UNIT – 2 📚 BIOCHEMISTRY - B. PHARMACY 2ND SEMESTER

POINTS TO BE COVERED IN THIS TOPIC

- ➤ CARBOHYDRATE METABOLISM
- ➤ BIOLOGICAL OXIDATION

🍯 CARBOHYDRATE METABOLISM

Carbohydrate metabolism encompasses the complex biochemical processes by which organisms convert carbohydrates into energy and building blocks for cellular functions. This metabolic pathway involves several interconnected processes including glycolysis, citric acid cycle, gluconeogenesis, and glycogen metabolism.

🔄 GLYCOLYSIS – PATHWAY, ENERGETICS AND SIGNIFICANCE

Glycolysis is the metabolic pathway that converts glucose into pyruvate through a series of enzyme-catalyzed reactions. This fundamental pathway occurs in the cytoplasm of cells and serves as the primary route for glucose catabolism.

PATHWAY OF GLYCOLYSIS

The glycolytic pathway consists of ten sequential enzymatic reactions divided into two main phases:

Phase 1: Energy Investment Phase (Preparatory Phase)

- Glucose is phosphorylated to glucose-6-phosphate by hexokinase
- Glucose-6-phosphate is converted to fructose-6-phosphate by glucose phosphate isomerase
- Fructose-6-phosphate is phosphorylated to fructose-1,6-bisphosphate by phosphofructokinase
- Fructose-1,6-bisphosphate is cleaved into two triose phosphates by aldolase
- Dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate by triose phosphate isomerase

Phase 2: Energy Generation Phase (Pay-off Phase)

- Glyceraldehyde-3-phosphate is oxidized and phosphorylated to 1,3-bisphosphoglycerate
- 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate with ATP generation
- 3-phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase
- 2-phosphoglycerate is dehydrated to phosphoenolpyruvate by enolase
- Phosphoenolpyruvate is converted to pyruvate by pyruvate kinase with ATP generation

ENERGETICS OF GLYCOLYSIS

The energy yield from glycolysis involves both ATP consumption and production:

Energy Investment:

- 2 ATP molecules consumed in the preparatory phase
- 1 ATP used for glucose to glucose-6-phosphate conversion
- 1 ATP used for fructose-6-phosphate to fructose-1,6-bisphosphate conversion

Energy Generation:

- 4 ATP molecules produced in the pay-off phase
- 2 ATP from 1,3-bisphosphoglycerate to 3-phosphoglycerate conversion
- 2 ATP from phosphoenolpyruvate to pyruvate conversion
- Net ATP yield: 2 ATP molecules per glucose molecule

SIGNIFICANCE OF GLYCOLYSIS

- Provides rapid energy production for cellular activities
- Functions under both aerobic and anaerobic conditions
- Serves as the initial step for glucose utilization in all cells
- Generates pyruvate for further oxidation in the citric acid cycle
- Produces NADH for electron transport and ATP synthesis
- Essential for red blood cell metabolism due to lack of mitochondria
- Provides intermediates for biosynthetic pathways

🌀 CITRIC ACID CYCLE – PATHWAY, ENERGETICS AND SIGNIFICANCE

The citric acid cycle, also known as the Krebs cycle or TCA cycle, is a series of chemical reactions that completely oxidize acetyl-CoA to carbon dioxide and water while generating high-energy compounds.

PATHWAY OF CITRIC ACID CYCLE

The cycle consists of eight enzymatic reactions occurring in the mitochondrial matrix:

- **Step 1:** Acetyl-CoA condenses with oxaloacetate to form citrate catalyzed by citrate synthase
- **Step 2:** Citrate is rearranged to isocitrate by aconitase through cis-aconitate intermediate
- **Step 3:** Isocitrate is oxidized to α-ketoglutarate by isocitrate dehydrogenase producing NADH and CO_2
- **Step 4:** α-ketoglutarate is oxidized to succinyl-CoA by α-ketoglutarate dehydrogenase complex producing NADH and CO_2
- **Step 5:** Succinyl-CoA is converted to succinate by succinate thiokinase generating GTP
- **Step 6:** Succinate is oxidized to fumarate by succinate dehydrogenase producing $FADH_2$
- **Step 7:** Fumarate is hydrated to malate by fumarase
- **Step 8:** Malate is oxidized to oxaloacetate by malate dehydrogenase producing NADH

ENERGETICS OF CITRIC ACID CYCLE

The energy yield per acetyl-CoA molecule entering the cycle:

