BIOCHEMISTRY

1 CELL/SUB ORGANELLES/TESTS TYPES OF BLOOD COLLECTION TUBES

Q. Which of the following pathways only takes place in a cell's cytoplasm? (FMGE Jan 2023) 00:01:13

A. Glycolysis
B. Beta Oxidation
C. TCA
D. Urea cycle

Explanation

- Usually oxidation process requires Oxygen → Thus happen in Mitochondria.
- Glycolysis- only oxidation that happens even in the absence of Oxygen.
- So, it can happen in Cytoplasm.
- Beta oxidation takes place in Mitochondria and Peroxisomes
- TCA cycle occurs in Mitochondria
- Urea cycle occurs partly in Mitochondria and partly in Cytoplasm

Pathways and Organelles 00:02:50

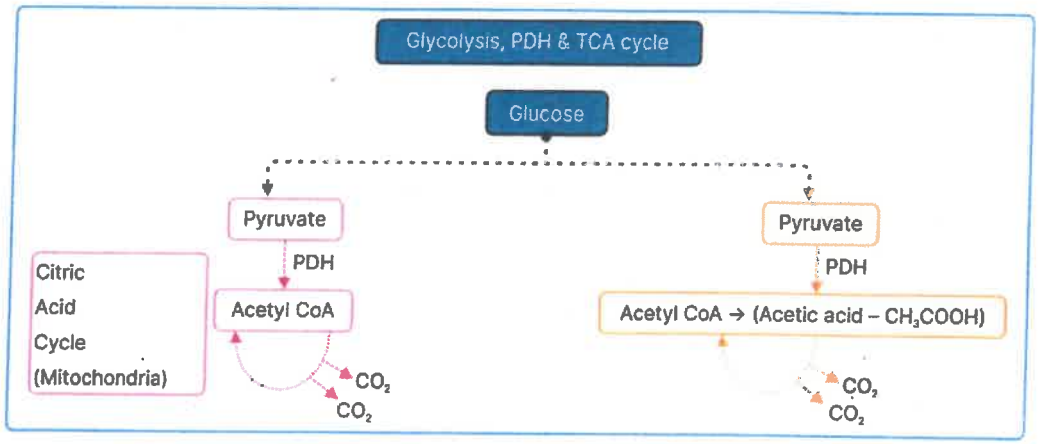
Pathways	Sub organelles
Glycolysis	Cytoplasm
PDH	Mitochondria
TCA cycle	Mitochondria
Glycogen metabolism: Glycogen synthesis and Glycogenolysis	Cytoplasm
Gluconeogenesis	Mitochondria, Cytoplasm and ER
HMP shunt	Cytoplasm
Fatty acid synthesis	Cytoplasm
Fatty acid oxidation	• Mitochondria • **Exception**: Very long chain fatty acids oxidise in Peroxisomes
Ketone body synthesis	Mitochondria
Cholesterol synthesis (Steroids)	Cytoplasm, Smooth Endoplasmic Reticulum
Bile acid synthesis (Steroids)	Smooth Endoplasmic reticulum
Urea cycle	Cytoplasm, Mitochondria
Heme synthesis	Cytoplasm, Mitochondria

Glycolysis: Process by which a six carbon compound called Glucose is split into two products

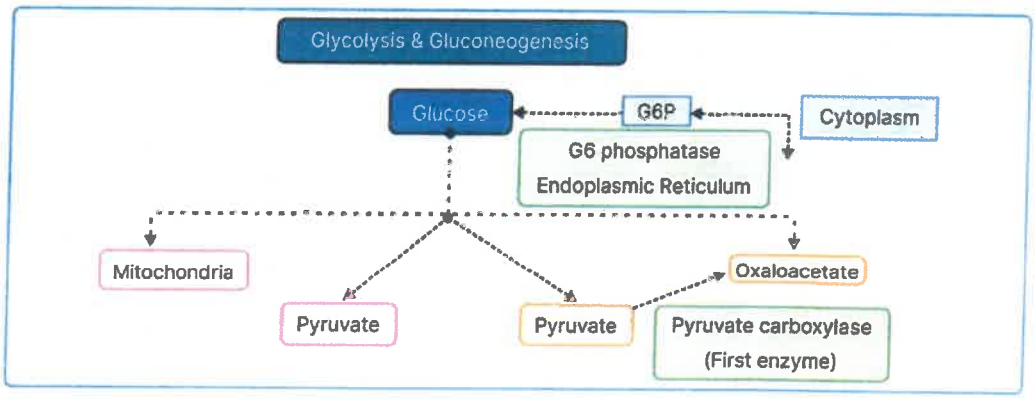
- The two products will differ depending upon whether the process occurs aerobically or anaerobically
  - On aerobic process: The products formed are 2 molecules of Pyruvate & 7 ATPs.
  - On anaerobic process: The products formed are 2 Lactate and 2 ATPs
- The only pathway that generates ATP in the absence of Oxygen

