Quick Review of BIOCHEMISTRY *for Undergraduates*

Questions and Answers

Krishnananda Prabhu
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Quick Review of Biochemistry for Undergraduates—Questions and Answers

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Preface

This book is specifically designed for a quick revision prior to examinations. Emphasis has been on examination-oriented topics and clinical applications, wherever relevant. The content has been designed for:

- Quick examination revision
- Easy and better recollection

For better focused study by the students, in each chapter, specific importance has been given to:

- Frequently asked questions in examinations
- Clinical applications
- Flow charts and concept maps
- Frequently asked viva questions
- Mnemonic (MN) created for better recollection.

Each topic is in the 'question and answer' format. At the end of each chapter, clinical applications and key points, which are important for viva and MCQs, have been mentioned.

This book can also be used by the Nursing, MSc and Allied Health Science students.

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1 Cell and Plasma Membrane

1. Name all cellular organelles with one function for each.

The function of different cellular organelles is given in Table 1.1.

Table 1.1: Different cellular organelles and their functions

Organelle	Functions
Plasma membrane	Protection, selective barrier and maintains shape of the cell
Endoplasmic reticulum	Translation and folding of new proteins (rough endoplasmic reticulum), synthesis of lipids (smooth endoplasmic reticulum) and metabolism of drugs
Golgi apparatus	Sorting and modification of proteins
Mitochondria	Energy production—ATP—from the oxidation of food substances
Nucleus	Maintenance of genetic material, deoxyribonucleic acid (DNA); controls all activities of the cell, ribonucleic acid (RNA) transcription
Nucleolus	Ribosome production
Lysosome	Breakdown of large molecules—carbohydrates, lipids, proteins, etc.
Peroxisome	Breakdown of peroxides
Ribosome	Translation of RNA into proteins

2. Compare and contrast prokaryotic cell with eukaryotic cell.

The comparison between prokaryotic cell and eukaryotic cell is given in Table 1.2.

Table 1.2: Comparison of prokaryotic and eukaryotic cells

Property	Prokaryotic cell	Eukaryotic cell
Size	Small	Large
Cell membrane	Rigid	Flexible
Nucleus	Not well-defined	Well-defined with nucleolus
Subcellular organelles	Absent	Present
Cytoplasm	Organelles and cytoskeleton absent	Organelles and cytoskeleton present
Cell division	Binary fission	Mitosis and meiosis
Transport system	Absent	Present

3. Draw a neat and labeled diagram of eukaryotic cell.

The structure of eukaryotic cell is shown in Figure 1.1.

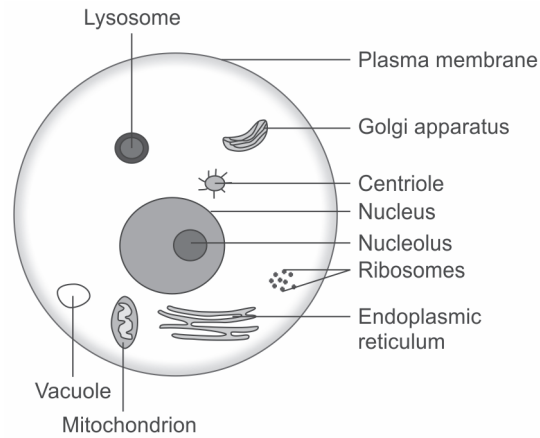


Fig. 1.1: Eukaryotic cell

4. Write short notes on fluid mosaic model of membrane.

As proposed by Singer and Nicolson in 1972, membrane is made up of **lipid bilayer** with embedded **proteins** (enzymes, transporters and receptors). Membrane lipids are amphipathic in nature, so they spontaneously form a bilayer in aqueous medium, by arranging their hydrophilic ends exposed to water and hydrophobic tails away from water (Fig. 1.2). **Membrane lipids** are mainly phospholipids, glycolipids and cholesterol.

- **Phospholipids:** Glycerophospholipids and sphingomyelin
- **Glycolipids:** Cerebrosides and gangliosides, present on the outer surface of the membrane
- **Cholesterol:** Provides fluidity to membrane.

Membrane lipids show lateral movements and flip-flop movements. Hence, membrane is **fluid in nature**. Hydrophobic interaction between the hydrocarbon tails in the phospholipids keeps the bilayer intact.

Factors Affecting Membrane Fluidity

- **Amount of unsaturated fatty acids:** More the unsaturated fatty acids, more will be the fluidity
- **Saturated fatty acids:** Decreases the membrane fluidity
- **Cholesterol:** Increases the membrane fluidity at low temperatures.

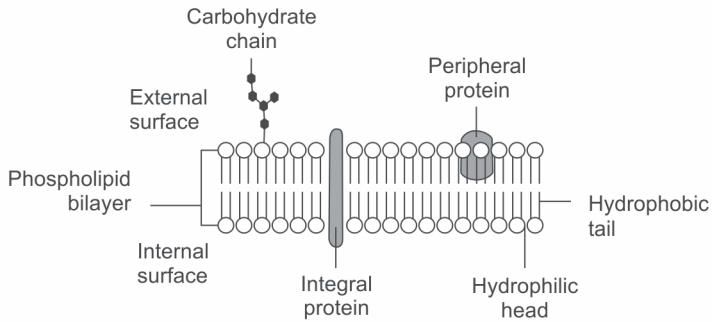


Fig. 1.2: Fluid mosaic model of membrane structure

Membrane Proteins

Peripheral membrane proteins: Are attached loosely to the surface of membrane bilayer.
Integral membrane proteins: Are deeply embedded in bilayer structure (proteins that extend all along the membrane bilayer are called transmembrane proteins).

Functions of Membrane Proteins

- Transport of molecules across the membrane
- Act as receptors
- Function as enzymes
- Components of respiratory chain.

Asymmetry in Membranes

The protein to lipid ratio varies in different membranes to suit their functions. For example, inner mitochondrial membrane, which has electron transport chain, is rich in proteins with protein and lipid ratio of 3.2, whereas in myelin sheath, which is designed to insulate the nerve fibers, this ratio is 0.23. Also, there is asymmetry with respect to distribution of phospholipids. For example, phosphatidylcholine, sphingomyelin are predominantly on the outer leaflet and phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine are predominantly on the inner leaflet.

5. Write the functions of plasma membrane.

Plasma membrane is a barrier with selective permeability. It is made up of lipids and proteins. It separates the cell from external environment and divides the interior of cell into different compartments. Fluid outside the membrane is called extracellular fluid and inside the cell is intracellular fluid.

Functions of Plasma Membrane

- Protects cytoplasm and organelles
- Maintains shape and size of the cell
- Selective barrier—permits transport of required substances in either direction
- Cell-cell interaction
- Signal transmission.

6. Describe the characteristics of facilitated diffusion. Mention two examples of transport by facilitated diffusion.

Definition: Movement of particles along the concentration gradient with the help of transport proteins. Facilitated diffusion does not require energy, e.g. transport of glucose, galactose, leucine and other amino acids.

Mechanism: Ping-pong Model

Carrier protein has two conformations—ping and pong conformation: Pong conformation of the carrier protein exposes it to higher concentration of molecules (solute) to be transported. Binding of molecules induces conformational change in the carrier protein to ping state, which exposes it to lower concentration of the molecules resulting in their release. Once the molecules are released, the conformation of the carrier protein reverts back to pong form (Fig. 1.3).

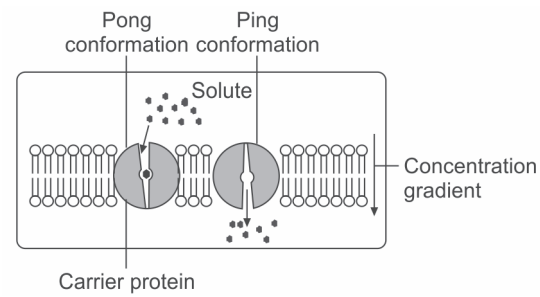


Fig. 1.3: Facilitated diffusion

7. Explain active transport with suitable examples.

Definition: Carrier-mediated transfer of molecules against the concentration gradient (from lower concentration to higher concentration) with the help of energy [adenosine triphosphate (ATP)]. Substances that are actively transported through cell membranes include Na^+, K^+, Ca^{2+}, Fe^{2+}, H^+, Cl^-, I^-. Active transport is susceptible to inhibition and this property is used for designing of drugs in some diseases.

Classification

i. **Primary active transport:** Transport of substrate against its concentration gradient with utilization of energy directly. For example, Na^+-K^+ ATPase, Ca^{2+}-pump, H^+-pump.
ii. **Secondary active transport:** ATP is used **indirectly** for transport.
For example,
Symport: Glucose-sodium cotransport, amino acid-sodium cotransport; two different substances are carried across the membrane in the same direction.
Antiport: Sodium-calcium cotransport, sodium-hydrogen pump; two different substances are carried across the membrane in the opposite direction.

Primary Active Transport

Na^+-K^+ ATPase: It pumps 3 Na^+ from inside to outside of the cell and brings in 2 K^+ from outside to inside of the cell against their concentration gradient, using energy provided by hydrolysis of one ATP molecule (Fig. 1.4).

Inhibitor of Na^+-K^+ ATPase and its significance:

Digoxin: Used in the treatment of congestive cardiac failure (CCF).

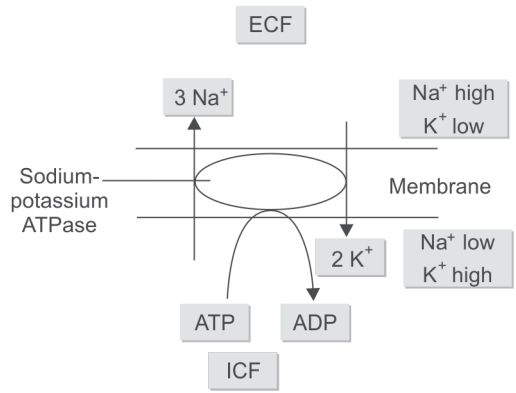


Fig. 1.4: Na^+-K^+ antiport (ECF, extracellular fluid; Na^+, sodium ion; K^+, potassium ion; ADP, adenosine diphosphate; ICF, intracellular fluid)

Secondary Active Transport

For example, Na^+-glucose cotransport (Fig. 1.5). The Na^+- K^+ ATPase in the basolateral membrane of the cell transports Na^+ out of the cell with the help of energy (ATP hydrolysis) creating a Na^+ gradient. This Na^+ gradient is used by sodium-glucose cotransporter—sodium moves

along its concentration gradient into the cell pulling glucose along with it against its gradient. Hence, energy is utilized indirectly.

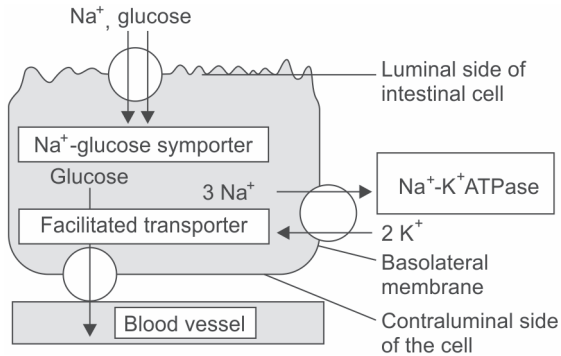


Fig. 1.5: Sodium-glucose cotransport

8. Describe transport processes across the membrane.

Membrane is a selectively permeable barrier. Non-polar substances gain easy access because of solubility in lipid bilayer, but polar substances cross the membrane selectively.

Selectivity of membrane transport depends upon:

i. **Size of molecules:** Small solutes pass through easily than larger ones.
ii. **Charge of the molecule:** Molecules with less charge pass through the membrane easily than one with more charges.
iii. **Transport proteins:** Specific proteins transport specific molecules.
iv. **Type of molecules:** Water readily traverses through the membrane.

Classification of transport mechanisms across the membrane:

i. Passive transport.
ii. Active transport.
iii. Endocytosis/exocytosis.
iv. Ionophores.

Passive transport: Simple diffusion, facilitated diffusion and transport through ion channels.

Simple diffusion

Definition: Movement of the particles across the membrane, along the concentration gradient, without any involvement of carrier proteins. Energy is not required for simple diffusion.

For example, small and lipophilic molecules like O_2, CO_2, N_2 and H_2O are transported by this process.

Facilitated diffusion (Refer question number 6)

Definition: Movement of the particles with the help of transport proteins along the concentration gradient. Facilitated diffusion does not require energy and is carried out by Ping-Pong mechanism (refer Fig. 1.3), e.g. glucose, galactose, leucine and other amino acids.

Ion channels

Ions pass through the ion channels, which open or close in response to a signal. Ion channels are:

i. *Voltage gated:* Open due to changes in membrane potential, e.g. Ca^{2+}, Na^+ and K^+ channels.
ii. *Ligand gated:* Binding of ligand to receptor site results in opening and closing of the channel, e.g. acetylcholine receptor.

Active transport (Refer question number 7)

Endocytosis and exocytosis

Endocytosis

Uptake of macromolecules into the cells. For example, uptake of low-density lipoproteins (LDL), polysaccharides, proteins and polynucleotides.

Two types:

i. *Pinocytosis:* Uptake of fluid and fluid contents by cell (cellular drinking).
ii. *Phagocytosis:* Ingestion of larger particles like bacterial cells and tissue debris by macrophages, which are further hydrolyzed by lysosome.

Exocytosis

Release of macromolecules from the cell to outside. For example, calcium-dependent secretion from vesicles (secretion of hormones).

Ionophores

Ionophores are the molecules that facilitate transport of ions across membranes.

Two types:

i. *Carrier ionophores:* They increase permeability for a particular ion, e.g. valinomycin transports K^+ and inhibits oxidative phosphorylation.
ii. *Channel-forming ionophores:* They facilitate passage of ions by forming channels, e.g. gramicidin A inhibits oxidative phosphorylation by facilitating movement of Na^+ and K^+ across the membrane.

Key Points

Hartnup disease: Defect in absorption of neutral amino acids in intestine and their defective reabsorption in kidney.

Cystinuria: Defect in reabsorption of cysteine in kidney.

Vitamin D-resistant rickets: Defective renal reabsorption of phosphate from kidney.

Myasthenia gravis: Defect in acetylcholine receptors (ligand-gated channels).

Cystic fibrosis: Due to mutation in chloride channels.

Digoxin: Inhibitor of sodium-potassium ATPase. Inhibition of this pump by digoxin will increase intracellular calcium concentration and myocardial contractility. So, digoxin is useful in the treatment of congestive cardiac failure.

Omeprazole: Inhibitor of hydrogen-potassium ATPase. Omeprazole inhibits gastric acid secretion, hence is used in the treatment of peptic ulcer.

Facilitated transporters: It can be classified with regard to direction of solute movement as:

- **Uniport:** Movement of one molecule at a time (bidirectional) by transporter, e.g. transport of fructose in intestine
- **Symport:** Movement of two different molecules simultaneously in the same direction, e.g. sodium-glucose transport in the intestine
- **Antiport:** Movement of two different molecules simultaneously in the opposite direction, e.g. chloride-bicarbonate transport in the red blood cell (RBC).

2 Enzymes

1. What are coenzymes? Explain with two examples.

Definition: The non-protein, organic, low-molecular weight substances associated with enzymes and required for their biological activity are called coenzymes.

For example,

$$\text{Pyruvate} \xrightarrow[\textit{NAD}^+,\ \textit{TPP, FAD, CoASH, lipoic acid}]{\text{Pyruvate dehydrogenase complex}} \text{Acetyl-CoA}$$

$$\alpha\text{-ketoglutarate} \xrightarrow[\textit{TPP, NAD}^+,\ \textit{FAD, CoASH, lipoic acid}]{\alpha\text{-ketoglutarate dehydrogenase complex}} \text{Succinyl-CoA}$$

Nicotinamide adenine dinucleotide (NAD^+), thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), CoASH and lipoic acid are coenzymes.

2. What are cofactors? Explain with two examples.

Definition: Inorganic groups that bind in a transient, dissociable manner either to the enzyme or to a substrate are called cofactors (Table 2.1).

Table 2.1: Enzymes and their cofactors

Enzyme	Cofactor required
Carbonic anhydrase, alcohol dehydrogenase	Zinc
Enolase	Manganese
Cytochrome oxidase, catalase, peroxidase	Iron
Xanthine oxidase	Molybdenum
Salivary amylase	Chloride
Kinase	Magnesium

3. Explain enzyme specificity with suitable examples.

Definition: Enzyme specificity is defined as the ability of an enzyme to bind to just one substrate from a group of similar compounds. Because of specificity for a substrate, more than one enzyme can exist in a cell without affecting the function of the other (Table 2.2).

Table 2.2: Types of specificity of enzymes

Classification	Definition and properties	Example
Absolute specificity	Act on only one substrate and catalyze one reaction	Glucose $\xrightarrow{\textit{Glucokinase}}$ Glucose-6-phosphate Lactose $\xrightarrow{\textit{Lactase}}$ Glucose + Galactose
Group specificity	Act on specific bond or group of substrates	*Phosphatase:* Hydrolyze organic phosphates *Exopeptidase:* Hydrolyze terminal peptide bonds
Reaction specificity	Enzymes are specific for a particular reaction even though the substrate is same for each reaction	Acetyl-CoA $\xleftarrow{1}$ Pyruvate $\xrightarrow{2}$ Lactate Pyruvate $\xrightarrow{3}$ Oxaloacetate Pyruvate $\xrightarrow{4}$ Alanine
Stereospecificity	Act on only one type of stereoisomer	L-amino acid $\xrightarrow{\textit{L-amino acid oxidase}}$ Keto acid D-amino acid $\xrightarrow{\textit{D-amino acid oxidase}}$ Keto acid

1, pyruvate dehydrogenase complex; 2, lactate dehydrogenase; 3, pyruvate carboxylase; 4, alanine transaminase.

4. Define and classify enzymes with suitable examples.

Definition: Enzymes are colloidal, thermolabile, biological catalysts, which are protein in nature. They are classified into six classes [Mnemonic (MN): OTH LIL = On The Heaven Life Is Luxurious].

i. **Oxidoreductases:** They catalyze oxidation-reduction reactions.

For example,

$$\text{Ethanol} + NAD^+ \xrightarrow{\textit{Alcohol dehydrogenase}} \text{Acetaldehyde} + NADH + H^+$$

$$\text{Lactate} + NAD^+ \xrightarrow{\textit{Lactate dehydrogenase}} \text{Pyruvate} + NADH + H^+$$

ii. **Transferases:** A group, other than hydrogen, is transferred from one substrate to other by transferases.

For example,

$$\text{Glucose + ATP} \xrightarrow{\textit{Hexokinase}} \text{Glucose-6-phosphate + ADP}$$

$$\text{Fructose + ATP} \xrightarrow{\textit{Fructokinase}} \text{Fructose-1-phosphate + ADP}$$

iii. **Hydrolases:** Catalyze hydrolysis of ester, ether, peptide, glycosidic bonds by addition of water.

For example,

$$\text{Lactose + } H_2O \xrightarrow{\textit{Lactase}} \text{Glucose + Galactose}$$

$$\text{Maltose + } H_2O \xrightarrow{\textit{Maltase}} \text{Glucose + Glucose}$$

iv. **Lyases:** Catalyze removal of groups or break bonds (without hydrolysis).

For example,

$$\text{Fructose-1,6-bisphosphate} \xrightarrow{\textit{Aldolase A}} \text{Glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate}$$

$$\text{2-phosphoglycerate} \xrightarrow{\textit{Enolase}} \text{Phosphoenolpyruvate (PEP)}$$

v. **Isomerases:** Catalyze optical, positional, geometrical isomerization of substrates.

For example,

$$\text{Glucose-6-phosphate} \xrightarrow{\textit{Phosphohexose isomerase}} \text{Fructose-6-phosphate}$$

$$\text{Glucose} \xrightarrow{\textit{Epimerase}} \text{Galactose}$$

vi. **Ligases:** Catalyze binding of two substrates by using energy (usually, hydrolysis of ATP).

For example,

$$\text{Acetyl-CoA + ATP + Biotin + } CO_2 \xrightarrow{\textit{Acetyl-CoA carboxylase}} \text{Malonyl-CoA + ADP + Pi + Biotin}$$

$$\text{Pyruvate + ATP + Biotin + } CO_2 \xrightarrow{\textit{Pyruvate carboxylase}} \text{Oxaloacetate + ADP + Pi + Biotin}$$

5. Write briefly on active site of an enzyme.

Definition: The active site of an enzyme is a three-dimensional structure that has amino acids or groups and occupies a small portion of the enzyme. It has substrate binding site (binds substrate non-covalently) and a catalytic site. It makes the reaction possible by:

- Bringing the reactive groups of substrate together (catalysis by proximity)
- Expelling water
- Stabilizing the transition state
- Lowering the activation energy.

Its specific interaction with substrate is explained by two theories:

i. Active site has a structure complementary to substrate **(lock and key theory)**.
ii. After binding to a substrate, the active site and enzyme undergo conformational change, which further facilitates the interaction **(induced fit theory)**.

Substrate Binding Site

Substrate binding site on the enzyme consists of certain groups (such as –OH, –SH, $–COO^-$), which recognize and bind to substrate to form enzyme-substrate (ES) complex.

Catalytic Site

Catalytic site enhances reaction rate by lowering energy of activation and converts the ES complex to enzyme + product (Fig. 2.1).

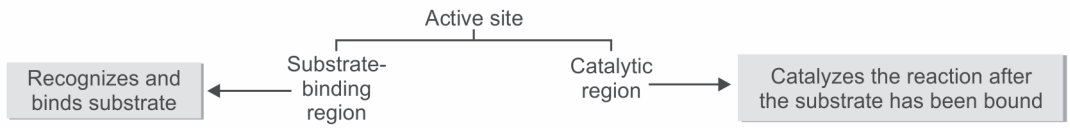


Fig. 2.1: Components of active site

6. Explain the models proposed for interaction of substrate with active site of an enzyme.

Lock and Key Model

Lock and key model was proposed by Emil Fisher in 1980. According to this model, the active site of an enzyme has a structure complementary to that of substrate (Fig. 2.2). The substrate binding site recognizes and binds the substrate through hydrophobic/electrostatic interactions or hydrogen bonds.

In this model, the interaction between substrate and the binding site is compared to a key fitting into a rigid lock. For example, most enzymes in carbohydrate metabolism can bind to D-isomers of hexoses, not L-isomers. This model does not explain interaction of the enzyme with allosteric modulators.

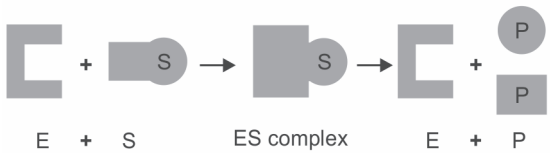


Fig. 2.2: Lock and key model (E, enzyme; S, substrate; P, product)

Induced Fit Model or Hand in Glove Model of Daniel E Koshland

According to this model, the shape of active site undergoes a change following binding of the substrate. Once the substrate binds to an enzyme, rapid conformational change occurs in the enzyme, which strengthens its interaction with the substrate (Fig. 2.3).

