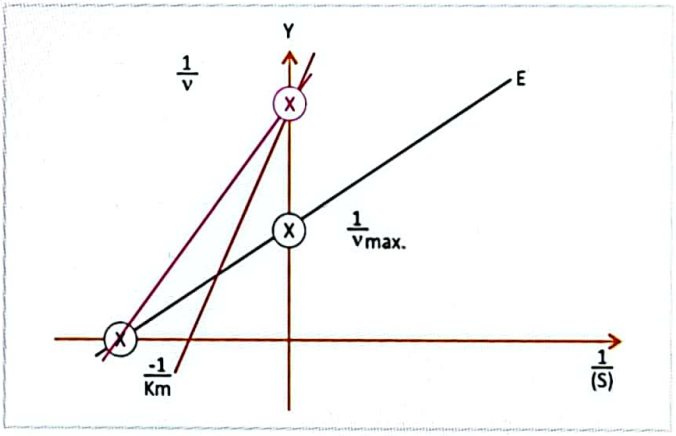
- Vmax is low, 1/Vmax is high
- Km is low, 1/Km is high
- Parallel lines indicates uncompetitive inhibition

Presence of a mixed Inhibitor



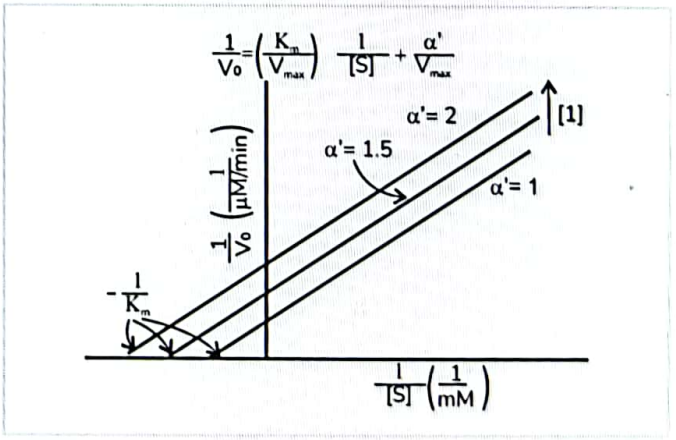
- Vmax is low, 1/Vmax is high
- Km is elevated, 1/Km is low

Presence of a non competitive inhibitor



- Km is normal, same as the point in the absence of the inhibitor
- Vmax is low, 1/Vmax is high

Q. What kind of inhibition is this?



a. Competitive inhibition
b. Uncompetitive inhibition
c. Non competitive inhibition
d. Mixed inhibition
Ans. Uncompetitive inhibition

Explanation: Parallel lines - Uncompetitive inhibition

Q. An enzyme was mixed with 4 mM substrate. The initial rate of product formation was 25% of Vmax. the Km of the enzyme is
a. 2 mM
b. 4 mM
c. 9 mM
d. 12 mM
Ans. 12 mM

Explanation
- [S] = 4 mM
- V = 25% Vmax
- V = Vmax / 4
- Km = substrate concentration at ½ Vmax
- V = Vmax [S] / Km + [S] = Michaelis menten equation
- Vmax / 4 = Vmax × 4 / Km + 4
- 1/4 = 4 / Kμ + 4
- Km + 4 = **16**
- Km = 16 - 4 = 12

Q. An enzyme - catalyzed reaction was carried out with the initial substrate concentration 1000 times greater than Km for that substrate. After 9 minutes, 1%of the substrate had been converted to the product, and the amount of product was 12 mmol. If one-third as much enzyme and twice as much of the substrate is combined, how long it would take for the same amount (12 mmol) of product to be formed?
a. 13.5 mins
b. 27 min
c. 3.8 mins
d. 4.9 mins
Ans. 27 min

Explanation
When,
- [S] = 1000 Km
- V = 12 mmol / 9 min

Now if,
- [S] = 2000 Km
- Enzyme concentration = ⅓ of initial
- V = Vmax [S] / Km + [S]

If [S] = Km
- V = Vmax Km / Km + Km
- V = Vmax Km / 2 Km
- V = Vmax / 2
- Or when substrate concentration is Km, velocity is 1/2 Vmax

If [S] = 10 Km
- V = Vmax 10 Km / Km + 10 Km
- V = 10/11 Vmax
- V = 0.9 Vmax

If [S] = 100 Km
- V = Vmax 100 Km / Km + 100 Km
- V = 100/101 Vmax
- V = 0.99 Vmax

To Remember
- When the substrate concentration is increased to 10 times km then velocity increases from 0.5 to 0.9 (linear increase in the velocity of the graph)
- So if substrate concentration is increased from 10 times to 100 times, there is hardly increase in velocity
- If substrate concentration is increased to 1000 times, velocity becomes 0.999 Vmax
- When substrate concentration is increased beyond 10 times Km, there is no change in velocity
- So here, the change in substrate concentration will not change the velocity
- It is the enzyme concentration which is the determinant of velocity
- It linearly affects the velocity
- Enzyme concentration decreases by ⅓
- Time taken will increase by 3 times
- 3 times (12mmol - 9min) = 27 minutes

Heme Synthesis and Porphyria 02:22:52

Q. A 35-year-old man has a history of intermittent abdominal pain and episodes of confusion and psychiatric problems. High amounts of delta-aminolevulinate and porphobilinogen are also detected in his urine analysis. The patient also has a mutation in the gene for uroporphyrinogen I synthase (porphobilinogen deaminase). The probable diagnosis of the patient is:
a. X-linked sideroblastic anemia
b. Acute intermittent porphyria
c. Congenital erythropoietic porphyria
d. Porphyria cutanea tarda
Ans. Acute intermittent porphyria

Explanation
- Patient has a sensory nerve defect with neuropsychiatric manifestations
- Uroporphyrinogen I synthase causes Acute intermittent porphyria

- **Other names**
  - Porphobilinogen deaminase
  - Hydroxymethylbilane synthase
  - Uroporphyrinogen I synthase
- **Porphyria are divided into three categories**
  - **Category 1:** Neuropsychiatric manifestations
  - **Category 2:** Photosensitivity
  - **Category 3:** Both neuropsychiatric manifestations and photosensitivity

Heme synthesis disorders

Enzyme Defect	Name of the disorder
Delta ALA Synthase II	X linked Sideroblastic anemia
ALA dehydratase	ALA dehydratase deficiency porphyria
Porphobilinogen deaminase or Hydroxymethylbilane synthase or Uroporphyrinogen I synthase	Acute intermittent porphyria
Uroporphyrinogen III synthase	Congenital erythropoietic porphyria
Uroporphyrinogen decarboxylase	Porphyria cutanea tarda
Coproporphyrinogen oxidase (CPO)	Hereditary Coproporphyria
Protoporphyrinogen oxidase (PPO)	Variegate porphyria
Ferrochelatase	Erythropoietic protoporphyria

Delta ALA II Synthase

- It is a complete defect
- Protoporphyrin and heme will not be synthesized
- So that Fe will not be chelated into the protoporphyrin
- As a result Fe will be accumulated in the mitochondria of RBC precursors
- Smear of the patients will show the RBC precursors surrounding the nucleus, multiple mitochondria will be accumulated
- This accumulated mitochondria will have the iron deposits
- Iron laden mitochondria surrounds the nucleus, it will look like a ring
- Hence it is known as the ring Sideroblast

Other Enzyme Defects

- They are partial defects

Initially heme will not be synthesized
↓
Feedback stimulation of the ALA synthase will occur
↓
Such that glycine and succinyl CoA will enter the pathway
↓
When the influx of the intermediates are increased, the block will be overcome
↓
As a result heme will be synthesized

- All the other disorders will not be presented with the significant anemia
- But to overcome the block, precursors have to accumulate.
- Based on the type of precursor accumulated, the presentation will be changed
- ALA dehydratase: ALA to Porphobilinogen (PBG)
- Uroporphyrinogen decarboxylase forms Coproporphyrinogen
- Congenital erythropoietic porphyria
  - It is a classical porphyria
  - Fear of photosensitivity
  - These patients will come out only in the night times (Nocturnal)
  - Side effects: Photosensitivity
    - → Blistering
    - → Scaring
    - → Hypertrichosis
  - Porphyrins will accumulate in the teeth
    - → Causes erythrodontia (Red fluorescence will appear in the teeth)

Classification of Porphyria

Categories	Conditions
• Neuropsychiatric manifestations	• ALA dehydratase deficiency porphyria • Acute intermittent porphyria
• Photosensitivity	• Congenital erythropoietic porphyria (Severe) • Porphyria cutanea tarda (Common) • Erythropoietic protoporphyria
• Both neuropsychiatric manifestations and photosensitivity	• Hereditary Coproporphyria • Variegate porphyria

Q. A 35-year-old man has a history of intermittent abdominal pain and episodes of confusion and psychiatric problems. High amounts of delta-aminolevulinate and porphobilinogen is also detected in his urine analysis. The patient also has a mutation in the gene for uroporphyrinogen I synthase (porphobilinogen deaminase). The probable diagnosis of the patient is:

a. X-linked sideroblastic anemia
b. Acute intermittent porphyria
c. Congenital erythropoietic porphyria
d. Porphyria cutanea tarda

Ans. Acute intermittent porphyria

Explanation

- X-linked sideroblastic anemia: ALA II Synthase (Only present with anemia)
  - There is no neuropsychiatric presentations
- Congenital erythropoietic porphyria:
  - There is no neuropsychiatric presentations, caused due to Uroporphyrinogen III synthase
- Porphyria cutanea tarda
  - Photosensitivity
  - Defective enzyme: Uroporphyrinogen decarboxylase

Q. A 15 year old boy presented with severe photosensitivity, atrophic scarring, mutilated fingers and port wine urine. There was no neurological involvement. The most probable enzyme defect is

a. Uroporphyrinogen III synthase
b. ALA dehydratase
c. Coproporphyrinogen oxidase
d. Protoporphyrinogen oxidase

Vesicles, scarring, pigmentation, hypertrichosis on the face and scleromalacia of the left eye

Vesicles, scarring, pigmentation, hypertrichosis of the face and mutliation of the ear lobe

Vesicles, scarring of the hands, multilating deformities of the fingers and dystrophic nails

Urine turning reddish pink on standing

Ans. Uroporphyrinogen III synthase

Explanation

- Condition: Congenital erythropoietic porphyria

4 ABG INTERPRETATION

All About ABG Interpretation in an hour

MCQ

Q1. Give your opinion regarding the acid base status of a blood sample that was taken from a person, who was acutely hysterical.

- Blood pH: 7.55
- pCO_2: 20 mmHg
- Plasma HCO_3: 20 mEq/L

a. Respiratory acidosis
b. Respiratory alkalosis
c. Metabolic acidosis
d. Metabolic alkalosis

Important Information

- A hysterical person hyper ventilated washes away carbon dioxide
- Carbon dioxide is a source of acid
  Person presents with alkalosis

Interpretation

Normal ranges

- Normal blood pH: 7.36-7.44
- Normal pCO2: 36-44 mmHg
- Normal HCO3: 21-27 mEq/L

Step 1: pH (2:55)

- **<7.36:** Acidosis
- **>7.44:** Alkalosis
- Normal range does not exclude an acid base disorder
- Example, Mixed conditions like Metabolic acidosis and alkalosis in Type 2 DM,
  - Outside eating causes vomiting
  - Vomiting leading to metabolic alkalosis
  - GI distress causes Diabetic ketoacidosis which causes metabolic acidosis

Step 2: Is it Metabolic or Respiratory?

- Interpret Bicarbonate and PCO_2 values
- Bicarbonate is a major plasma buffer, neutralizes metabolic acids
  - Low HCO_3 levels leads to Metabolic acidosis
  - High HCO_3 levels leads to Metabolic alkalosis
- Carbon dioxide is a source of an acid
  - High pCO_2 levels causes Respiratory acidosis
  - Low pCO_2 levels causes Respiratory alkalosis

MCQ

Q1. Give your opinion regarding the acid base status of a blood sample that was taken from a person, who was acutely hysterical.