PDH

- Pyruvate dehydrogenase complex is a link between Glycolysis and Citric acid cycle
- It converts Pyruvate product of Glycolysis into Acetyl CoA
- Acetyl CoA will enter into Citric acid cycle and comes out as Co_2
- Product of Citric acid cycle: Co_2
- Every metabolic pathway has one aim
  - To push every fuel that we ingest into Co_2
- Citric acid cycle occurs in Mitochondria
- So, PDH is present in Mitochondria



- **Gluconeogenesis:** Reversal of glycolysis
- Gluconeogenesis starts with 2 molecules of Pyruvate and condense to form one molecule of glucose
- Step 1: Pyruvate is converted to Oxaloacetate by Pyruvate carboxylase
- First enzyme: Pyruvate carboxylase
- Pyruvate carboxylase is present in Mitochondria
- So, first step of Gluconeogenesis occurs in Mitochondria
- Then Oxaloacetate reaches Cytoplasm
- In Cytoplasm, the remaining steps of Gluconeogenesis occurs until Glucose-6-Phosphate is formed
- Glucose-6-phosphatase converts Glucose-6-Phosphate into Glucose
- Glucose-6-Phosphatase is present in Endoplasmic Reticulum
  - It is a microsomal enzyme marker.



Functions of Peroxisomes

00:12:41

1. Oxidation of Very long chain fatty acids
2. Oxidation of Branched chain fatty acids
3. Glycine and Taurine conjugation of bile acids

- All bile acids are derivatives of Cholesterol
- Bile acids undergo conjugation with Glycine and Taurine

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Formation of Bile salts

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Help in Lipid digestion and absorption

4. Ether lipid synthesis
   - Endoplasmic Reticulum
     - Smooth ER: It is related to Steroid synthesis
     - Rough ER: It is related to protein synthesis
   - Since Cholesterol is a steroid, It is synthesized partly in Smooth ER and Cytoplasm
   - Ketone bodies: Products of incomplete oxidation of fatty acids
   - Fatty acid oxidation: Suppose we take nCFA (Some carbon atoms containing Fatty acid); E.g.: Palmitic acid(16 carbon atoms)

When this fatty acid is subjected to Beta oxidation, it is converted to Acetyl CoA (contains 2 carbon atoms)

↓

From n carbon atom containing fatty acid, we will get n/2 Acetyl CoA

↓

From 16 carbon atoms, we will get 8 Acetyl CoA molecules

↓

Acetyl CoA molecules enter Citric acid cycle

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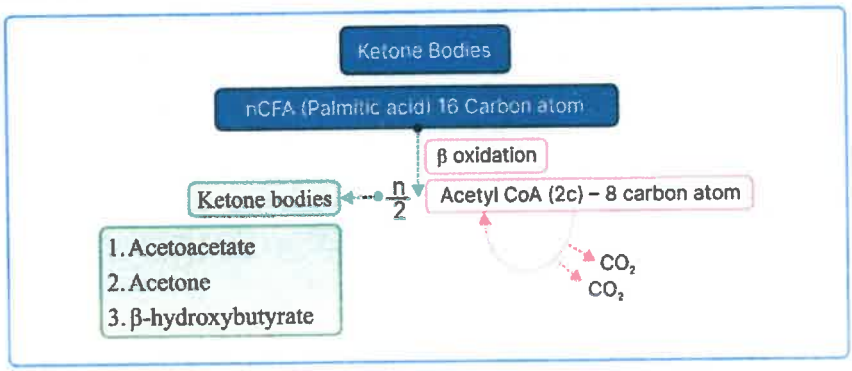
These molecules come out as Co_2

