1. **Define the following (Any Four): (1x4=4)**
    - (i) Anaplerotic reaction:
        - Anaplerotic reactions are chemical reactions that form intermediates of a metabolic pathway.
        - They are crucial for replenishing the intermediates of the citric acid cycle (TCA cycle) that are drawn off for various biosynthetic pathways.
        - A key anaplerotic reaction is the carboxylation of pyruvate to oxaloacetate, catalyzed by pyruvate carboxylase.
    - (ii) Shuttle system:
        - A shuttle system is a mechanism that transports specific molecules or their equivalents across a membrane when the membrane is impermeable to the original molecule.
        - In biochemistry, these systems are often involved in transferring reducing equivalents (e.g., NADH) from the cytosol into the mitochondria for oxidative phosphorylation, as NADH cannot directly cross the inner mitochondrial membrane.
        - Examples include the malate-aspartate shuttle and the glycerol-3-phosphate shuttle.
    - (iii) Ketosis:
        - Ketosis is a metabolic state characterized by an elevated level of ketone bodies in the blood and urine.
        - It occurs when the body primarily uses fat for energy instead of carbohydrates, leading to increased fatty acid oxidation and the production of ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone).

- Ketosis can be a normal physiological response to fasting, prolonged exercise, or low-carbohydrate diets, but it can also be pathological in conditions like diabetic ketoacidosis.

- (iv) Fermentation:
  - Fermentation is a metabolic process that produces chemical changes in organic substrates through the action of enzymes.
  - It is an anaerobic process where energy is released from glucose or other organic molecules in the absence of oxygen.
  - The primary purpose of fermentation is to regenerate NAD^+ from NADH, allowing glycolysis to continue and produce a small amount of ATP. Examples include lactic acid fermentation and alcoholic fermentation.
- (v) Oxidative phosphorylation:
  - Oxidative phosphorylation is the metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing energy which is used to reform ATP.
  - In eukaryotes, this process occurs in the mitochondria.
  - It involves two main stages: the electron transport chain (ETC) and chemiosmosis, where the energy from the electron flow is used to pump protons, creating a proton gradient that drives ATP synthase.

2. **Differentiate between the following (Any three): (2x3=6)**
   - (i) Substrate level phosphorylation and Oxidative Phosphorylation:
     - **Substrate level phosphorylation:**
       - Direct transfer of a phosphate group from a high-energy substrate molecule to ADP to form ATP.

- Occurs in the cytoplasm (glycolysis) and mitochondrial matrix (TCA cycle).
- Does not require oxygen.
- Produces a small amount of ATP.

- **Oxidative Phosphorylation:**
  - ATP synthesis driven by the energy released from the oxidation of reduced coenzymes (NADH and $FADH_2$) through the electron transport chain.
  - Occurs in the inner mitochondrial membrane.
  - Requires oxygen as the final electron acceptor.
  - Produces a large amount of ATP.

- (ii) Transamination and Deamination:
  - **Transamination:**
    - The transfer of an amino group from an α-amino acid to an α-keto acid, forming a new amino acid and a new α-keto acid.
    - Catalyzed by aminotransferases (transaminases), which often require pyridoxal phosphate (PLP) as a coenzyme.
    - Crucial for the synthesis and degradation of amino acids and for interconverting amino acids.
  - **Deamination:**
    - The removal of an amino group from a molecule, typically from an amino acid.
    - The amino group is usually released as ammonia (NH_3).

- Examples include oxidative deamination, which is catalyzed by glutamate dehydrogenase, and is a key step in amino acid catabolism for energy production or glucose synthesis.

- (iii) Hexokinase and Glucokinase:
  - **Hexokinase:**
    - An enzyme that phosphorylates hexoses (six-carbon sugars), including glucose, fructose, and mannose, at their C-6 position.
    - Has a high affinity (low K_m) for glucose, allowing it to efficiently phosphorylate glucose even at low concentrations.
    - Found in most tissues and is inhibited by its product, glucose-6-phosphate.
  - **Glucokinase:**
    - An isoenzyme of hexokinase specifically found in the liver and pancreatic β-cells.
    - Has a low affinity (high K_m) for glucose, meaning it becomes active only when glucose concentrations are high.
    - Not inhibited by glucose-6-phosphate, allowing the liver to take up and phosphorylate large amounts of glucose after a meal.
    - Plays a crucial role in regulating blood glucose levels.
- (iv) Acyl CoA and Acetyl CoA:
  - **Acyl CoA:**

- A generic term for a fatty acid molecule that is activated by being linked to coenzyme A (CoA) via a thioester bond.
- The acyl group can vary in length, representing different fatty acids (e.g., palmitoyl CoA, stearoyl CoA).
- These molecules are intermediates in fatty acid synthesis and β-oxidation.

▪ **Acetyl CoA:**

- A specific type of acyl CoA where the acyl group is an acetyl group (CH_3CO-), consisting of only two carbon atoms.
- A central molecule in metabolism, serving as a key intermediate connecting glycolysis, fatty acid oxidation, and the citric acid cycle.
- Its acetyl group can be used for energy production in the TCA cycle or for synthesis of fatty acids, cholesterol, and ketone bodies.

3. **Expand the following terms (Any Four): (½x4=2)**
   - (i) PFK: Phosphofructokinase
   - (ii) PLP: Pyridoxal phosphate
   - (iii) UDP Glucose: Uridine diphosphate glucose
   - (iv) HMG CoA: Hydroxymethylglutaryl-Coenzyme A
   - (v) EMP: Embden-Meyerhof-Parnas pathway (Glycolysis)
   - (vi) PEP: Phosphoenolpyruvate
4. **Name the cofactor/coenzyme required for the following enzymes: (1x3=3)**

- (i) Pyruvate dehydrogenase Complex: Thiamine pyrophosphate (TPP), Lipoamide, FAD, NAD^+, Coenzyme A (CoA)
- (ii) Hexokinase: Mg^{2+} (as a cofactor for ATP)
- (iii) Cytochrome oxidase: Heme (Fe), Copper (Cu)

5. **With the help of chemical structures describe Tricarboxylic Acid Cycle. And write its energetics involved per cycle. (10)**

   The user requested not to make diagrams or structures. Therefore, a description of the cycle with its energetics will be provided.