- Blood pH: 7.55
- pCO_2: 20 mmHg
- Plasma HCO3: 20 mEq/L

Explanation

- pH (7.36-7.44) is high, Alkalosis
- HCO_3 (21-27) is low, it cause acidosis but not alkalosis
- PCO_2 (36-44) is low, causes alkalosis

Ans: Respiratory alkalosis

MCQ

Q2. Interpret ABG report

- Blood pH: 7.30
- pCO_2: 29 mmHg
- Plasma HCO_3: 14 mEq/L

a. Compensated metabolic acidosis
b. Uncompensated metabolic acidosis
c. Compensated respiratory acidosis
d. Uncompensated respiratory acidosis

Step 3: Basis of compensation 00:09:58

- If primary defect is metabolic , compensation is done by lungs
- Example, If primary defect is Metabolic acidosis (low HCO_3), lungs reduce PCO_2 levels
- In acidosis, Central chemoreceptors in medulla gets stimulated
- Which activates respiratory center and hyperventilates
- CO2 is washed out by hyperventilation

Important Information

- Kussmaul's respiration, in DKA shows Metabolic acidosis
- Decline in pH, HCO_3 leads to stimulation of respiratory center which causes hyperventilation
- Excessive CO_2 is removed from body

- **Rule 1**
  - Compensation is always parallel
  - If primary defect is decrease in HCO_3, compensation effect is decrease in PCO_2 and Vice versa
  - If primary defect is increase in PCO_2, compensation effect is increase in HCO_3 and Vice versa

- **Rule 2**
  - Only for respiratory disorders there is always an acute and chronic compensation
  - In respiratory acidosis, low pH, increased PCO_2 compensation is by increase in HCO_3
  - First response by Chemical equilibrium shift by Buffer system (Acute compensation)
    → $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$
    → For every mole increase in CO_2, there is a corresponding increase in HCO_3
  - Second response by kidneys (Chronic compensation)
    → In acidosis, kidney reclaim more amounts of HCO_3

Important Information

- Reclamation of HCO_3, PCT reclaims all HCO_3 from tubular fluid

Formulae

a. Metabolic disorders

Condition	pH	HCO_3^-	Compensation	Expected PCO_2
Metabolic acidosis	Low	Low	Reduce PCO_2	PCO_2=1.5× HCO3+8
Metabolic alkalosis	High	High	Increase PCO_2	PCO_2=0.9× HCO3+16

- Example, In a patient ABG,
  - pH=7.34 (acidosis)
  - HCO_3=20 mEq/L (Metabolic)
  - Expected PCO2=1.5×20+8=38 ± 2 mmHg (36-40)
  - If PCO_2 is 41 mmHg, it is Uncompensated
  - If PCO_2 is 38 mmHg, it is Compensated
  - If PCO_2 is 30 mmHg, it is not called Over compensation
    → There is a Hidden Acid Base disorder (Respiratory alkalosis) due washed out CO_2 (Hyperventilation)
    → This condition has both Metabolic acidosis+Respiratory alkalosis

Important Information

- Body never over compensate
- It is always in Uncompensated or compensated stage

- Example, Patient with ABG of
  - HCO3=18 mEq/L
  - Expected PCO_2= 1.5 × 18+8=35±2 mmHg (33-37)
  - If actual PCO_2 =40 mmHg (Uncompensated Metabolic acidosis)
  - If actual PCO_2 =35 mmHg (Compensated Metabolic acidosis)
  - If actual PCO_2 =30 mmHg (both Metabolic acidosis+ Respiratory alkalosis)

b. Respiratory disorders

- Acute and chronic compensation

Disorder	Acute	Chronic
Respiratory acidosis	1	3.5
Respiratory alkalosis	2	5

- In Respiratory acidosis, PCO_2 is increased
  - For every 10 mmHg rise in PCO_2
  - HCO_3 value
    → Acute: Elevated by 1 mEq/L
    → Chronic: Elevated by 3.5 mEq/L

- Example, In a COPD patient (accumulation of CO_2) with PCO_2= 60 mmHg
  - Expected HCO_3= Increased by 7= 24+7=31±1 mEq/L (30-32)
  - If actual PCO_2 is 24 mmHg: Uncompensated Chronic Respiratory acidosis
  - If actual PCO_2 is 30 mmHg: Compensated Chronic Respiratory acidosis
  - If actual PCO_2 is 35 mmHg: Hidden acid base disorder (Chronic Respiratory acidosis with Metabolic alkalosis)

- In Respiratory alkalosis, PCO_2 is decreased
  - For every 10 mmHg decline in PCO_2
  - HCO_3 value reduced by
    → Acute: 2 mEq/L
    → Chronic: 5 mEq/L

MCQ

Q2. Interpret the ABG report

- Blood pH: 7.30
- PCO_2: 29 mmHg
- Plasma HCO_3: 14 mEq/L

a. Compensated metabolic acidosis
b. Uncompensated metabolic acidosis
c. Compensated respiratory acidosis
d. Uncompensated respiratory acidosis

Ans: a. Compensated metabolic acidosis

Important Information

- Expected PCO_2 in Compensated metabolic acidosis calculated by Winter's formula

Explanation

- Expected PCO_2= 1.5*HCO3+8=1.5*14+8=29 mmHg
- Step 1: Acidosis or alkalosis, Low pH
- Step 2: Primary metabolic disorder (Metabolic or respiratory), low HCO_3
- Step 3: Hidden disorder (calculate compensation), 29 mmHg
- Step 4: Only if Metabolic acidosis, calculate Anion gap

MCQ

Q3. Interpret the ABG report

- Blood pH: 7.30
- PCO_2: 29 mmHg
- Plasma HCO_3: 14 mEq/L
- Na^+: 130 mEq/L
- Cl^-: 90 mEq/L

a. Compensated increased anion gap metabolic acidosis
b. Uncompensated increased anion gap metabolic acidosis
c. Compensated normal anion gap metabolic acidosis
d. Uncompensated normal anion gap metabolic acidosis

Explanation

- Expected PCO_2= 1.5*HCO3+8=1.5*14+8=29 mmHg
- Step 1: Acidosis or alkalosis, Low pH
- Step 2: Primary metabolic disorder (metabolic or respiratory), low HCO_3
- Step 3: Hidden disorder (calculate compensation), 29 mmHg
- Step 4: Calculate Anion gap (Only if Metabolic acidosis)

Step 4: Anion gap

00:30:50

- In any physiological solution, sum of anion is equal to sum of cations
- Cations of plasma
  - Na^+ (majority)
  - K^+ (negligible)
  - Unmeasured cations

- Anions in plasma
  - Cl^-
  - HCO_3^-
  - Unmeasured anions
- Cations [(Na^+) + (Unmeasured cations)] = Anions [(Cl^-) + (HCO_3^-)] (Unmeasured anions)]
- Anion gap is the difference between Unmeasured anions - Unmeasured cations
- Anion gap = [Na] - [(Cl^-)+(HCO_3^-)]
- It is used find the cause of Metabolic acidosis

a. Decrease in HCO_3

- Increased utilization of HCO_3, due to abnormal acid production
- Loss of HCO_3

1. Increased utilization HCO_3

- Abnormal acids represented by HA
- $HA + HCO_3 \rightarrow H_2CO_3 + A^-$
- Every mole of Acid (HA) needs 1 mole of HCO_3
- Unmeasured anion (UMA) concentration increases
- In DKA, ketone bodies (abnormal acids) are produced
  - Dissociate into Acetoacetate anion + H^+
  - Acetoacetate anion concentration increases
- In lactic acidosis, Lactate anion concentration increases
- Increased in abnormal acid production increases UMA
- This increased UMA increases Anion gap (UMA-UMC)

- **Sources of abnormal acids:**
  - Ketoacidosis
    - → DKA: increased acetoacetate concentration
    - → Starvation ketosis: increased Beta-hydroxybutyrate concentration
  - Lactic acidosis
  - Uric acidosis
  - Alcohol poisoning
    - → Methanol: Formaldehyde → Formic acid→Formate anion
    - → Ethanol: Acetaldehyde → Acetic acid → Acetate anions
    - → Ethylene glycol (Antifreeze): Alcohol dehydrogenase → Aldehyde dehydrogenase → Oxalate anions
  - Kidney failure, fails to secrete metabolic acids, corresponding anions increases
- "MUDPILES" mnemonic

2. Loss of HCO_3

- Loss is by GIT or kidney
- GIT loss is due to
  - Diarrhea
    - → Intestinal mucus rich in HCO_3 is lost
    - → Loss of major plasma buffer causes metabolic acidosis

To Remember: Vomiting causes Metabolic alkalosis

  - Ureterosigmoidostomy
    - → Ureter is transplanted in sigmoid colon
    - → Excess mucus production causing loss of bicarbonate through GIT

- Renal loss is due to Renal tubular acidosis
- In PCT acidosis or Fanconi syndrome, reclamation of HCO_3 is absent
- To maintain osmotic balance, body reclaims chloride anions

- In diarrhea, intestinal cells secrete more HCO_3 into intestinal lumen
- This secretion is through HCO_3-Cl Antiporter
- Plasma chloride concentration increases
- In PCT acidosis
  - PCT fails to reclaim HCO_3
  - Na^+ reabsorption also fails
  - Increases Na^+ concentration in ascending limb of loop of Henle
  - Reabsorption of Cl^- along with Na^+ in early DCT
  - Plasma Cl^- concentration increases
  - Hyperchloremic Metabolic Acidosis (GIT or kidney) is seen, anion gap is normal

Hyperchloremic Metabolic Acidosis

- Loss of HCO_3 by GIT or kidney
- Metabolic acidosis is due to increased elimination of HCO_3
- Compensated by Chloride ions
- In ABG report, Anion gap is normal

Important Information

- Anion gap is considered only in Metabolic acidosis

- Increased gap, production of abnormal acids
  - Ketoacids
  - Lactic acids
  - Uric acids
  - Alcohol (Methanol, Ethanol, Ethylene glycol)
  - Salicylic acids
  - Chronic kidney disease

Important Information

In salicylate poisoning

- Salicylate is an uncoupler of ETS (Electron Transport Chain)
- When ETS is inhibited, Oxidative phosphorylation is terminated
- Only anaerobic metabolism occurs
- This increases lactic acid metabolism- Lactic acidosis
- This leads to an increased anion gap, due to production of lactic acid

- Normal anion gap, due to GI or urinary loss
  - GI loss of HCO_3
    → Diarrhea
    → Ureterosigmoidostomy
  - Urinary loss of HCO_3
    → Renal tubular acidosis (Type I, II, IV)

MCQ

Q4. Interpret the ABG report

- Blood pH: 7.30
- PCO_2: 29 mmHg
- Plasma HCO_3: 14 mEq/L
- Na^+: 130 mEq/L
- Cl^-: 90 mEq/L

a. Compensated increased anion gap metabolic acidosis
b. Uncompensated increased anion gap metabolic acidosis
c. Compensated normal anion gap metabolic acidosis
d. Uncompensated normal anion gap metabolic acidosis

Ans: a. Compensated increased anion gap metabolic acidosis

Explanation

- Normal anion gap, 12 ± 2, if K+ is not included
- Anion gap= 130- (90+14)= 26 mEq/L
- 26>12 ± 2, increased anion gap

Important Information

ABG reports helps in

- Difference between
  - Calculated HCO_3 plasma
  - Calculated HCO_3 standard
- Significance of Base excess
- How to interpret in normal pH values
  - PCO_2
  - HCO_3
  - Anion gap
- If pH, PCO_2, HCO_3 and Anion gap are normal, exclude an acid base disorder
- If any of pH, PCO_2, HCO_3 and Anion gap are abnormal, identify acid base disorder