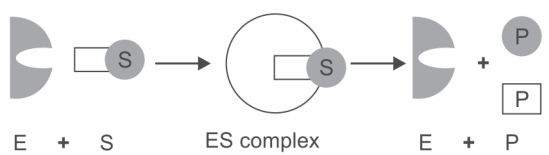


Fig. 2.3: Induced fit model (E, enzyme; S, substrate; P, product)

7. What are the various factors affecting enzyme activity? Explain with suitable diagrams.

Substrate Concentration

At low substrate concentration [S], most of the enzymes will be in unbound form (active site is free), so rate of reaction will be proportional (first-order kinetics) to (S). This reaches a point beyond which, any increase in substrate concentration causes a minimal increase in V; plateau/steady state is reached. This is called zero-order kinetics. At V_{max}, most of the enzymes will be in bound form (ES) and enzymes available for binding is very few or zero. In this state, a further increase in [S] does not have any effect on the rate of the reaction (Fig. 2.4).

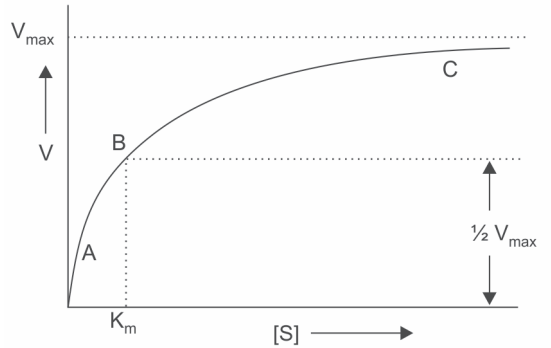


Fig. 2.4: Effect of substrate concentration on velocity of a reaction (A, first order; B, mixed order; C, zero-order kinetics; [S], substrate concentration; V, velocity of reaction)

Enzyme Concentration

When saturating amount of substrate is present, the velocity of a reaction is directly proportional to the amount of enzyme (Fig. 2.5).

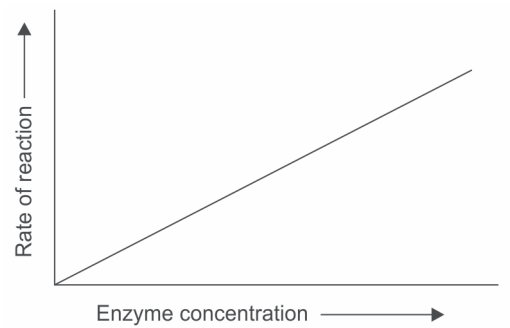


Fig. 2.5: Effect of enzyme concentration on velocity of reaction

Effect of pH on Enzyme Activity

Every enzyme has an optimum pH and activity of enzyme is highest at this pH. Above and below optimum pH, enzyme activity is decreased. Optimum pH varies from enzyme-to-enzyme. For example, optimum pH of pepsin is 2 and that of trypsin is 8. A bell curve is obtained on plotting enzyme activity against pH (Figs 2.6 and 2.7).

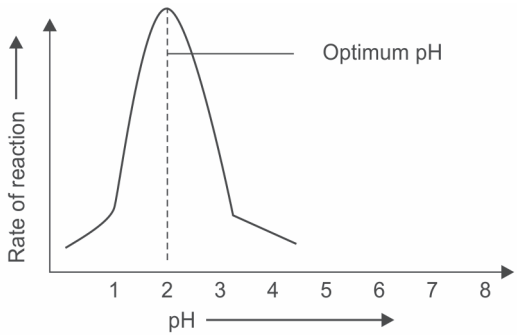


Fig. 2.6: Effect of pH on velocity of reaction

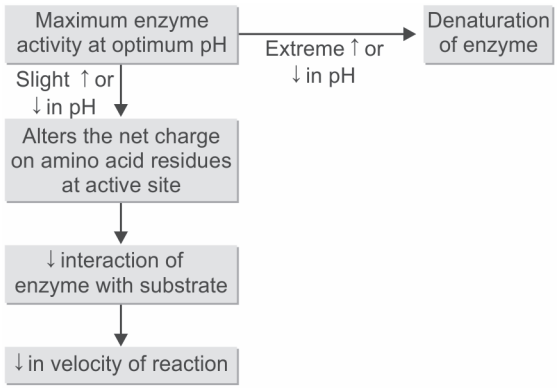


Fig. 2.7: Effect of pH on enzyme substrate interaction (↑, increase; ↓, decrease)

Effect of Temperature on Enzyme Activity

At extreme temperature, enzyme activity is lost. All human enzymes have maximum activity at body temperature (Figs 2.8 and 2.9).

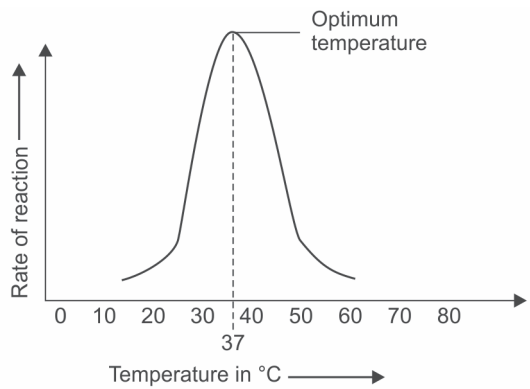


Fig. 2.8: Effect of temperature on velocity of reaction

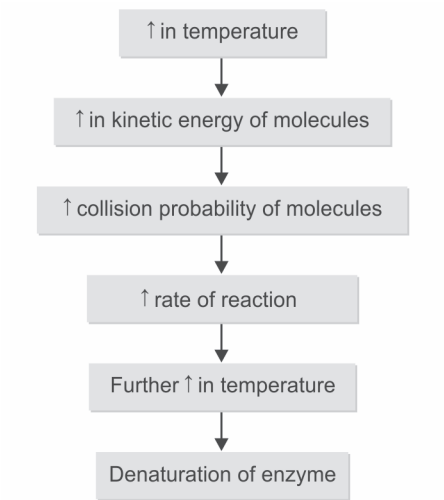


Fig. 2.9: Schematic diagram showing the effect of temperature on velocity of reaction

8. What is Michaelis-Menten equation? What is its significance?

Definition: It is an equation showing relationship between initial reaction velocity V_i and substrate concentration [S].

$$V_i = \frac{V_{max}\,[S]}{K_m + [S]}$$; V_{max} = Maximum velocity, K_m = Michaelis constant.

It can be used to calculate K_m or V_{max} of a reaction.

9. What is Michaelis constant? What does it signify?

Definition: Michaelis constant, denoted as K_m, is equal to the substrate concentration at half the maximal velocity of a reaction. It is inversely proportional to affinity of the enzyme for its substrate. This means higher is the K_m, lower is the affinity of the enzyme for the substrate (refer Fig. 2.4).

10. What is enzyme inhibition? What is its significance?

Definition: Enzyme inhibitors are molecules that interact with enzymes, thus decreasing the rate of enzymatic reaction. They can be substrate analogs, drugs, toxins or metal complexes.

The study of enzyme inhibition is important for understanding enzyme regulation, action of drugs and toxic agents on biological system.

Significance [MN: MATS]

- To elucidate the **M**etabolic pathways in cells
- To understand the nature of functional group at **A**ctive site of an enzyme and its mechanism of catalysis
- **T**herapeutic applications: **A**ntibiotics like penicillins, sulfonamides, antiviral drugs like acyclovir, anticancer drugs like 5-fluorouracil and methotrexate act by inhibiting enzymes
- To understand **S**ubstrate specificity of enzymes.

Types of Enzyme Inhibition

Enzyme inhibition is of different types as shown in Figure 2.10.

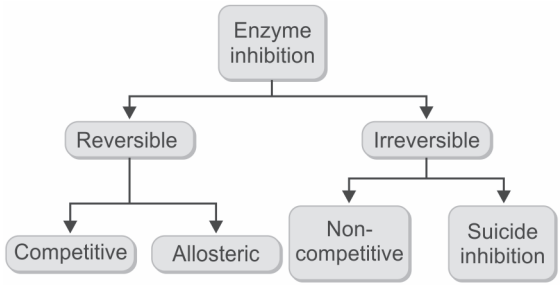


Fig. 2.10: Types of enzyme inhibition

Reversible Inhibition

Reversible inhibitors bind to enzyme through non-covalent bonds or by weak interaction. Activity of enzyme is restored when more substrate is added.

Irreversible Inhibition

Irreversible inhibitors bind to enzyme covalently or by strong interactions and inhibit the activity of enzyme, which cannot be restored.

11. Enlist the features of competitive inhibition. Give two clinically relevant examples.

Competitive inhibition

- Competitive inhibitor is similar to substrate in structure
- It binds to active site of the enzyme
- It competes with substrate for the active site of the enzyme
- Inhibition is reversible: Inhibition can be overcome by increasing substrate concentration
- During competitive inhibition, K_m is increased (affinity for substrate decreases as some active sites are occupied by the inhibitor), but V_{max} remains same.

For example,

$$\text{HMG-CoA} \xrightarrow{\textit{HMG-CoA reductase}} \text{Cholesterol}$$

This enzyme is competitively inhibited by Lovastatin, a drug used to treat hypercholesterolemia.

$$\text{Folic acid} \xrightarrow{\textit{Folate reductase}} \text{Tetrahydrofolic acid}$$

This enzyme is competitively inhibited by methotrexate, an anticancer drug.

12. Enlist the features of non-competitive inhibition. Give two examples.

Non-competitive inhibition

- The inhibitor is not similar to substrate in structure
- It does not bind to active site; it does not compete with substrate for binding to enzyme
- Inhibition is usually irreversible: Inhibition cannot be overcome by increasing substrate concentration
- During non-competitive inhibition, K_m is not altered (no change in substrate affinity), but V_{max} is reduced.

For example,

$$\text{2-phosphoglycerate} \xrightarrow{\textit{Enolase}} \text{Phosphoenolpyruvate}$$

Fluoride is a non-competitive inhibitor of enolase.

$$\text{Acetylcholine} \xrightarrow{\textit{Acetylcholinesterase}} \text{Acetate + Choline}$$

Acetylcholinesterase is inhibited by diisopropyl fluorophosphate (DIFP).

13. What is suicide inhibition? Explain with two examples.

Definition: It is a type of irreversible inhibition of an enzyme. After binding with active site of the enzyme, the inhibitor is converted to a more potent compound resulting in irreversible inhibition of the enzyme. This is also called mechanism-based inactivation (Table 2.3).

Table 2.3: Examples for suicide inhibition

Inhibitor	Target enzyme	Application
5-fluorouracil	Thymidylate synthase	Cancer treatment
Aspirin	Cyclooxygenase	Anti-inflammatory agent
Penicillin	Bacterial transpeptidase	Antibacterial agent
Deprenyl	Monoamine oxidase	Antidepressant, Parkinson's disease
Disulfiram	Aldehyde dehydrogenase	Alcohol de-addiction

14. Explain enzyme regulation.

Short-term Regulation

Short-term regulation is a quick regulation mechanism, which is based on altering the activities of existing enzymes. It is of two types (Fig. 2.11):

a. Allosteric regulation.
b. Covalent modification.

Allosteric Regulation

In allosteric regulation, the inhibitor is not a structural analog of substrate. Inhibition is partially reversible when concentration of substrate is increased. The inhibitor causes an increase in K_m and a decrease in V_{max}. This can be further subdivided into feedback inhibition and feedforward regulation.

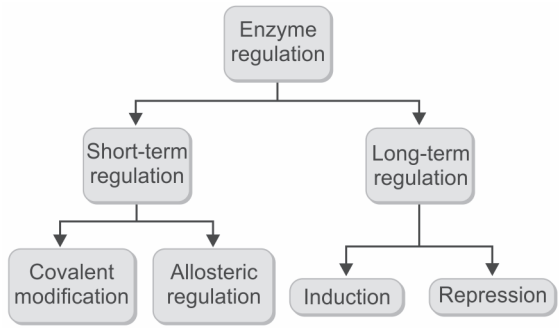


Fig. 2.11: Types of enzyme regulation

- Feedback allosteric inhibition: In multienzyme system, the first enzyme of the sequence of reactions can be inhibited by the end product (Fig. 2.12).

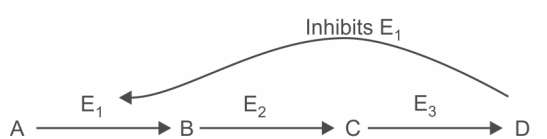


Fig. 2.12: Schematic diagram of feedback inhibition (E, enzyme)

For example,

i. In heme synthesis, end product heme inhibits first enzyme (ALA synthase).

ii. Cholesterol inhibits its own synthesis by blocking HMG-CoA reductase (Table 2.4).

Table 2.4: Allosteric enzymes and their modulators

Pathway	Enzyme	Inhibitor	Activator
Glycolysis	Phosphofructokinase-1	ATP and citrate	AMP
TCA cycle	Isocitrate dehydrogenase	ATP	ADP
Glycogenolysis	Glycogen phosphorylase	ATP	AMP
Gluconeogenesis	Pyruvate carboxylase	AMP	ATP, citrate, acetyl-CoA

- Feedforward regulation: Initial reactants in a reaction enhance their own metabolism by inducing downstream enzymes. Usually associated with metabolism of drugs, alcohol, poisons, etc.

  For example, alcohol intake ⟶ induction of cytochrome P4502E1 ⟶ increased metabolism of alcohol ⟶ tolerance to alcohol.

Covalent Modification

Covalent modification is the regulation of enzyme activity by addition of phosphate groups to specific serine, threonine or tyrosine residues of the enzyme or removal of attached phosphate from the above residues. Depending on specific enzyme, phosphorylation/dephosphorylation may lead to its activation/inactivation. For example, phosphorylation of glycogen phosphorylase increases its activity, whereas addition of phosphate to glycogen synthase decreases its activity (Fig. 2.13).

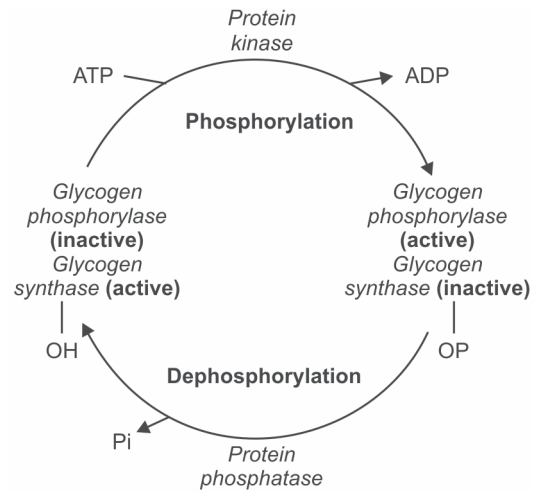


Fig. 2.13: Reversible covalent modification

Long-term Regulation

Long-term regulation involves altering concentration of enzyme by increasing *(induction)* or decreasing *(repression)* enzyme synthesis at genetic level.

Induction (Derepression)

Here, enzyme activity is increased by 1,000 or million times by increasing synthesis of the enzyme. For example, induction of lactose operon in *Escherichia coli* by lactose.

Other examples:

- *Glucose:* (+) Glucokinase
- *Barbiturates:* (+) ALA synthase
- *Glucocorticoids:* (+) Transaminase.

Note: (+) = Induction.

Repression

Here, enzyme activity is decreased by 1,000 to million times by stopping translation of enzyme at genetic level. For example,

- Heme: (–) ALA synthase
- Cholesterol: (–) HMG-CoA reductase.

Note: (–) = Repression.

15. What are isoenzymes? Explain their clinical importance with an example.

Definition: Different molecular forms of an enzyme synthesized from different organs and catalyzing the same reaction are called isoenzymes, e.g. isoenzymes of lactate dehydrogenase and creatine phosphokinase.

Lactate Dehydrogenase

Normal serum level is 100–200 U/L. LDH is a tetramer containing two types of polypeptide chains—M (muscle) type and H (heart) type. It has five isoenzymes (Table 2.5).

Table 2.5: Various isoenzymes of lactate dehydrogenase

Type	Composition	Location	Concentration as % of total	Electrophoretic mobility	Elevated in
LDH1	HHHH	Heart	30	Fast moving	Myocardial infarction
LDH2	HHHM	RBC, kidneys	35	–	Anemia, renal disease
LDH3	HHMM	Brain	20	–	Leukemia
LDH4	HMMM	Lungs, spleen	10	–	Pulmonary infarction
LDH5	MMMM	Liver, muscle	5	Slowest	Liver/muscle disease

Creatine Phosphokinase

Normal serum level is 15–100 IU/L. Creatine kinase is a dimer and is made up of two types of polypeptide chains—M (muscle) type and B (brain) type. It exists as three different isoenzymes (Table 2.6).

Table 2.6: Various isoenzymes of creatine kinase					
Type	**Composition**	**% of total**	**Location**	**Electrophoretic mobility**	**Elevated in**
CK1	BB	1	Brain	Fast moving	–
CK2	MB	5	Heart	–	Myocardial infarction
CK3	MM	80	Skeletal muscle	Slow moving	Muscular dystrophy

16. Explain the role of enzymes in diagnosis of acute myocardial infarction.

Serum enzyme levels during acute myocardial infarction is shown in Figure 2.14. The role of isoenzymes in diagnosis of acute myocardial infarction is given in Table 2.7.

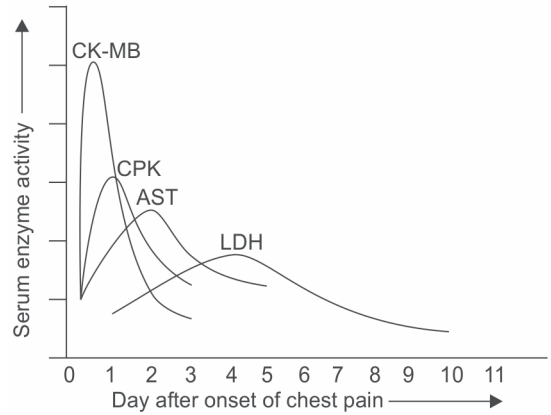


Fig. 2.14: Serum enzymes during acute myocardial infarction

Table 2.7: Serum enzymes in acute myocardial infarction			
Enzyme	**Starts increasing (after onset of chest pain)**	**Peak level**	**Normalizes by**
CPK	Within 3–6 hour	12–24 hour	3rd day
AST	Within 6–12 hour	48 hour	4th–5th day
LDH	After 2nd day	3rd–4th day	10th day

17. Enlist some enzymes and conditions where they are elevated.

The enzymes in various clinical conditions is given in Table 2.8.

Table 2.8: Enzymes in clinical diagnosis

Enzyme	Condition where it is elevated
Amylase	Acute pancreatitis
AST*	Myocardial infarction, hepatitis
ALT†	Hepatitis
ALP‡	Obstructive jaundice, bone disease
ACP§	Prostate cancer
LDH‖	Myocardial infarction, liver/muscle disease
CPK¶	Myocardial infarction, muscle disease
5'-nucleotidase	Obstructive jaundice
GGT**	Alcoholic liver disease
Lipase	Acute pancreatitis

*AST, aspartate transaminase; †ALT, alanine transaminase; ‡ALP, alkaline phosphatase; §ACP, acid phosphatase; ‖LDH, lactate dehydrogenase; ¶CPK, creatinine phosphokinase; **GGT, γ-glutamyl transferase.

Key Points

Q_{10}: Q_{10} or temperature coefficient is the factor, by which the rate of a biologic process increases for a 10°C increase in temperature. For temperature range over which enzymes are stable, the rates of most biologic processes typically double for a 10°C rise in temperature (Q_{10} = 2).

Substrate: The molecule acted upon by the enzyme to form product.

Apoenzyme: It is the protein part of the enzyme without any prosthetic groups.

Prosthetic group: It is non-protein part of an enzyme bound to an apoenzyme.

Holoenzyme: Cofactor or prosthetic group + apoenzyme.

Maturity onset diabetes of the young (MODY): Occurs due to decreased glucokinase activity that results in lower insulin secretion for a given blood glucose level.

Febuxostat: It is a non-purine substrate analogue of xanthine oxidase, which is used in the treatment of gout.

Fluoride: Non-competitively inhibits enolase of glycolysis in RBC and is used as an additive when collecting blood for glucose estimation.

Organophosphorus compounds: Accidental or suicidal ingestion of DIFP, nerve gases (sarin, tabun) and insecticides act like poison and non-competitively inhibit acetylcholinesterase $\rightarrow\uparrow$ acetylcholine $\rightarrow\uparrow$ bronchosecretion, intestinal motility and salivation; bradycardia, hypotension, constriction of pupil, etc.

Methanol poisoning: Methanol is a common adulterant in spurious liquor. This can lead to blindness, organ failure, acidosis and finally death. Methanol gets metabolized to formic acid, which is very toxic. This can be prevented by administering ethanol to the patient which competitively blocks methanol metabolism.

Alcohol de-addiction: Disulfiram is a suicide inhibitor of aldehyde dehydrogenase. It blocks conversion of acetaldehyde to acetic acid. Whenever a person on such a drug consumes alcohol, acetaldehyde accumulates, which causes lot of unpleasant side effects like vomiting, hypotension, headache, flushing (aldehyde syndrome). This makes the person abstain from alcohol.

People from Asian origin are more sensitive to alcohol as compared to people from West: Asians have decreased activity of mitochondrial acetaldehyde dehydrogenase as compared to Western population → slow metabolism of acetaldehyde → dizziness, headache, flushing.

Streptokinase and urokinase (tissue plasminogen activator): Cleave Arg-Val bond in plasminogen to form active plasmin. They are useful for the treatment of myocardial infarction.

Uncompetitive inhibition: Inhibitor binds only to enzyme substrate complex. It decreases both V_{max} and K_m. For example, placental alkaline phosphatase is inhibited by phenylalanine.

Competitive inhibition: K_m is increased and V_{max} is unaltered in competitive inhibition (Table 2.9).

Table 2.9: Examples for competitive inhibitors

Inhibitor (analog)	Natural substrate	Target enzyme	Application
Malonate	Succinate	Succinate dehydrogenase	–
Sulfonamides	PABA	Pteroid synthetase	Antimicrobial agent
Dicumarol	Vitamin K	Epoxide reductase	Anticoagulant
Allopurinol	Xanthine	Xanthine oxidase	Gout
Methanol	Ethanol	Alcohol dehydrogenase	Methanol poisoning
Enalapril, captopril	Angiotensin I	Angiotensin-converting enzyme	Hypertension
Neostigmine	Acetylcholine	Acetylcholinesterase	Myasthenia gravis

Non-competitive inhibition: K_m is unaltered and V_{max} decreases in non-competitive inhibition (Table 2.10).

Table 2.10: Non-competitive inhibitors

Inhibitor	Enzyme
Arsenite	Glyceraldehyde-3-phosphate dehydrogenase
Fluoride	Enolase
Omeprazole	H^+-K^+ ATPase
Cyanide	Cytochrome oxidase
Diisopropyl fluorophosphate (DIFP)	Acetylcholinesterase

Specific proteolytic cleavage: Type of enzyme regulation in which enzymes are synthesized as zymogens and are subsequently activated by cleavage of one or few specific peptide bonds (irreversible covalent modification). For example, formation of pepsin from pepsinogen; chymotrypsin from chymotrypsinogen.

3 Chemistry of Carbohydrates

1. Define and classify carbohydrates with suitable examples.

Definition: Carbohydrates are polyhydroxy aldehydes (HC=O) or ketones (C=O) with an empirical formula $C_n(H_2O)_n$ (Fig. 3.1).

Exceptions: Deoxy sugars, e.g. deoxyribose—formula is $C_5H_{10}O_4$.