- Fatty acid oxidation is known as Complete oxidation because CO_2 is formed which is exhaled out
- Sometimes Acetyl CoA will not enter the Citric acid cycle
- In that case, Acetyl CoA molecules condense to form ketone bodies
  - Acetoacetate (2 molecules of Acetyl CoA get condensed)
  - Acetone (Acetoacetate undergoes decarboxylation)
  - Betahydroxy butyrate



- **Urea cycle:**

Whenever amino acids undergo oxidation

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Amino group is released as Ammonia (Toxic)

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Toxic ammonia is converted to Non toxic urea by Urea cycle

Q. The marker enzyme of Microsome is:

A. Galactosyl transferase

B. Cathepsin

C. Lactate dehydrogenase

D. Glucose-6-Phosphatase

Explanation

- Glucose- 6-phosphatase is the last enzyme of Glucuneogenesis
- Galactosyl transferase - marker enzyme for Golgi complex
- Cathepsin - marker enzyme for Lysosomes
- Lactate dehydrogenase- marker enzyme for Cytoplasm

Other Marker Enzymes

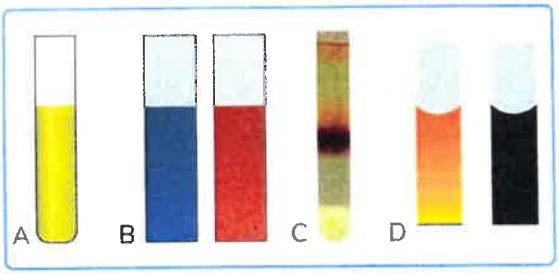
00:24:41

PYQ: FMGE 2020, 2023

Sr. No.	Organelle		Marker Enzyme
1	Nucleus		DNA Polymerase/RNA Polymerase
2	Endoplasmic Reticulum		Glucose-6-Phosphatase
3	Golgi complex (related to Glycoprotein synthesis)		Glucosyl transferase/Galactosyl transferase
4	Mitochondria	Outer Membrane (OM)	• Mono amino Oxidase (MAO)
		Inner Membrane	• Complex 2 of ETC/SDH or Complex 5 of ETC/ATP synthase
5	Lysosome		Cathepsins
6	Cytoplasm (Glycolysis)		Lactate dehydrogenase
7	Peroxisome		Catalase

- Nucleus is where chromosomes are present
- Chromosomes/DNA can undergo Replication/Transcription
- Replication is done by DNA Polymerase
- Transcription is done by RNA Polymerase
- **Inner membrane of Mitochondria**: Electron transport chain complexes are present along the inner side of Inner mitochondrial membrane
- **Three fates of Pyruvate:**
  1. Pyruvate can become Acetyl CoA by Pyruvate dehydrogenase complex (Citric acid cycle)
  2. Pyruvate can enter into Gluconeogenesis
     - Pyruvate carboxylase converts Pyruvate to Oxaloacetate
  3. Pyruvate can become Lactate
     - Lactate dehydrogenase converts Pyruvate to Lactate
- Peroxisome is called so because Hydrogen peroxide keeps generated in it.
  - Catalase enzyme detoxifies Hydrogen peroxide

Q. On prolonged starvation, which of the following tests will be positive? (INI CET Nov 2021) 00:32:41

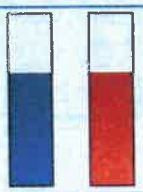


Explanation

Correct Option C

- On prolonged starvation → Ketoacidosis
- Ketone bodies in urine answer Rothera's test (purple ring)
- Rothera's test - done to detect Ketone bodies
- It is seen in Starvation and Diabetic patients

Key

Choice	Image	Name of Test	Significance
A		Xanthoproteic acid test (Yellow colour reaction)	Answered by any aromatic amino acids: Phenylalanine, Tyrosine, Tryptophan
B		Benedict's test	To detect the presence of reducing substances in the urine sample
C		Rothera's test	Positive in Diabetic ketoacidosis or Starvation ketosis
D		Benzidine test	• Alkaptonuria • Heme present in urine (haematuria or Hemoglobinuria)

Tests done in urine to detect Abnormal Constituents 00:37:05

Sr. No.	Abnormal Constituents	Colour Reaction	Observation
1	Reducing substances	Benedict's test (Semi quantitative test)	Blue → Green → Yellow → Orange → Red
2	Ketone bodies	Rothera's test	Purple ring
3	Protein	Sulphosalicylic acid test	White colour precipitate
4	Blood/Hemoglobin	Benzidine test	Yellow → Dark green/Black
5	Bile salts	Hay's test	Sulfur powder sinking to the bottom
6	Bile pigments	Fouchet's test	Bluish or Green precipitate on the filter paper