MCQ

Q5. Interpret following Venous ABG report and identify a probable cause after considering the spot urine values

- pH: 7.411
- PO_2: 72.7 mmHg
- PCO_2: 23.7 mmHg
- Base excess: 7.7 mmol/L
- Base excess (ECF): 9.9 mmol/L
- $cHCO_3$: 17.5 mmol/L
- $cHCO_3$ (std): 20.1 mmol/L
- Na^+: 138 mEq/L
- K^+: 2.56 mEq/L
- Cl^-: 109 mEq/L
- Spot urine
  - Na^+: 20 mEq/L
  - K^+: 5.6 mEq/L
  - Cl^-: 22 mEq/L
  - pH: 7

a. Diarrhea
b. Distal Renal tubular acidosis
c. Proximal Renal tubular acidosis
d. Lactic acidosis

Ans: b. Distal Renal tubular acidosis

Explanation

- Normal pH: 7.36-7.44
- $cHCO_3$, $cHCO_3$ (std) calculated using Henderson Hesselbach equation
- Calculated plasma is interpreted, Normal: 21-27 mEq/L
- Normal PCO_2: 36-44 mmHg
- Normal pH, low HCO_3 and PCO_2
- If low PCO_2 is primary disorder, it is respiratory alkalosis with metabolic compensation
- If low HCO_3 is primary disorder, it is metabolic acidosis with respiratory compensation
- If pH is normal, check Base excess
- Normal Base excess is between -2 to 2
- If Base excess is more negative than -2, it means there is no base and it is Acidosis
- If Base excess is more positive than 2, it means there is adequate base buffer and it is Alkalosis
- In this case, Base excess is -7.7, which much less than -2
- Hence it is an Metabolic Acidosis with respiratory compensation condition
- Calculate compensation using Winter's formula
  - Expected PCO_2= 1.5*HCO_3+8=1.5*18+8=35 mmHg
- PCO_2 is less than Expected value, Hidden acid base disorder is present (Respiratory alkalosis)
- Difference between $cHCO_3$ and $cHCO_3$ (std)

Important Information

- $cHCO_3$ is calculated by Henderson Hesselbach equation
- $cHCO_3$ (std) is concentration in blood when equilibrated with normal PCO_2 and normal PO_2
- No respiratory disorder causes the difference
- Purely a metabolic disorder causes the difference $cHCO_3$ and $cHCO_3$ (std)

- Hidden respiratory disorder (metabolic acidosis with respiratory alkalosis) is present
- Anion gap is to be calculated for Metabolic acidosis
- Anion gap= 138-(109+17.5)=11.5
- Normal anion gap: 12 ± 2
- Lactic acidosis is present in increased lactic acid levels
- Calculate Urinary anion gap for distinguishing GI or Urinary loss

Recall

- Plasma concentration of Na+ is more than K^+
- Hence, K^+ can be neglected

Urinary Anion Gap

- Sum of cations is equal to sum of anions in urine
- Urinary cations
  - Na^+
  - K^+
  - UMC
- Urinary anions
  - Cl^-
  - UMA
- K^+ is secreted more in urine compared to Na^+
- K^+ cannot be neglected
- HCO_3 is completely reabsorbed in PCT so urinary HCO3- is zero
- Urinary anion gap= $(Na^+)+(K^+)-(Cl^-)$
- Plasma anion gap= $(Na^+)-(HCO_3)-(Cl^-)$
- If urinary anion gap is negative, it is Gut loss (Diarrhea)
- If urinary anion gap is positive, it is Renal tubular acidosis
- UAG: 20+5.6-22=Positive, Renal Tubular acidosis
- In Distal Renal Tubular acidosis, kidney is unable to produce acidic pH in urine
- If Urinary pH is much higher than limiting pH of urine (4.4) it is Distal Renal Tubular acidosis
- No acidification of urine is seen in this case

MCQ

Q6. Identify the mixed acid base disorder observed in a patient using the following ABG report

- pH: 7.176
- PCO_2: 13.7 mmHg
- PO_2: 95 mmHg
- Na^+: 143 mEq/L
- K^+: 3.2 mEq/L
- Cl^-: 118 mEq/L
- $cHCO_3$(p): 4.7 mmol/L
- $cHCO_3$ (std): 4.6 mmol/L

a. Increased anion gap metabolic acidosis and respiratory alkalosis
b. Increased anion gap metabolic acidosis and normal anion gap metabolic acidosis
c. Increased anion gap metabolic acidosis and metabolic alkalosis
d. Increased anion gap metabolic acidosis and respiratory acidosis

Ans: b. Increased anion gap metabolic acidosis and normal anion gap metabolic acidosis

- D/D ratio: 20-12/24-5= 0.5 (<1)

Explanation

- Low pH
- Low PCO_2, not respiratory defect
- cHCO3 (p) is very low, metabolic acidosis
- Compensation = 1.5*5+8 = 15.5 ± 2, within the range
- Compensated metabolic acidosis
- Anion gap = 143-(118+4.7)=20, normal 12 ± 2, increased anion gap metabolic acidosis

Step 5: Delta Delta Ratio 01:07:39

- In increased anion gap metabolic acidosis, can hide normal metabolic acidosis
- Example, a known diabetic with diarrhea causes normal anion gap metabolic acidosis
- This causes DKA, increased anion gap metabolic acidosis which hides normal anion gap metabolic acidosis
- It can also hide metabolic alkalosis, in vomiting patient with increased anion gap metabolic acidosis
- Delta Delta ratio: Anion gap-12/ 24-HCO3
- In just Increased anion gap metabolic acidosis, any decrease in bicarbonate should be compensated by increase in anion gap
- If Delta Delta Ratio is between 1-2, there is just Increased anion gap metabolic acidosis
- If more than 2, denominator is less, HCO3 is high, which is not accounted by increase in anion gap
- Hidden disorder (metabolic alkalosis) increases bicarbonate
- If >2 ratio, Both Increased anion gap metabolic acidosis and hidden metabolic alkalosis
- If <1, denominator is high, HCO3 is too low, which is not accounted by anion gap, there is also a normal anion gap metabolic acidosis
- In <1, combination of increased anion gap metabolic acidosis and Normal anion gap metabolic acidosis

Recall

- Just Increased anion gap metabolic acidosis (IAGMA), D/D: 1-2
- D/D: >2, IAGMA+ Met. Alkalosis
- D/D: <1, IAGMA + NAGMA

Ans: b. Increased anion gap metabolic acidosis and normal anion gap metabolic acidosis

- D/D ratio: 20-12/24-5= 0.5 (<1)

Summary

- **Step-01:** pH (acidosis or alkalosis)
- **Step-02:** HCO3 and PCO2 (respiratory or metabolic)
- **Step-03:** Compensation (Hidden acid base disorder)
- **Step-04:** Only if metabolic acidosis (Anion gap)
  - Anion gap High- abnormal acids
  - Anion gap Normal- loss of HCO3
    → Urinary loss
    a. Negative: Gut loss
    b. Positive: Urinary loss
- **Step-05:** IAGMA
  - Just IAGMA, D/D: 1-2
  - D/D: >2, IAGMA+ Met. Alkalosis
  - D/D: <1, IAGMA + NAGMA
- All the above steps are done if pH is abnormal
- If pH is normal,
  - HCO3 and PCO2 measured
  - Acidosis or alkalosis, check base excess
    → <-2 - Acidosis
    → >2 - Alkalosis
  - Hidden respiratory disorder
    → cHCO3 (p)
    → cHCO3 (std)

Salicylate Poisoning 01:15:33

- Presented with
  - IAGMA
  - Respiratory alkalosis
- Salicylate is an uncoupler of ETS (Electron Transport Chain)
- When ETS is inhibited, Oxidative phosphorylation is terminated
- Only anaerobic metabolism occurs
- This increases lactic acid metabolism, Lactic acidosis
- This leads to an increased anion gap, due to production of lactic acid
- Salicylates stimulates Fatty acid oxidation, which produces Acetyl CoA
- Acetyl CoA condense to form ketone bodies
- IAGMA is due to
  - Impact on ETS
  - Stimulating Fatty acid oxidation
- Salicylates also stimulates respiratory center, hyperventilates to cause Respiratory alkalosis
- Adults, both IAGMA and Respiratory alkalosis
- Children, IAGMA alone

PREVIOUS YEAR QUESTIONS

NEET PG 2022

Q. Identify the acid base disorder in a patient with the following values

- pH: 7.2
- PCO_2: 80 mmHg
- PO_2: 90 mmHg
- HCO3: 35 mEq/L

a. Metabolic Alkalosis
b. Metabolic Acidosis
c. Respiratory Alkalosis
d. Respiratory Acidosis
Ans: d. Respiratory Acidosis

Explanation

- Low pH, acidosis
- HCO3 high, not metabolic
- PCO2 high, Respiratory acidosis

NEET PG 2021

Q. A 60 year old diabetic patient with repeated vomiting following a recent dine out. Her blood pressure was 90/60 mmhg

- pH: 7.3
- HCO3: 18 mEq/L
- PCO_2: 35 mmHg

Identify the acid base disorder
a. Metabolic Acidosis
b. Metabolic Alkalosis
c. Respiratory Alkalosis
d. Respiratory Acidosis

Ans: a. Metabolic Acidosis

Explanation

- Vomiting, metabolic alkalosis
- Vomiting reduces BP
- pH, acidosis
- Diabetic with stress, DKA

Important Information

- Vomiting causes metabolic alkalosis
- Due to dehydration, low chloride delivery to DCT (hypochloremia)
- Activate RAAS, this lead to secondary hyperaldosteronism
- NA+ and water reabsorption
- K+ (Hypokalemia) and H+ (metabolic alkalosis) secretion

NEET PG 2021

Q. A 7 week old baby was brought by the mother with complaints of repeated projectile vomiting and pellet stools. The probable metabolic disturbance is

a. Normal anion gap metabolic acidosis
b. Hypochloremic hypokalemic metabolic alkalosis
c. Hyperchloremic hypokalemic metabolic alkalosis
d. Respiratory acidosis
Ans: b. Hypochloremic hypokalemic metabolic alkalosis

NEET PG 2021

Q. A hyperventilating hysterical woman presents with carpopedal spasm. The cause is:

a. High total calcium
b. Low total calcium
c. Alkalosis
d. Acidosis
Ans: c. Alkalosis

Explanation

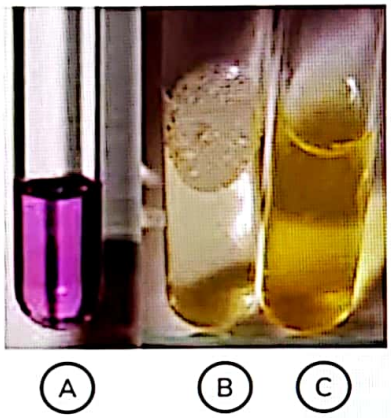
- Respiratory alkalosis is seen
- pH is high
- All plasma proteins buffers, neutralize alkaline pH
- Proteins lose H+, negatively charged
- Calcium has bound or free form
- Bound to either inorganic anions or plasma proteins
- Free calcium is the active form which stabilizes resting membrane potential
- In respiratory alkalosis,because all plasma proteins are negatively charged (because albumin is negatively charged) binds more calcium
- Free calcium concentration reduce, neurons are hyperstimulated
- This causes carpopedal spasm

5 AMINOACID AND PROTEIN METABOLISM/ VITAMINS

Amino Acid and Proteins

MCQ

Q. 1 mL of ninhydrin reagent was taken in 3 test tubes. Few drops of solution A, B and C were added to the three tubes and were kept in a boiling water bath. The resultant color is shown. Solutions A, B and C respectively are?