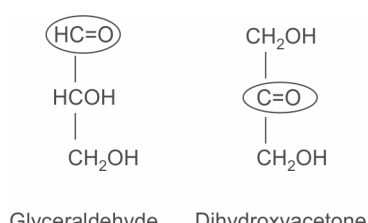


Fig. 3.1: Carbohydrates: Aldose (glyceraldehyde) and ketose (dihydroxyacetone)

Classification

i. **Monosaccharides:** Are simplest sugars, which cannot be hydrolyzed further to a simpler form of sugar, e.g. glucose, fructose.

ii. **Disaccharides:** Made up of two monosaccharide units joined by a glycosidic bond, e.g. sucrose, trehalose, lactose, maltose.

iii. **Oligosaccharides:** Made up of 3–10 monosaccharide units joined by glycosidic bonds, e.g. raffinose (glucose + galactose + fructose), stachyose (2 galactose + glucose + fructose), verbascose (3 galactose + glucose + fructose).

iv. **Polysaccharides:** Made up of more than 10 monosaccharide units joined by glycosidic bonds (Table 3.1). They are of two types.

a. **Homopolysaccharides:** Made up of **same** monosaccharide units joined by glycosidic bonds, e.g. starch, cellulose, glycogen, dextrin, inulin.
b. **Heteropolysaccharides (glycosaminoglycan):** Made up of **different** monosaccharide units joined by glycosidic bonds, e.g. heparin, chondroitin sulfate, dextran.

Table 3.1: Composition and importance of some polysaccharides

Sugar	Composition and importance
Inulin	Homopolysaccharide; used for measuring glomerular filtration rate
Hyaluronic acid	N-acetylglucosamine + glucuronic acid, not covalently attached to protein; lubricant, shock absorber—present in synovial fluid, vitreous humor, umbilical cord, loose connective tissue
Chondroitin sulfate	N-acetylgalactosamine + glucuronic acid; present in cartilage, tendons, ligaments
Dextran	Heteropolysaccharide; used as plasma volume expander
Dermatan sulfate	N-acetylgalactosamine + L-iduronic acid; present in skin, blood vessels, heart valves
Heparin	Glucosamine + glucuronic acid or iduronic acid; present in liver, lung, spleen; anticoagulant
Keratan sulfate	N-acetylglucosamine + galactose; present in cartilage, cornea; only glycosaminoglycan not having uronic acid

2. What are the various functions of carbohydrates?

[MN: TERN]

- Part of connective **T**issue, e.g. hyaluronic acid in joints
- Source of **E**nergy: 65% of the calorie requirement comes from carbohydrates
- Fiber (**R**oughage): Relieves constipation, e.g. cellulose
- Part of **N**ucleic acids: Ribose in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

3. Explain different types of carbohydrate isomers with examples.

Definition: Compounds, which have **same molecular formula**, but:

i. Have different structures are called structural isomers (Fig. 3.2).

For example, glucose, galactose and fructose.

ii. Have different spatial configuration are called stereoisomers (refer Fig. 3.2).

a. *Epimers:* Stereoisomers, which differ in orientation of H and OH around only one carbon. For example, glucose and galactose (4 epimer of glucose).

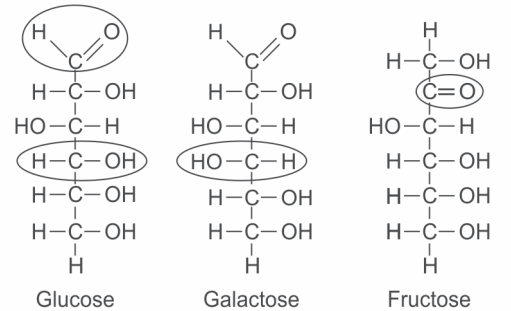


Fig. 3.2: Isomers of glucose

b. *Enantiomers:* Stereoisomers, which are mirror images of each other. For example, D- and L-glucose (Fig. 3.3).

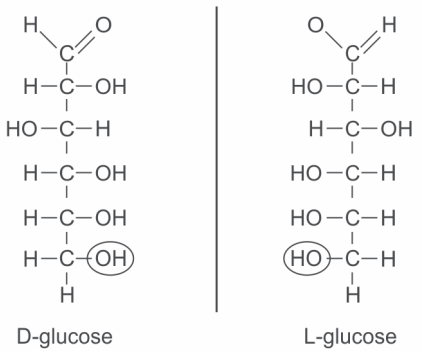


Fig. 3.3: Enantiomers

c. *Anomers:* Stereoisomers which differ in orientation of H and OH around anomeric carbon (Fig. 3.4, p. 28). For example, α-glucose and β-glucose.

iii. Have different optical rotation are called optical isomers: dextro (d or +) and levo (l or –).

- The rotation of polarized light either to the right or left determines whether an isomer is dextro (d) or levorotatory (l) respectively (Fig. 3.5).

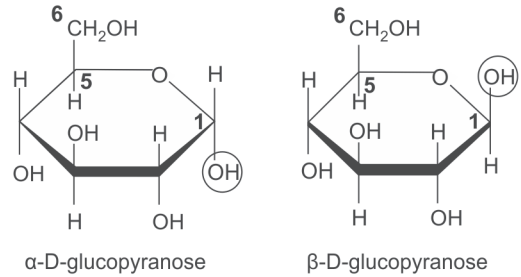


Fig. 3.4: Anomers [MN: **A**lpha-OH is not **A**bove, **B**eta-OH is not **B**elow]

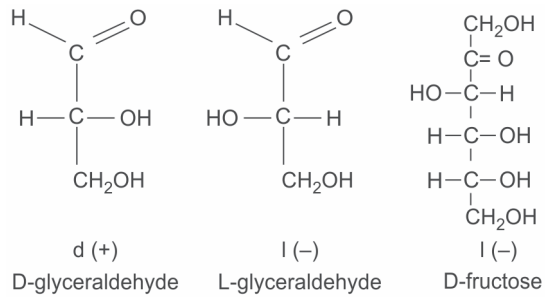


Fig. 3.5: Optical isomers

4. What are the modified sugars encountered in our metabolism?

Modified sugars are formed when the primary or secondary alcohol groups of sugars get oxidized/reduced or replaced by amino group to form sugar acids/sugar alcohols or amino sugars respectively. Some are often seen in our body tissues and genetic materials (Table 3.2 and Fig. 3.6).

Table 3.2: Modified sugars

Name	Modification	Examples
Deoxy sugars	OH group is reduced to H	Deoxyribonucleic acid (DNA)
Amino sugars	OH group replaced with amino group	Glucosamine, galactosamine
Sugar acid	Alcohol group of first carbon of glucose is oxidized to COOH	Gluconic acid

Contd...

Contd...

Name	Modification	Examples
Uronic acid	Last carbon of glucose is oxidized to COOH	Glucuronic acid
Saccharic acid	Alcohol groups of first and last carbon atoms of glucose are oxidized to COOH	–
Sugar alcohol	Reactive aldehyde group of sugar is reduced to alcohol	Sorbitol, mannitol, glycerol

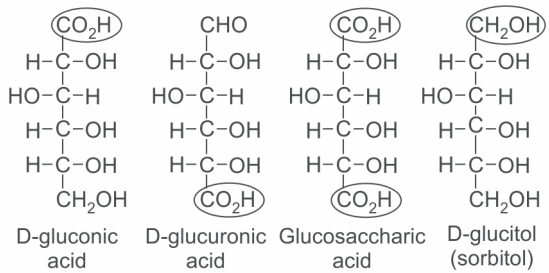


Fig. 3.6: Modified sugars

5. What is mutarotation? Explain with an example.

Definition: Mutarotation is defined as the change in specific optical rotation, with time, of a sugar in solution to reach an equilibrium mixture.

For example, glucose in solution has two forms α (+ 112.2°) and β (+ 18.7°); with time, an equilibrium mixture of the two is formed with net rotation of (+ 52.7°).

6. What is a glycoside? Give some examples.

Definition: Glycoside is a molecule formed when the hemiacetal or hemiketal hydroxyl group of anomeric carbon of a monosaccharide reacts with hydroxyl/amine group of another carbohydrate or a non-carbohydrate (methanol, phenol, glycerol, sterol) with removal of water. The non-carbohydrate moiety in a glycoside is referred to as aglycone (Table 3.3).

For example, streptomycin, erythromycin, ouabain, digitalis are glycosides.

Table 3.3: Glycoside and its components

Glycoside	Carbohydrate	Aglycone
Phlorizin (rose bark)	Glucose	Phloretin
Digitonin (foxglove)	Galactose/xylose	Digitogenin
Indican (stain)	Glucose	Indoxyl

7. Compare the structure of starch and glycogen.

The comparison between starch and glycogen is given in Table 3.4.

Table 3.4: Starch and glycogen—structural features

Starch	Glycogen
Reserve carbohydrate of plant kingdom	Reserve carbohydrate of animal kingdom
10%–20% amylose and 80% amylopectin	Predominantly amylopectin-like structure
15–18 glucose residues/branch	6–7 glucose residues/branch
Branch every 8–9 residue	Branch every 3–4 residues
Gives blue color with iodine	Red-brown/violet color with iodine

8. What are glycoproteins? Give some examples.

Definition: These are proteins to which short chain of oligosaccharides are attached. Unlike glycosaminoglycans, they lack uronic acids. They have comparatively less carbohydrates (protein > carbohydrate) than glycosaminoglycans.

For example,

- *Structural glycoproteins:* Collagen
- *Enzymes:* Ribonuclease, prothrombin
- *Glycoproteins in transport:* Ceruloplasmin, transferrin
- *Hormone:* Thyroid stimulating hormone (TSH)
- *Glycoproteins in immune system:* Blood group antigen, immunoglobulins
- *Membrane glycoprotein:* Glycophorin of red blood cells (RBCs)
- *Glycoprotein as lubricant:* Mucin
- *Receptor:* Hormone receptors and receptors on surface of viruses.

Key Points

Lactose: Lactose is a milk sugar, which is digested by the enzyme lactase. A deficiency of this enzyme can lead to lactose intolerance where the person cannot tolerate lactose in the diet.

Sucrose and trehalose: They are non-reducing sugars because anomeric hydroxyl group of individual subunits in both of them are involved in formation of glycosidic bonds. Non-reducing sugars will not answer Benedict's test, Barfoed's test and osazone test.

Inulin: It is a homopolysaccharide used for measuring glomerular filtration rate.

Hyaluronic acid: It is a heteropolysaccharide that is not covalently attached to protein. Hyaluronidase enzyme is present in sperm head—it breaks this sugar (present on the outer layer of human egg) and helps in entry of sperm. Absence of this enzyme on sperm head can lead to infertility.

Keratan: It is the only glycosaminoglycan not having uronic acid.

Dextran: It is a heteropolysaccharide used as plasma expander.

Sorbitol: It accumulates in lens in diabetics resulting in cataract.

Mannitol: It is an osmotic diuretic, which can be used to treat cerebral edema.

Olestra: It is an artificially synthesized glycoside using sucrose + fatty acids. It can be used as a fat substitute that adds no fat or calories.

Digitalis: It is a glycoside used to treat congestive cardiac failure.

4 Digestion, Absorption and Metabolism of Carbohydrates

1. Explain digestion and absorption of carbohydrates.

Digestion of Carbohydrates

Digestion of carbohydrate occurs in three phases: salivary phase, pancreatic phase and intestinal phase (Figs 4.1 and 4.2).

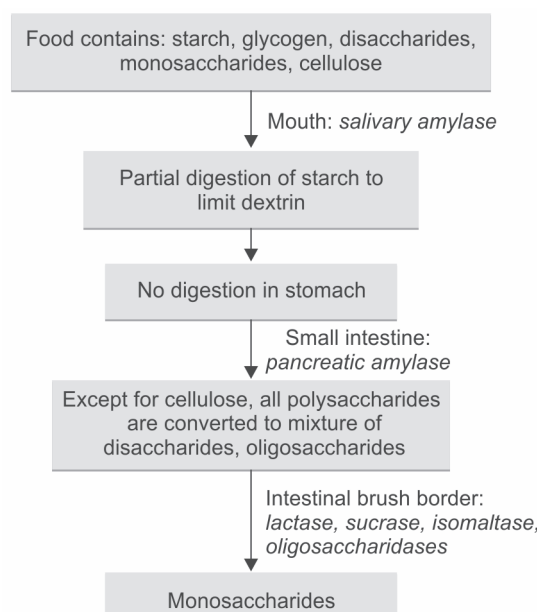


Fig. 4.1: Digestion of carbohydrates

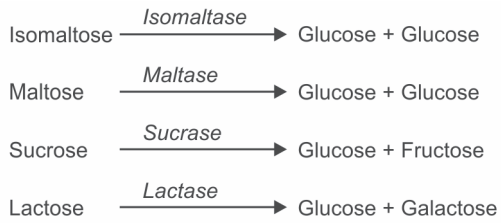


Fig. 4.2: Intestinal brush border disaccharidases

Absorption

All carbohydrates, except cellulose, are digested to respective monosaccharides.

Glucose, galactose and fructose (monosaccharides formed as end products of digestion) are initially absorbed by a common transporter GLUT-5 (passive facilitated diffusion) from luminal side into the intestinal cell.

Glucose and galactose are also absorbed by sodium-dependent glucose transporter-1 (SGLUT-1) (Fig. 4.3). Cellulose cannot be digested and absorbed and acts as a dietary fiber.

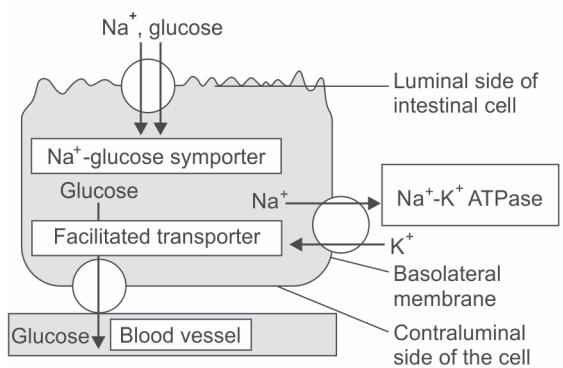


Fig. 4.3: Absorption of carbohydrates in the intestine (Na^+, sodium ion; K^+, potassium ion; ATPase, adenosine triphosphatase)

2. Write the individual reactions of glycolysis. Add a note on its energetics.

Glycolysis (Embden-Meyerhof pathway)

Definition: Glycolysis is a major pathway for glucose metabolism—glucose is converted to pyruvate or lactate.

Significance of Glycolysis

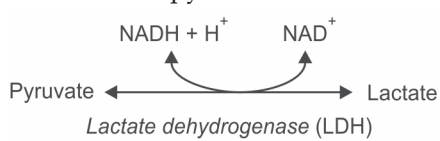
- Principal route for metabolism of glucose, fructose, galactose and other carbohydrates
- Operates in both aerobic and anaerobic conditions
- Lactate generated in anaerobic glycolysis is used for gluconeogenesis in the liver
- Anaerobic glycolysis is a major pathway that provides energy for skeletal muscles during strenuous exercise
- Hexokinase is allosterically inhibited by product glucose-6-phosphate, which favors storage of glucose in the liver as glycogen
- Deficiency of glycolytic enzymes (e.g. pyruvate kinase) results in hemolytic anemia
- Anaerobic glycolysis is the only pathway that provides energy for mature red blood cells (RBCs).

Requirements for Glycolysis

- **Site:** All the cells in the body
- **Subcellular site:** Cytosol
- **Starting material:** Glucose
- **End product:** Pyruvate in aerobic and lactate in anaerobic glycolysis
- **Coenzymes:** Nicotinamide adenine dinucleotide hydrogen (NADH), adenosine triphosphate (ATP)
- **Steps:** Divided into two phases (Fig. 4.4, p. 35)
  - **Energy investment phase:** Chemical priming phase requiring energy in the form of ATP
  - **Energy yielding phase:** Energy is generated in the form of ATP.

Anaerobic Glycolysis

For steps refer Figure 4.4 + conversion of pyruvate to lactate shown below:



In anaerobic conditions, NADH generated is not used for ATP generation in electron transport chain. NADH is used for conversion of pyruvate to lactate. This results in regeneration of nicotinamide adenine dinucleotide (NAD^+), which is required for continuation of glycolysis. So, in anaerobic glycolysis 4 ATPs are generated, while 2 ATPs are used up—hence, net yield is 2 ATPs.

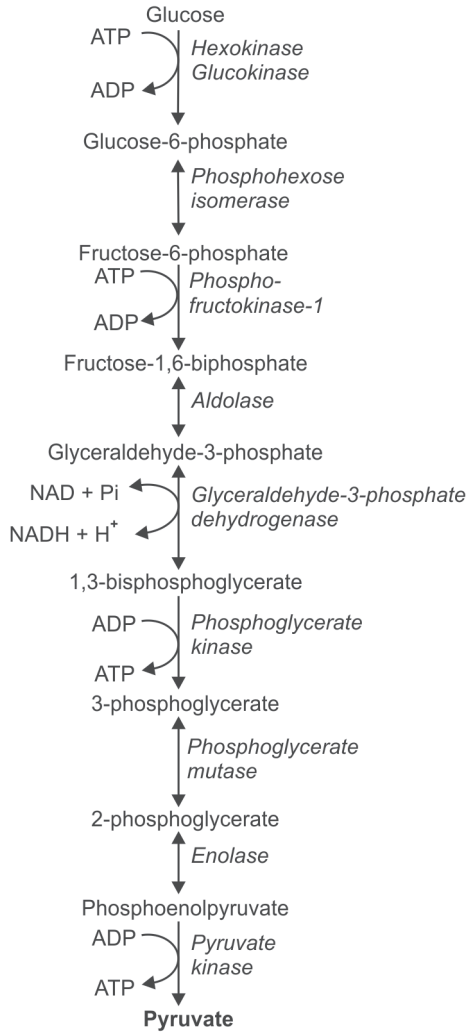


Fig. 4.4: Glycolysis (ATP, adenosine triphosphate; ADP, adenosine diphosphate; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; H^+, hydrogen ion)

Energetics of glycolysis: The number of ATPs generated per molecule of glucose in aerobic glycolysis is given in Table 4.1.

Table 4.1: Energetics of glycolysis (aerobic conditions)

Reaction	Enzyme	ATP formed (+) or utilized (–)
Glucose → Glucose-6-phosphate	Hexokinase/glucokinase	(–) 1 ATP
Fructose-6-phosphate → Fructose-1,6-bisphosphate	Phosphofructokinase	(–) 1 ATP
Glyceraldehyde-3-phosphate → 1,3-bisphosphoglycerate	Glyceraldehyde-3-phosphate dehydrogenase	(+) 5 ATP
1,3-bisphosphoglycerate → 3-phosphoglycerate	1,3-bisphosphoglycerate kinase	(+) 2 ATP
Phosphoenolpyruvate → Pyruvate	Pyruvate kinase	(+) 2 ATP
	Total	**7 ATPs**

(+) ATP, ATP yielding phase; (–) ATP, ATP investment phase.

3. Briefly write about the regulation of glycolysis.

Regulation of glycolysis is given in Table 4.2.

Table 4.2: Regulation of glycolysis

Key enzymes	Activated by (+)	Inhibited by (–)
Hexokinase	Insulin,	Glucagon,
Glucokinase	AMP*,	cAMP†, ATP,
Phosphofructokinase	NAD^+,	NADH + H^+,
Pyruvate kinase	CoASH	Acetyl-CoA,
Pyruvate dehydrogenase		Citrate

*AMP, adenosine monophosphate; †cAMP, cyclic adenosine monophosphate.

4. What is substrate level phosphorylation? Give any three examples for it.

Substrate level phosphorylation is a process by which ATPs are formed directly without going through electron transport chain. Examples for substrate level phosphorylation is given in Table 4.3.

Table 4.3: Substrate level phosphorylation		
Reaction	**Enzyme**	**ATP yield**
1,3-bisphosphoglycerate → 3-phosphoglycerate	1,3-bisphosphoglycerate kinase	1 ATP
Phosphoenolpyruvate → pyruvate	Pyruvate kinase	1 ATP
Succinyl-CoA → Succinate	Succinate thiokinase	1 ATP

5. Enlist inhibitors of glycolysis.

Inhibitors of glycolysis is given in Table 4.4.

Table 4.4: Inhibitors of glycolysis		
Inhibitor	**Enzyme inhibited**	**Outcome**
Fluoride	Enolase	Prevents glycolysis in RBCs—used during blood collection for glucose estimation
Arsenic	Pyruvate dehydrogenase	Lactic acidosis
Iodoacetate	Glyceraldehyde-3-phosphate dehydrogenase	Block in glycolysis and ATP formation

6. Explain metabolic disorders associated with glycolysis.

Metabolic disorders and its causes are given in Table 4.5.

Table 4.5: Disorders of glycolysis	
Disorders	**Causes**
Lactic acidosis	Diabetes mellitus, alcoholism, hypoxia, strenuous exercise, deficiency of pyruvate dehydrogenase/pyruvate carboxylase/thiamine
Hemolytic anemia	Deficiency of pyruvate kinase, hexokinase and aldolase A
Muscular hypotonia	Deficiency of pyruvate dehydrogenase complex/muscle phosphofructokinase
Neuronal loss, fatigue	–

7. What is BPG shunt (Rapoport-Luebering shunt)? What is its significance?

Bisphosphoglycerate (BPG) shunt pathway is shown in Figure 4.5.

- **Site:** Red blood cells (RBCs)
- **Subcellular site:** Cytosol
- **Significance:** *Important regulator of oxygen affinity of hemoglobin.* The levels of 2,3 BPG in RBC is increased during hypoxia. It helps to release oxygen from hemoglobin especially in cases of hypoxia or at high altitude, where oxygen saturation is less.

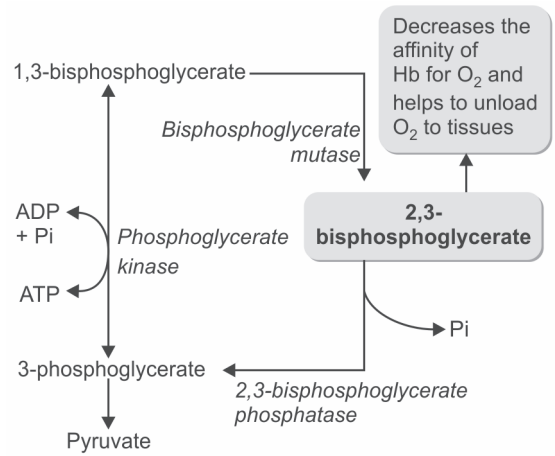


Fig. 4.5: Bisphosphoglycerate (BPG) shunt

8. **Write the reactions of tricarboxylic acid (TCA) cycle (Krebs cycle). Explain its energetics.**

Definition: It is the final common pathway for the oxidation of carbohydrates, lipids and proteins. It is the major pathway, which provides energy (Figs 4.6A and B).

- **Site:** All cells with mitochondria (except RBC)
- **Subcellular site:** Mitochondria
- **Starting material:** Acetyl-coenzyme A + Oxaloacetate
- **End product:** CO_2 + H_2O + ATP
- **Coenzymes:** NAD^+, flavin adenine dinucleotide (FAD), guanosine diphosphate (GDP), CoASH.