- Bile salts and pigments are seen in Obstructive jaundice

In Obstructive jaundice
↓
Obstruction of biliary tract → bile accumulates in the biliary duct
↓
Bile overflows from the biliary duct
↓
Hepatic sinusoids

- Bile salts and pigments overflow into the hepatic sinusoids → Systemic circulation
- Thus the blood has bile salts and pigments
- Anything present in the blood is filtered into the urine
- Urine will have bile salts and pigments

Q. The additive that is used in the given blood collection tube is?

A. Sodium Fluoride
B. K2EDTA
C. Heparin
D. Silica

Explanation

- Red colour top
  - **RSS:** Red top tube is used for Serum separation that has got Silica as additive (Clot activator)

Differences Between Serum and Plasma 00:44:20

Serum	Plasma
• If a blood sample is collected in a simple tube → negative charges of the tube will stimulate in vitro clotting. • The component that separates above after clotting is Serum	• If an anticoagulant is placed in a blood sample, the component that separates above is Plasma
Does not have clotting factors, Fibrinogen	All clotting factors including Fibrinogen are present
• It is used for all biochemical estimation • For E.g.: Renal function test, Liver function tests, Amylase, Lipase, Hormone estimation • Tube top is red coloured: Serum	• Plasma is used for whole blood samples o For E.g.: CBC, HbA1C o K2EDTA is an anticoagulant used for CBC (Tube top is Lavender coloured)

Important Information

- **If by mistake K2 EDTA is used for biochemical estimation → If Potassium level is estimated → Potassium will be elevated**
- **In this case, if Calcium is estimated → Calcium level is low → All enzyme activities will be falsely low.**
- **Hence, K2EDTA tubes or lavendar topped tubes are not be used for biochemical analysis**

Types of Blood Collection Tubes PYQ: INICET 2023 PYQ: FMGE 2023 00:49:37

1. Gray Colored Top Tube

- Tube with gray coloured cap is used for Glucose estimation.
- The additive used is Sodium Fluoride and Potassium Oxalate
  - Because Fluoride inhibits enolase of RBCs
  - As long as RBCs are in touch with plasma, it will continue to utilise glucose from plasma

After sometime when we estimate plasma glucose

↓

Left over glucose

↓

False low values

↓

To avoid getting false low values

↓

Fluoride is added in the tube

↓

Fluoride does not allow RBCs to use glucose.

↓

Thus we get true values of plasma glucose

2. Lavender Coloured Top Tube

00:52:03

- Lavender top tube: K2EDTA/K3EDTA
- EDTA chelates Calcium
- It acts as an Anticoagulant
- So, we will get a plasma sample that is used for Whole blood samples
- E.g.: CBC, HbA1C
- It is also used for estimation of labile parameters (PTH, ACTH, Ammonia) → Degraded by Protease in the sample
- Proteases are inhibited by chelating Calcium
- Calcium is chelated by EDTA
- E.g.: Patient of CKD → For estimation of PTH → Lavender top tube is used

3. Blue Coloured Top Tube

00:53:36

- Citrated tube
- Additive: 3.2% Sodium citrate
- It is used for Coagulation assays
  - For estimation of PT, aPTT, Fibrinogen
- 3.8% Sodium citrated tube (Black top tube) - For estimation of ESR

4. Green Coloured Top Tube

- Heparinised tubes
- It is used for stat biochemical analysis
- For E.g.: Cardiac Trop T
- Also used for Molecular diagnostics and Cytogenetics
  - Where RNA and DNA need to be extracted

5. Yellow Coloured Top Tube

- Yellow top tube
- Gel and a clot activated tube - Gel is present in the bottom
- Use:
  - For transportation of blood samples or storage of blood samples
  - Advantage: Even if the cell is contaminated, serum is not contaminated because of the presence of Intracellular fluid
  - On blood centrifugation, gel acts as a mechanical barrier between the cells and the serum

Difference between Red and Yellow coloured top tube

- **Red coloured top tube**
  - Cells in the bottom after clotting will be in touch with the Serum
  - In due course, when the cells undergo lysis, there will be contamination of Serum
  - It cannot be used if the sample has to be transported or stored
- **Yellow coloured top tube**
  - It is used by Private laboratory chains wherein multiple collection centres collect the blood samples and has to be transported to the main laboratory
  - In the laboratory, if the sample has to be retaked for 24-48 hours we use Gel and clot activator tube.