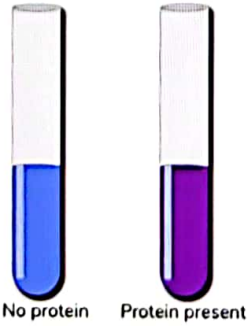


A. Arginine, glucose, lysine
B. Lysine, glucose, Arginine
C. Lysine, fructose, Proline
D. Hydroxyproline, fructose and Lysine

Important Information

- Ninhydrin reagent is capable of reacting with any amino acids within Alpha amino group to form purple coloured complex.
- Proline is not an amino acid & is not having an Alpha group

Q. This is a test used to detect the presence of proteins. Which of the following is true?



A. The composition is Biuret and copper sulfate
B. Proline gives a positive result
C. It is not answered by dipeptides
D. Urea does not answer this test

Explanation:

- To detect proteins, Biuret test is done except when it is CSF or urine which uses Sulphosalicylic acid test
- In the Biuret test the blue color solution becomes purple
- Compounds containing 2 or more peptide linkages will be able to react with Biuret agents to form a purple coloured complex
- Biuret test is not answered by free amino acids and it is answered by compounds containing at least 2 peptide linkages which means there are 3 amino acids
- Principle of Biuret test - compounds with 2 peptide linkages reacts with CuSo4 in strong alkaline medium to form purple complex
- Sodium Hydroxide which provides alkaline medium
- Sodium potassium tartrate is there which stabilizes the solution

Benedict vs Biuret Reagent 00:06:36

Function	Benedict's reagent	Biuret reagent
Cu	CuSo4	CuSo4
Alkaline	Na2Co3	NaOH
Stabilizing sol.	Sodium citrate	Sodium Potassium Tartrate

Important Information

- Urea directly cannot answer Biuret test
- It's known as Biuret because Urea on heating, 2 molecules of urea connects with each other known as Biuret

Q. The group present in Tryptophan is
A. Benzene
B. Phenol
C. Indole
D. Imidazole

Refer Table 5.1

Q. A pair of 19-year-old female identical twins was referred to our hospital with progressive visual loss. They exhibited bilateral chorioretinal atrophy. Based on these observations and biochemical findings, the patients were diagnosed with gyrate atrophy of the choroid and retina. Which is true about this disorder?

A. Responds to Arginine supplementation
B. Responds to PLP supplementation
C. Low Ornithine levels
D. Enzyme defective is Ornithine transcarbamoylase

Gyrate atrophy of retina

- Defect of ornithine transaminase
- Ornithine is break down by ornithine aminotransferase and is converted to Gamma Glutamate Semialdehyde → Glutamate → alpha ketoglutarate → citric acid cycle and comes out as Co2.
- If there is defect of ornithine aminotransferase, the ornithine accumulates and it is toxic to retinal pigment epithelium and that causes Gyrate atrophy of retina
- All transaminases require Pyridoxal Phosphate. If it is defected, when you supplement Pyridoxal Phosphate, this enzyme activity is boosted
- Ornithine is not coded by a gene (codon).
- Major source of ornithine is Arginine
- In urea cycle arginase acts on arginine, where urea is released & ornithine gets back.
- If we restrict arginine in the diet, arginine levels will be low and Gyrate atrophy of retina will not grow
- Ornithine transcarbamoylase defect leads to type 2 hyperammonemia

Q. Source of Ammonia in Urine
A. Glutamine
B. Urea
C. Ornithine
D. Asparagine

How does glutamine give rise to ammonia?

- This happens in proximal convoluted tubule
- In PCT, we have glutamine acted upon by glutaminase
- Glutaminase converts glutamine into Glutamate & 1 ammonia is released into tubular lumen
- Now, glutamate is converted to alpha ketoglutarate by glutamate dehydrogenase & 1 more ammonia is released into Urine
- The 2 ammonia released in tubular lumen will react with any hydrogen ion which is secreted in the tubules
- The hydrogen ion will drop the urinary pH & once it reaches the limiting pH, no further hydrogen ion can be secreted
- But as we don't need to stop secretion, we need renal buffers (ammonia)
- Ammonia immediately reacts with hydrogen ions and forms ammonium ions

Limiting pH

- 4.4 or 4.5 for urine and for glomerular filtrate, it is 7.4
- This means the tubules can acidify the urine till the pH for glomerular filtrate reaches 4.4
- If all metabolic acids are not secreted and accumulated in the body, it leads to metabolic acidosis, that is why we need to secrete all and for that we need ammonia as major urinary buffers

Q. Glucogenic amino acids give rise to all of the following intermediates of citric acid cycle except?
A. Isocitrate
B. Alpha ketoglutarate
C. Succinyl CoA
D. Fumarate

Glucogenic AA to TCA cycle intermediates 00:27:19

- **Glycolytic intermediate:**
  Glucogenic amino acids are those amino acids which on catabolism give rise to one of the glycolytic intermediates or Citric acid cycle intermediate other than acetyl CoA. Among this, Glucogenic amino acids give rise to:
  - Pyruvate by amino acid - Glycine / Alanine / Serine / Threonine / hydroxyproline/ Cystine
- **TCA cycle intermediate:** Glucogenic amino acids gives rise to:
  - Alpha ketoglutarate by Glutamate, Glutamine, Histidine, Proline, Arginine
  - Succinyl CoA by Valine, Isoleucine, Methionine
  - Fumarate by Phenylalanine,Tyrosine
  - Oxaloacetate by Aspartate, Asparagine
- Purely ketogenic amino acids are:
  - Leucin
  - Lysine
- Amino acids which are both ketogenic and Glucogenic:
  - Phenylalanine
  - Tyrosine
  - Tryptophan
  - Isoleucine

Q. An infant had white hair, blue eyes, hyperpnoea, convulsions, mental retardation, recurrent diarrhea. The urine had a characteristic and unique odor like that of dried hops flower. Ferric chloride test was positive. The probable defect is

A. Defective conversion of phenylalanine to tyrosine
B. Defective absorption of Tryptophan
C. Defective absorption of Methionine
D. Defective reabsorption cysteine

Explanation:

- Phenylalanine hydroxylase converts phenylalanine to tyrosine & when it is defective, it's known as phenylketonuria

Features of Phenylketonuria 00:35:57

- Mental Retardation
  - Whenever phenylalanine hydroxylase is defective, phenyl ketones accumulates and these will compete with other neutral amino acids to cross the blood brain barriers and because neutral amino acids are not able to cross, brain will not develop resulting in mental retardation
- Hypopigmentation
  - When phenylalanine hydroxylase is Defective, tyrosine is not synthesized
  - Without tyrosine, melanin cannot be synthesized resulting in hypopigmentation
  - Tyrosinase acts on Tyrosine → Melanin
- Mousy odor
  - One of phenyl ketone accumulated is phenyl acetate which is having a mousy odor
- Urine - ferric chloride test
  - It is because in urine if ketone is present, they will react with FeCl3 to form a green colored complex (discoloration)
  - Not a specific test
- Guthrie's Test
  - Done is blood
  - Based on the fact that an organism B. Subtilis needs phenyl ketones for its growth
  - For this test, we take a colony of B. Subtilis which has a number of colonies. Into this colony we add drops of patients blood. If patient is suffering from phenylketonuria, there will be source of Phenyl ketone in colonies now.
- Screening Test used now is HPLC with TMS

Important Information

- Defective absorption of Tryptophan is Tryptophan malabsorption syndrome and it is also known as Hartnup's Disease

Hartnup's Disease

- It is due to the defect of neutral amino acid transporter which is specific for tryptophan and this transporter is associated with Tryptophan absorption of intestine and Renal tubules as well.
- In Hartnup's disease these processes do not take place and if tryptophan is not absorbed from diet, there will be tryptophan Deficiency.
- Tryptophan is a precursor for Niacin (60mg tryptophan= 1 mg niacin)
- When tryptophan is not synthesized, Niacin Deficiency is seen which is also known as Pellagra.

Features of Hartnup's Disease

- 3Ds of Pellagra
- Diarrhea
- Dermatitis
- Dementia
- Aminoaciduria (because of Renal Tryptophan not synthesized)
  - In this case, the person's urine has Tryptophan and Tryptophan has an indole ring.
  - On exposure to air, indole ring → Skatole Compounds (blue color)

Important Information

- Defective reabsorption of Cysteine is known as Cystinuria.

Cystinuria

- Caused by Defect of neutral amino acids which is specific to reabsorbing 4 amino acids along Renal Tubules:
  - Cysteine
  - Ornithine
  - Lysine
  - Arginine
- These 4 together get reabsorbed along Renal Tubule through a common transporter
- When the common transporter is defective, there is supersaturation of Cysteine
- As cysteine has got a Cys-SH Group, 2 molecules of cysteine comes together & form disulfide bridge known as Cystine
- Cystine accumulates in Renal tubules and causes renal stones
- Cystinuria will present as Recurrent renal stones (Hexagonal)

Facts about Oasthouse Syndrome

- Aka Methionine malabsorption syndrome
- Diarrhea:
  - Methionine is not getting absorbed in the intestine & stays back. It attracts water and causes Diarrhea.
- Mental Retardation:
  - Unabsorbed methionine reaches colon and is converted to Alpha Hydroxybutyrate (Ketone body)
  - Alpha Hydroxybutyrate is absorbed along colon and reaches the circulation
  - Now, there is excess alpha Hydroxybutyrate which competes with NAA to cross blood brain barrier
  - Brain not gets nutritional amino acids and that causes mental Retardation
- Oasthouse Odor / Beer baby syndrome / Dried hops odor:
  - Now, the alpha Hydroxybutyrate gets excreted by sweat and it has a characteristic odor

- The odor is of dried hops which is used to add flavor to beer
- That's why the patient odors like that dried hops

- Ferric Chloride Test +ve
  - Alpha Hydroxybutyrate being a Ketone body shows Ferric Chloride Test
- Hypopigmentation Methionine Malabsorption:
  - Because Methionine is necessary as a activator of Tyrosinase (it converts tyrosine to melanin)
  - In methionine malabsorption, there is no methionine & no Melanin is activated

- There are Oasthouses
- There is blue sky & white clouds which means child has blue eyes, white skin & white hair

Phenylketonuria vs Oasthouse Syndrome

S. No	Characteristics	Phenylketonuria	Oasthouse Syndrome
1	Hypopigmentation	Mild	Pronounced
2	Diarrhea	Not a feature	Feature
3	Odor	Mousy	Dried hops odor
4	Methionine load test	Alpha Hydroxybutyrate level is undetectable	Alpha Hydroxybutyrate level is high

Amino Acid Metabolism Disorders 00:56:18

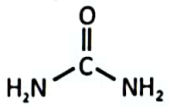
Refer Table 5.2

Urine Odor & Diagnosis

S. No	Odor	Disorder
1	Mousy Odor	Phenylketonuria
2	Fruit odor	Diabetic Ketoacidosis
3	Cabbage Odor	Type I Tyrosinemia
4	Boiled Cabbage Odor	Hypermethioninemia
5	Oasthouse / Beer odor	Methionine Malabsorption syndrome
6	Swimming Pool odor	Hawkinsinuria
7	Sweaty Feet Odor	Isovaleric Acidemia
8	Tom Cat Urine Odor	Multiple Carboxylase Deficiency

Urea Cycle- Facts to Remember 01:16:43

- Helps in conversion of Ammonia → Urea
- Takes place in Liver in both Mitochondria & Cytoplasm
- Needs ammonia in non toxic form
- M/c non toxic form of ammonia is Glutamate
  - In Neurons, we have Glutamine
  - In Muscle, we have Alanine
- Two steps of Urea cycle in Mitochondria-
  - CPS I (carbamoyl phosphate synthetase I)
  - OTC (ornithine transcarbamylase)
- Urea formula-



  - N1 donor of urea is ammonia
  - N2 donor of urea is Aspartate
- Every Urea Cycle, Net ATP used is 1.5 & 2 Ammonia are detoxified
- Urea cycle & Citric Acid Cycle are linked by Fumarate
  - In urea cycle, Fumarate is formed and it enters directly into Citric Acid Cycle

Urea Cycle Disorders

- All disorders present with Hyperammonemia
- When Urea cycle is defective Ammonia cannot be converted to Urea
- Clinical features for Hyperammonemia:
  - CNS depression manifestations and CNS Stimulation
    → Vomiting
    → Seizures
  - Child depressed & apathetic
  - EEG abnormalities (slowing of waves)

- Ammonia formed reacts with Alpha ketoglutarate and forms glutamate (excitatory neurotransmitters) which is responsible for CNS Manifestations
- Ammonia is called an osmolyte because Ammonia formed within a cell reacts with hydrogen ions and forms Ammonium ions which attract water.
- Respiratory alkalosis is also seen
- CNS Depression manifestations:
  - Ammonia causes hydrogen ion Deficiency within cell and this inhibits the ETC, so ATP cannot be produced
  - Another reason is ammonia reacts with Alpha ketoglutarate which causes its Deficiency and Citric acid cycle stops, which again stops the formation of ATP
- The features will be stop, if the child is given glucose only diet & not mixed diet
- X-linked recessive disorder includes:
  - Carbohydrate metabolism → G6PD Deficiency
  - Lipid metabolism → Fabry's disease
  - Protein metabolism → Type II hyperammonemia
  - Purine Salvage pathways → HGPRTase Deficiency or Lesch-Nyhan syndrome
  - MPS → Hunter's ds.