Product	Quantity	ATP Equivalent
NADH	3 molecules	9 ATP
$FADH_2$	1 molecule	2 ATP
GTP	1 molecule	1 ATP
Total	-	**12 ATP**

SIGNIFICANCE OF CITRIC ACID CYCLE

- Complete oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins
- Central hub of metabolism connecting catabolic and anabolic pathways
- Generates reducing equivalents (NADH and $FADH_2$) for electron transport
- Provides intermediates for biosynthetic reactions
- Regulates cellular energy production through allosteric control
- Essential for aerobic ATP production in mitochondria

🔀 HMP SHUNT AND ITS SIGNIFICANCE

The Hexose Monophosphate (HMP) shunt, also called the pentose phosphate pathway, is an alternative route for glucose oxidation that generates NADPH and ribose-5-phosphate.

PATHWAY OF HMP SHUNT

The pathway consists of two phases:

Oxidative Phase:

- Glucose-6-phosphate is oxidized to 6-phosphogluconolactone by glucose-6-phosphate dehydrogenase producing NADPH
- 6-phosphogluconolactone is hydrolyzed to 6-phosphogluconate by lactonase
- 6-phosphogluconate is oxidized to ribulose-5-phosphate by 6-phosphogluconate dehydrogenase producing NADPH and CO_2

Non-oxidative Phase:

- Ribulose-5-phosphate is isomerized to ribose-5-phosphate and xylulose-5-phosphate
- Series of transketolase and transaldolase reactions regenerate glucose-6-phosphate
- Net result: regeneration of glucose-6-phosphate for continued cycling

SIGNIFICANCE OF HMP SHUNT

- Primary source of NADPH for reductive biosynthesis
- Provides ribose-5-phosphate for nucleotide synthesis
- Essential for fatty acid and steroid synthesis
- Maintains glutathione in reduced state for antioxidant protection
- Critical in rapidly dividing cells and tissues with high biosynthetic activity

- Important in red blood cells for maintaining membrane integrity

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⚠️ GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY

G6PD deficiency is an inherited enzyme deficiency affecting the first step of the HMP shunt, resulting in decreased NADPH production.

CHARACTERISTICS OF G6PD DEFICIENCY

- Most common enzyme deficiency worldwide affecting over 400 million people
- X-linked genetic disorder primarily affecting males
- Results in reduced NADPH production in red blood cells
- Leads to increased susceptibility to oxidative stress
- Can cause hemolytic anemia under certain conditions

CLINICAL MANIFESTATIONS

- Acute hemolytic episodes triggered by oxidative stress
- Favism (hemolysis after eating fava beans)
- Drug-induced hemolysis with certain medications
- Neonatal jaundice in severe cases
- Chronic non-spherocytic hemolytic anemia in severe variants

BIOCHEMICAL BASIS

- Reduced glucose-6-phosphate dehydrogenase activity

- Decreased NADPH regeneration
- Impaired glutathione reduction
- Increased susceptibility to oxidative damage
- Red blood cell membrane instability and hemolysis

🏪 GLYCOGEN METABOLISM

Glycogen metabolism involves the synthesis (glycogenesis) and breakdown (glycogenolysis) of glycogen, the storage form of glucose in animals.

GLYCOGEN SYNTHESIS (GLYCOGENESIS)

The pathway for glycogen synthesis involves several key enzymes:

- **UDP-glucose formation:** Glucose-1-phosphate reacts with UTP to form UDP-glucose catalyzed by UDP-glucose pyrophosphorylase
- **Primer formation:** Glycogenin serves as a protein primer for initial glucose attachment
- **Chain elongation:** Glycogen synthase adds glucose units from UDP-glucose to the growing chain
- **Branch formation:** Branching enzyme creates α-1,6-glycosidic bonds every 8-12 glucose residues

GLYCOGEN BREAKDOWN (GLYCOGENOLYSIS)

The pathway for glycogen breakdown involves:

- **Phosphorylysis:** Glycogen phosphorylase cleaves α-1,4-glycosidic bonds producing glucose-1-phosphate

- **Debranching:** Debranching enzyme removes α-1,6-branch points
- **Glucose-1-phosphate conversion:** Phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate
- **Glucose release:** Glucose-6-phosphatase (in liver) converts glucose-6-phosphate to free glucose

REGULATION OF GLYCOGEN METABOLISM

- **Hormonal control:** Insulin promotes synthesis, glucagon and epinephrine promote breakdown
- **Allosteric regulation:** ATP inhibits phosphorylase, AMP activates phosphorylase
- **Covalent modification:** Phosphorylation activates phosphorylase and inhibits synthase

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🏥 GLYCOGEN STORAGE DISEASES (GSD)

Glycogen storage diseases are inherited disorders characterized by defects in enzymes involved in glycogen metabolism.