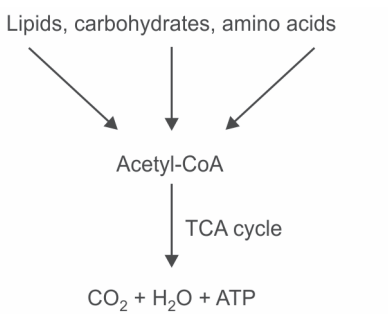


Fig. 4.6A: TCA cycle final common pathway

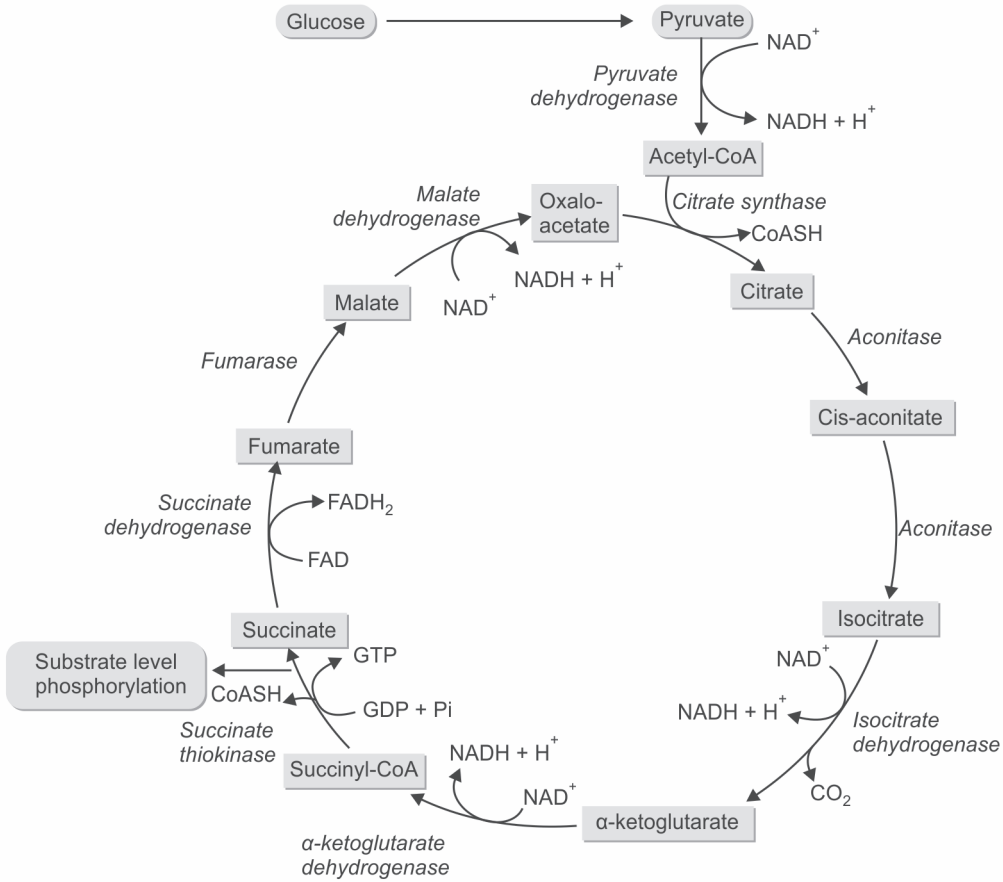


Fig. 4.6B: Tricarboxylic acid cycle (TCA cycle)

- **Energetics of TCA cycle** is given in the Table 4.6.

Table 4.6: Energetics of TCA cycle

Reaction	Enzyme	ATP formed (+)
Isocitrate → α-ketoglutarate	Isocitrate dehydrogenase	(+) 2.5 ATPs
α-ketoglutarate → Succinyl-CoA	α-ketoglutarate dehydrogenase	(+) 2.5 ATPs

Contd...

Contd...

Reaction	Enzyme	ATP formed (+)
Succinyl-CoA → Succinate	Succinate thiokinase	(+) 1 ATP
Succinate → Fumarate	Succinate dehydrogenase	(+) 1.5 ATPs
Malate → oxaloacetate	Malate dehydrogenase	(+) 2.5 ATPs
	Total	***10 ATPs**

*Per molecule of acetyl-CoA

9. Write a short note on the regulation of TCA cycle.

Regulation of TCA cycle is given in Table 4.7.

Table 4.7: Regulation of TCA cycle

Key enzymes	Inhibited by (–)	Activated by (+)
Pyruvate dehydrogenase complex	ATP,	AMP, ADP,
Citrate synthase	Citrate,	CoASH,
Isocitrate dehydrogenase	$NADH + H^+$,	NAD^+,
α-ketoglutarate dehydrogenase	Succinyl-CoA	Ca^{2+}

10. Enlist the inhibitors of TCA cycle.

List of inhibitors of TCA cycle are given in Table 4.8.

Table 4.8: Inhibitors of TCA cycle

Inhibitor	Enzyme inhibited	Type of inhibition
Fluoroacetate	Aconitase	Suicide inhibition
Arsenite	α-ketoglutarate dehydrogenase	Non-competitive inhibition
Malonate	Succinate dehydrogenase	Competitive inhibition

11. Explain the amphibolic role of TCA cycle.

Amphibolic means both anabolic and catabolic. TCA cycle, other than energy production (catabolic), is also involved in providing raw materials for biosynthesis of various compounds (anabolic). Examples are given in the Table 4.9.

Table 4.9: Anabolic reactions of TCA cycle

Anabolic reactions	Enzyme	Use
Pyruvate → Oxaloacetate	Pyruvate carboxylase	Gluconeogenesis
Citrate → Acetyl-CoA → Malonyl-CoA	Citrate lyase and acetyl-CoA carboxylase	Fatty acid biosynthesis
α-ketoglutarate → Glutamate	AST* and ALT†	Purine synthesis
Succinyl-CoA + glycine → ALA‡	ALA synthase	Heme synthesis
Oxaloacetate → Aspartate	AST	Pyrimidine synthesis

*AST, aspartate aminotransferase; †ALT, alanine aminotransferase; ‡ALA, aminolevulinic acid.

12. What are the anaplerotic reactions of TCA cycle?

Since TCA cycle is both anabolic and catabolic, the intermediates of this cycle are used up continuously. The reactions that can replenish these intermediates of TCA cycle are called anaplerotic reactions (Table 4.10).

Table 4.10: Anaplerotic reactions of TCA cycle

Anaplerotic reactions	Enzyme
Pyruvate + CO_2 + ATP → Oxaloacetate	Pyruvate carboxylase
Pyruvate + CO_2 + NADPH → Malate	Malic enzyme
Aspartate → Oxaloacetate	Aspartate transaminase (AST)
Glutamate → α-ketoglutarate	AST and alanine transaminase

13. Define gluconeogenesis. Write the reactions of this pathway.

Gluconeogenesis (Fig. 4.7) is the formation of glucose or glycogen from non-carbohydrate precursors.

- **Site:** Liver, kidneys
- **Subcellular site:** Mitochondria and cytosol
- **Starting material:** Lactate, pyruvate, glucogenic amino acids, glycerol, propionyl-CoA
- **End product:** Glucose
- **Coenzymes:** Guanosine triphosphate (GTP), NADH, NAD^+
- **Energy requirement:** To generate one molecule of glucose, 6 ATPs are required.

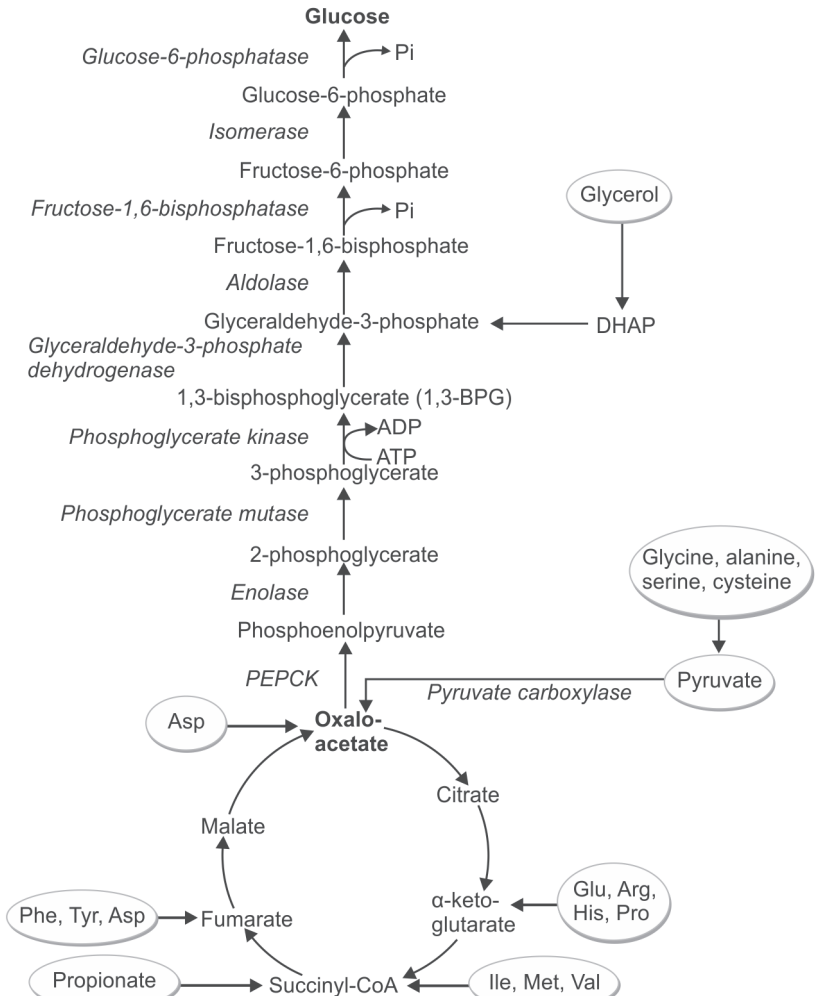


Fig. 4.7: Gluconeogenesis (First reaction occurs in mitochondria and rest in the cytosol. Oxaloacetate formed inside the mitochondria is transported to cytosol by malate shuttle) (PEPCK, phosphoenolpyruvate carboxykinase). Encircled are the substrates for gluconeogenesis.

Key Enzymes in Gluconeogenesis

- Pyruvate carboxylase: Pyruvate ⟶ Oxaloacetate
- Phosphoenolpyruvate carboxykinase (PEPCK): Oxaloacetate ⟶ Phosphoenol-pyruvate (PEP)
- Fructose-1,6-bisphosphatase: Fructose-1,6-biphosphate ⟶ Fructose-6-phosphate
- Glucose-6-phosphatase: Glucose-6-phosphate ⟶ Glucose.

14. What is Cori cycle?

The process of conversion of lactate formed in the muscles (under anaerobic conditions) to glucose in the liver, which is then converted to lactate in the muscle is called Cori cycle (Fig. 4.8).

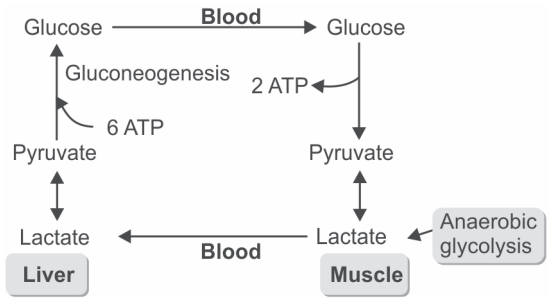


Fig. 4.8: Cori cycle

15. Explain the regulation of gluconeogenesis.

i. Allosteric regulation (Table 4.11).

Table 4.11: Allosteric regulation of gluconeogenesis

Key enzyme	Inhibited by (–)	Activated by (+)
Pyruvate carboxylase	ADP, AMP,	Citrate,
Phosphoenolpyruvate carboxykinase	CoASH,	Acetyl-CoA,
Fructose-1,6-bisphosphatase	NAD^+, insulin,	NADH + H^+,
Glucose-6-phosphatase	Fructose-2,6-bisphosphate	Anti-insulin hormones

ii. Regulation by compartmentalization.

First step of gluconeogenesis is inside mitochondria and rest of the steps are in cytosol.

16. Mention the non-carbohydrate substrates for gluconeogenesis.

Non-carbohydrate substrates of gluconeogenesis is given in Table 4.12.

Table 4.12: Substrates of gluconeogenesis

Substrate	Comment
Pyruvate and lactate	They are the primary substrates for gluconeogenesis
Amino acids	All except leucine and lysine
Propionate	From odd chain fatty acids and amino acids like methionine, isoleucine, valine
Alanine	During starvation, alanine released from muscles gets transaminated to pyruvate
Glycerol	From triacylglycerol breakdown in adipose tissue

17. Write the reactions of glycogenolysis.

Definition: Breakdown of glycogen into glucose-1-phosphate (Figs 4.9 and 4.10).

Requirements

- **Site:** Liver, muscle, kidney, intestine, brain
- **Subcellular site:** Cytosol
- **Starting material:** Glycogen
- **End product:** Glucose-1-phosphate (liver, muscle) and glucose (liver)
- **Key enzyme:** Glycogen phosphorylase.

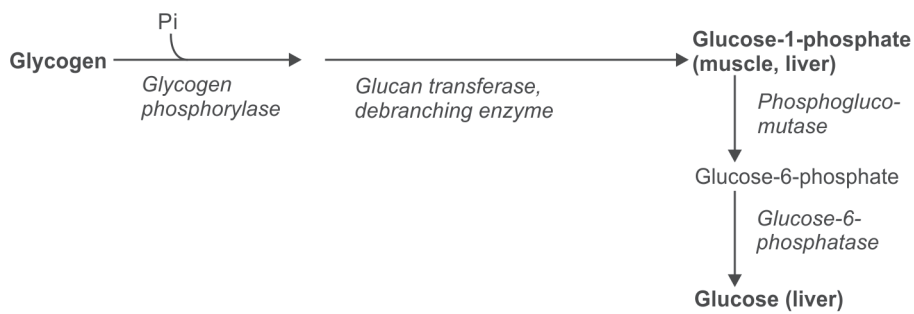


Fig. 4.9: Glycogenolysis

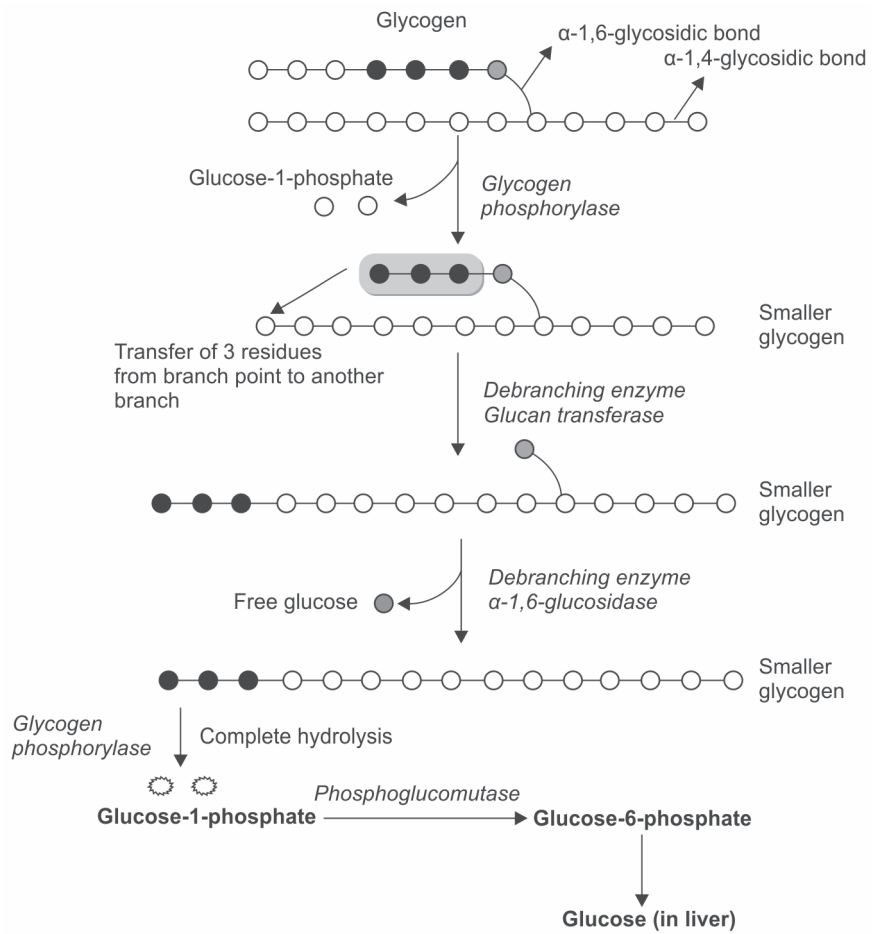


Fig. 4.10: Steps in glycogenolysis

18. Write the reactions of glycogenesis. Add a note on regulation of glycogen metabolism.

Definition: Synthesis of glycogen from glucose for storage in liver and muscle (Figs 4.11 and 4.12) is glycogenesis.

- **Site:** Liver, muscle
- **Subcellular site:** Cytosol—smooth endoplasmic reticulum
- **Starting material:** Glucose
- **End product:** Glycogen
- **Requirements:** Glycogenin, glycogen primer, uridine triphosphate (UTP)
- **Key enzyme:** Glycogen synthase.

Glucose has to be converted to UDP-glucose. Glycogen is formed by addition of glucose molecules on existing glycogenin to form a glycogen primer.

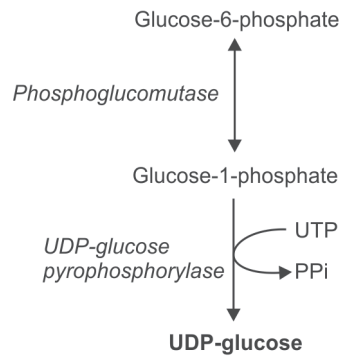


Fig. 4.11: Formation of UDP-glucose (UTP, uridine triphosphate; UDP, uridine diphosphate)

Regulation of glycogen metabolism: Hormones like glucagon, epinephrine and insulin affect glycogen metabolism (Table 4.13, Figs 4.13A and B, p. 48).

- Regulatory enzyme in glycogenolysis: Glycogen phosphorylase
- Regulatory enzyme in glycogenesis: Glycogen synthase.

Table 4.13: Allosteric regulation of glycogen metabolism

Enzyme	Activator	Inhibitor
Glycogen synthase	Glucose-6-phosphate	–
Glycogen phosphorylase	AMP (muscle isoform)	Glucose-6-phosphate, ATP (muscle and liver isoform)

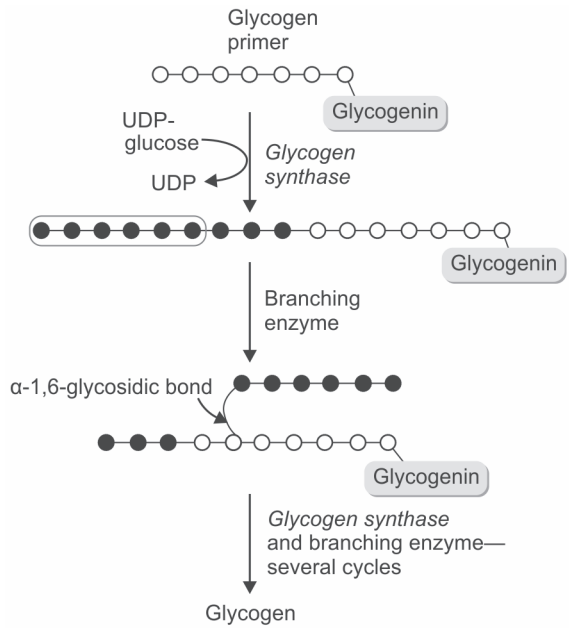


Fig. 4.12: Glycogen synthesis

19. Enlist the types of glycogen storage disorders with respective metabolic defects.

The types of glycogen storage disorders with respective metabolic defects are given in Table 4.14.

Table 4.14: Glycogen storage disorders

Disorder	Enzyme defect	Clinical features
Von Gierke (type I)	Glucose-6-phosphatase	Hepatomegaly, fasting hypoglycemia, lactic acidosis, hyperuricemia, ketosis
Pompe's disease (type II)	1,4-glucosidase	Cardiomegaly and early death
Cori's disease (type III)	Debranching enzyme	Hepatomegaly, hypoglycemia
Anderson's disease (type IV)	Branching enzyme	Hepatomegaly, cirrhosis of liver, early death due to liver and heart failure
McArdle's disease (type V)	Muscle phosphorylase	Muscle glycogen is high, exercise intolerance
Hers' disease (type VI)	Liver phosphorylase	Hepatomegaly, hypoglycemia

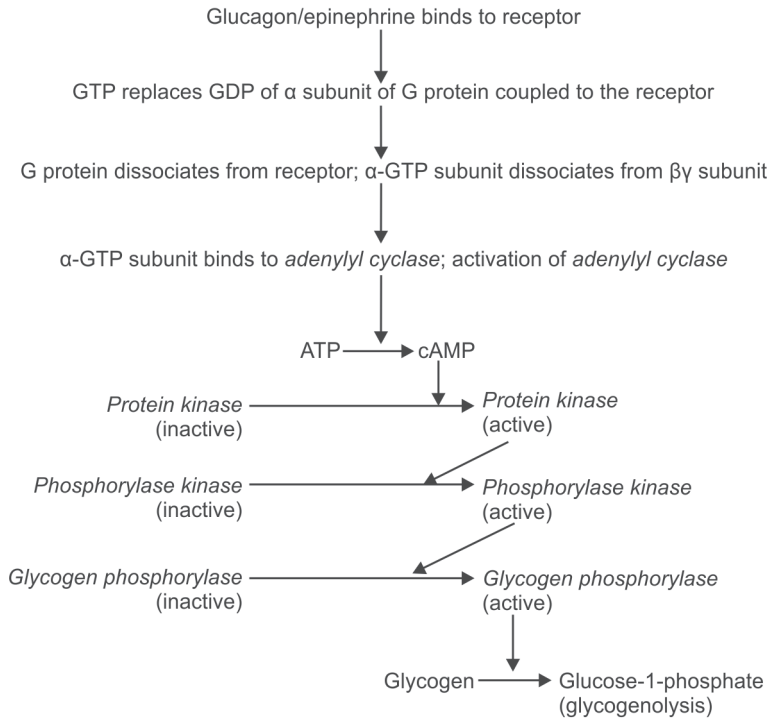


Fig. 4.13A: Regulation of glycogenolysis

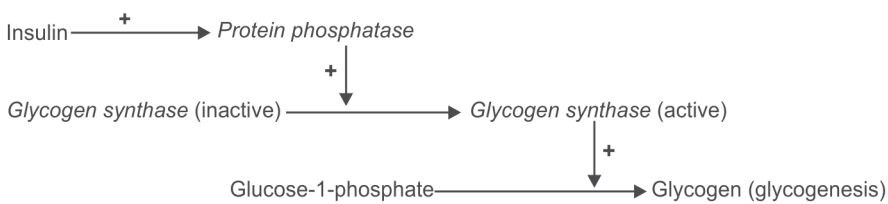


Fig. 4.13B: Regulation of glycogenesis

20. Write the reactions of pentose phosphate pathway (HMP shunt/direct oxidative pathway). Add a note on its significance.

Definition: Alternative pathway for glucose metabolism with production of NADPH and pentose sugars (Fig. 4.14).