   The Tricarboxylic Acid (TCA) Cycle, also known as the Krebs Cycle or Citric Acid Cycle, is a central metabolic pathway that completes the oxidation of acetyl CoA, derived from carbohydrates, fats, and proteins, into carbon dioxide. It is a series of eight enzyme-catalyzed reactions that occur in the mitochondrial matrix in eukaryotes.

   Overview of Reactions:

   - **Step 1: Citrate Synthase:** Acetyl CoA combines with oxaloacetate to form citrate. This is an irreversible condensation reaction.
   - **Step 2: Aconitase:** Citrate is isomerized to isocitrate via an intermediate, cis-aconitate.
   - **Step 3: Isocitrate Dehydrogenase:** Isocitrate is oxidatively decarboxylated to α-ketoglutarate. This reaction produces the first molecule of NADH and releases a CO_2.
   - **Step 4: α-Ketoglutarate Dehydrogenase Complex:** α-Ketoglutarate is oxidatively decarboxylated to succinyl CoA. This complex reaction produces the second NADH and releases another CO_2.
   - **Step 5: Succinyl CoA Synthetase:** Succinyl CoA is converted to succinate, accompanied by the substrate-level phosphorylation of GDP to GTP (which can be readily converted to ATP).

- **Step 6: Succinate Dehydrogenase:** Succinate is oxidized to fumarate. This reaction uses FAD as a coenzyme, reducing it to $FADH_2$. Succinate dehydrogenase is embedded in the inner mitochondrial membrane and is part of Complex II of the electron transport chain.
- **Step 7: Fumarase:** Fumarate is hydrated to malate.
- **Step 8: Malate Dehydrogenase:** Malate is oxidized back to oxaloacetate, regenerating the starting molecule for the cycle. This reaction produces the third molecule of NADH.

Energetics involved per cycle (starting with one molecule of Acetyl CoA):

For each turn of the TCA cycle (per acetyl CoA molecule):

- **ATP/GTP produced directly:**
  - 1 molecule of GTP (which is equivalent to 1 ATP) is produced during the conversion of succinyl CoA to succinate by substrate-level phosphorylation.
- **Reduced coenzymes produced:**
  - 3 molecules of NADH are produced (from isocitrate, α-ketoglutarate, and malate).
  - 1 molecule of $FADH_2$ is produced (from succinate).

ATP yield from oxidative phosphorylation (assuming standard values):

- Each NADH molecule, when oxidized via the electron transport chain, yields approximately 2.5 ATP.
  - 3 NADH × 2.5 ATP/NADH = 7.5 ATP
- Each $FADH_2$ molecule, when oxidized via the electron transport chain, yields approximately 1.5 ATP.
  - 1 $FADH_2$ × 1.5 ATP/$FADH_2$ = 1.5 ATP

Total ATP yield per Acetyl CoA from the TCA cycle:

- 1 (GTP/ATP) + 7.5 (from NADH) + 1.5 (from $FADH_2$) = **10 ATP**

Therefore, the complete oxidation of one molecule of acetyl CoA through the TCA cycle yields approximately 10 molecules of ATP.

6. **What are ketone bodies? Add a note on it. (5)**

   - **What are ketone bodies?**

     - Ketone bodies are water-soluble molecules produced by the liver from fatty acids during periods of low carbohydrate intake, prolonged fasting, or starvation.
     - They serve as an alternative fuel source for various tissues, especially the brain, heart, and skeletal muscles, when glucose availability is limited.
     - The three main ketone bodies are:
       - Acetoacetate (β-ketoacid)
       - β-Hydroxybutyrate (an alcohol, derived from acetoacetate)
       - Acetone (a volatile ketone, formed by spontaneous decarboxylation of acetoacetate, exhaled from the body)

   - **Note on Ketone Bodies:**

     - **Synthesis (Ketogenesis):** Ketone bodies are synthesized in the mitochondrial matrix of liver cells. The process begins with the condensation of two acetyl CoA molecules to form acetoacetyl CoA, followed by the addition of another acetyl CoA to form HMG-CoA. HMG-CoA is then cleaved to acetoacetate, which can be reduced to β-hydroxybutyrate or spontaneously decarboxylated to acetone.

- **Utilization (Ketolysis):** Tissues like the brain, heart, and skeletal muscles can utilize ketone bodies for energy. β-Hydroxybutyrate is converted back to acetoacetate, which is then converted to two molecules of acetyl CoA. These acetyl CoA molecules can then enter the TCA cycle for energy production. The liver, despite producing ketone bodies, cannot utilize them because it lacks the enzyme β-ketoacyl CoA transferase (thiophorase), which is essential for converting acetoacetate back to acetoacetyl CoA.
- **Physiological Significance:**
  - **Fuel during starvation/fasting:** After glycogen stores are depleted, fatty acids become the primary energy source. Ketone bodies are crucial as the brain cannot directly utilize fatty acids due to the blood-brain barrier.
  - **Diabetes Mellitus:** In uncontrolled Type 1 diabetes, insulin deficiency leads to increased fatty acid mobilization and ketone body production, resulting in diabetic ketoacidosis (DKA), a life-threatening condition characterized by high levels of ketone bodies and acidosis.
  - **Ketogenic Diets:** Low-carbohydrate, high-fat diets induce a state of nutritional ketosis, where the body primarily uses fats and ketone bodies for energy. Such diets are sometimes used for weight loss, epilepsy management, and other therapeutic purposes.
- **Regulation:** Ketone body synthesis is regulated by the availability of fatty acids, which are increased during low insulin-to-glucagon ratios. Increased glucagon promotes lipolysis, providing substrates for ketogenesis.