S no	Disorder	Enzyme Defect	Clinical Features
1	Type I Hyperammonemia	CPS I	Hyperammonemia, High Glutamate & Glutamine
2	Type II Hyperammonemia - X-linked recessively inherited	Ornithine transcarba moylase (allows CP to form citrulline)	Elevation of ammonia, glutamate, Glutamine, orotic acid, Uracil
3	Citrullinemia	argininosu ccinate synthetase	High Glutamate, ammonia, glutamine, Uracil, Citrullin
4	Argininosuccinic aciduria	ASL (Converts ASA → Arginine)	High ammonia, Glutamate, Glutamine, Uracil, OA, Citrullin, ASA (Condition is known as Trichorrhexis nodosa)
5.	Argininemia	Arginase	2 yrs of life (delayed presentation); Spastic Diplegia & quadreplegia
6.	HHH Syndrome	Ornithine Citrulline Trans-porter	Hyperammonemia, Hyperornithinemia, Homocitrullinemia

CPS I vs CPS II

CPS I	CPS II
Enzyme of Urea cycle	Enzyme of Pyrimidine synthesis
Present in Mitochondria	Present Cytoplasm
Amino group donor is Ammonia	Amino group donor is Glutamine
Stimulate by N acetyl glutamate	Not stimulated by N acetyl glutamate

Vitamin Deficiency Diagnosis 01:39:22

S. No.	Vitamin	Investigation	Patient preparation
1.	B1	RBC Transketolase activity	
2.	B2	Glutathione Reductase activity	
3.	B3 / Niacin Deficiency	RBC Niacinamide concentration	
4.	B6	Xanthurenic acid levels	Load Tryptophan
5.	B12	MMA level	Overnight fast
6.	B9 / Folate Deficiency	FIGLU level	Histidine load

Table 5.1

S. No.	Amino Acid	Group	Color reaction	Color
1	Phenylalanine	Benzene	Xanthoproteic acid test	Yellow→ orange/ red (on alkaline presence)
2	Tyrosine	Phenol	Xanthoproteic acid test	Yellow→ orange/ red (on alkaline presence)
			Millan Test	Red complex
3	Tryptophan	Indole	Aldehyde test	Purple
4	Histidine (HIP)	Imidazole	Paulys test	Red
5	Arginine (AGS)	guanidinium	Sakaguchi test	Red
6	Proline	Immino	Ninhydrin	Yellow

Table 5.2

S. No.	Disorder	Enzyme Defect	Clinical Features
1	Type I Tyrosinemia Aka Hepatorenal Syndrome	FAH Deficiency FAA accumulates→ Succinyl acetone (toxic to liver/ cells) → inhibit delta aminolevulinate dehydratase	Jaundice, Porphyria Hepatomegaly Hepatic Cirrhosis Hepatocellular carcinoma Fanconi Syndrome Chronic Kidney Dis.
2	Type II Tyrosinemia	Tyrosine Transaminase defect	Oculocutaneous syndrome- Painful Corneal erosion, palmar hyperkeratosis Richner Hanhart Syndrome
3	Type III Tyrosinemia	PHPP Dioxygenase (loss of function mutation)	Recurrent Seizures, Intermittent Ataxia
4	Hawkinsinuria: Autosomal Dominant	PHPP Dioxygenase (gain of function mutation)	Metabolic Acidosis in neonatal period, Swimming Pool odor
5	Alkaptonuria	HGO (Homogentisate oxidase)	Homogentisic acid (oxidation) → Benzoquinone acetate (Polymerisation) → Melanin like fibres which cause cartilage destruction Ochronosis Pigmentation of skin & mucous membrane Urine turns dark on standing Osler Sign (1st sign) Benedict Test +ve
6	Albinism	Tyrosinase	Hypopigmentation
7	Type I Homocystinuria (responds to B6 administration)	Cystathionine Beta Synthase (helps in Homocysteine → Cysteine)	Accelerated Atherosclerosis / Thrombosis, Skeletal Deformities, low Cysteine & High Methionine
8	Type II Homocystinuria (Responds to B12 and folate administration)	Methionine Synthase (helps in Homocysteine → Methionine)	Accelerated Atherosclerosis / Thrombosis, Low methionine & high Cysteine

6 GENETICS

Genetics

Q. Pyrimidine ring is derived from all the following except:

A. Carbon dioxide
B. Glutamine
C. Aspartate
D. Glycine

Important Information

Differences between purines and pyrimidines

- Purines have 2 rings and 9 atoms.
- Pyrimidines have 1 ring and 6 atoms.

Pyrimidine synthesis

00:01:32

- 6 atoms

CPS II or carbamoyl phosphate synthetase II

- 1st enzyme of pyrimidine synthesis
- Carbon dioxide, glutamine and ATP.
- Carbon dioxide acts as a C2 source.
- Glutamine acts as an N3 source.

Aspartyl transcarbamoylase

- 2nd enzyme in synthesis.
- Aspartic acid + carbamoyl phosphate.
- Aspartic acid is a source of:
  - N1, C4, C5 and C6.

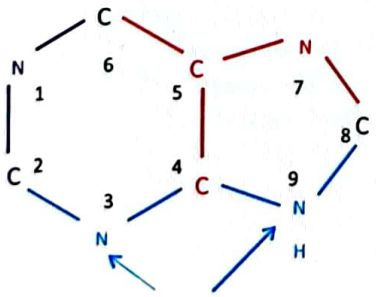
Important Information

Rate limiting enzymes

- Rate limiting enzyme for pyrimidine synthesis - Carbamoyl phosphate synthetase II.
- Rate limiting enzyme for purine synthesis - Glutamine PRPP aminotransferase.

Sources of atoms in purine ring

00:04:15



- N1 - Aspartic acid.
- N3 and N9 - Glutamine.
- C2 and C8 - Tetrahydrofolate derivatives.
  - C2 - N_{10} formyltetrahydrofolate.
  - C8 - N_5, N_{10}-methenyltetrahydrofolate.
- C4, C5 and N7 - Glycine.
- C6 - Carbon dioxide.

MCQ

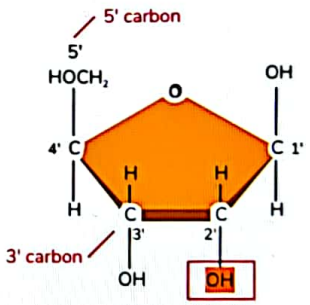
Q. A 39 year old person presents with colon cancer. Tissue biopsy was sent to detect presence of mismatch repair defect and microsatellite stability index, which was high. The geneticist however detected multiple base excision in the DNA. The linkage that is broken to cause a base excision is:

A. Phosphoester
B. β-N glycosidic linkage
C. 3'5' phosphodiester linkage
D. 5'3' phosphodiester linkage

Nucleoside

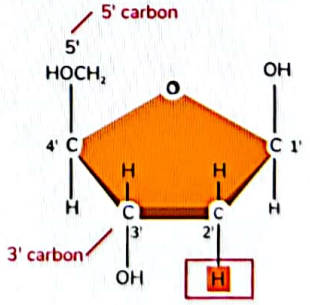
- Base + Sugar - Nucleoside
- Sugar can be ribose or deoxyribose

Ribose and deoxyribose



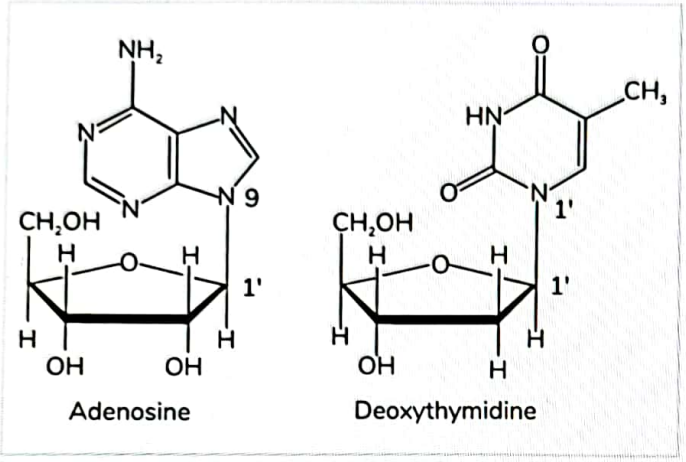
Ribose

- **Ribose** - Pentose sugars (5 carbons).



2- Deoxyribose

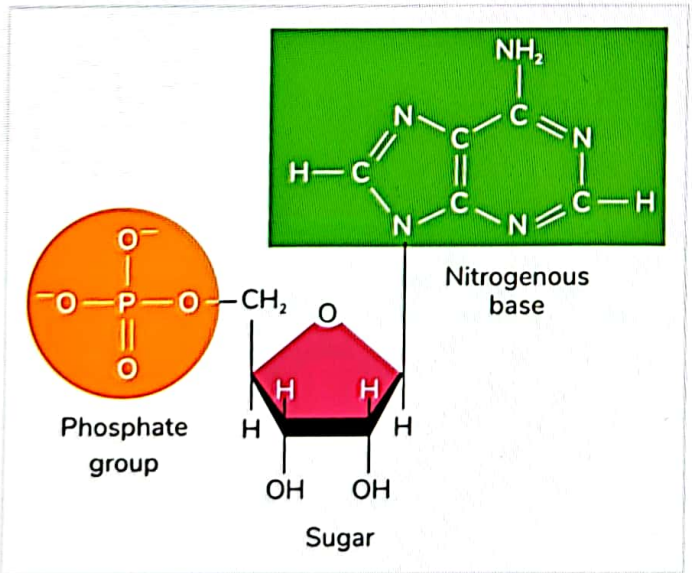
- **Deoxyribose** -C2 hydroxyl (OH) loses its oxygen atom.
- 1" OH group of ribose and deoxyribose attaches to N9 of purine or N1 of pyrimidine.
- Forms a nucleoside.



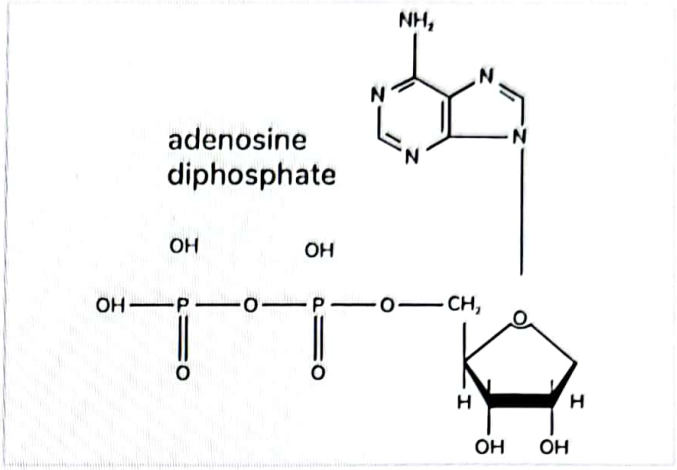
- Linkage formed between the base and sugar is known as β-N glycosidic linkage.