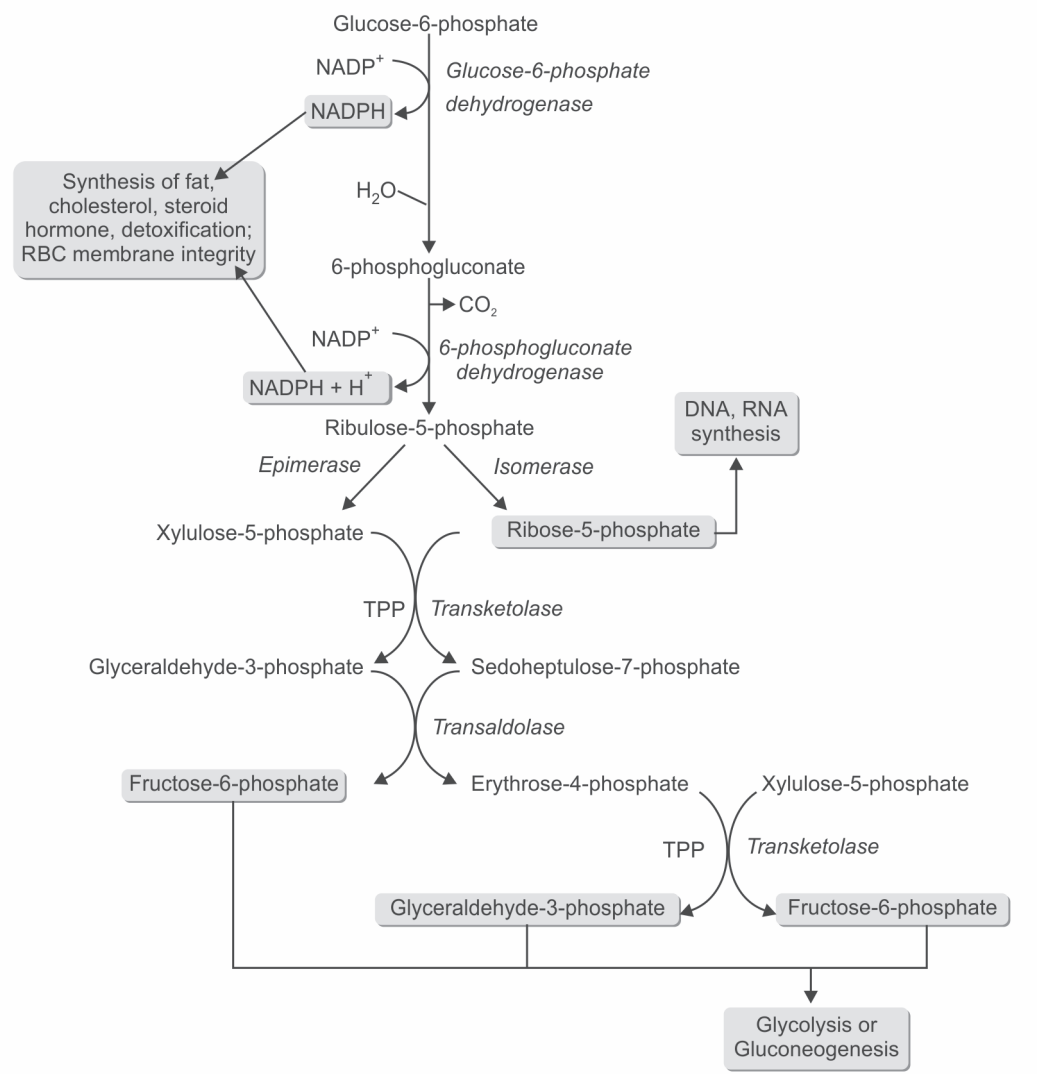


Fig. 4.14: Hexose monophosphate shunt

- **Site:** RBC, liver, intestine, lens, adrenal cortex, adipose tissue, gonads
- **Subcellular site:** Cytosol
- **Starting material:** Glucose
- **End products:** Pentose sugar and NADPH
- **Coenzymes:** NADP, thiamine pyrophosphate (TPP)
- **Cofactor:** Magnesium ion (Mg^{2+})
- **Regulatory enzyme:** Glucose-6-phosphate dehydrogenase.

i. Allosteric regulation (Table 4.15).
ii. Hormonal regulation: Insulin induces glucose-6-phosphate dehydrogenase.

Table 4.15: Allosteric mode of regulation

Enzyme	Activator	Inhibitor
Glucose-6-phosphate dehydrogenase	$NADP^+$	NADPH

Significance:

- NADPH generated is required for synthesis of fatty acids, cholesterol, steroid hormones
- It also helps to maintain glutathione in reduced state, which is required for integrity of RBC membrane
- NADPH also helps in detoxification of drugs (CYP450 of liver)
- WBC depends on NADPH (NADPH oxidase) for killing of bacteria
- Ribose-5-phosphate is required for synthesis of nucleotides and nucleic acids.

21. Discuss the metabolic defects of HMP shunt.

Glucose-6-phosphate Dehydrogenase (G6PD) Deficiency

- Defect: X-linked recessive disorder characterized by hemolytic anemia (Fig. 4.15)
- Clinical features: Hemolytic anemia, jaundice.

Precipitating Conditions

- Drugs [MN: AAA]: **A**ntimalarial—primaquine, **A**ntibiotic—sulfonamides and **A**ntipyretic—paracetamol
- Fava beans (favism): When a patient with G6PD deficiency consumes fava beans, he/she develops hemolysis, jaundice, hemoglobinuria, pallor, etc.
- Infections.

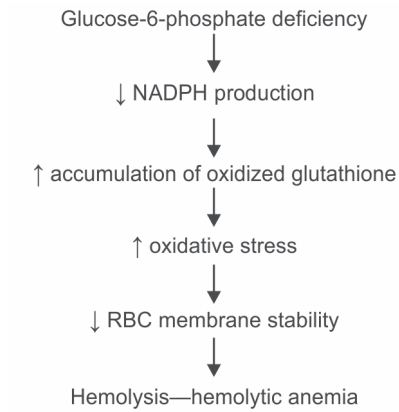


Fig. 4.15: Mechanism of hemolysis in G6PD deficiency

Thiamine Deficiency (Beriberi)

Thiamine deficiency results in decreased RBC transketolase activity leading to decreased NADPH production.

- Clinical features: Hemolytic anemia and features of beriberi
- Treatment: Supplementation of thiamine.

Wernicke-Korsakoff Syndrome

Wernicke-Korsakoff syndrome occurs due to deficiency of thiamine (required for transketolase) in alcoholics or decreased affinity of enzyme transketolase (of HMP pathway) for thiamine.

- Clinical features: Psychosis (encephalopathy), ataxia, ophthalmoplegia, nystagmus, etc.
- Treatment: Supplementation of thiamine.

22. Enlist the metabolic disorders associated with fructose and galactose metabolism.

Metabolic disorders associated with fructose and galactose metabolism are listed in Table 4.16.

Table 4.16: Metabolic disorders of fructose and galactose

Name of disorder	Enzyme defect	Comment
Essential fructosuria	Fructokinase	Benign metabolic disorder, no treatment required
Hereditary fructose intolerance	Aldolase B	Lethal disorder associated with hypoglycemia, vomiting, hepatomegaly, hyperlipidemia, etc. Avoidance of fructose in diet

Contd...

Contd...

Name of disorder	Enzyme defect	Comment
Galactosemia (classical)	Galactose-1-phosphate uridyltransferase	Lethal; symptoms same as above, there will be associated mental retardation and congenital cataract; avoidance of lactose in diet till age of 5 years
Galactosemia (non-classical)	Galactokinase	Benign disorder; does not require any treatment

23. What is the significance of uronic acid pathway? What is the metabolic disorder associated with glucuronic acid pathway.

Definition: Alternative pathway for glucose oxidation.

Significance:

- **Synthesis of UDP-glucuronic acid:** For detoxification; synthesis of glycosaminoglycans (heparin, hyaluronic acid, dermatan sulfate)
- **Synthesis of UDP-glucose:** For synthesis of glycogen, lactose and galactose
- **Synthesis of ascorbic acid:** In lower animals. Ascorbic acid is not synthesized in humans due to lack of enzyme L-gulonolactone oxidase.

Metabolic Disorder

Essential Pentosuria

Benign disorder in glucuronic acid pathway due to deficiency of enzyme xylitol dehydrogenase.

24. Explain regulation of blood glucose level.

Regulation of blood glucose level is given in Table 4.17.

Net effect of insulin is decrease in blood glucose level; glucagon and anti-insulin hormones increase blood glucose level.

Table 4.17: Regulation of blood glucose

Biochemical pathway	Stimulated by (+)	Inhibited by (–)
• Glycolysis • Glycogenesis • Lipogenesis • Protein biosynthesis	Insulin	Glucagon, Adrenal hormones, Thyroid hormones, Anterior pituitary hormones
• Ketogenesis • Lipolysis • Gluconeogenesis • Glycogenolysis	Glucagon, Adrenal hormones, Thyroid hormones, Anterior pituitary hormones	Insulin

25. List the actions of insulin and glucagon.

Actions of Insulin (Fig. 4.16)

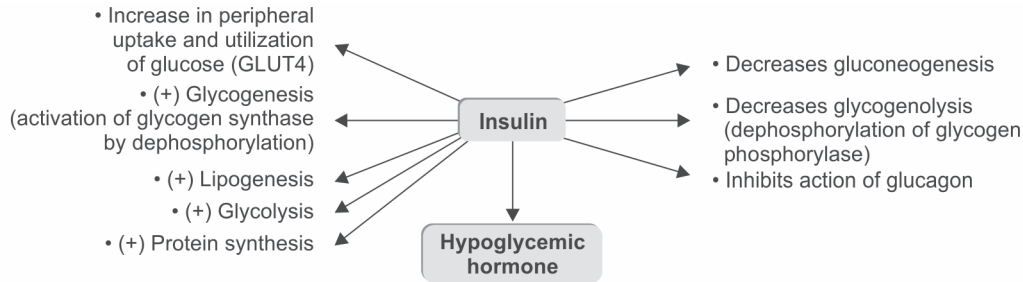


Fig. 4.16: Actions of insulin [(+), stimulates]

Actions of Glucagon (Fig. 4.17)

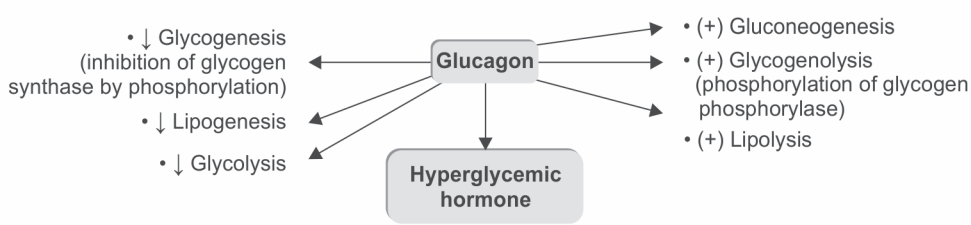


Fig. 4.17: Actions of glucagon [↓, decrease; (+), stimulates]

26. Define hypoglycemia and hyperglycemia. Mention some causes for the same.

Hypoglycemia

When plasma glucose level is less than 40 mg/dL.

Causes

- Prolonged fasting
- Alcoholism
- Overdose of insulin
- Insulinoma (insulin-secreting tumor)
- Hypoactivity of anti-insulin hormones.

Hyperglycemia

When a person's fasting glucose is greater than 100 mg/dL and postprandial glucose greater than 140 mg/dL, then it is called hyperglycemia.

Causes

- Diabetes mellitus: Decreased insulin production/action [fasting plasma glucose (FPG) > 126 mg/dL or postprandial plasma glucose (PPG) > 200 mg/dL or random plasma glucose (RPG) > 200 mg/dL with signs and symptoms of hyperglycemia]
- Hyperactivity of anti-insulin hormones
- Glucagonoma
- Prolonged treatment with steroid hormones.

27. Define diabetes mellitus. Add a note on types and clinical features of the same.

Definition: It is a metabolic disease leading to hyperglycemia due to absolute or relative insulin deficiency. The classification of diabetes is given in Table 4.18.

Table 4.18: Classification of diabetes mellitus

Type I	Type II
Juvenile diabetes	Adult onset diabetes
Due to lack of insulin	Due to insulin resistance or relative deficiency of insulin
5%–10% of all diabetics fall under this category	90%–95% of all diabetics fall under this category
Common complications: Ketosis	*Common complications:* Non-ketotic hyperosmolar coma

Clinical Features

- Excessive thirst (polydipsia)
- Frequent urination (polyuria)
- Extreme hunger or constant eating (polyphagia)
- Unexplained weight loss
- Presence of glucose in urine (glycosuria)
- Tiredness or fatigue.

Diagnosis (WHO Criteria)

- Fasting plasma glucose > 126 mg/dL OR
- Postprandial plasma glucose > 200 mg/dL OR
- Random plasma glucose > 200 mg/dL with signs and symptoms of hyperglycemia.

Complications

- Diabetic retinopathy
- Diabetic neuropathy
- Diabetic nephropathy
- Atherosclerosis and coronary artery disease
- Gangrene of foot
- Ketoacidosis.

Treatment

- Diet regulation
- Exercise
- Antidiabetic agents: Insulin, sulfonylureas, biguanides, etc.

Key Points

Reducing substances in urine: Glucose, fructose, lactose, galactose, pentose, homogentisic acid, salicylates, ascorbic acid and glucuronides of drugs.

Maturity-onset diabetes of young (MODY): There is a mutation in pancreatic glucokinase, which has decreased K_m and V_{max} for glucose. This results in decreased release of insulin from pancreas. Interestingly, however, these patients are somewhat resistant to long-term complications of chronic hyperglycemia.

Fructose intake can lead to obesity: Fructose enters glycolysis by phosphorylation to fructose-1-phosphate and it bypasses the main regulatory step (hexokinase), resulting in formation of more pyruvate (and acetyl-CoA) than is required for ATP formation. Also, there is increased availability of dihydroxyacetone phosphate (DHAP), which generates glycerol-3-phosphate, a starting material for triacylglycerol formation in the liver and adipose tissue, leading to increased lipogenesis.

Aldolase A: Present in most tissues, but **aldolase B** is present in liver and kidney.

2,3-BPG: Its concentration decreases in stored blood in blood bank and such blood may become unsuitable for transfusion. This can be prevented by adding inosine to the blood.

Arsenate will not block glycolysis: It can substitute phosphate in the reaction by glyceraldehyde-3-phosphate dehydrogenase forming highly unstable 1-arseno-3-phosphoglycerate, which readily hydrolyses to form 3-phosphoglycerate.

Arsenite (trivalent): Works by forming a stable complex with enzyme-bound lipoic acid. Thus, it can inhibit pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and branched chain α-ketoacid dehydrogenase. Arsenic was commonly used as a homicidal poison as it is a colorless, odorless and tasteless compound. Chronic arsenic poisoning can be diagnosed by finding the compound in hairs and finger nails of the victim.

Enolase is inhibited by fluoride: When blood samples are taken for measurement of glucose, it is collected in tubes containing fluoride to inhibit glycolysis. Fluoride binds with Pi to form fluorophosphates, which binds with Mg^{2+} bound enzyme and prevents the formation of metal bridge enzyme-substrate-complex (E-M-S).

Iodoacetate: Is an alkylating agent, which acts on cysteine residues in proteins. It is often used to modify SH-groups to prevent the reformation of disulfide bonds. It binds covalently and alkylates SH groups of enzymes and prevents enzyme-substrate complex formation (irreversible).

Benefit of G6PD deficiency: It protects the person against falciparum malaria. Malaria parasite requires reduced glutathione (GSH) and the products of the pentose phosphate pathway for survival, and is unable to complete the life cycle due to early lysis of RBCs.

Succinate thiokinase (succinyl-CoA synthetase): This is the only example of substrate level phosphorylation in the citric acid cycle.

Succinate dehydrogenase: This is bound to the inner surface of the inner mitochondrial membrane.

Alcohol ingestion can lead to hypoglycemia: Alcohol metabolism generates NADH, which converts oxaloacetate to malate. So, oxaloacetate (an initial raw material required for gluconeogenesis) is depleted leading to hypoglycemia.

Cancer cachexia: Conversion of lactate (formed in large amount in cancer cells) to glucose in the liver requires 6 ATPs, which is responsible for much of the hypermetabolism and weight loss seen in cancer cachexia.

Hereditary fructose intolerance (HFI): The most common defect is a single missense mutation in exon 5 (G → C), resulting in an amino acid substitution (alanine → proline). As a result of this substitution, a catalytically impaired aldolase B is synthesized.

Secondary diabetes: May be due to endocrinopathies, Cushing's syndrome, thyrotoxicosis, acromegaly or drug induced (steroids), pancreatitis.

Glycated Hb (HbA_{1c}): For every 60 mg/dL rise in blood glucose level, approximately there is a rise of HbA_{1c} by 2%. Glycated Hb levels indicate average blood glucose levels during past 1–4 months period. 4%–7% is considered as normal range for this parameter.

Fructosamine (glycated albumin): Provide average blood glucose levels during past 1–3 weeks.

Neotame: Is an artificial sweetener that is between 8,000 and 13,000 times sweeter than sucrose.

Sucralose: Is an artificial sweetener that is approximately 600 times sweeter than sucrose.

Glycogen has highly branched structure: It provides a large number of sites (non-reducing ends) for glycogenolysis (glycogen phosphorylase), permitting rapid release of glucose.

Diabetic Prone States

Gestational diabetes mellitus (GDM): Pregnant woman having abnormal glucose tolerance, which resolves after delivery. These patients are at high risk of developing frank diabetes.

Impaired glucose tolerance (IGT): In this condition, fasting blood glucose is more than 100 and less than 126 mg/dL; postprandial blood glucose is more than 140 and less than 200 mg/dL.

Impaired fasting glycemia (IFG): In this condition, fasting glucose is more than 100 and less than 126 mg/dL, but postprandial sugar is within normal limits (< 140 mg/dL).

5 Chemistry of Lipids

1. Define and classify lipids with suitable examples.

Definition: Lipids are organic substances, relatively insoluble in water and soluble in organic solvents like alcohol, ether, etc. They are mainly compounds of C, H and O, but also carry P, N and S in some cases.

Classification

Lipids are classified (Fig. 5.1) as following.

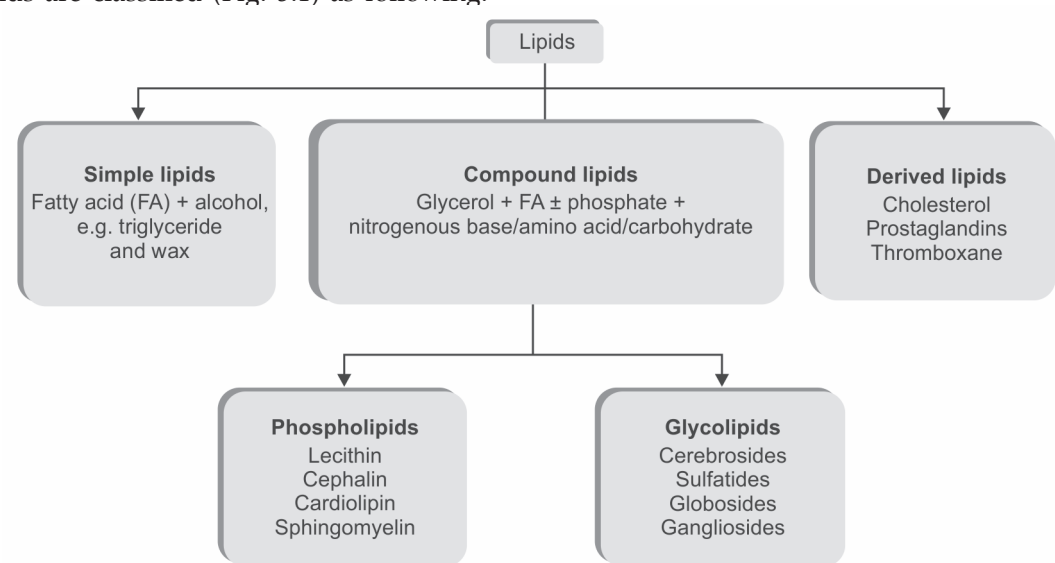


Fig. 5.1: Classification of lipids

a. **Simple lipids:** Esters of fatty acid with alcohol.
   - Fats or oils (triacylglycerol): Esters of fatty acids with glycerol
   - Waxes: Esters of fatty acids with long-chain aliphatic alcohols other than glycerol, e.g. cetyl alcohol. These are solid at room temperature.

b. **Compound lipids:** Esters of fatty acids with alcohol, carrying additional groups such as phosphate and nitrogenous group or carbohydrate. Compound lipids are of two types:
   - *Phospholipids:* For example, glycerophospholipids, sphingophospholipids
   - *Glycolipids:* For example, cerebrosides, sulfatides, gangliosides, globosides.

c. **Complex lipids:** For example, lipoproteins—very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and chylomicrons.

d. **Derived lipids:** Obtained by hydrolysis of simple or compound lipids. For example, steroids (cholesterol), prostaglandins, leukotrienes, etc.

2. Give a brief account of functions of lipids.

- Source of energy
- Concentrated fuel reserve (adipose tissue)
- Constituent of membranes and regulate membrane permeability. For example, phospholipids, glycolipids, lipoproteins and sterols
- As compounds of inner mitochondrial membrane, lipids participate in electron transport chain
- Phospholipids, glycolipids and sterols are constituents of plasma lipoprotein, which transport fat
- Source of fat-soluble vitamins and bile acids
- Metabolic regulators—steroid hormones and prostaglandins
- Protect internal organs, serve as insulating materials.

3. Define and classify fatty acids.

Definition: Fatty acids are monocarboxylic acids with hydrocarbon side chain. They are simplest form of lipids.

Classification

a. Depending on nature of hydrocarbon chain

- *Saturated fatty acids:* Do not contain double bonds in their hydrocarbon chain, e.g. palmitic and stearic acid

- *Unsaturated fatty acids:* Contain one (monounsaturated) or more (polyunsaturated) double bonds in their hydrocarbon chain, e.g. oleic acid, linoleic acid, linolenic acid, arachidonic acid.

b. Depending on number of carbon atoms

- *Even-chain fatty acids:* Natural lipids with even number of carbon atoms in their chain, e.g. palmitic acid (16), stearic acid (18)
- *Odd-chain fatty acids:* Propionic acid (3C) and valeric acid (5C).

c. Depending upon length of hydrocarbon chain of fatty acid

- *Short-chain fatty acids:* Chain contains six or less than six carbons, e.g. propionic acid, butyric acid, etc.
- *Medium-chain fatty acids:* Chain contains 8–14 carbons, e.g. caprylic acid, myristic acid, etc.
- *Long-chain fatty acids:* Chain contains 16–22 carbons, e.g. palmitic acid, stearic acid, etc.

d. Depending upon nutritional importance

- *Essential fatty acids:* Not synthesized in the body, therefore should be taken in the diet, e.g. linoleic acid, linolenic acid and arachidonic acid
- *Non-essential fatty acids:* These can be synthesized in the body, e.g. palmitic acid, stearic acid, etc.

4. Write a short note on essential fatty acids.

The essential fatty acids (EFA) are those which cannot be synthesized by the body, hence they should be supplied in the diet. Chemically, they are polyunsaturated fatty acids (PUFA).

Biochemical basis for essentiality: Linoleic and linolenic acids are essential fatty acids—humans lack the enzymes that can introduce double bonds beyond carbon 9 of fatty acids.