7. **Explain the sequence of reactions involved when one molecule of C-16 fatty acid is to be oxidized. (10)**

The complete oxidation of a C-16 fatty acid, such as palmitic acid, occurs through a process called β-oxidation. This process takes place in the mitochondrial matrix and involves a repeating sequence of four reactions that shorten the fatty acyl CoA by two carbon atoms in each cycle, producing one acetyl CoA, one NADH, and one $FADH_2$.

Preparation:

- **Activation:** Before β-oxidation can begin, the fatty acid must be activated to fatty acyl CoA. This occurs in the outer mitochondrial membrane, catalyzed by acyl CoA synthetase (thiokinase), consuming 2 ATP equivalents (ATP to AMP + 2 Pi).
  - Fatty acid + CoA + ATP → Fatty Acyl CoA + AMP + PPi
- **Carnitine Shuttle:** Long-chain fatty acyl CoAs cannot directly cross the inner mitochondrial membrane. They are transported into the mitochondrial matrix via the carnitine shuttle system.
  - **Carnitine palmitoyltransferase I (CPT I):** Transfers the fatty acyl group from CoA to carnitine, forming acylcarnitine. This occurs on the outer mitochondrial membrane.
  - **Carnitine-acylcarnitine translocase:** Transports acylcarnitine across the inner mitochondrial membrane into the matrix, while simultaneously transporting free carnitine out.
  - **Carnitine palmitoyltransferase II (CPT II):** Transfers the fatty acyl group from carnitine back to CoA, regenerating fatty acyl CoA in the matrix and releasing free carnitine.

β-Oxidation Cycle (for one turn): Once inside the mitochondrial matrix, the fatty acyl CoA undergoes a repetitive four-step cycle:

a. **Oxidation by Acyl CoA Dehydrogenase:**
   - Fatty acyl CoA is oxidized by acyl CoA dehydrogenase, introducing a double bond between the α (C-2) and β (C-3) carbons.

- This reaction reduces FAD to $FADH_2$.
- Product: Trans-Δ^2-Enoyl CoA.

b. **Hydration by Enoyl CoA Hydratase:**

- Water is added across the double bond of trans-Δ^2-enoyl CoA, forming a β-hydroxyacyl CoA.
- Product: L-3-Hydroxyacyl CoA.

c. **Oxidation by β-Hydroxyacyl CoA Dehydrogenase:**

- The hydroxyl group at the β-carbon is oxidized to a keto group by β-hydroxyacyl CoA dehydrogenase.
- This reaction reduces NAD^+ to NADH.
- Product: β-Ketoacyl CoA.

d. **Thiolytic Cleavage by Thiolase:**

- β-Ketoacyl CoA is cleaved by β-ketoacyl CoA thiolase (thiolase) in the presence of a new molecule of CoA.
- This reaction releases one molecule of Acetyl CoA (2 carbons).
- The remaining fatty acyl CoA is now two carbons shorter than the original, and it re-enters the β-oxidation pathway.
- Products: Acetyl CoA + shortened Fatty Acyl CoA.

Oxidation of a C-16 fatty acid (Palmitic Acid): Palmitic acid (C-16) requires 7 cycles of β-oxidation to be completely oxidized.

- **Cycle 1:** C-16 Palmitoyl CoA → C-14 Myristoyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH
- **Cycle 2:** C-14 Myristoyl CoA → C-12 Lauroyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH

- **Cycle 3:** C-12 Lauroyl CoA → C-10 Caproyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH
- **Cycle 4:** C-10 Caproyl CoA → C-8 Capryloyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH
- **Cycle 5:** C-8 Capryloyl CoA → C-6 Caproyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH
- **Cycle 6:** C-6 Caproyl CoA → C-4 Butyroyl CoA + 1 Acetyl CoA + 1 $FADH_2$ + 1 NADH
- **Cycle 7:** C-4 Butyroyl CoA → 2 Acetyl CoA + 1 $FADH_2$ + 1 NADH

Total products from complete oxidation of C-16 fatty acid:

- 7 $FADH_2$
- 7 NADH
- 8 Acetyl CoA (7 from the cycles + 1 from the final C-4 molecule)

Energy Yield (assuming 2.5 ATP/NADH and 1.5 ATP/$FADH_2$, and 10 ATP/Acetyl CoA):

- From 7 $FADH_2$: $7 \times 1.5 = 10.5$ ATP
- From 7 NADH: $7 \times 2.5 = 17.5$ ATP
- From 8 Acetyl CoA (entering TCA cycle): $8 \times 10 = 80$ ATP
- Total ATP generated: 10.5 + 17.5 + 80 = 108 ATP
- Net ATP (subtracting 2 ATP for activation): 108 - 2 = **106 ATP**

Thus, the complete oxidation of one molecule of palmitic acid (C-16) yields a substantial amount of ATP, making fatty acids a highly efficient fuel source.

8. **Comment upon chemiosmotic hypothesis. (5)**

The chemiosmotic hypothesis, proposed by Peter Mitchell in 1961, explains how the energy released during the electron transport chain (ETC) is coupled to the synthesis of ATP in oxidative phosphorylation. It describes the fundamental mechanism by which a proton gradient across a membrane drives ATP synthesis.