Order of Draw 01:01:20

- Dependant on two variables:
  1. How sensitive is the parameter to contamination (collected first)
  2. How notorious is the additive in the tube is to cause contamination (collected at last)
- Order of Draw is as follows:
  1. Culture blood samples (Yellow bottles) - Collected first
  2. Blue top tube - for coagulation assays
  3. Red coloured top tube (clot activated tube)/ Yellow coloured top tube (gel and clot activated tube)
  4. Green coloured top tube: Heparinised tube
  5. Lavender top tube: CBC/HbA1C
  6. Black top tube: ESR estimation
  7. Gray top tube: Fluoride is too notorious because of inhibition of many assays

Q. The most widely used technique for HbAIC estimation? PYQ: INICET 2021

A. Ion Exchange Chromatography
B. Affinity Chromatography
C. Immunoassay
D. Electrophoresis

Explanation

- The most widely used technique for HbA1C estimation is Ion exchange Chromatography
- Affinity Chromatography, Immunoassay, Electrophoresis are also used forHBA1C estimation

Glycated Hemoglobin 01:04:46

- It is a measure of irreversible non enzymatic glycation of N terminal Valine of beta globin chain of Adult Haemoglobin
- Irreversible: Long term glycemic status of an individual needs to be estimated
- Non enzymatic: The variable that determines the proportion of glycated Hemoglobin is only the glucose level in the blood
  - Not affected by enzymatic activity
- Globin chain: Lifespan of RBCs is 120 days
  - Once glycated, it remains glycated for the entire 120 days
  - Not affected by short term glycemic changes of an individual
  - It measures the latest 6-8 weeks glycemic control of an individual
- Methods used to measure glycated Hemoglobin
  1. Ion exchange Chromatography
  2. Affintiy Chromatography
  3. Electrophoresis based assays
  4. Immunoassays

Ion exchange Chromatography

- The most frequently used is Ion exchange Chromatography
  - Because it was used since the parameter was introduced
  - ADA treatment goal is based on the value estimated by ion exchange chromatography

Glycated Hemoglobin - Limitations 01:08:18

- In Iron deficiency anemia, the Iron level in the body is low
  - Life span of RBC is prolonged
  - Thus HbA1C value is falsely elevated
- In Hemolytic anemia, RBC lifespan is reduced
  - Thus HbA1C value is falsely decreased
- In these scenarios, always estimate Complete blood count before analyzing HbA1C values
  - If the patient has got Iron deficiency anemia, always interpret the value with a guard
- In these scenarios, Fructosamine can be used

Fructosamine 01:10:33

- Irreversible non enzymatic glycation of Albumin
- So, its not affected by Lifespan of RBCs
- Lifespan of Albumin is not as high as RBCs
- Limitation:
  - If glycated hemoglobin can give you an information on Glycemic status for the last 6-8 weeks.
  - Fructosamine can give you an information on glycemic status for the last 3-4 weeks only.
- Immunoassays are used for estimating Fructosamine

Q. Long term glycemic control in a person with hemolysis is assessed by estimating Fructosamine. Fructosamine is a/an?

A. Glycosaminoglycan
B. Urea
C. Fructose
D. Protein

Explanation

- Fructosamine is a glycated albumin
- Albumin is a protein
- So, the correct option is Protein

Q. The group present in Tryptophan is:

A. Benzene
B. Phenol
C. Indole
D. Imidazole

Explanation

- Tryptophan has an indole ring that answers Aldehyde tests (Mnemonic: TIA)

Aminoacid Mnemonics 01:13:41

- HIP: Histidine has an Imidazole group and it answers Pauly's test
- TIA: Tryptophan has an indole ring that answers Aldehyde tests
- AGS: Arginine has got Guanidinium group and it answers Sakaguchi tests
- MPS: Millon's test, Pauly's test, Sakaguchi test - These tests give red colour reaction

Amino Acids, Groups & Colour Reactions 01:15:23

Sr. No.	Aminoacid	Group	Color reaction	Color
1	Phenylalanine	Benzene	Xanthoproteic acid test	Yellow → Orange
2	Tyrosine	Phenol	• Xanthoproteic acid test • Millon's test	• Yellow → Orange • Millon's test gives red colour