Functions of essential fatty acids: It is required for the following:

- Membrane structure and function
- Transport of cholesterol
- Formation of lipoproteins
- Synthesis of eicosanoids—prostaglandins, thromboxane, leukotrienes
- Protective effect against fatty liver.

Deficiency of EFA: The deficiency of EFA results in phrynoderma, hair loss and poor wound healing.

5. What are the types of rancidity? How can it be prevented?

Definition of rancidity: It is the deterioration of fats and oils resulting in unpleasant taste and smell.

Types of rancidity

- *Hydrolytic rancidity:* Due to bacterial hydrolytic enzymes, which results in the formation of short-chain fatty acids. It can be prevented by heat deactivation, proper handling and storage
- *Oxidative rancidity:* It is due to oxidation of fat by free radicals resulting in formation of compounds such as dicarboxylic acids, aldehydes, ketones, etc.

  Prevention: By antioxidants, e.g. tocopherol, hydroquinone, gallic acid and food preservatives like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc.

6. Define iodine number. Mention its significance.

Definition: Iodine number is defined as number of grams of iodine absorbed by 100 g of fat. A higher iodine number indicates greater number of double bonds.

Significance: It measures average degree of unsaturation, so it is useful in detecting adulteration of polyunsaturated fatty acid (PUFA) with saturated fatty acid. For example, iodine number of coconut oil is 6–10 and sunflower oil is 141–155.

7. Define and write the significance of saponification number.

Definition: The number of milligrams (mg) of potassium hydroxide (KOH) required to saponify 1 g of fat is saponification number.

Significance: It measures the average molecular weight of fat.

8. Define and classify phospholipids. Write its compositions and one example for each class.

Definition: Phospholipids are lipids containing phosphoric acid + fatty acids + alcohol ± nitrogenous base. They contain both polar and non-polar groups and are called amphipathic lipids.

Classification

Glycerophospholipids: Contain glycerol as alcohol (Table 5.1).

Table 5.1: Composition of individual phospholipids

Name	Composition	Function
Lecithin	Glycerol + fatty acids + phosphoric acid + choline	• Membrane component, nerve transmission • Source of choline and methyl group
Dipalmitoyl lecithin	Glycerol + palmitic acid (2) + phosphoric acid + choline	• Lung surfactant • Deficiency causes respiratory distress syndrome

Contd...

Contd...

Name	Composition	Function
Cephalin	Glycerol + fatty acids + phosphoric acid + ethanolamine	• Structural component of membranes
Plasmalogens	Alcohol + fatty acids + phosphoric acid + nitrogenous base + glycerol	• Structural component of membranes in brain and muscle
Platelet-activating factor	Contains alkyl linkage in first carbon of glycerol. Second carbon contains acetyl group. Third carbon is attached with phosphate group to choline.	• Mediator of hypersensitivity, acute inflammatory reactions and anaphylactic shock
Cardiolipin	Phosphatidic acid + glycerol + phosphatidic acid	• Component of inner mitochondrial membrane has antigenic properties
Phosphatidylinositol	Glycerol + fatty acids + phosphate + inositol	• Phosphatidylinositol 4, 5-bisphosphate is second messenger for hormone action • Anchors the glycoproteins to membranes

Sphingophospholipids: Contain sphingosine as alcohol.

- *For example,* sphingomyelins—major component of membranes of nervous tissue
- *Composition:* Ceramide (sphingosine + fatty acid) + phosphoric acid + choline
- *Niemann-Pick disease:* Due to excessive accumulation of sphingomyelin as a result of deficiency of enzyme sphingomyelinase.

Functions of Phospholipids

- Constituent of cell membrane, myelin sheath
- Constituent of surfactant (dipalmitoyl lecithin) in lungs
- Second messenger (phosphatidylinositol)
- Source of arachidonic acid—for prostaglandin synthesis
- Components of lipoproteins (transport of lipids)
- Absorption of fat from the intestine (micelles and chylomicrons)
- Acts as lipotropic factor and prevents fatty liver
- Responsible for maintaining the conformation of electron transport chain in mitochondria.

9. Mention the composition of glycolipids and give examples.

Definition: Glycolipids are carbohydrates—lipid complexes having amphipathic nature. They are the important components of cell membrane and nervous tissue.

Composition: Ceramide (sphingosine + fatty acid) + carbohydrate. Do not have phosphoric acid.

Classification: Depending on the nature of attached carbohydrate chain.

Cerebrosides: Galactocerebroside (ceramide + galactose).

Sulfatides: Ceramide + galactose + sulfate.

Globosides: Ceramide + more than one hexose or hexosamine.

Gangliosides: Ceramide + oligosaccharides + NANA (N-acetylneuraminic acid).

10. What are prostaglandins? Mention their functions.

Definition: Unsaturated hydroxyl and ketosubstituted C_{20} fatty acids (Table 5.2).

Synthesis: Formed from arachidonic acid by cyclooxygenase pathway.

Significance: Act as local hormones and mediators of inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, are the inhibitors of cyclooxygenase.

Table 5.2: Functions of prostaglandins

Compounds	Functions
PGE_2	• Mediator of pain and fever • Vasodilatation, relaxation of smooth muscle
$PGF_{2\alpha}$	• Used for induction of labor • Vasoconstriction • Contraction of uterine smooth muscle
PGE_1	• Decreases gastric acid secretion
PGI_2	• Produced by endothelium of vessels • Vasodilatation • Inhibits platelet aggregation
TXA_2	• Produced by platelets • Platelet aggregation • Vasoconstriction

PG, prostaglandin; PGI, prostacyclin; TX, thromboxane.

11. Mention four biological functions of cholesterol.

Functions

- Constituent of cell membrane
- Formation of bile salts (liver)
- Synthesis of steroid hormones (cortisol, aldosterone, testosterone, estrogen, progesterone)
- Precursor of vitamin D.

12. What is the name of cholesterol ring? Mention the compounds derived from cholesterol.

- **Name of the ring:** Cyclopentanoperhydrophenanthrene ring
- **Compounds derived from cholesterol**: Vitamin D_3, bile acids, testosterone, estrogens, cortisol and aldosterone.

Key Points

Essential fatty acids: Linoleic, linolenic, arachidonic acid.
Trans fatty acids: They are found in partially hydrogenated vegetable oils and increase the risk of atherosclerosis and cancer.
Omega-6 fatty acids: Linoleic and arachidonic acid.
Omega-3 fatty acids: Linolenic acid. High levels of omega-6 fatty acids and low omega-3 fatty acids can lead to atherosclerosis and cancer.
Iodine number: Used for detection of adulteration of unsaturated fat with saturated fat.
Saponification number: Used for measure of average molecular weight of fat.
Palmitic acid: Most common saturated fatty acid in diet; metabolized in the body.
Rancidity: Deterioration of oils and fat resulting in an unpleasant smell.
Food preservatives: Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT).
Dipalmitoyl lecithin: Lung surfactant. Deficiency causes respiratory distress syndrome.
Phosphatidylinositol 4, 5 bisphosphate: Acts as a second messenger in hormone action.
Niemann-Pick disease: Due to deficiency of sphingomyelinase.
Arachidonic acid: Acts as a source for synthesis of prostaglandins.
Choline: Lipotropic factor and prevents fatty liver.
PGE_2: Used for dilatation of cervix during labor.
$PGF_{2\alpha}$: Induction of labor by uterine contraction.
Aspirin and NSAIDs: Inhibitors of cyclooxygenase; used as anti-inflammatory and analgesic agents.
Steroids: Block the enzyme phospholipase A_2—prevent the release of arachidonic acid, hence act as anti-inflammatory agents.
LDL: Increases the risk of atherosclerosis.
HDL: Decreases the risk of atherosclerosis.
Cholesterol: Normal serum level is 140–200 mg/dL. Vitamin D_3, bile acid, steroid hormone (cortisol, testosterone and estrogens) are derived from cholesterol.
Leukotrienes: Formed from arachidonic acid by lipo-oxygenase pathway. They mediate chemotaxis, allergy and inflammation. They are components of slow reacting substance of anaphylaxis.

6 Digestion, Absorption and Metabolism of Lipids

1. How are lipids in our diet digested and absorbed in gastrointestinal tract?

Digestion of lipids is a process by which there is breakdown of large and complex lipid molecules into smaller and relatively water-soluble molecules, which can be easily absorbed by the gastrointestinal (GI) tract.

Sources of lipids in diet: Meat, animal fat, butter, milk, cheese, egg yolk, cooking oil and ghee. About 20%–30% of total energy is obtained in the form of lipids.

Starting materials and end products of lipid digestion are given in Table 6.1.

Table 6.1: Starting materials and end products of lipid digestion

Starting material	End product
Triglycerides	Free fatty acids + 2-monoacylglycerols
Cholesterol esters	Free cholesterol + free fatty acids
Phospholipids	Free fatty acids + lysophospholipid

Digestion and Absorption of Lipids

In the mouth: Due to lack of favorable conditions, minimal digestion of lipid occurs in the mouth. Lingual lipase acts on short-chain triglycerides.

In the stomach: In humans, initiation of fat digestion occurs in stomach with mechanical emulsification followed by the action of gastric lipase. Gastric lipase hydrolyzes dietary triglycerides containing medium- and short-chain fatty acids (SCFAs) into diacylglycerol and free fatty acids. In infants and patients with pancreatic insufficiency, gastric lipases play an important role in fat digestion. Lingual lipase also acts in the stomach on short-chain triglycerides.

In the intestine: Bile salts (sodium and potassium salts of glycocholic and taurocholic acids) form emulsions with lipids by reducing the surface tension.

Digestion, absorption, transport of lipids in small intestine: Small intestine is the major site of lipid digestion due to pancreatic lipase—it requires colipase and bile salts for its activity (Fig. 6.1).

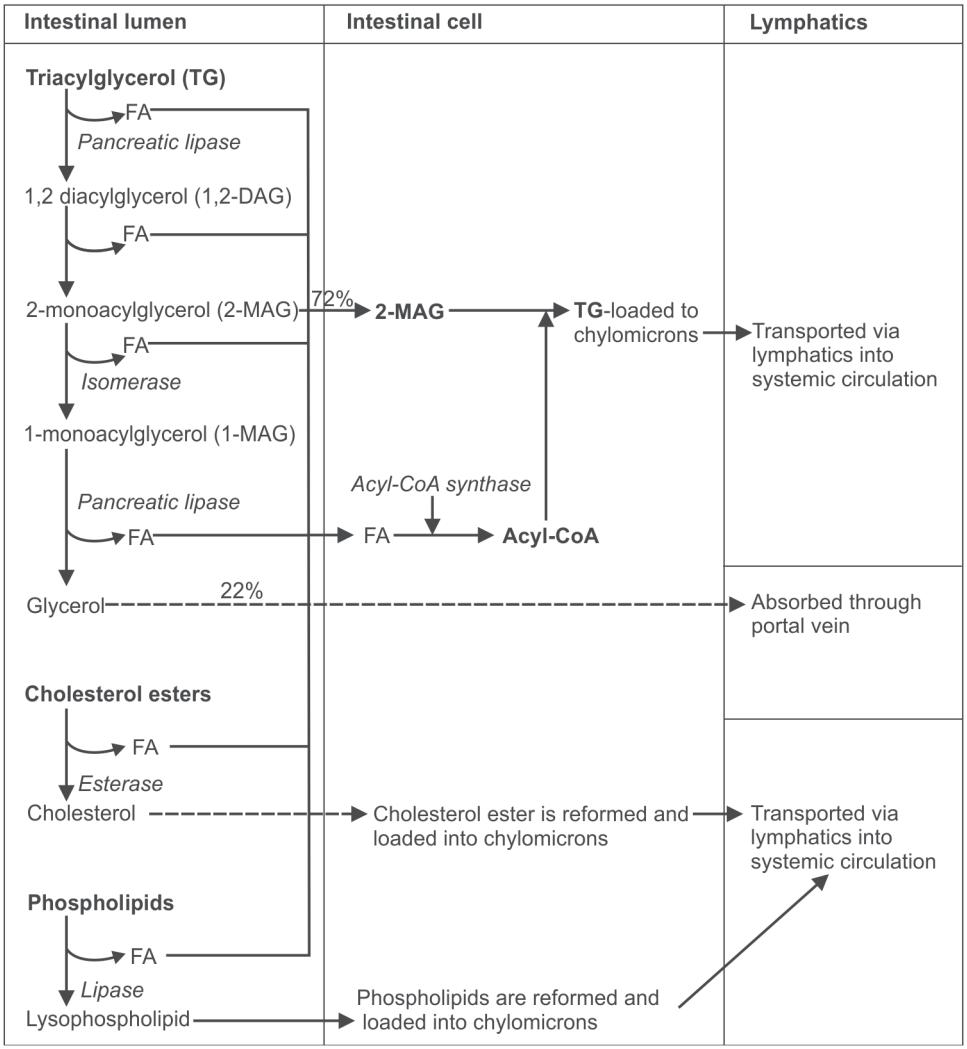


Fig. 6.1: Digestion, absorption and transport of lipids (FA, fatty acid)

Long-chain fatty acids are re-esterified in the intestinal cell to triglycerides. Short-chain fatty acids directly enter into the blood vessels.

Hormonal Control of Lipid Digestion

Cholecystokinin: It is a peptide secreted by duodenal and jejunal mucosal cells; secreted in response to lipids in duodenum. It acts on gallbladder to release bile and on pancreas to release pancreatic lipase and other enzymes.
Secretin: It stimulates pancreas to release bicarbonate, thereby helps to neutralize acidic pH.

Utilization of Lipids

- Chylomicrons in the circulation is acted upon by lipoprotein lipase and forms free fatty acids, glycerol + chylomicron remnants
- Free fatty acids are taken up by tissues for energy or stored in the adipose tissue
- Glycerol is converted to glycerol phosphate in liver and used for lipid storage or it can enter into glycolysis or gluconeogenesis
- Chylomicron remnants are taken up by liver and metabolized.

2. Explain β-oxidation of saturated fatty acids (or palmitic acid) under following headings: a. Oxidation. b. Energetics.

Definition: β-oxidation is defined as oxidation of fatty acids in which two carbon fragments are successively removed at 'β' position from the carboxyl end.

Requirements for β-oxidation

Site: Mitochondria of all cells in the body.
Starting material: Fatty acids, mobilized from adipose tissues (lipolysis) by glucagon-mediated activation of enzyme hormone sensitive lipase. Activated lipase converts the triacylglycerol into free fatty acids and glycerol. Glycerol enters into gluconeogenesis.
End products: Acetyl-CoA, NADH and $FADH_2$.
Coenzymes: NAD^+ and FAD.

Steps in β-oxidation

a. Activation of fatty acids into fatty acyl-CoA in the cytosol of cell.

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \xrightarrow{\textit{Thiokinase}} \text{Fatty acyl-CoA} + \text{PPi} + \text{AMP}$$

b. Transport of fatty acyl-CoA from cytosol to mitochondria is carried out by carnitine shuttle. Long-chain fatty acids (LCFA) cannot cross mitochondrial membrane, hence they need transport system. Carnitine transports LCFA through inner mitochondrial membrane.

c. β-oxidation involves four steps (Fig. 6.2):

- Oxidation
- Hydration
- Oxidation
- Cleavage.

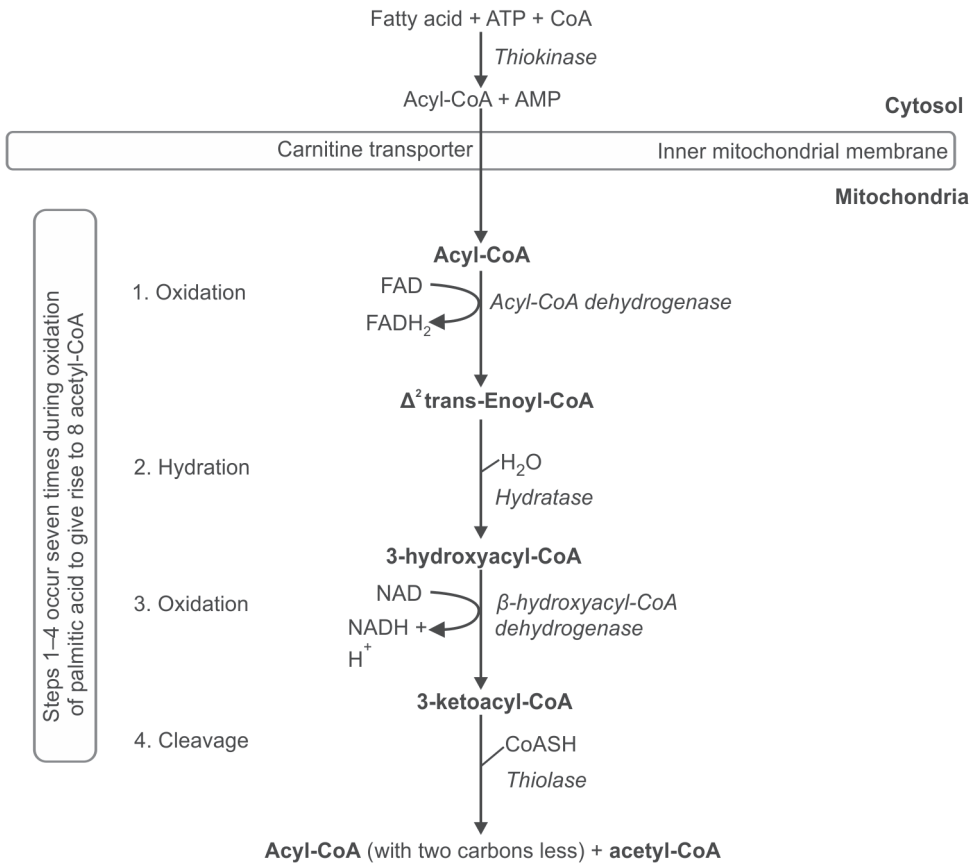


Fig. 6.2: β-oxidation of fatty acids

Energetics in β-oxidation

Palmitic acid (16C) undergoes seven cycles of β-oxidation to produce:
8 acetyl-CoA → TCA cycle → 10 ATP × 8 = +80 ATP
7 $FADH_2$ → ETC → 1.5 ATP × 7 = +10.5 ATP
7 NADH → ETC → 2.5 ATP × 7 = +17.5 ATP.
Total = 108 ATPs.
Activation of fatty acid = –2 ATP.
Net ATP yield = 108 – 2 = 106.

Net ATP production on complete oxidation of palmitic acid is 106 ATP.

Regulation of β-oxidation

Energy status: In well-fed state, β-oxidation is blocked and fatty acid synthesis is active. During starvation, β-oxidation is active and fatty acid synthesis is blocked.
Hormones: Insulin stimulates lipogenesis and blocks lipolysis, whereas glucagon stimulates lipolysis and blocks lipogenesis.
Malonyl-CoA (precursor for fatty acid synthesis): It blocks the entry of fatty acids from cytosol to mitochondria by inhibiting carnitine palmitoyltransferase I.

3. Write briefly on oxidation of odd-chain fatty acids.

Oxidation of odd-chain fatty acid: It gives acetyl-CoA (depending on number of carbons in the fatty acid), one propionyl-CoA, NADH and $FADH_2$ as end products. Propionyl-CoA enters into tricarboxylic acid (TCA) cycle after being converted into succinyl-CoA.

For example: Valeric acid is a fatty acid containing 5 carbons. It undergoes 1 cycle of β-oxidation producing 1 acetyl-CoA + 1 propionyl-CoA.

Fate of acetyl-CoA: Enters into TCA cycle and generates 10 ATPs.

Fate of propionyl-CoA: Enters into TCA cycle as a precursor for gluconeogenesis after getting converted into succinyl-CoA (Fig. 6.3).

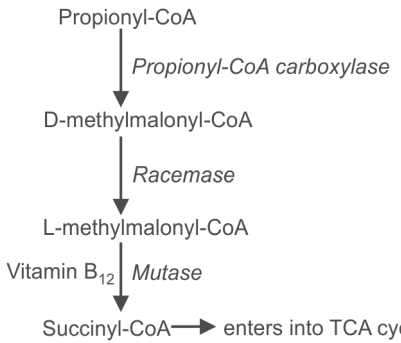


Fig. 6.3: Fate of propionyl-coenzyme A (TCA, tricarboxylic acid)

4. Briefly mention the disorders associated with oxidation of fatty acids.

Disorders associated with oxidation of fatty acids and their clinical features are given in Table 6.2.

Table 6.2: Disorders of fatty acid oxidation

Disorder	Defect	Clinical features
Carnitine deficiency	CPT-I and CPT-II deficiency	• Severe hypoglycemia • If not treated, coma and death
Zellweger's syndrome	Deficiency of peroxisomes	• Severe neurological symptoms
Dicarboxylic aciduria	Medium-chain acyl-CoA dehydrogenase	• Hypoglycemia, acidosis
Jamaican vomiting sickness	Consumption of unripe ackee fruit, which contain toxin hypoglycin that inhibits acyl-CoA dehydrogenase	• Hypoglycemia and excretion of medium- and short-chain dicarboxylic acids in urine

CPT, carnitine palmitoyltransferase

5. Describe the de novo synthesis of fatty acids. Add a note on role of citrate in this process.

Definition: Defined as synthesis of palmitate from acetyl-CoA in the cytosol.

Site: Liver, kidney, brain, mammary gland, adipose tissue.

Cellular site: Cytosol.

Coenzymes/cofactors: NADPH (from HMP shunt), ATP, manganese, biotin, HCO_3^-.

Starting material: Acetyl-CoA.

End product: Palmitate.

Enzyme: Fatty acid synthase complex (Fig. 6.4), which is a dimer with seven enzymes and acyl carrier protein on each subunit.

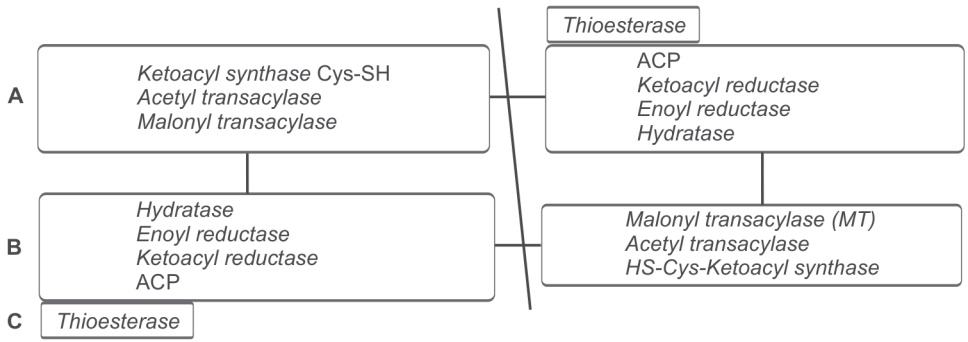


Fig. 6.4: Fatty acid synthase complex (A = condensing unit; B = reduction unit; C = releasing unit; oblique line shows arbitrary division between individual units of the dimer; ACP, acyl carrier protein).

Steps in the synthesis of fatty acids (Figs 6.5 and 6.6)

- Transport of acetyl-CoA from mitochondria to cytosol (as citrate)

  Role of citrate: Acetyl-CoA is formed by pyruvate in the mitochondria and transferred to the site of synthesis (cytosol) after getting converted into citrate (Fig. 6.5).