- **Core Principle:** The central idea is that the flow of electrons through the electron transport chain complexes (Complexes I, III, and IV) in the inner mitochondrial membrane results in the pumping of protons (H^+) from the mitochondrial matrix into the intermembrane space. This creates an electrochemical proton gradient across the inner mitochondrial membrane.
- **Proton-Motive Force:** This gradient has two components:
  - **Chemical Potential Energy:** Due to the difference in proton concentration (higher $[H^+]$ in the intermembrane space, lower $[H^+]$ in the matrix), creating a pH gradient.
  - **Electrical Potential Energy:** Due to the charge separation (more positive charge in the intermembrane space, more negative charge in the matrix), creating a membrane potential.
  - Together, these components constitute the proton-motive force (Δp), which represents the stored energy available to do work.
- **ATP Synthesis:** The inner mitochondrial membrane is largely impermeable to protons. The only pathway for protons to flow back into the mitochondrial matrix is through a specific protein complex called ATP synthase (Complex V).
  - ATP synthase acts as a molecular motor. The flow of protons down their electrochemical gradient through the F_0 subunit of ATP synthase causes it to rotate.
  - This rotational energy is then transmitted to the F_1 subunit, inducing conformational changes that drive the synthesis of

ATP from ADP and inorganic phosphate (P_i). This process is known as chemiosmotic coupling.

- **Significance:**
  - **Unified Mechanism:** The chemiosmotic hypothesis provided a unified explanation for ATP synthesis in both oxidative phosphorylation (mitochondria) and photophosphorylation (chloroplasts), where light energy drives proton pumping.
  - **Energy Transduction:** It elegantly explains how the energy from redox reactions is transduced into the chemical energy of ATP without direct chemical coupling between electron transfer and ATP synthesis.
  - **Regulation:** The proton-motive force is tightly regulated. Uncouplers, such as dinitrophenol, can dissipate the proton gradient, allowing electron transport to continue without ATP synthesis, leading to heat production.
  - **Foundation for Bioenergetics:** The chemiosmotic theory is a cornerstone of modern bioenergetics, providing a framework for understanding energy transformations in living systems.

9. **Explain the reactions and significance of the Pentose Phosphate Pathway. Describe it role in NADPH generation. (10)**

   Pentose Phosphate Pathway (PPP) / Hexose Monophosphate Shunt (HMP Shunt)

   The Pentose Phosphate Pathway is an alternative pathway for glucose oxidation that runs parallel to glycolysis. It takes place in the cytoplasm of cells and is particularly active in tissues involved in biosynthesis (e.g., liver, adipose tissue, adrenal cortex, red blood cells). The pathway has two main phases: an oxidative phase and a non-oxidative phase.

   Reactions of the Pentose Phosphate Pathway:

I. Oxidative Phase (Irreversible): This phase generates NADPH and converts glucose-6-phosphate into ribulose-5-phosphate.

a. **Glucose-6-phosphate Dehydrogenase (G6PD):**

- Glucose-6-phosphate is oxidized to 6-phosphoglucono-δ-lactone.
- NADPH is produced from $NADP^+$.
- This is the rate-limiting and committed step of the PPP.

b. **6-Phosphogluconolactonase:**

- 6-Phosphoglucono-δ-lactone is hydrolyzed to 6-phosphogluconate.

c. **6-Phosphogluconate Dehydrogenase:**

- 6-Phosphogluconate is oxidatively decarboxylated to ribulose-5-phosphate.
- Another molecule of NADPH is produced from $NADP^+$, and CO_2 is released.

II. Non-Oxidative Phase (Reversible): This phase interconverts various phosphorylated sugars, allowing the synthesis of ribose-5-phosphate (for nucleotide synthesis) or the regeneration of glycolytic intermediates (fructose-6-phosphate and glyceraldehyde-3-phosphate).

d. **Ribulose-5-phosphate Isomerase:**

- Ribulose-5-phosphate is isomerized to ribose-5-phosphate (an aldopentose).

e. **Ribulose-5-phosphate Epimerase:**

- Ribulose-5-phosphate is epimerized to xylulose-5-phosphate (a ketopentose).

f. **Transketolase (requires Thiamine Pyrophosphate - TPP):**

- Transfers a two-carbon unit from xylulose-5-phosphate to ribose-5-phosphate, forming glyceraldehyde-3-phosphate and sedoheptulose-7-phosphate.

g. **Transaldolase:**

- Transfers a three-carbon unit from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate, forming fructose-6-phosphate and erythrose-4-phosphate.

h. **Transketolase (second reaction):**

- Transfers a two-carbon unit from another xylulose-5-phosphate to erythrose-4-phosphate, forming fructose-6-phosphate and glyceraldehyde-3-phosphate.

Significance of the Pentose Phosphate Pathway:

The PPP serves two major functions crucial for cellular metabolism:

i. **Production of NADPH:** This is the most significant output of the oxidative phase.

- NADPH is a crucial reducing agent for biosynthetic reactions, distinct from NADH, which is primarily used for ATP generation.
- It is essential for:
  - **Reductive biosynthesis:** Synthesis of fatty acids, cholesterol, and steroid hormones. Tissues actively involved in these processes (e.g., liver, adipose tissue, mammary glands, adrenal cortex) have a high PPP activity.
  - **Detoxification and defense against reactive oxygen species (ROS):** NADPH is required by glutathione reductase to maintain a high concentration of reduced glutathione (GSH). GSH is then used by glutathione peroxidase to neutralize harmful peroxides by converting

them into water. This is particularly vital in red blood cells to prevent oxidative damage to hemoglobin and cell membranes. Individuals with G6PD deficiency experience hemolytic anemia when exposed to oxidative stress (e.g., certain drugs, fava beans).

- **Phagocytosis:** NADPH oxidase in phagocytic cells (e.g., neutrophils, macrophages) uses NADPH to generate superoxide radicals, which are part of the respiratory burst that kills bacteria.

j. **Synthesis of Ribose-5-phosphate:**

- Ribose-5-phosphate is a precursor for the synthesis of nucleotides (ATP, GTP, CTP, UTP), nucleic acids (DNA and RNA), and coenzymes (NAD^+, FAD, CoA).
- Rapidly dividing cells (e.g., bone marrow, intestinal mucosa, tumors) have a high demand for nucleotide synthesis and thus exhibit high PPP activity to provide ribose-5-phosphate.