Nucleotide

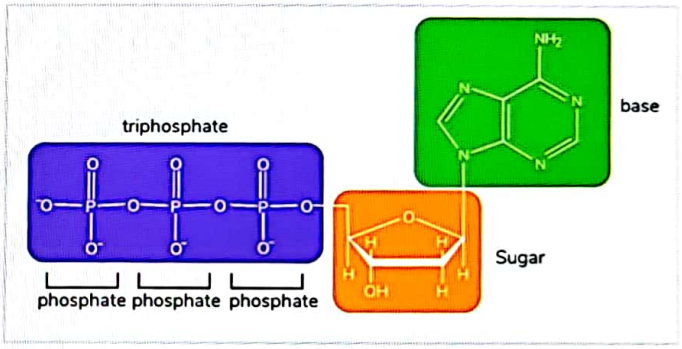
- Hexose monophosphate pathway (HMP) shunt acts as a source of ribose-5-phosphate.
- Ribose-5-phosphate is essential in nucleotide synthesis.
- Phosphate is attached to a 5' OH group of nucleosides, forming a monophosphate nucleotide.



- Nitrogenous base + Sugar + Phosphate group.
- Linkage between phosphate group and sugar in monophosphate nucleotide is known as phosphoester linkage.



- Two phosphates attached to sugar.
- Linkage between two phosphate groups in a diphosphate nucleotide is known as acid anhydride linkage.
- Acid anhydride linkages are rich in energy.

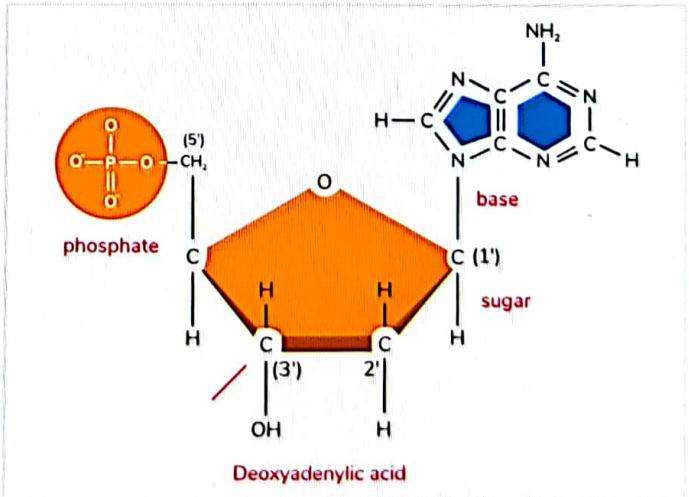


- Triphosphate nucleotides with two acid anhydride linkages act as an energy source e.g. ATP.
- Monophosphate nucleotides do not act as an energy source due to the lack of acid anhydride linkages.

Significance

- Monophosphate nucleotides present in polynucleotide chains e.g. RNA or DNA.

Polynucleotide formation



- 3'OH group is always free in ribose and deoxyribose sugars.
- 3' OH of one nucleotide links with the 5' OH group of adjacent nucleotides.
- Linkage between 3' OH and 5' OH is known as 3'5' phosphodiester linkage.
- 5'end - 5' phosphate group is free.
- 3'end - 3'OH group is free.
- All polymerases can synthesize a new strand only in the 5'-3' direction.
- Template should lie in the 3'-5' direction.

Double stranded DNA

Refer image 6.1

- Linkage between strands known as the hydrogen bond.
- 2 hydrogen bonds between A and T.
- 3 hydrogen bonds between G and C.
- Breaking hydrogen bonds leads to the unwinding of the double stranded DNA to form 2 single strands.
  - This is denaturation or unwinding.
- Breaking the β-N glycosidic linkage is known as base excision
  - Backbone is maintained with loss of the base.
- Breaking the 3'5'-phosphodiester linkage is known as double stranded DNA break.

Important Information

- Breaking the β-N glycosidic linkage is known as base excision.
  - Backbone is maintained with loss of the base.
- Breaking the 3 '5'-phosphodiester linkage is known as double stranded DNA break.

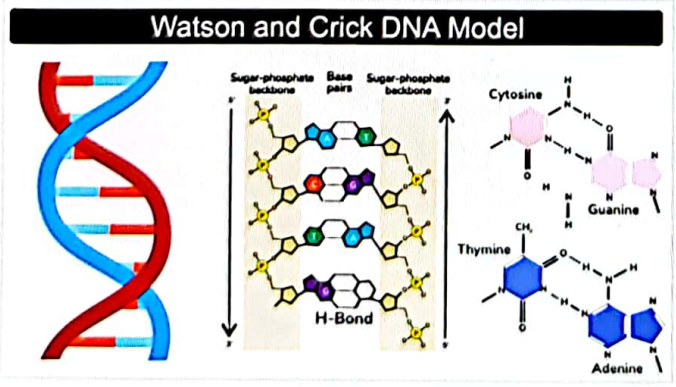
Q. DNA is extracted from a blood sample and is subjected to dehydration. The form of DNA that is expected is:

A. B DNA
B. A DNA
C. Z DNA
D. C DNA

Basic DNA structure

Basic DNA structure

- Watson and Crick model.



- The two strands are complementary and antiparallel.
- Adenine (A) - Thymine (T).
- Guanine (G) - Cytosine ©.
- One strand is 3'5' while the other strand is 5'3'.
- Two strands oriented in the ladder-like fashion.
- Backbone - Phosphodiester linkages and ribose or deoxyribose sugars.
- Steps - Nitrogen bases linked by hydrogen bonds.
- 2 hydrogen bonds between A and T.
- 3 hydrogen bonds between G and C.

Conformations of DNA

B DNA

- Most common physiological conformation.
- Right-handed coiled conformation.
- 10 base pairs per turn.
- Distance within each base pair is 3.4 Angstroms.
- Total turn length is 34 Angstroms.
- Width is 20 Angstroms.
- Each full turn has a major and minor groove.

A DNA

- Dehydration of B-type DNA.
- DNA-RNA hybrid.
- RNA-RNA hybrid.
- 11 base pairs per turn.
- Right-handed coiled conformation.
- Lack of major and minor grooves as all grooves are of the same dimension.

Z DNA

- Left-handed coiled conformation.
- 12 base pairs per turn.
- Parts of chromosomes rich in G-C sequences.

Q. A person is working on finding out polymorphisms of the albumin gene. He wants a fragment of albumin gene which include 5th to 9th exon of the gene. He knows that human chromosomes can be unwound at 95°. He constructed two primers – the melting temperature of the forward primer is 64° and that of the reverse primer is 60°. He uses Bacillus Smithii DNA polymerase enzyme. The elongation step temperature that he would use for this setup is:

A. 62°
B. 72°
C. 57°
D. 60°

Explanation:

- LAMP assays do not involve thermocyclers necessary for PCR.
- They use 60° for all the steps.

Gene/Transcription unit

- Segment of the chromosome.

- Codes for protein or RNA.
- Contains coding exon and non-coding intervening sequences (introns).

Primary transcription

- 1st transcript formed after gene transcription i.e. Primary transcript/Heteronuclear RNA.
- It is formed in the nucleus.

Post-transcriptional modification

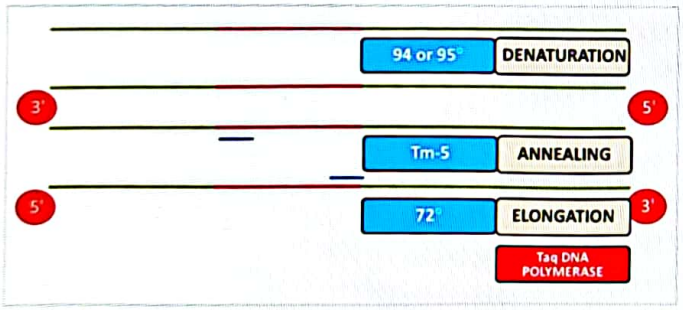
- Occurs in the nucleolus.
- Addition of 7-methylguanosine cap to 5' end.
- Addition of poly (A) tail to 3' end.
- Splicing i.e. removal of introns.
- End result is a functional mRNA.

Translation

- Nucleotide sequences of mRNA translated as amino acid sequences of a polypeptide chain.
- Occurs in cytoplasm.
- Functional mRNA read by ribosomes from 5' end to 3' end.
- Recruitment of complementary anticodons containing tRNA.
- tRNA drags amino acids along with it

Polymerase chain reaction (PCR) 00:39:58

- In-vitro amplification of a desired fragment of DNA.



Denaturation

- 1st step of PCR.
- Unwinding of double stranded DNA.
- Temperature- 94-95°.

Annealing

- Primers chosen should be complementary to the 3' flanking sequences of the desired fragment.
- 2 primers added.
- Primers complementary to 5' flanking sequence leads to undesired results, as elongation occurs in 5'-3' direction.
- Temperature -3-4° (low)
  - Primers do not follow Watson and Crick base pairing rules.
  - A pair with T while G pairs with C.
  - Annealing of primers somewhere else - Non-specific amplification.
- Optimum temperature is 5° less than the melting temperature of the primers.

Elongation

- DNA polymerase used should be thermostable to withstand cyclical temperature rise to 94-95°.
- Thermus aquaticus (Taq) DNA polymerase preferred.
- Taq DNA polymerase optimum temperature is 72°.
- Thermocycler used to control cyclical temperature rise.

Isothermal amplification techniques

- LAMP assay
- Loop-mediated amplification assay.
- All steps carried out at 60°.
- Thermocycler not used.
- Incubator capable of maintaining temperature at 60°.
- Bacillus stearothermophilus (BST) DNA polymerase used.
- Advantage of BST DNA polymerase is its very high strand invasion ability.

Q. A child was brought to the clinic for evaluation of recurrent hemiparesis and seizures. Lab investigations revealed lactic acidosis and muscle biopsy followed by Gomori trichrome stain showed ragged red fibres What is the likely mode of inheritance of this disorder?

A. It is not inherited
B. Autosomal dominant
C. X-linked dominant
D. Maternal

Myoclonic epilepsy with ragged red fibres caused by mitochondrial DNA mutations.

Mitochondrial DNA 00:54:28

- Close, circular, double stranded DNA.
- Plasmids of prokaryotes
- Mitochondria is considered a prokaryote that develops into eukaryote.
- Mitochondrial DNA codes:
  - 13 proteins of electron transport chain.
  - 22 tRNAs
  - 2 rRNAs
- Contains 37 genes
- Genetic code is different
  - UGA codes for tryptophan (Try).
  - AGA and AGG are stop codons.
- Mutations are more common.
  - DNA polymerase gamma that synthesizes mitochondrial DNA lacks proofreading and repair mechanisms.
- Maternally inherited
- Heteroplasmy

Heteroplasmy

- One site mutation causes a spectrum of disorders.
- Neuropathy, ataxia and retinitis pigmentosa (NARP) caused by mutation of ATP 6 gene.
  - ATP 6 gene is a mitochondrial gene.
  - Leigh's syndrome

- Definition - Existence of more than one type of genome in an individual, occurring due to mitochondrial DNA mutations.
- Sperm mitochondrial DNA effects are generally not seen in progeny.
- No. of sperm mitochondrial DNA is more in this progeny the effect of ova mitochondrial DNA mutation will be diluted
  - Individual presents with mild mental retardation.
- If there are few or no copies of sperm mitochondrial DNA in the zygote, ova mitochondrial DNA mutations have severe effects e.g. Leigh's syndrome.