Type	Enzyme Deficiency	Clinical Features
Type I (von Gierke)	Glucose-6-phosphatase	Hepatomegaly, hypoglycemia, growth retardation
Type II (Pompe)	α-1,4-glucosidase	Cardiomegaly, muscle weakness, respiratory problems
Type III (Cori)	Debranching enzyme	Hepatomegaly, hypoglycemia, muscle weakness
Type IV (Andersen)	Branching enzyme	Cirrhosis, liver failure, early death
Type V (McArdle)	Muscle phosphorylase	Exercise intolerance, muscle cramps, myoglobinuria

PATHOPHYSIOLOGY OF GSD

- Accumulation of structurally abnormal or excessive amounts of glycogen
- Impaired glucose homeostasis and energy metabolism
- Tissue-specific manifestations depending on enzyme distribution
- Metabolic complications including hypoglycemia and lactic acidosis

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🔄 GLUCONEOGENESIS – PATHWAY AND SIGNIFICANCE

Gluconeogenesis is the metabolic pathway that results in the generation of glucose from non-carbohydrate substrates such as amino acids, lactate, and glycerol.

PATHWAY OF GLUCONEOGENESIS

The pathway essentially reverses glycolysis with key bypass reactions:

Key Substrates:

- Lactate from muscle and red blood cells
- Amino acids from protein catabolism
- Glycerol from fat metabolism
- Propionate from odd-chain fatty acids

Bypass Reactions:

- **Pyruvate to phosphoenolpyruvate:** Pyruvate carboxylase and phosphoenolpyruvate carboxykinase bypass pyruvate kinase
- **Fructose-1,6-bisphosphate to fructose-6-phosphate:** Fructose-1,6-bisphosphatase bypasses phosphofructokinase
- **Glucose-6-phosphate to glucose:** Glucose-6-phosphatase bypasses hexokinase

REGULATION OF GLUCONEOGENESIS

- **Hormonal control:** Glucagon and cortisol stimulate, insulin inhibits
- **Substrate availability:** Increased amino acids and lactate promote pathway
- **Allosteric regulation:** ATP and citrate stimulate key enzymes
- **Enzyme induction:** Prolonged fasting increases enzyme synthesis

SIGNIFICANCE OF GLUCONEOGENESIS

- Maintains blood glucose during fasting and starvation
- Provides glucose for glucose-dependent tissues (brain, red blood cells)
- Recycles lactate produced by anaerobic glycolysis
- Utilizes amino acids during protein catabolism
- Essential for survival during carbohydrate restriction
- Important in metabolic adaptation to various physiological states

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🎯 HORMONAL REGULATION OF BLOOD GLUCOSE LEVEL

Blood glucose homeostasis is maintained through the coordinated action of several hormones that regulate glucose production, utilization, and storage.

KEY HORMONES IN GLUCOSE REGULATION

Insulin:

- Secreted by pancreatic β-cells in response to elevated blood glucose
- Promotes glucose uptake by muscle and adipose tissue
- Stimulates glycogen synthesis and lipogenesis
- Inhibits gluconeogenesis and glycogenolysis
- Primary hormone for glucose lowering

Glucagon:

- Secreted by pancreatic α-cells during hypoglycemia
- Stimulates hepatic glycogenolysis and gluconeogenesis

- Promotes lipolysis and ketogenesis
- Counter-regulatory hormone to insulin

Epinephrine:

- Released from adrenal medulla during stress
- Stimulates glycogenolysis in liver and muscle
- Promotes gluconeogenesis and lipolysis
- Inhibits insulin secretion

Cortisol:

- Secreted by adrenal cortex
- Stimulates gluconeogenesis from amino acids
- Promotes protein catabolism
- Antagonizes insulin action

Growth Hormone:

- Secreted by anterior pituitary
- Promotes lipolysis and gluconeogenesis
- Antagonizes insulin action on glucose uptake