Role in NADPH Generation:

The primary role of the oxidative phase of the Pentose Phosphate Pathway is the generation of NADPH. Two molecules of NADPH are produced for every molecule of glucose-6-phosphate that enters the pathway and undergoes complete oxidation to ribulose-5-phosphate.

- The first NADPH is generated in the reaction catalyzed by **glucose-6-phosphate dehydrogenase (G6PD)**, where glucose-6-phosphate is oxidized.
- The second NADPH is generated in the reaction catalyzed by **6-phosphogluconate dehydrogenase**, where 6-phosphogluconate is oxidatively decarboxylated.

This continuous supply of NADPH is critical for maintaining cellular redox balance, supporting anabolic pathways, and protecting cells from oxidative

damage, particularly in tissues with high biosynthetic activity or those frequently exposed to oxidative stress.

10. **"Gluconeogenesis is not just the reversal of glycolysis", justify the statement. (5)**

The statement "Gluconeogenesis is not just the reversal of glycolysis" is fundamentally true because while gluconeogenesis does synthesize glucose from non-carbohydrate precursors, it bypasses three irreversible steps of glycolysis. This bypass mechanism is crucial for energetic feasibility and for independent regulation of the two pathways.

Here's the justification:

a. **Overcoming Irreversible Steps:** Glycolysis has three highly exergonic (irreversible) reactions that prevent a simple reversal:

- **Hexokinase/Glucokinase step:** Phosphorylation of glucose to glucose-6-phosphate.
- **Phosphofructokinase-1 (PFK-1) step:** Phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate.
- **Pyruvate Kinase step:** Conversion of phosphoenolpyruvate (PEP) to pyruvate.

b. **Bypass Reactions in Gluconeogenesis:** Gluconeogenesis employs different enzymes and reactions to bypass these irreversible steps, making the overall pathway energetically favorable in the direction of glucose synthesis:

- **Bypass of Pyruvate Kinase:**
  - Pyruvate is first converted to oxaloacetate by **pyruvate carboxylase** (requires ATP and biotin, occurs in mitochondria).
  - Oxaloacetate is then converted to phosphoenolpyruvate (PEP) by **PEP carboxykinase (PEPCK)** (requires GTP, can occur in mitochondria or cytosol depending on the

species). This two-step process bypasses the single, irreversible step catalyzed by pyruvate kinase in glycolysis.

- **Bypass of Phosphofructokinase-1:**
    - Fructose-1,6-bisphosphate is dephosphorylated to fructose-6-phosphate by **fructose-1,6-bisphosphatase** (a hydrolysis reaction, releasing Pi). This bypasses the PFK-1 reaction.
- **Bypass of Hexokinase/Glucokinase:**
    - Glucose-6-phosphate is dephosphorylated to glucose by **glucose-6-phosphatase** (a hydrolysis reaction, releasing Pi). This enzyme is primarily found in the liver and kidney, explaining why these organs are the main sites of gluconeogenesis.

c. **Energetic Considerations:** If gluconeogenesis were a simple reversal of glycolysis, it would require a large input of energy to overcome the highly exergonic steps of glycolysis, making it energetically unfavorable. By using different enzymes for the irreversible steps, gluconeogenesis ensures that the overall ΔG of the pathway is negative (or close to zero), making it thermodynamically favorable. For example, gluconeogenesis consumes 4 ATP, 2 GTP, and 2 NADH per glucose molecule synthesized, while glycolysis yields 2 ATP and 2 NADH.

d. **Independent Regulation:** Having different enzymes for these key steps allows for independent and reciprocal regulation of glycolysis and gluconeogenesis. When glucose is abundant, glycolysis is active, and gluconeogenesis is inhibited. When glucose is scarce, gluconeogenesis is activated, and glycolysis is inhibited. This prevents a "futile cycle" where both pathways would be active simultaneously, leading to net ATP hydrolysis without productive output. For instance, PFK-1 is allosterically activated by AMP and fructose-2,6-

bisphosphate, while fructose-1,6-bisphosphatase is inhibited by these molecules.

In conclusion, gluconeogenesis is not a simple reversal of glycolysis but rather a distinct pathway that shares many reversible steps with glycolysis while employing unique bypass reactions at the irreversible steps. This design ensures both the energetic feasibility and the precise regulation necessary for maintaining glucose homeostasis.

11. **Give a detailed explanation of the Urea cycle with structural formulae, highlighting the events that occur in the mitochondria and cytosol. (10)**

The user requested not to make diagrams or structures. Therefore, a description of the cycle with its events in mitochondria and cytosol will be provided.

The Urea Cycle (also known as the Ornithine Cycle) is the primary pathway for the excretion of excess nitrogen from the body, mainly in the form of urea. It converts highly toxic ammonia (NH_3), primarily derived from amino acid catabolism, into less toxic urea. This cycle occurs predominantly in the liver, with specific steps taking place in the mitochondria and others in the cytosol.

Overall Reaction: $2\ NH_3 + CO_2 + 3\ ATP + H_2O \rightarrow Urea + 2\ ADP + 4\ Pi + AMP + 2\ H^+$

Steps of the Urea Cycle (highlighting location):

I. Mitochondrial Reactions (First two steps):

a. **Formation of Carbamoyl Phosphate:**

- **Enzyme:** Carbamoyl Phosphate Synthetase I (CPSI)
- **Reactants:** Ammonia (NH_3) + Bicarbonate (HCO_3^-) + 2 ATP
- **Products:** Carbamoyl Phosphate + 2 ADP + Pi
- **Details:** This is the rate-limiting step of the urea cycle and requires N-acetylglutamate (NAG) as an allosteric activator.