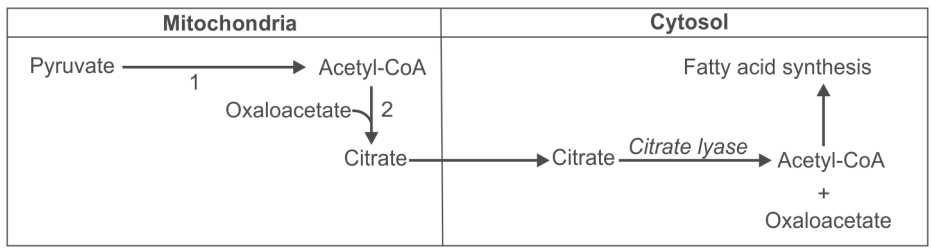


Fig. 6.5: Transport of acetyl-CoA from mitochondria to cytosol (1 = pyruvate dehydrogenase; 2 = citrate synthase)

- Formation of malonyl-CoA

$$\text{Acetyl-CoA} \xrightarrow[CO_2,\ \text{biotin, ATP}]{\textit{Acetyl-CoA carboxylase}} \text{Malonyl-CoA}$$

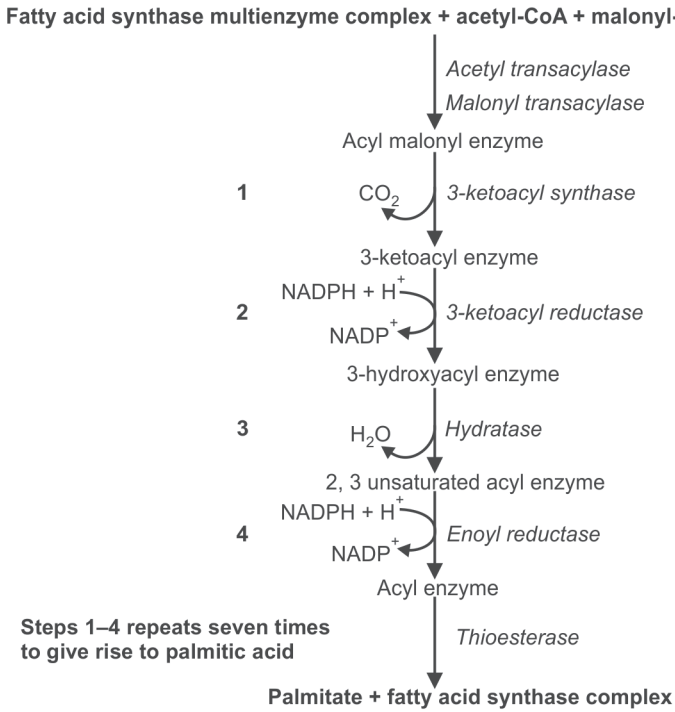


Fig. 6.6: Synthesis of fatty acid

To synthesize one molecule of palmitate: 1 acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H^+ is required. Regulation of fatty acid synthesis is given in Table 6.3.

Table 6.3: Regulation of fatty acid synthesis

Enzymes	Activator	Inhibitor
Acetyl-CoA carboxylase	Citrate, insulin	Acyl-CoA, glucagon
Carnitine palmitoyltransferase I	–	Malonyl-CoA

6. How are ketone bodies formed and utilized in our body? Add a note on ketosis.

Definition: Ketone body metabolism occurs during the high rates of fatty acid oxidation, primarily in the liver. The large amount of acetyl-CoA generated, which exceeds the capacity of the TCA cycle, results in the synthesis of ketone bodies (ketogenesis). Ketone bodies are acetoacetate, β-hydroxybutyrate and acetone (Fig. 6.7).

Site: Liver.

Subcellular site: Mitochondria.

Starting material: Acetyl-CoA.

End products: Acetoacetate, β-hydroxybutyrate and acetone.

Coenzymes: NADH, Coenzyme A (CoASH).

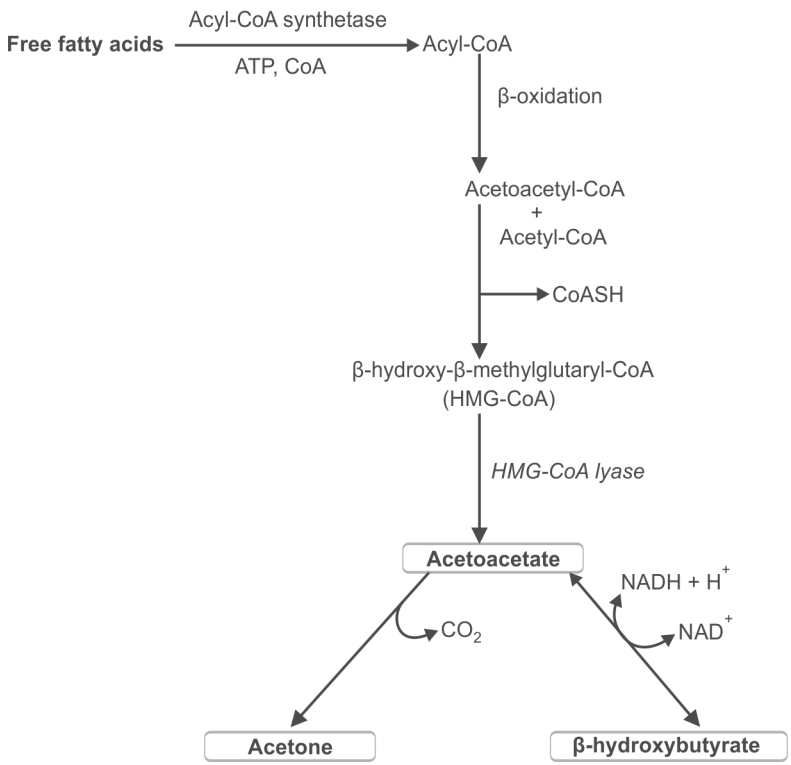


Fig. 6.7: Ketogenesis (ATP, adenosine triphosphate; CoA, coenzyme A)

Transport: Ketone bodies are transported through the blood into peripheral tissues and metabolized.

Utilization of ketone bodies (ketolysis): Enzyme thiophorase is present in all tissues except the liver, so **liver cannot utilize ketone bodies**. Utilization of ketone bodies is shown in Figure 6.8 and regulation of ketogenesis is given in Table 6.4.

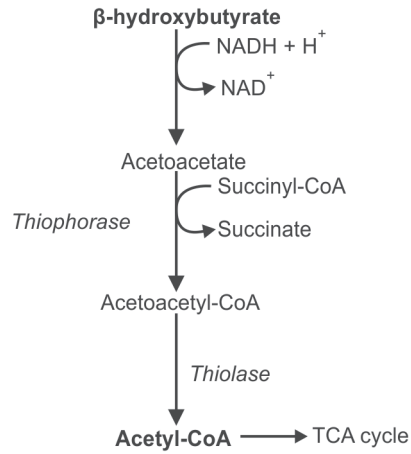


Fig. 6.8: Utilization of ketone bodies (NADH, nicotinamide adenine dinucleotide (reduced); TCA, tricarboxylic acid)

Table 6.4: Regulation of ketogenesis

Inhibitor	Stimulator
Acyl-CoA, malonyl-CoA	Glucagon
Insulin	

Ketosis: Elevated ketone bodies in the blood (**ketonemia**), which gets excreted in urine **(ketonuria)**.

Causes: The following are the causes of ketosis:

a. Diabetic ketoacidosis.
b. Prolonged starvation.

Mechanism of ketosis: The mechanism of ketosis is shown in Figure 6.9.

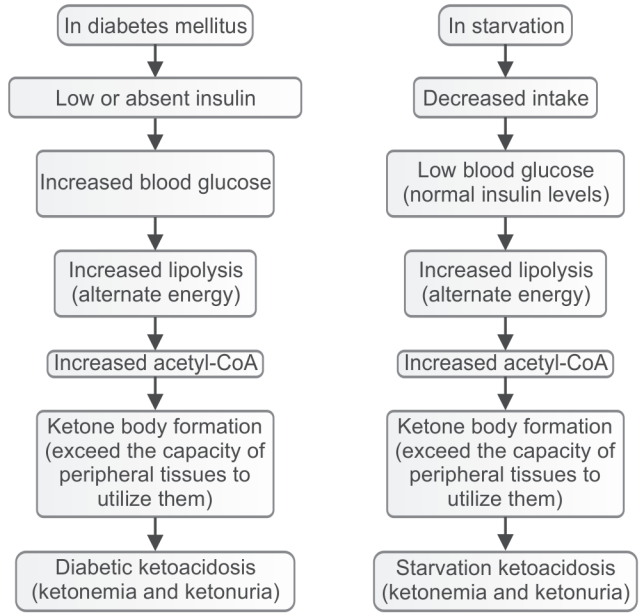


Fig. 6.9: Mechanism of ketosis

7. Outline the pathway for synthesis of cholesterol. What is its importance?

Definition: Cholesterol is an animal sterol present in tissues and lipoproteins in either esterified or free form. Cholesterol is a 27 carbon compound having cyclopentanoperhydrophenanthrene ring.

Starting material: Acetyl-CoA.

End product: Cholesterol.

Site: Liver, adrenal gland and gonads.

Subcellular site: Cytosol.

Coenzymes: NADPH and ATP.

Steps in cholesterol synthesis

Biosynthesis of cholesterol is shown in Figure 6.10.

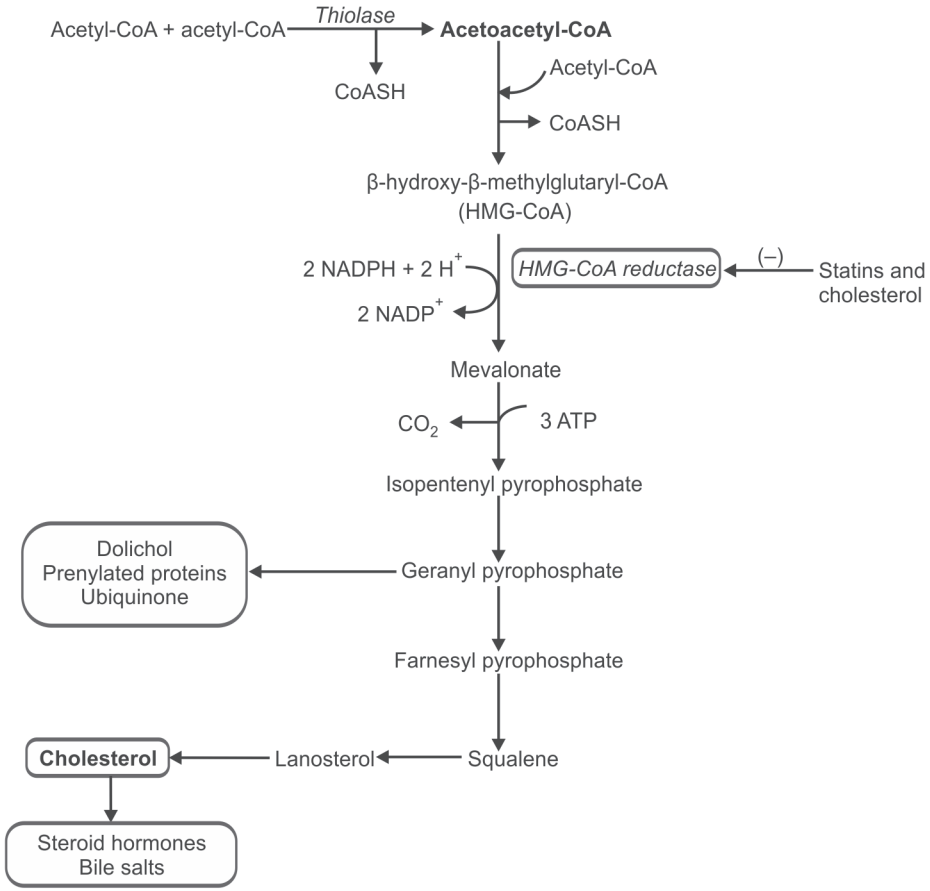


Fig. 6.10: Biosynthesis of cholesterol (– = inhibition)

Regulation of cholesterol synthesis

HMG-CoA reductase: It is rate limiting enzyme, which is inhibited by high cholesterol levels and stimulated by low cholesterol levels (at gene level).

Hormonal regulation: Insulin and thyroxine increase cholesterol synthesis. Glucagon and glucocorticoids decrease cholesterol synthesis.

Drugs: Statins, competitive inhibitors of HMG-CoA reductase, are used in the treatment of hypercholesterolemia, e.g. Lovastatin, simvastatin, atorvastatin.

Bile acids: Inhibit HMG-CoA reductase.

8. How are bile acids synthesized? What are their functions?

Bile acids: These are 24 carbon compounds essential for lipid digestion. Bile acid is the route of excretion of cholesterol.

Classification

- **Primary:** Cholic acid and chenodeoxycholic acid
- **Secondary:** Deoxycholic acid and lithocholic acid.

Synthesis of Bile Acids (Fig. 6.11, P. 77)

Site: Liver.

Starting material: Cholesterol.

End product: Bile acids.

Coenzymes: NADPH.

Steps: Process involves following modifications in cholesterol structure (Fig. 6.11):

- Insertion of hydroxyl groups in specific positions
- Saturation of double bond in the B ring of cholesterol
- Hydrocarbon chain is shortened by 3 carbons and introduction of carboxyl group.

Regulation: The rate limiting enzyme is 7α-hydroxylase, which is inhibited by bile acids (feedback inhibition).

Functions: Bile salts decrease the surface tension and cause emulsification of fat. They also help to form micelles, which are important for fat absorption. They help in absorption of fat-soluble vitamins A, D, E and K.

9. Write a note on enterohepatic circulation of bile salts.

Definition: Process involving secretion of bile acids from liver → intestine → reabsorption of secreted bile acids and salts from intestine → transport to liver → re-excretion in bile. About 98%–99% of bile acids secreted are reabsorbed and this process is known as enterohepatic circulation (Fig. 6.12, p. 77).

About 15–30 g of bile salt is secreted from liver to intestine per day. About 0.5 g is lost per day and remaining reabsorbed by enterohepatic circulation. Approximately 0.5 g of cholesterol is synthesized per day to replace the loss.

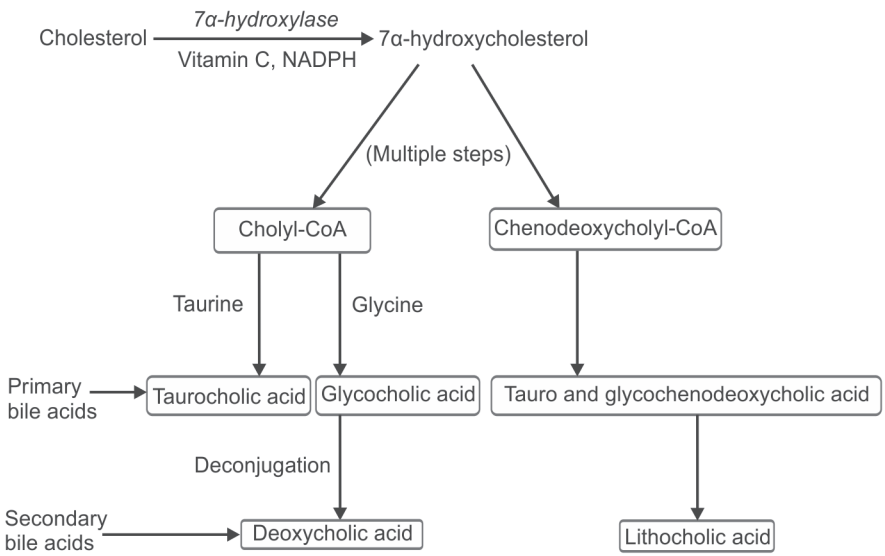


Fig. 6.11: Synthesis of bile acids (NADPH, reduced nicotinamide adenine dinucleotide phosphate)

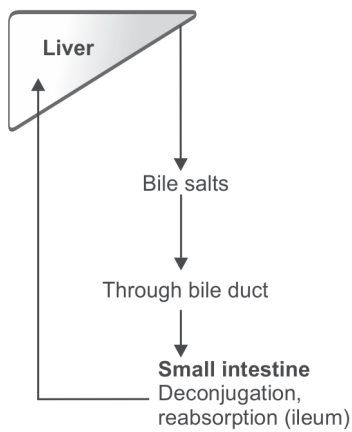


Fig. 6.12: Enterohepatic circulation of bile salts

10. Classify lipoproteins with their composition and functions.

Lipoproteins: These are complex lipids having lipids and specific proteins (apoproteins). Lipoproteins are helpful in transport of lipids in plasma.

Structure: Neutral lipid core (containing triacylglycerol and cholesterol ester) surrounded by a shell of amphipathic apolipoproteins, phospholipids and non-esterified cholesterol (Fig. 6.13).

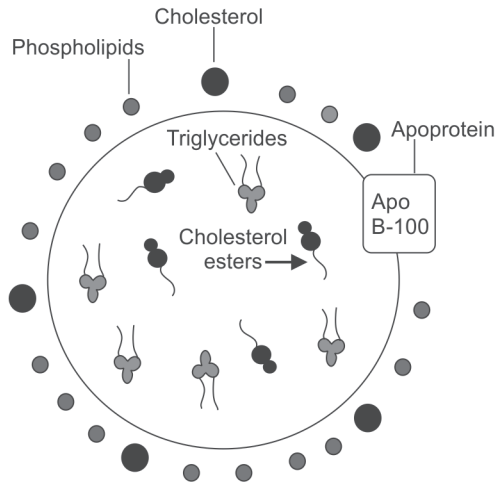


Fig. 6.13: Structure of lipoproteins

Classification and functions: Depending on the electrophoretic mobility and density, lipids are classified as given in Table 6.5.

Table 6.5: Classification of lipoproteins

Class	Diameter (nm)	Source and functions	Major apolipoproteins
Chylomicrons	500	• Intestine: Transport of dietary triacylglycerol (TAG) to tissues.	A, B-48, C-II, E
Very-low-density lipoproteins (VLDL)	43	• Liver: Transport of TAG from liver to peripheral tissues.	B-100, C-II, E
Low-density lipoproteins (LDL)	22	• IDL: Transport of cholesterol from liver to peripheral tissues.	B-100
High-density lipoproteins (HDL)	8	• Transport of cholesterol from peripheral tissues to liver (reverse cholesterol transport). Donates apolipoproteins to chylomicrons and VLDL.	A, C (I, II, III), E

11. What is the normal serum cholesterol levels? Briefly describe the metabolism of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL).

Normal serum cholesterol level is 140–200 mg/dL.

Metabolism of very-low-density lipoproteins (Fig. 6.14)

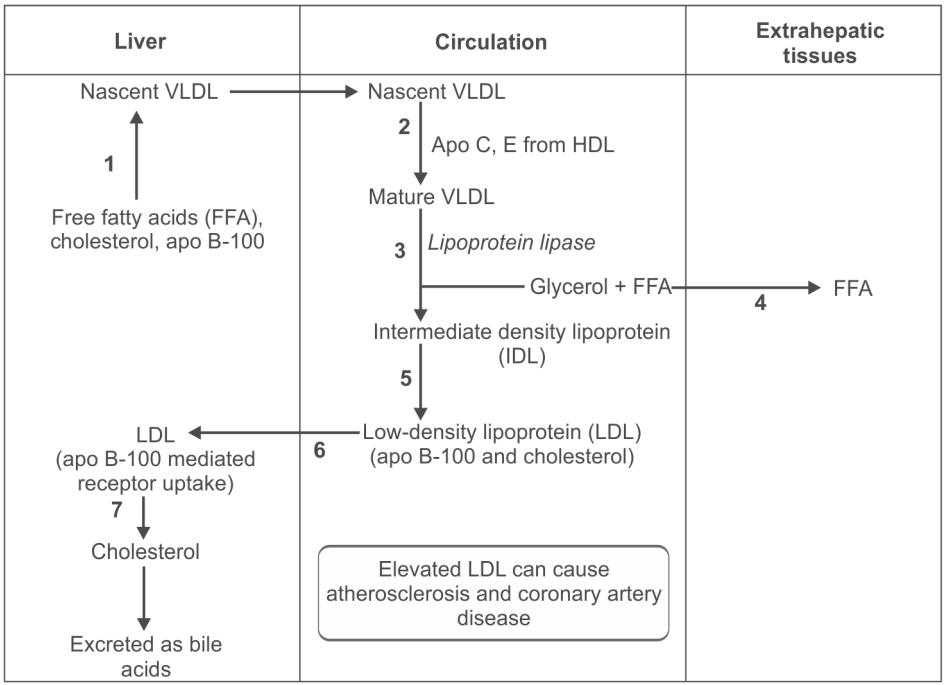


Fig. 6.14: Metabolism of very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL)

- **Step 1:** Very-low-density lipoprotein (VLDL) is synthesized in the liver as nascent VLDL; Nascent VLDL will transport endogenous triacylglycerol and cholesterol
- **Step 2:** In the circulation, it acquires apolipoproteins (C and E) from HDL and becomes mature VLDL
- **Step 3:** Lipoprotein lipase (on endothelium of capillaries) metabolizes triacylglycerol of VLDL releasing free fatty acids (FFA) and glycerol. VLDL is converted to IDL
- **Step 4:** FFA released is taken up by extrahepatic tissues (adipose tissue and muscle)

- **Step 5:** Intermediate density lipoproteins (IDL) lose triglycerides to form LDL
- **Step 6:** LDL, apo B-100 containing lipoprotein, is responsible for transport of cholesterol from liver to peripheral tissues. Most of the LDL is formed from VLDL in the circulation. From the circulation, cholesterol rich LDL is taken up by liver via apo B-100 mediated uptake through LDL receptor
- **Step 7:** In the liver, LDL is hydrolyzed; cholesterol is secreted into intestine either directly or after getting converted into bile acids.

12. Give an account of metabolism of chylomicrons.

Metabolism of chylomicrons

Chylomicrons are synthesized in the intestine; they transport dietary lipids from intestine to peripheral tissues (Fig. 6.15).

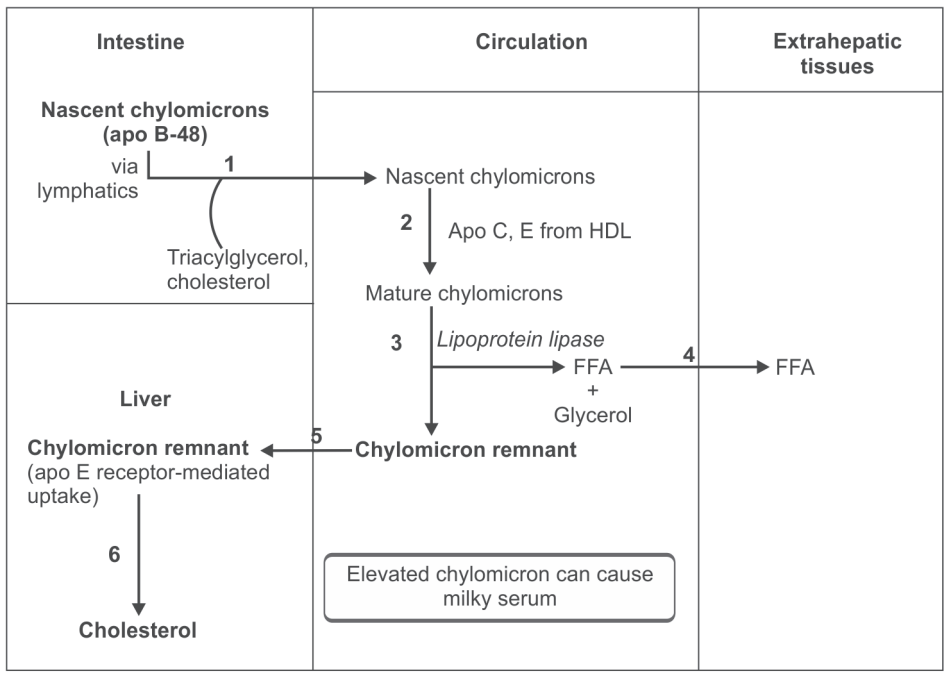


Fig. 6.15: Metabolism of chylomicrons (HDL, high-density lipoproteins; FFA, free fatty acid)

- **Step 1:** Nascent chylomicrons are synthesized in the intestinal cells, which take up dietary triacylglycerol, cholesterol and transport them into circulation via lymphatics
- **Step 2:** In the circulation, it acquires apolipoproteins (C and E) from HDL and becomes mature chylomicron
- **Step 3:** Lipoprotein lipase will act on chylomicrons to release free fatty acids and glycerol; chylomicron is converted to chylomicron remnants
- **Step 4:** FFA released from chylomicrons is taken up by peripheral tissues
- **Step 5:** Chylomicron remnants are taken up by apo E-mediated uptake by liver
- **Step 6:** Cholesterol from chylomicron remnants is secreted into bile either directly or after getting converted into bile acids.