Q. The process of gene regulation involve condensation and uncondensation of chromosomes. This occurs by binding of a variety of proteins on DNA by charge interactions. At physiological pH, the charge on DNA is:

A. Positive charge
B. Negative charge
C. Both
D. No charge

Histones

- Positively charged.
- Segment of negatively-charged, double stranded DNA bound around histones, facilitating condensation.
- 5 types of histones: H1, H2A, H2B, H3 and H4.
- All types except H1 forms dimers that is called octamers.
- Histone octamers lie centrally on the chromosome.
- Nucleosome - string on bead appearance.
- Several nucleosomes connected by a linker fragment containing H1 histones.
  - Examples include 10 nm fibril, 30 nm fibril - The 10 nm fibril gets folded on itself to form the 30 nm fibril.
- 30 nm fibril forms loops on the 46 chromosome scaffolds to form the highly compacted chromosome containing a centromere, short arm and long arm.
  - Scaffolds are centrally located proteins that help in chromosome formation.
- Specific segments undergo uncondensation and unwinding when required.

Q. Barr body is an example of:

A. Euchromatin
B. Constructive heterochromatin
C. Facultative heterochromatin
D. Hypersensitive heterochromatin

Types of Chromatin

Euchromatin	Heterochromatin
Active	Inactive
Uncondensed	Condensed
Less densely stained	More densely stained

Giemsa staining

- Alternating dark and light bands seen.
- Dark bands - Condensed, inactive parts.
- Lighter bands - Active, uncondensed parts.

Types of heterochromatin

- Constitutive heterochromatin
  - Always inactive.
  - Found in centromere and telomere regions.
  - Centromere undergoes longitudinal breakage during metaphase, hence the importance of transcriptionally - inactive, non-coding sequences in this region.
  - Telomere undergoes progressive shortening after every cell division.
- Facultative heterochromatin
  - Occasionally active
  - Found in X-chromosomes
  - Two X chromosomes in the cell:
    → One is transcriptionally active.
    → The other one is inactive, highly condensed and densely stained i.e. Barr body.
  - Random inactivation of X-chromosome - X-chromosome active in one somatic cell will be inactive in another and vice versa.

Epigenetic alterations

- Stable, inheritable changes in the DNA.
- Not related to sequence changes.
- Chromatin remodelling.
- Part of the chromosome becomes heterochromatin causing gene silencing.
  - Histone deacetylation.
  - Methylation of cytidine residues of CG islands.

Histone deacetylation

- Positively charged histones are acetylated, losing their positive charge.
- No interaction with DNA and uncondensation occurs.
- Part of DNA uncondensed is expressed.

Q. CG islands are important targets for gene regulation by genomic imprinting. Which of the following chemical modifications occurs in CG islands to cause genomic imprinting?

A. Methylation
B. Acetylation
C. Phosphorylation
D. Replication

- Gene imprinting refers to gene silencing.

Q. DNA polymerase requires all the following except:
A. RNA primer
B. 3' to 5' strand to act as a template
C. d-NTP
D. 5' to 3' strand as a template

DNA polymerases 01:20:50
- Require 3' to 5' template strands
- Requires primer
- Requires all 4 deoxynucleotide triphosphates.
  - All polynucleotide chains have d-NMP.
  - d-NTP used as an energy source to form 3'5' phosphodiester linkages.
- Divalent cations e.g. magnesium or manganese acts as a catalyst.
- Buffer maintains pH

Properties
- Proofreading and repair activity.
- Synthesis of new strand in 5' to 3' direction.
- Proofreading and repair activity - 3' to 5' exonuclease activity.
- Exception:
  - DNA polymerase I has both 3'5' exonuclease activity and 5'3' exonuclease activity.

Q. DNA polymerase require all except:
A. RNA primer
B. 3' to 5' strand to act as a template
C. d NTP
D. 5'to 3'strand as a template

Q: All of the following are true about Klenow fragment except:
A. It is a product of DNA polymerase I.
B. It has 5'3' polymerase activity.
C. It has 5'3' exonuclease activity.
D. It has 3'5' exonuclease activity.

Klenow fragment
- Fragment used in PCR i.e. Taq DNA polymerase I fragment.
  - 5'3' polymerase activity
  - 3'5' exonuclease activity
  - 5'3' exonuclease activity
- Unfavourable for PCR
- Subtilisin enzyme breaks fragments responsible for 5'3' exonuclease activity.
- Remaining fragment is known as the Klenow fragment.

Q. The strand of DNA used as the template for transcription has the base sequence GATCTAC. What is the base sequence of RNA product?
A. CTAGATG
B. GTAGATC
C. GAUCUAC
D. GUAGAUC

Replication and transcription
- Unwinding occurs in both processes.
- The single strands act as a template for synthesis of two new strands.

Transcription
- The 3'5' strand acts as a template.
- Synthesis of new RNA in 5' to 3' direction.
- 5'3' strand formed after unwinding does not take part in transcription.
  - Known as the coding strand.
- Coding strand - Has the same polarity and sequence as newly synthesized strand, except T in coding strand replaced by U in RNA.

Replication
- Both single strands take part in replication.

Template strand
- Polarity should be oriented in 3'5' direction.
- Rule - Every polynucleotide chain or oligonucleotide chain should be written in 5'3 direction.

Coding strand
- Maintain polarity and retain the sequence.
- Replace T by U
- Gene sequence.
  - Coding strand sequence.

Q. Following CRISPR-mediated gene nicks, which of the following can result in gene knock in?
A. Non-homologous end joining
B. Homologous DNA repair
C. Interference
D. Ku helicase mediated repair
- Interference phenomenon causes gene knock-down.

CRISPR
- Emmanuelle Charpentier.
- Gene editing by double stranded DNA break.
- Chromosomal repair by:
  - Non-homologous end joining (NHEJ).
  - Homologous DNA repair (HDR).
- NHEJ mediated by Ku helicase leading to loss of gene sequence - Gene knock-out.
- HDR mediated by homologous DNA sequences -Gene knock-in.

Gene knock-out and knock-in
- Gene knock-out - Removal of defective gene.
- Gene knock-in - Replacement of defective gene with normal gene.

Q. A 21 year old man wants a molecular diagnosis of sickle cell anemia as three of his maternal cousins are diagnosed with sickle cell anemia. The intern knows a few steps involved in molecular diagnostics in a jumbled way. Help him choose appropriate steps and arrange them in the right sequence.

1. RT-PCR
2. Sample collection
3. FISH
4. RFLP
5. Cytogenetics
6. Conventional PCR
7. DNA extraction

A. 2,7,1,3
B. 2,7,6,4
C. 7,2,1,3
D. 2,6,7,3

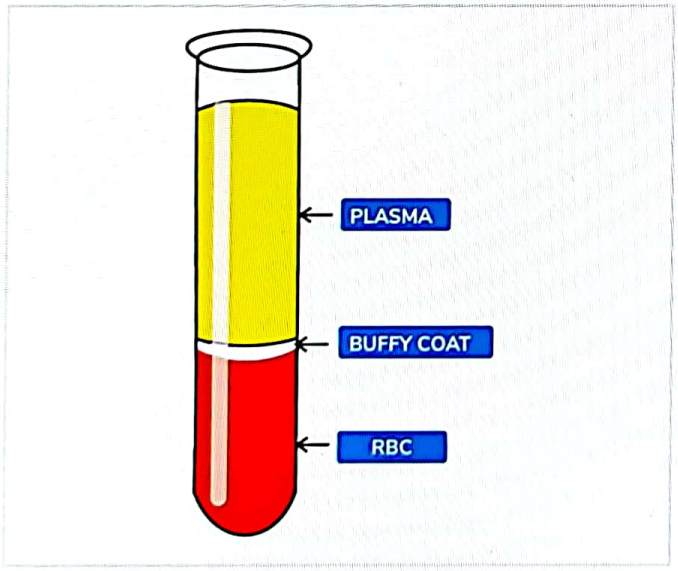
- Cytogenetics and FISH and molecular diagnostic tests done at the chromosome level.
- Reverse transcriptase-PCR (RT-PCR) is a molecular diagnostic test done for RNA estimation.

Steps of molecular diagnostics of known mutation site

Sample collection

- Preferred sample is blood.

DNA extraction



- Centrifugation of blood sample.
- Extraction of buffy coat.
- Transfer extract of buffy coat into test tube containing WBC's
- High salt method used to employ cell lysis
  - 6M NaCl or guanidinium isothiocyanate.
- 46 chromosomes with all genes extracted.

PCR

- Primers specific for fragments of β-globin gene that includes the 6th codon.
- 1.4kb β-globin gene obtained.
- Next step may be sequencing or RFLP.

Sequencing

- Determine whether the fragment is normal or abnormal.
- Expensive

RFLP

- Used for molecular diagnosis of a disorder of known mutation site.
- Restriction enzyme.
  - 6th codon is the restriction site of enzyme MstII.
- Specific sites.
- Determine whether the codon is cut or uncut.
  - MstII cuts the segment if it is normal.
  - Gives rise to 0.2 kb and 1.2 kb fragments.

Important Information

Other techniques that can be used aside from RFLP and sequencing:

- Single stranded conformation polymorphism (SSCP).
- Denaturing gradient gel electrophoresis (DGGE).

Biochemistry MCQs - Medley

Q. A child presents with dermatitis, thinning of hair and alopecia. The family gave history of eating raw egg white for a long time. Which of the following vitamin deficiency is it likely to be:

A. Vitamin A
B. Vitamin B12
C. Biotin
D. Iron deficiency

Biotin deficiency 01:51:54

- Egg whites have avidin that binds biotin with high affinity, resulting in biotin malabsorption.
- Related to thinning of hair and brittle nails.

Co-enzyme activity

- Biotin is a coenzyme necessary for carboxylases.
- Vitamin B6 or pyridoxal phosphate is a coenzyme necessary for decarboxylases and transaminases.
- Thiamine is a coenzyme necessary for oxidative decarboxylation reaction e.g. PDH complex.

Q. Which of the following plays a role in collagen maturation?

A. Copper and zinc
B. Ascorbic acid and copper
C. Proline
D. Phenylalanine

Vitamin C and copper activity in collagen

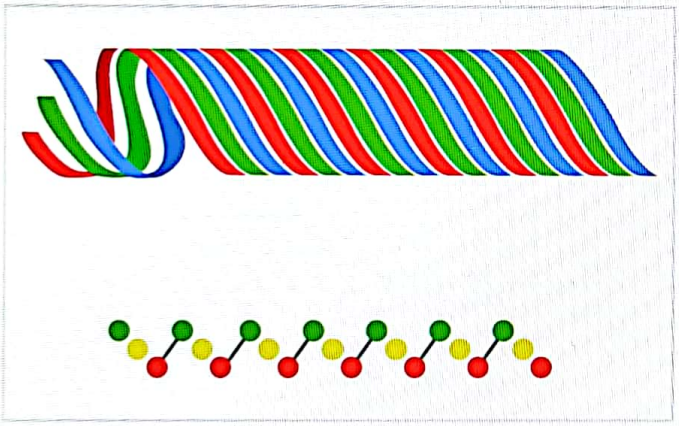
- Vitamin C is a coenzyme necessary for prolyl hydroxylase and lysyl hydroxylase.
  - These enzymes are involved in post-translational modification of collagen.
- Copper is a coenzyme for lysyl oxidase.
  - Lysyl oxidase involved in collagen cross linkage formation.

Collagen synthesis

- Triple helix. It has three polypeptide chains in a helical fashion.
- Each polypeptide chain has 1000 amino acids.
- Multiple units of Gly-X-Y.
- Most abundant amino acid of collagen - Glycine.
- X and Y are most commonly hydroxyproline and hydroxylysine.