Ammonia for this reaction primarily comes from oxidative deamination of glutamate.

b. **Formation of Citrulline:**

- **Enzyme:** Ornithine Transcarbamoylase (OTC)
- **Reactants:** Carbamoyl Phosphate + Ornithine
- **Products:** Citrulline + Pi
- **Details:** Ornithine, an amino acid, enters the mitochondria from the cytosol. Citrulline is then transported out of the mitochondria into the cytosol.

II. Cytosolic Reactions (Last three steps):

c. **Formation of Argininosuccinate:**

- **Enzyme:** Argininosuccinate Synthetase
- **Reactants:** Citrulline + Aspartate + ATP
- **Products:** Argininosuccinate + AMP + PPi
- **Details:** Aspartate provides the second nitrogen atom for urea synthesis. ATP is hydrolyzed to AMP and PPi, making this step energetically costly (equivalent to 2 ATP). Aspartate is generated in the mitochondria from oxaloacetate via transamination with glutamate, catalyzed by mitochondrial aspartate aminotransferase.

d. **Cleavage of Argininosuccinate:**

- **Enzyme:** Argininosuccinase (Argininosuccinate Lyase)
- **Reactants:** Argininosuccinate
- **Products:** Arginine + Fumarate
- **Details:** Fumarate is released into the cytosol. It can then enter the TCA cycle (via malate) or be used in gluconeogenesis.

e. **Hydrolysis of Arginine (Urea Formation):**

- **Enzyme:** Arginase
- **Reactants:** Arginine + H_2O
- **Products:** Urea + Ornithine
- **Details:** This final step produces urea, which is excreted, and regenerates ornithine, which is transported back into the mitochondria to continue the cycle.

Summary of Events by Location:

- **Mitochondrial Matrix:**
  - Synthesis of carbamoyl phosphate from ammonia, bicarbonate, and ATP (CPSI).
  - Condensation of carbamoyl phosphate with ornithine to form citrulline (OTC).
  - Import of ornithine into the matrix.
  - Export of citrulline out of the matrix.
  - (Indirectly involved: Generation of aspartate from glutamate and oxaloacetate).
- **Cytosol:**
  - Condensation of citrulline with aspartate to form argininosuccinate (Argininosuccinate Synthetase).
  - Cleavage of argininosuccinate to arginine and fumarate (Argininosuccinase).
  - Hydrolysis of arginine to urea and ornithine (Arginase).
  - Export of ornithine back into the mitochondria.

- (Indirectly involved: Metabolism of fumarate and production of aspartate).

The efficient functioning of the urea cycle requires coordinated transport of intermediates between the mitochondria and cytosol, demonstrating the intricate compartmentalization of metabolic pathways within the cell.

12. **Give a detailed account of oxidative deamination with examples. (5)**

Oxidative Deamination

Oxidative deamination is a biochemical reaction that involves the removal of an amino group (usually from an amino acid) as ammonia, coupled with the oxidation of the molecule. This process typically uses NAD^+ or $NADP^+$ as an electron acceptor and often involves a dehydrogenase enzyme. It is a critical step in the catabolism of amino acids, especially for amino acids whose carbon skeletons are destined for energy production or conversion into glucose or fatty acids.

Mechanism (General):

The general reaction of oxidative deamination involves two main steps, though they are often catalyzed by a single enzyme:

a. **Dehydrogenation:** The amino acid loses two hydrogen atoms (one from the α-carbon and one from the amino group) to an electron acceptor (NAD^+ or $NADP^+$), forming an imino acid intermediate.

b. **Hydrolysis:** The imino acid is then spontaneously or enzymatically hydrolyzed, releasing ammonia (NH_3) and forming an α-keto acid.

Key Example: Glutamate Dehydrogenase

The most prominent and physiologically significant example of oxidative deamination is the reaction catalyzed by **glutamate dehydrogenase (GDH)**. This enzyme is unique because it can use either NAD^+ or $NADP^+$ as a coenzyme, and it is crucial for channeling nitrogen from various amino acids into the urea cycle.

- **Reaction:** Glutamate + $NAD(P)^+$ + H_2O ⇌ α-Ketoglutarate + NAD(P)H + NH_3
- **Location:** Glutamate dehydrogenase is found in the mitochondrial matrix of eukaryotic cells, particularly abundant in the liver and kidney.
- **Significance:**
  - **Central Role in Nitrogen Metabolism:** Glutamate is formed by transamination reactions involving almost all amino acids. Therefore, glutamate dehydrogenase provides a central pathway for releasing the α-amino nitrogen from most amino acids as free ammonia.
  - **Ammonia for Urea Cycle:** The ammonia produced by GDH in the liver is a direct substrate for the urea cycle (specifically for carbamoyl phosphate synthesis).
  - **Interconversion of Metabolites:** The reaction is reversible. In the reverse direction, it can incorporate ammonia into α-ketoglutarate to synthesize glutamate, which is important for amino acid synthesis and for "fixing" ammonia when its levels are high.
  - **Link to TCA Cycle:** α-Ketoglutarate is an intermediate of the TCA cycle. Thus, oxidative deamination connects amino acid catabolism to central energy metabolism.
  - **Regulation:** GDH activity is allosterically regulated. It is activated by ADP and GDP (indicating low energy charge, promoting amino acid catabolism for energy) and inhibited by ATP and GTP (indicating high energy charge, conserving amino acids).

Other Examples (less common or indirect):

- **Amino Acid Oxidases (L-amino acid oxidase, D-amino acid oxidase):** These enzymes catalyze the oxidative deamination of L- or

D-amino acids, respectively, producing an α-keto acid and ammonia, with the reduction of FAD to $FADH_2$ (which then reacts with oxygen to produce H_2O_2). These are less prominent in the overall nitrogen metabolism of mammals compared to glutamate dehydrogenase.