MECHANISMS OF GLUCOSE HOMEOSTASIS

- **Fed State:** Insulin dominates, promoting glucose storage as glycogen and fat
- **Fasting State:** Counter-regulatory hormones maintain glucose through glycogenolysis and gluconeogenesis

- **Stress Response:** Epinephrine provides rapid glucose mobilization
- **Long-term Regulation:** Cortisol and growth hormone provide sustained glucose production

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💉 DIABETES MELLITUS

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

TYPES OF DIABETES MELLITUS

Type 1 Diabetes:

- Autoimmune destruction of pancreatic β-cells
- Absolute insulin deficiency
- Usually develops in childhood or young adults
- Requires lifelong insulin therapy
- Associated with genetic susceptibility and environmental triggers

Type 2 Diabetes:

- Insulin resistance combined with relative insulin deficiency
- Most common form (90-95% of cases)
- Usually develops in adults over 40
- Associated with obesity, sedentary lifestyle, and genetics
- Initially managed with lifestyle modifications and oral medications

Gestational Diabetes:

- Glucose intolerance during pregnancy
- Usually resolves after delivery
- Increases risk of Type 2 diabetes later in life
- Associated with large birth weight babies

PATHOPHYSIOLOGY OF DIABETES

- **Hyperglycemia:** Elevated blood glucose due to impaired glucose utilization
- **Glucosuria:** Glucose spillage into urine when renal threshold exceeded
- **Polyuria:** Excessive urination due to osmotic diuresis
- **Polydipsia:** Excessive thirst due to fluid loss
- **Polyphagia:** Increased appetite due to cellular glucose starvation

METABOLIC CONSEQUENCES

- **Protein catabolism:** Increased proteolysis and amino acid oxidation
- **Lipolysis:** Enhanced fat breakdown and ketone production
- **Ketoacidosis:** Dangerous accumulation of ketone bodies (Type 1)
- **Hyperosmolar state:** Severe dehydration and electrolyte imbalance (Type 2)

BIOLOGICAL OXIDATION

Biological oxidation encompasses the processes by which cells extract

energy from nutrients through controlled oxidation reactions, primarily occurring in mitochondria through the electron transport chain and oxidative phosphorylation.

ELECTRON TRANSPORT CHAIN (ETC) AND ITS MECHANISM

The electron transport chain is a series of protein complexes embedded in the inner mitochondrial membrane that facilitates the transfer of electrons from NADH and $FADH_2$ to oxygen, coupled with proton pumping.

COMPONENTS OF ELECTRON TRANSPORT CHAIN

Complex I (NADH-CoQ Oxidoreductase):

- Contains flavin mononucleotide (FMN) and iron-sulfur clusters
- Accepts electrons from NADH
- Transfers electrons to coenzyme Q (ubiquinone)
- Pumps 4 protons across the inner membrane

Complex II (Succinate-CoQ Oxidoreductase):

- Contains FAD and iron-sulfur clusters
- Accepts electrons from $FADH_2$ (succinate dehydrogenase)
- Transfers electrons to coenzyme Q
- Does not pump protons

Complex III (CoQ-Cytochrome c Oxidoreductase):

- Contains cytochromes b and c_1, and iron-sulfur clusters

- Accepts electrons from reduced coenzyme Q
- Transfers electrons to cytochrome c
- Pumps 4 protons through the Q-cycle mechanism

Complex IV (Cytochrome c Oxidase):

- Contains cytochromes a and a_3, and copper centers
- Accepts electrons from cytochrome c
- Reduces oxygen to water
- Pumps 2 protons across the membrane

MECHANISM OF ELECTRON TRANSPORT

Electron Flow Pathway:

- NADH → Complex I → CoQ → Complex III → Cytochrome c → Complex IV → O_2
- $FADH_2$ → Complex II → CoQ → Complex III → Cytochrome c → Complex IV → O_2

Proton Pumping:

- Complexes I, III, and IV pump protons from matrix to intermembrane space
- Creates electrochemical gradient (proton-motive force)
- Energy stored as both electrical and chemical potential

Oxygen Reduction:

- Terminal electron acceptor in aerobic respiration

- Reduced to water at Complex IV
- Essential for maintaining electron flow

🔋 OXIDATIVE PHOSPHORYLATION AND ITS MECHANISM

Oxidative phosphorylation is the process by which ATP is synthesized using the energy derived from the electron transport chain through chemiosmotic coupling.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Chemiosmotic Theory:

- Proton gradient across inner mitochondrial membrane drives ATP synthesis
- Electrochemical gradient provides driving force
- ATP synthase harnesses proton flow to synthesize ATP

ATP Synthase Structure and Function:

- **F_0 Component:** Membrane-embedded proton channel
- **F_1 Component:** Catalytic unit projecting into matrix
- **Mechanism:** Proton flow through F_0 causes rotation of central stalk
- **Conformational Changes:** Binding change mechanism drives ATP synthesis

Coupling of Oxidation and Phosphorylation:

- Electron transport creates proton gradient

- Proton gradient drives ATP synthesis
- Tight coupling ensures efficient energy conversion

P/O RATIOS

The phosphorylation efficiency is measured by P/O ratios:

Substrate	Entry Point	ATP Yield	P/O Ratio
NADH	Complex I	~3 ATP	~3
$FADH_2$	Complex II	~2 ATP	~2
Ascorbate + TMPD	Complex IV	~1 ATP	~1

⚖️ SUBSTRATE PHOSPHORYLATION

Substrate-level phosphorylation is the direct transfer of a phosphate group from a high-energy substrate to ADP, forming ATP without involvement of the electron transport chain.

CHARACTERISTICS OF SUBSTRATE PHOSPHORYLATION

- Direct phosphorylation of ADP to ATP
- Does not require oxygen or electron transport
- Occurs in glycolysis and citric acid cycle
- Less efficient than oxidative phosphorylation
- Can occur under anaerobic conditions

EXAMPLES OF SUBSTRATE PHOSPHORYLATION

- **Glycolysis:** 1,3-bisphosphoglycerate to 3-phosphoglycerate and phosphoenolpyruvate to pyruvate
- **Citric Acid Cycle:** Succinyl-CoA to succinate (forming GTP, equivalent to ATP)

🚫 INHIBITORS OF ETC AND OXIDATIVE PHOSPHORYLATION

Various compounds can inhibit electron transport and oxidative phosphorylation at different sites, providing insights into the mechanism and serving as experimental tools.

CLASSIFICATION OF INHIBITORS

Complex I Inhibitors:

- **Rotenone:** Natural insecticide that blocks electron transfer from iron-sulfur clusters to CoQ
- **Barbiturates:** Some barbiturates inhibit Complex I
- **Piericidin A:** Antibiotic that competes with CoQ binding

Complex II Inhibitors:

- **Malonate:** Competitive inhibitor of succinate dehydrogenase
- **Oxaloacetate:** Product inhibition of succinate dehydrogenase

Complex III Inhibitors:

- **Antimycin A:** Blocks electron transfer from cytochrome b to cytochrome c_1
- **Myxothiazol:** Inhibits at the Q_o site of the Q-cycle

Complex IV Inhibitors:

- **Cyanide (CN^-):** Binds to cytochrome a_3, preventing oxygen reduction
- **Carbon Monoxide (CO):** Competes with oxygen for binding to cytochrome oxidase
- **Azide (N_3^-):** Inhibits cytochrome oxidase

ATP Synthase Inhibitors:

- **Oligomycin:** Blocks proton flow through F_0 component
- **DCCD:** Covalently modifies F_0 subunit c

🔓 UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

Uncouplers are compounds that dissipate the proton gradient without ATP synthesis, separating electron transport from phosphorylation.

MECHANISM OF UNCOUPLING

- Allow protons to cross inner membrane without passing through ATP synthase
- Dissipate proton gradient as heat
- Electron transport continues but ATP synthesis is inhibited
- Energy is released as heat instead of being captured in ATP bonds

TYPES OF UNCOUPLERS

Chemical Uncouplers:

- **2,4-Dinitrophenol (DNP):** Lipophilic weak acid that shuttles protons across membrane
- **FCCP:** Fluorocarbonyl cyanide phenylhydrazone, potent uncoupler
- **Pentachlorophenol:** Industrial chemical with uncoupling properties

Physiological Uncoupling:

- **Brown Adipose Tissue:** Contains uncoupling protein 1 (UCP1) for thermogenesis
- **Muscle Thermogenesis:** UCP2 and UCP3 may play roles in heat production
- **Regulated Heat Production:** Important for maintaining body temperature

CONSEQUENCES OF UNCOUPLING

- Increased oxygen consumption without ATP synthesis
- Heat generation and hyperthermia
- Rapid depletion of energy stores
- Potentially fatal if severe
- Therapeutic potential in obesity (historical use of DNP)