Intracellular collagen synthesis

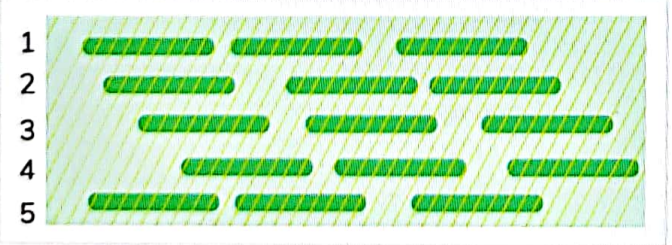
- Prolyl and lysyl hydroxylase convert proline and lysine respectively to hydroxyproline and hydroxylysine – require vitamin C
- Glycosylation in the Golgi complex.
- Procollagen - Helical structure formed.



- No helix formation at the carboxy terminal end.
- Formation of procollagen occurs intracellularly and then reaches extracellular matrix

Extracellular collagen synthesis

- N terminal and C terminal ends are cleaved.
- Procollagen converted to tropocollagen.
- Tropocollagen forms quarter-staggered alignment.
  - Increased tensile strength.



- Lysyl oxidase convert amino groups of lysine residues to aldehyde groups.
- Aldimine linkage formation.

Vitamin C deficiency/Scurvy

- Weak collagen linkages.
- Weak subendothelial connective tissue leading to weak blood vessels and bleeding gums.

Q. Provided are images of the sweet pea flower and its seeds. Many cases of osteolathyrism were reported in MP following consumption of sweet pea seeds as a staple diet among farmers. The toxin found in the seed and the enzyme inhibited by the toxin are:

A. β-aminopropionitrile, Lysyl oxidase
B. β-oxalyamino-L-alanine, Lysyl oxidase
C. β-aminopropionitrile, Lysyl hydroxylase
D. β-oxalyamino-L-alanine, Lysyl hydroxylase

β-oxalyamino-L-alanine (BOAA)

- Toxin present in sweet pea.
- Causes neurolathyrism.

Lathyrism

Neurolathyrism	Osteolathyrism
Lathyrus sativus seeds	Lathyrus odoratus seeds
BOAA	BAPN
Interferes with glutamate release and activity	Inhibit lysyl oxidase

Treatment

- Vitamin C given as a prophylactic supplement for people consuming lathyrus odoratus.

Q. There is a history of village people consuming a crop as a staple diet. Many of the villagers are now presenting with paresis and are using a stick to stand. Which of the following vitamins would help?

A. Vitamin A
B. Vitamin D
C. Vitamin C
D. Vitamin K

Q. Which of the following micronutrient deficiencies can cause poor wound healing?
A. Copper
B. Zinc
C. Selenium
D. Iron

Q. The biochemical basis of scurvy is?
A. Impaired collagen synthesis.
B. Increased keratinization of epithelium.
C. Inhibition of clotting factors.
D. Low calcium.

Q. A 14 month old child presented with erythematous scaly and pustular lesions in the perioral region and diarrhoea. The probable mineral deficiency is?
A. Copper
B. Iron
C. Zinc
D. Calcium

- Acrodermatitis enteropathica due to zinc malabsorption presenting with growth retardation.

Q. Which of the following is not a microelement?
A. Copper
B. Calcium
C. Zinc
D. Selenium

Micronutrients
- Requirement of < 100 mg/day.
- Vitamins, iron, copper, zinc, molybdenum and manganese.

Macronutrients
- Requirement of > 100 mg/day.
- Calcium (400 mg/day), magnesium, potassium and phosphorous.

Q. Which immunoglobulin is present in serum/plasma at the highest concentration?
A. IgG
B. IgA
C. IgM
D. IgE

IgG	IgM
Secondary immune response.	Primary immune response.
Monomer.	Pentamer.
Can cross placenta.	Cannot cross placenta.

Q. Iron absorption in the form of ferrous is facilitated by which of the following vitamins?
A. Vitamin A
B. Vitamin C
C. Vitamin D
D. Vitamin E

Iron absorption 02:09:19
- Iron absorption is in the form of ferrous (Fe^{2+}).
- Iron is bound to transferrin or ferritin in the form of ferric (Fe^{3+}).
- 2 sources of iron:
  - Heme source.
  - Non-heme source.
- Heme-source iron can be easily absorbed along the apical side.
  - With the help of a heme transporter.
- Non-heme source iron transported along the apical side with the help of divalent metal transporter I.
  - Only ferrous form taken up.
- Conversion of Fe3+ to Fe2+ by duodenal cytochrome b reductase.
- Iron absorption on the basolateral side occurs with the help of ferroportin as Fe2+.
- Conversion of Fe2+ to Fe3+ on the basolateral side by hephaestin.
- Hepcidin from the liver suppresses basolateral side transport of iron.

Q. A 32 year old male presented with malaise and fatigue. He is a non-smoker and non-alcoholic. Routine investigations revealed elevated high AST and ALT. No other abnormalities were observed. As he insisted on further workup, iron studies were performed.
- Serum iron: 140 µg/dl (Reference: 50-150 µg/dl).
- Serum TIBC: 225 µg/dl (Reference 250-450 µg/dl).
- Serum ferritin: 1020 ng/ml (Reference: 250-450 µg/dl).

His transferrin saturation % and probable diagnosis are?
A. 13.7%, Anemia of chronic disease
B. 62%, Anemia of chronic disease
C. 13.7%, Hereditary hemochromatosis
D. 62%, Hereditary hemochromatosis

Transferrin saturation
- Percentage of transferrin that is saturated with iron.
  Transferrin = Serum iron/Serum transferrin X 100
  Transferrin = Serum iron/Serum TIBC X 100
- Normal levels - 25-45%.

Applications
- Differentiate iron deficiency anaemia and anaemia of chronic disease.
- Diagnose primary hemochromatosis.

Biochemical basis of anaemia of chronic disease

- Iron is an essential micronutrient for many microbes.
- Inflammation is presumed to occur due to an infection.
- Reduced iron availability.
  - Reduce iron absorption - Increased hepcidin.
  - Reduce iron transport - Decreased transferrin.
  - Reduce iron uptake into tissues - Decreased soluble transfer receptor availability
  - Increase iron sequestration - Increased ferritin.
- Transferrin and albumin are negative acute phase reactants.
- Ferritin is a positive acute phase reactant.
- Reduction in serum iron.
- Reduction in total iron binding capacity (TIBC).
- Normal transferrin saturation.

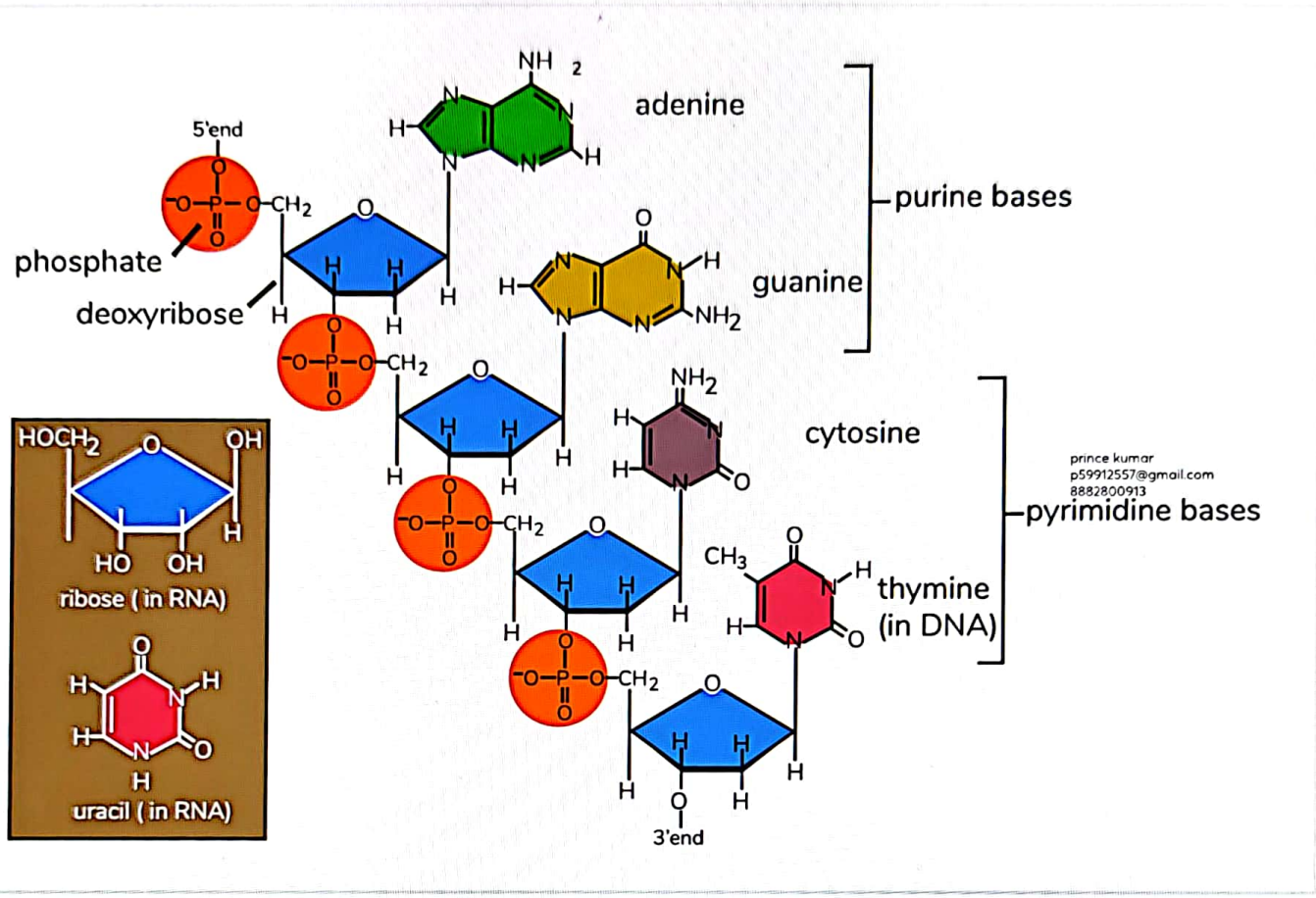
Iron deficiency anaemia

- Reduced serum iron.
- Compensatory increase in transferrin release.
- Low transferrin saturation.

Hereditary hemochromatosis

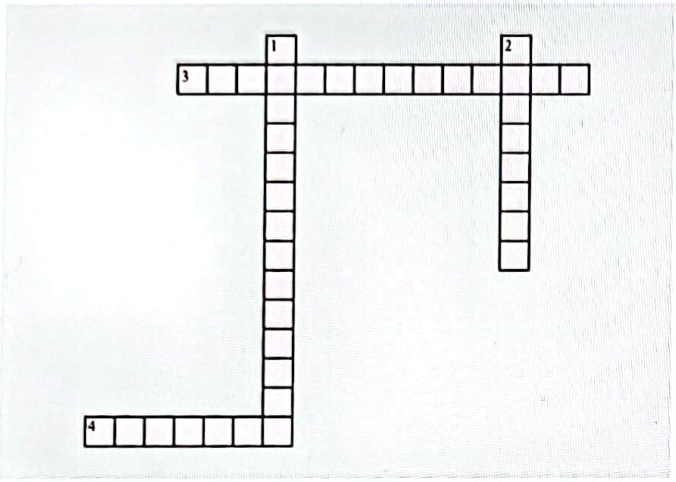
- Increased iron absorption along the intestine.
- Perpetual activation of HFE gene.
- HFE protein suppresses hepcidin release.
- Upregulation of ferroportin and increased iron absorption.
- Increased serum iron.
- Compensatory decrease in transferrin.
- Increased transferrin saturation.
  - > 60% of men.
  - > 50% of women.

Image 6.1



CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Inhibit lysyl oxidase.
4. Not considered as a microelement

Down

1. Interferes with glutamate release and activity
2. Given as a prophylactic supplement for people consuming lathyrus odoratus

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