- Example (L-amino acid oxidase): L-Amino acid + FAD + H_2O $\rightarrow$ α-Keto acid + NH_3 + $FADH_2$
- $FADH_2 + O_2 \rightarrow FAD + H_2O_2$

In summary, oxidative deamination, particularly via glutamate dehydrogenase, is a critical process for removing nitrogen from the body by converting amino acid nitrogen into ammonia, which can then be detoxified through the urea cycle. It also plays a vital role in linking amino acid metabolism with the TCA cycle.

13. **Write short notes on Any Three: (5x3=15)**

- **(i) Malate aspartate shuttle**
  - **Purpose:** The malate-aspartate shuttle is a highly efficient mechanism that facilitates the transfer of reducing equivalents (electrons) from cytosolic NADH (produced, for example, during glycolysis) into the mitochondrial matrix, where they can be oxidized by the electron transport chain (ETC) to generate ATP. The inner mitochondrial membrane is impermeable to NADH itself.
  - **Mechanism:** This shuttle involves four key enzymes and two membrane transporters.
    1. **Cytosolic Malate Dehydrogenase:** Reduces oxaloacetate to malate, using NADH (cytosolic NADH is oxidized to NAD^+).
    2. **Malate-α-Ketoglutarate Antiporter:** Malate is transported into the mitochondrial matrix in exchange for α-ketoglutarate.

3. **Mitochondrial Malate Dehydrogenase:** Oxidizes malate back to oxaloacetate inside the matrix, reducing NAD^+ to NADH (mitochondrial NADH).
4. **Mitochondrial Aspartate Aminotransferase (transaminase):** Converts oxaloacetate (in the matrix) to aspartate, using glutamate as an amino donor (forming α-ketoglutarate).
5. **Aspartate-Glutamate Antiporter:** Aspartate is transported out of the matrix into the cytosol in exchange for glutamate.
6. **Cytosolic Aspartate Aminotransferase (transaminase):** Converts aspartate (in the cytosol) back to oxaloacetate, using α-ketoglutarate as an amino acceptor (forming glutamate).

- **Net Result:** Cytosolic NADH is effectively "transferred" to mitochondrial NADH. Since mitochondrial NADH enters Complex I of the ETC, it yields approximately 2.5 ATP per NADH, making this shuttle energetically efficient. It is predominant in the heart, liver, and kidney.

- **(ii) Glycogenolysis**
  - **Definition:** Glycogenolysis is the biochemical pathway by which glycogen, a stored form of glucose, is broken down into glucose-1-phosphate and ultimately into glucose or glucose-6-phosphate. This process is crucial for maintaining blood glucose levels, especially between meals or during periods of increased energy demand.
  - **Location:** Occurs primarily in the liver (to maintain blood glucose for other tissues) and skeletal muscles (to provide glucose for their own energy needs).
  - **Key Enzymes and Steps:**

1. **Glycogen Phosphorylase:** This is the rate-limiting enzyme. It catalyzes the sequential removal of glucose residues from the non-reducing ends of glycogen branches, phosphorylating them to form glucose-1-phosphate. This reaction breaks α-1,4 glycosidic bonds. Phosphorylase stops 4 glucose units from an α-1,6 branch point.
2. **Debranching Enzyme (Glucan Transferase and α-1,6-Glucosidase):** This bifunctional enzyme is required to handle the branch points.
    - **Transferase activity:** Moves a block of three glucose residues from a branch to a non-reducing end of another chain.
    - **α-1,6-Glucosidase (debranching activity):** Hydrolyzes the single remaining glucose residue at the α-1,6 branch point, releasing it as free glucose.
3. **Phosphoglucomutase:** Converts glucose-1-phosphate to glucose-6-phosphate.
4. **Glucose-6-phosphatase (Liver only):** In the liver, glucose-6-phosphate is dephosphorylated by glucose-6-phosphatase to free glucose, which can then be released into the bloodstream. Muscle cells lack this enzyme, so glucose-6-phosphate directly enters glycolysis for energy production within the muscle.

- **Regulation:** Glycogenolysis is tightly regulated by hormones:
    - **Glucagon:** Released by the pancreas during low blood glucose, primarily acts on the liver to stimulate glycogen breakdown.

- **Epinephrine (Adrenaline):** Released during stress or exercise, stimulates glycogen breakdown in both liver and muscle.
- These hormones act via cAMP-dependent protein kinase, which activates glycogen phosphorylase and inhibits glycogen synthase.

- **(iii) Cascade of metabolic events in fasting and starvation**
  - **Fasting (Short-term, e.g., overnight or up to 24-48 hours):**
    - **Initial Response (first few hours):** Blood glucose levels begin to fall.
    - **Hormonal Shift:** Insulin levels decrease, while glucagon levels increase. This low insulin-to-glucagon ratio is the primary trigger for metabolic changes.
    - **Glycogenolysis:** Liver glycogen is rapidly broken down (glycogenolysis) to release glucose, which is the primary source of blood glucose during the initial hours of fasting. Muscle glycogen is used by muscles for their own energy.
    - **Gluconeogenesis:** As liver glycogen stores deplete (after 12-24 hours), gluconeogenesis (synthesis of glucose from non-carbohydrate precursors like amino acids, lactate, glycerol) becomes increasingly important to maintain blood glucose for the brain and red blood cells. Amino acids from muscle protein breakdown are a major substrate.
    - **Lipolysis:** Adipose tissue breaks down triglycerides into fatty acids and glycerol. Fatty acids become a major fuel for most tissues (muscle, heart, liver), sparing glucose for the brain. Glycerol is used by the liver for gluconeogenesis.

- **Ketogenesis:** As fatty acid oxidation increases in the liver, acetyl CoA accumulates, leading to the production of ketone bodies (acetoacetate and β-hydroxybutyrate). These are initially used by heart and muscle.

▪ **Starvation (Long-term, beyond 2-3 days):**

- **Continued Gluconeogenesis:** Gluconeogenesis continues, but the body attempts to spare muscle protein.
- **Increased Ketogenesis:** Ketone body production significantly increases, and the brain adapts to use ketone bodies as a major fuel source (up to 75% of its energy needs). This adaptation greatly reduces the brain's demand for glucose, thereby sparing muscle protein from being broken down for gluconeogenesis.
- **Fatty Acid Dominance:** Fatty acids remain the primary fuel for most tissues.
- **Metabolic Slowdown:** Basal metabolic rate may decrease to conserve energy.
- **Clinical Features:** Prolonged starvation leads to significant weight loss, muscle wasting (if ketone body utilization isn't sufficient to spare protein), and eventual organ failure.

▪ **Overall Goal:** The metabolic adaptations during fasting and starvation are aimed at maintaining glucose supply for glucose-dependent tissues (brain, red blood cells) while shifting the primary fuel source for other tissues to fatty acids and ketone bodies, thereby conserving protein.

○ **(iv) Complexes of Electron Transport Chain**

▪ **Overview:** The Electron Transport Chain (ETC), also known as the respiratory chain, is a series of protein complexes embedded

in the inner mitochondrial membrane. Its primary function is to transfer electrons from reduced coenzymes (NADH and $FADH_2$), generated from metabolic pathways like glycolysis, TCA cycle, and fatty acid oxidation, to molecular oxygen, releasing energy that is used to pump protons across the membrane, establishing a proton-motive force for ATP synthesis.

- **The Four Major Complexes:**

1. **Complex I (NADH Dehydrogenase / NADH:Ubiquinone Oxidoreductase):**
    - Accepts electrons from NADH.
    - Contains FMN and iron-sulfur (Fe-S) clusters.
    - Catalyzes the transfer of electrons from NADH to Coenzyme Q (ubiquinone).
    - Pumps 4 protons (H^+) from the mitochondrial matrix to the intermembrane space per NADH oxidized.
2. **Complex II (Succinate Dehydrogenase / Succinate:Ubiquinone Oxidoreductase):**
    - Unique because it is the only enzyme of the TCA cycle (succinate dehydrogenase) that is also part of the ETC.
    - Accepts electrons directly from $FADH_2$ (generated from succinate oxidation).
    - Contains FAD and Fe-S clusters.
    - Catalyzes the transfer of electrons from $FADH_2$ to Coenzyme Q.

- **Does NOT pump protons** directly across the membrane, contributing less to the proton gradient than NADH.

3. **Complex III (Cytochrome bc1 Complex / Ubiquinone:Cytochrome c Oxidoreductase):**
   - Accepts electrons from reduced Coenzyme Q (QH_2).
   - Contains cytochromes b, c1, and Fe-S clusters.
   - Transfers electrons from QH_2 to cytochrome c.
   - Pumps 4 protons (H^+) from the matrix to the intermembrane space per QH_2 oxidized (via the Q cycle).
4. **Complex IV (Cytochrome c Oxidase):**
   - Accepts electrons from cytochrome c.
   - Contains cytochromes a, a3, and copper (Cu) centers.
   - Transfers electrons to the final electron acceptor, molecular oxygen (O_2), forming water (H_2O).
   - Pumps 2 protons (H^+) from the matrix to the intermembrane space per 2 electrons (which ultimately originate from 1/2 O_2).

- **Mobile Electron Carriers:**
  - **Coenzyme Q (Ubiquinone):** A lipid-soluble quinone that carries electrons from Complexes I and II to Complex III.
  - **Cytochrome c:** A small, water-soluble protein that carries electrons from Complex III to Complex IV.

- **Significance:** The coordinated action of these complexes creates the proton-motive force, which is then used by ATP Synthase (Complex V) to synthesize ATP through oxidative phosphorylation.

- **(v) ω-oxidation of fatty acid**

  - **Definition:** ω-oxidation (omega-oxidation) is a minor pathway for fatty acid degradation, distinct from the primary β-oxidation pathway. It involves the oxidation of the methyl group (CH_3) at the ω-carbon (the carbon furthest from the carboxyl group) of a fatty acid, rather than the β-carbon.

  - **Location:** Occurs primarily in the endoplasmic reticulum (microsomes) of liver and kidney cells.

  - **Process:**

    1. **Hydroxylation:** The ω-carbon is hydroxylated by a mixed-function oxidase system (cytochrome P450 enzymes) requiring NADPH and O_2. This forms an ω-hydroxy fatty acid.

    2. **Oxidation to Carboxyl Group:** The hydroxyl group is then successively oxidized to an aldehyde group by an alcohol dehydrogenase, and subsequently to a carboxyl group by an aldehyde dehydrogenase. This results in the formation of a dicarboxylic acid (a fatty acid with carboxyl groups at both ends).

  - **Significance:**

    - **Minor Pathway:** Under normal physiological conditions, ω-oxidation contributes very little to overall fatty acid catabolism.

    - **Alternative in β-oxidation defects:** It becomes more important when β-oxidation is impaired (e.g., due to

genetic defects in β-oxidation enzymes or carnitine deficiency). In such cases, fatty acids cannot be adequately metabolized through the primary pathway, leading to their accumulation, and ω-oxidation provides an alternative route for their partial degradation.

- **Excretion of Dicarboxylic Acids:** The dicarboxylic acids produced can then undergo β-oxidation from both ends, generating shorter-chain dicarboxylic acids that are more water-soluble and can be excreted in the urine. This process is particularly notable in conditions like dicarboxylic aciduria.
- **Regulation:** The activity of ω-oxidation enzymes can be induced in response to high fatty acid loads or during conditions of metabolic stress.