13. How is HDL metabolized?

High-density lipoprotein (HDL) is responsible for transport of cholesterol from peripheral tissues to liver and this process is termed as **reverse cholesterol transport**. Low HDL levels are associated with increased incidence of atherosclerosis and coronary artery disease.

Metabolism of high-density lipoprotein (Fig. 6.16)

- **Step 1 and 2:** Synthesis of nascent HDL occurs in the intestine and liver—it is then released into circulation; it is discoidal in shape
- **Step 3:** Nascent HDL takes up cholesterol from peripheral tissue

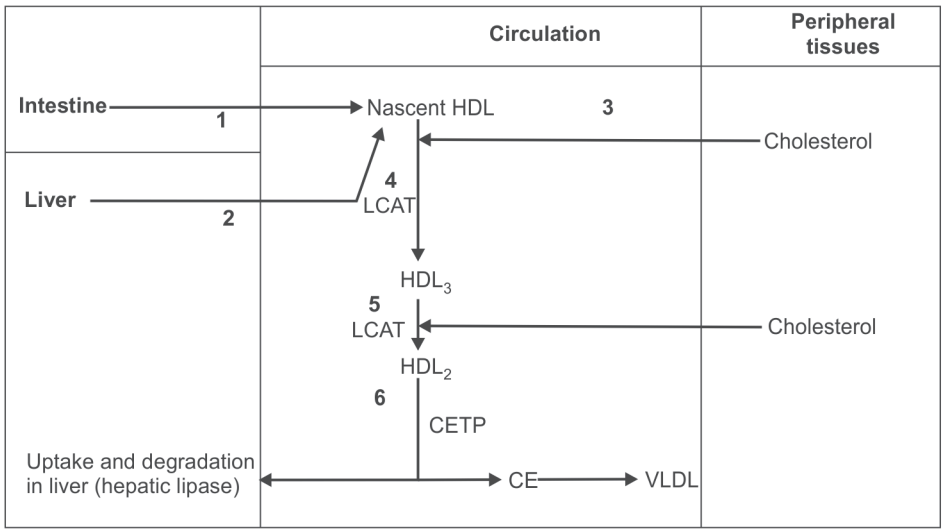


Fig. 6.16: Metabolism of high-density lipoprotein (CETP, cholesterol ester transfer protein; CE, cholesterol ester)

- **Step 4:** Lecithin cholesterol acyl transferase (LCAT) binds to discoidal HDL and esterifies free cholesterol → formation of spherical HDL_3
- **Step 5:** HDL_3 accepts more cholesterol (from tissues), which is esterified by LCAT→ formation of larger HDL_2
- **Step 6: Cholesterol ester transfer protein (CETP):** Transfers the cholesterol esters from HDL_2 to VLDL in exchange for triacylglycerol from VLDL. HDL is taken up by liver and degraded by hepatic lipase.

14. Enlist the functions of important apolipoproteins.

The functions of important apolipoproteins are listed in Table 6.6.

Table 6.6: Functions of apolipoproteins

Apolipoprotein	Functions
Apo A-I	Structural component of HDL; activates LCAT
Apo B-100	Structural component of VLDL and LDL; ligand for LDL receptor
Apo C-II	Activates lipoprotein lipase
Apo E	Ligand for hepatic receptors—promotes uptake of chylomicron remnants
Lp(a)	Inhibits fibrinolysis; elevated Lp(a) levels increases the risk for atherosclerosis

15. Name the tests done under lipid profile and give their normal values.

Lipid profile tests and their normal values are given in Table 6.7.

Table 6.7: Normal lipid profile (as per ATP III guidelines)

Test	Normal values	Method
Total cholesterol	140–200 mg/dL	Cholesterol oxidase
Triglycerides	60–150 mg/dL	Enzymatic
LDL cholesterol	50–130 mg/dL	Total cholesterol—(HDL + TG/5)
HDL cholesterol	40–65 mg/dL	Enzymatic after precipitation of other lipoproteins
Total cholesterol/HDL cholesterol ratio	< 5	(Calculation)

16. Classify hyperlipidemia with suitable examples.

Definition: Increase in serum lipids either due to genetic defect or due to underlying diseases.

Classification

a. **Primary hyperlipidemia (Frederickson's classification):** It is due to genetic defect in metabolism or clearance of lipoproteins (Table 6.8).

Table 6.8: Primary hyperlipidemia

Type	Defect	Clinical features
Type I	Lipoprotein lipase deficiency	• Increased chylomicrons, creamy plasma with elevated triglycerides; xanthomas and pancreatitis are the characteristic features
Type IIa	LDL receptor defect	• Increased cholesterol rich LDL with high risk for coronary artery disease
Type IIb	Apo B overproduction	• Increased VLDL and LDL with high risk for coronary artery disease
Type III	Abnormality in Apo E	• Increased chylomicrons and VLDL with xanthomas and high risk for coronary artery disease
Type IV	Overproduction of VLDL	• Increased VLDL usually associated with diabetes and hyperinsulinemia
Type V	Secondary to disorders	• Increased chylomicrons and VLDL with increased risk for coronary artery disease

b. **Secondary hyperlipidemia:** It is characterized by elevated cholesterol or triglycerides or both. It is due to underlying diseases:
   - Nephrotic syndrome
   - Obstructive jaundice
   - Hypothyroidism
   - Diabetes mellitus.

General Principles of Treatment in Hyperlipidemia

- **Diet:** Balanced diet with low cholesterol, supplementation of poly- and mono-unsaturated fatty acids and fiber rich diet
- **Exercise:** Increased physical activity in the form of exercise
- **Drugs:** Hypolipidemic drugs like statins, fibrates, bile acid binding resins, niacin, etc.

17. Explain lipotropic factors with examples.

Lipotropic factors: These are compounds, which prevent fatty liver by proper mobilization and utilization of fat reaching/produced in the liver.

For example: Choline, betaine, inositol and methionine (vitamin B_{12}, glycine and serine are required for synthesis of lipotropic factors).

Functions

- Choline is required for synthesis of phospholipid lecithin and prevents accumulation of excessive amount of fat, thereby protects against fatty liver
- Betaine and methionine contribute methyl group for synthesis of choline and carnitine. Carnitine favors increased movement of fatty acids into the mitochondria during β-oxidation, thus prevents accumulation of fat.

18. Write a note on fatty liver.

Definition: Excessive accumulation of triacylglycerol in the liver either due to increased production or due to decreased clearance of fat. In untreated cases, chronic accumulation of fat in the liver can cause fibrotic changes, which can progress to cirrhosis.

Causes

- *Starvation, high fat diet or uncontrolled diabetes mellitus:* All these conditions lead to increased plasma free fatty acids (from adipose tissue or extrahepatic tissues), which are taken up by liver and esterified. Entry of free fatty acids to the liver exceeds the capacity of VLDL to secrete fat from liver leading to fatty liver.
- *Deficiency of lipotropic factors like choline, betaine, methionine, etc.* leads to decreased synthesis of phospholipids in lipoproteins, causing decreased clearance of fat from liver.
- *Chronic alcoholism:* Alcohol metabolism causes increased $NADH/NAD^+$ ratio, which blocks TCA cycle and β-oxidation. Also, alcohol provides acetyl-CoA, which stimulates lipogenesis and cholesterol synthesis.
- *Carbon tetrachloride, chloroform, lead and arsenic:* Chronic exposure to these compounds, which are hepatotoxic, can impair lipoprotein synthesis and secretion of fat from liver leading to fatty liver.
- *Essential fatty acid deficiency:* They can contribute to development of fatty liver, as they are components of phospholipids.

19. Explain lipid storage disorders or sphingolipidoses.

Sphingolipidoses: Group of inherited lipid storage disorders due to gene mutations leading to defective synthesis of specific lysosomal hydrolytic enzymes responsible for breakdown of lipids (Table 6.9).

Table 6.9: Sphingolipidoses

Disease	Defective enzyme	Clinical features
Tay-Sachs disease	Hexosaminidase A	• **S**pot in macula (Blindness) **A**shkenazic jews **C**NS degeneration (mental retardation) **H**exosaminidase A deficiency. **S**torage disease (GM_2 gangliosidosis) [MN: Sachs]
Sandhoff disease	Hexosaminidase A and B	• Mental retardation, blindness, muscle weakness
Fabry disease	α-galactosidase	• Renal failure; death at young age
Gaucher's disease	β-glucosidase	• Hepatosplenomegaly, long bone erosion, mental retardation
Krabbe disease	β-galactosidase	• Mental retardation
Niemann-Pick disease	Sphingomyelinase	• Hepatosplenomegaly, mental retardation

20. Explain briefly about alcohol metabolism.

Metabolism of alcohol

Alcohol metabolism (Fig. 6.17, p. 86) generates NADH, which inhibits TCA cycle. Increased NADH also converts oxaloacetate to malate (inhibits gluconeogenesis and causes hypoglycemia). Acetyl-CoA generated is the raw material for lipogenesis and cholesterol synthesis.

21. What is the significance of brown adipose tissue?

Brown adipose tissue metabolism generates heat rather than ATP due to presence of natural uncoupler of oxidative phosphorylation (thermogenin). Thermogenin can act as a proton conductance pathway through the inner mitochondrial membrane and thereby uncouples oxidation from phosphorylation, which generates heat.

Significance

People with significant amount of brown adipose tissue have less chance to develop obesity. Brown adipose tissue is very active in hibernating animals and in newborn.

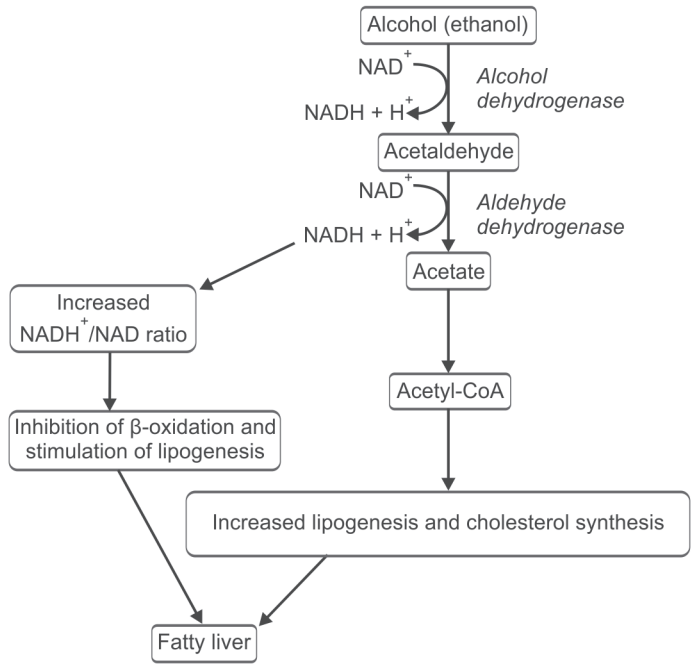


Fig. 6.17: Metabolism of alcohol

22. What is atherosclerosis? Mention the causes for it.

Definition: Atherosclerosis is a complex disease characterized by thickening or hardening of arteries due to accumulation of lipids. Events in atherosclerosis are given in Figure 6.18.

Predisposing Causes of Atherosclerosis and Coronary Artery Disease

- Diabetes mellitus
- Hyperlipoproteinemias
- Nephrotic syndrome
- Hypothyroidism
- Obesity, smoking, hypertension.

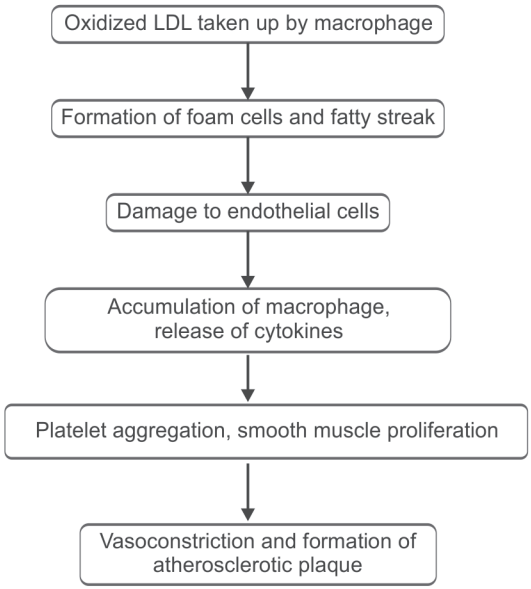


Fig. 6.18: Events in atherosclerosis

Key Points

Lipases: Pancreatic lipase, gastric lipase, lipoprotein lipase and hormone sensitive lipase.

End products of lipid digestion: Free fatty acids, 2-monoacylglycerol, 1-monoacylglycerol and cholesterol.

Steatorrhea: When a person is unable to digest fat, his stool becomes bulky, glistening, yellow brown and foul smelling and floats on the surface of the water. Seen in pancreatic insufficiency, obstructive jaundice (defective bile salts secretion), cystic fibrosis, shortened bowel and colipase deficiency (> 6 g of fat excreted in stool/day).

Chyluria: Characterized by excretion of milky urine due to abnormal connection between urinary tract and lymphatics.

Chylothorax: Characterized by milky pleural fluid in pleural space due to abnormal connection between pleural space of lungs and lymphatics of small intestine.

Congenital abetalipoproteinemia: Characterized by accumulation of triglycerides in the intestinal cells due to lack of **apo B-48** required for lipoprotein formation (chylomicrons).

Gastric lipase: Digests triacylglycerol containing medium- and short-chain fatty acids. This has an important role in digestion of lipids in infants and also in patients with pancreatic insufficiency. This is the basis for treatment of such patients with triacylglycerol containing medium- and short-chain fatty acids.

Enterohepatic circulation of bile salts: Reabsorption of bile salts from the intestine into liver, followed by resecretion into intestine.

Gallstones: Formed due to increased cholesterol levels in bile, which reduces its ability to solubilize in bile. This can be treated with chenodeoxycholic acid, an inhibitor of HMG CoA reductase.

Medium-chain fatty acids: These are absorbed into portal circulation as free fatty acids.

Orlistat: It is a drug, which inhibits pancreatic lipase—used in the treatment of obesity.

Cholecystokinin and secretin: These are hormones which stimulate secretion of enzymes of digestion and HCO_3^- (neutralizes the acidic chyme) respectively.

Glycerol is not utilized by adipocytes: Due to lack of glycerol kinase, it enters into liver and is converted into dihydroxyacetone phosphate (DHAP), which may enter into gluconeogenesis or glycolysis.

Carnitine (made up of lysine and methionine): Transports the long-chain fatty acids from cytosol to mitochondria for β-oxidation. It has three enzymes, carnitine palmitoyltransferase I (CPT I), carnitine palmitoyltransferase II (CPT II), translocase. Patients undergoing dialysis can lose carnitine, which may predispose to fatty liver.

Malonyl-CoA: Inhibits CPT I.

Number of ATPs generated after complete oxidation of palmitic acid (16C): 106.

β-oxidation of odd-chain fatty acid (valeric acid): Gives rise to acetyl-CoA and propionyl-CoA. Propionyl-CoA is converted into succinyl-CoA and enters into TCA cycle.

Methyl-malonic acidemia: Due to deficiency of enzyme methyl malonyl-CoA mutase or vitamin B_{12}, which is a coenzyme for conversion of L-methyl malonyl-CoA to succinyl-CoA.

Zellweger's syndrome: Lack of peroxisomes leading to accumulation of very long-chain fatty acids.

Refsum's disease: Autosomal recessive disorder characterized by accumulation of phytanic acid due to deficiency of α-oxidase (enzyme for α-oxidation).

Jamaican vomiting sickness: Due to consumption of akee fruit (containing hypoglycin A), which inhibits medium-chain fatty acyl-CoA dehydrogenase.

Omega oxidation: Minor pathway for oxidation of fatty acids, catalyzed by hydroxylase enzymes involving cytochrome P_{450}. The $-CH_3$ in omega position is converted to $-CH_2OH$ and then oxidized to –COOH thus forming dicarboxylic acid, which further undergoes β-oxidation.

Dicarboxylic aciduria: In deficiency of acyl-CoA dehydrogenase, fatty acids undergo omega oxidation and produce large amounts of dicarboxylic acids, which are excreted in urine.

Lipotropic factors: Choline, betaine, methionine (prevent fatty liver).

Sources of NADPH: HMP shunt and malic enzyme.

Thiophorase: Is absent in liver, so it cannot utilize ketone bodies.

Rothera's test: Acetone and acetoacetic acid can form purple color complex with sodium nitroprusside in the presence of ammonia.

Ferric chloride test: Less commonly done test for ketone body detection. It can be used for detection of phenylpyruvic acid in case of phenylketonuria.

Causes for ketoacidosis (> 0.2 mmol/L): Uncontrolled diabetes and prolonged starvation.

Tay-Sach's disease: Due to deficiency of β-hexosaminidase A leading to accumulation of gangliosides (GM_2) and neurological manifestations.

Niemann-Pick disease: Due to deficiency of sphingomyelinase and characterized by accumulation of sphingomyelin, hepatosplenomegaly and neurological manifestations.

Gaucher's disease: Deficiency of β-glucosidase leading to accumulation of glucocerebrosides, hepatosplenomegaly and neurological manifestations.

Statins (atorvastatin, lovastatin): Drugs, which reduce the serum cholesterol levels by inhibiting HMG CoA reductase (rate limiting enzyme).

Bile acids: Cholic acid, chenodeoxycholic acid, deoxycholic acid and lithocholic acid.

7α-hydroxylase: Is rate limiting enzyme in bile acid synthesis.

Cholestyramine: Is a drug, which binds to bile acids and prevents their reabsorption (treatment of hypercholesterolemia).

Fibrate: Is a drug, which increases the lipoprotein lipase activity and thereby reduces the triglyceride levels in blood.

Abetalipoproteinemia: Hypolipoproteinemia due to defect in triglyceride transfer protein, leading to an inability to load apo B with lipid. As a result, chylomicrons or VLDL are not formed and triacylglycerol accumulates in liver and intestine.

Type IIa hyperlipoproteinemia: Due to LDL receptor defect—increased risk for coronary artery disease.

Lipoprotein (a): Structurally similar to LDL particle with apoprotein a (apo a). Increased lipoprotein (a) can inhibit fibrinolysis and increase risk for development of atherosclerosis.

Free radicals: Can cause oxidation of LDL particles, which triggers the formation of foam cells and atherosclerotic plaque.

7 Amino Acid and Protein Chemistry

1. Classify amino acids with examples.

Based on nature of side chain

Non-polar amino acids: Alanine, Phenylalanine, Valine ('al' is common to them).

Uncharged (neutral), polar amino acids: Serine, Threonine, Tyrosine (OH containing).

Acidic amino acids: Aspartic acid, Glutamic acid.

Basic amino acids: Arginine, Lysine, Histidine.

Based on nutritional requirement

Essential amino acids: **M**ethionine, **A**rginine, **T**hreonine, **T**ryptophan, **V**aline, **I**soleucine, **L**eucine, **P**henylalanine, **H**istidine, **L**ysine [MN: MATT VIL PHLy].

Non-essential amino acids: **G**lutamic acid, **A**spartic acid, **G**lutamine, **A**sparagine, **G**lycine, **A**lanine [MN: GA GA GA].

Based on metabolic fate

Glucogenic amino acids: **G**lutamic acid, **A**spartic acid, **G**lutamine, **A**sparagine, **G**lycine, **A**lanine [MN: GA GA GA].

Ketogenic amino acids: Leucine.

Both glucogenic and ketogenic: **P**henylalanine, **L**ysine, **I**soleucine, **T**yrosine and **T**ryptophan [MN: SPLITT].

2. Define various orders of protein structures by giving examples.

Primary structure: It is defined as the number and sequence of amino acids in a protein. It also includes the position of disulfide bonds.

Secondary structure: Local folding (regular/irregular) of short segments of polypeptides results in secondary structure. For example, α-helices, β-pleated sheets and random coils.

Tertiary structure: It is the folding and linking between secondary structural elements of a protein to give it a three-dimensional structure. For example, β-barrel in pyruvate kinase, four-helix bundle in cytochromes.

Quaternary structure: It refers to the regular association of two or more polypeptide chains to form a complex. It is stabilized by weak interactions. Each polypeptide is called subunit. For example, hemoglobin and immunoglobulin.

3. Explain secondary structures of proteins with examples.

Definition: Local folding (regular/irregular) of short segments of polypeptides results in secondary structure. For example, α-helices, β-pleated sheets and random coils.

Alpha-helix

Structure: It is formed by regular folding of polypeptide chain to give a right-handed helical conformation having 3.6 amino acids per turn with a pitch of 5.4 Å and distance between adjacent amino acid is 1.5 Å (Fig. 7.1). For example, α-helix is present in hemoglobin and myoglobin.

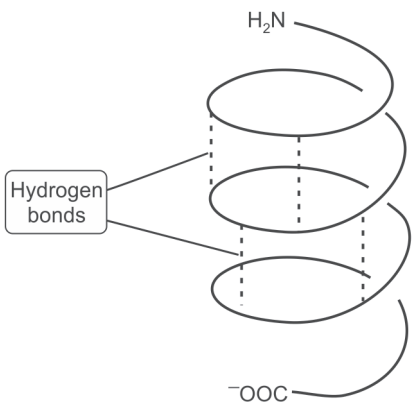


Fig. 7.1: α-helix formed by coiling of polypeptide chain

Common amino acids in α-helix: **M**ethionine **AL**anine and **L**eucine [MN: MALL].

Amino acid disrupting α-helix: **P**roline, **A**rginine, **G**lutamic acid, **A**spartic acid, **L**ysine [MN: PAGAL].

Bonds stabilizing α-helix: Hydrogen bonds between adjacent amino acids.

Beta-pleated sheet

Structure: It is a regular folding of polypeptide chain to give a pleated-sheet like appearance. It is fully extended polypeptide chain with 3.5 Å distance per amino acid. For example, carbonic anhydrase and silk fibroin.

Bonds stabilizing β-pleated sheet: Interchain or intrachain hydrogen bonds stabilize β-pleated sheets. Disulfide bonds play a key role in U bends.

Direction of β-pleated sheet: Strands may be parallel (N-termini of both strands at the same end) or antiparallel (Figs 7.2a and b).

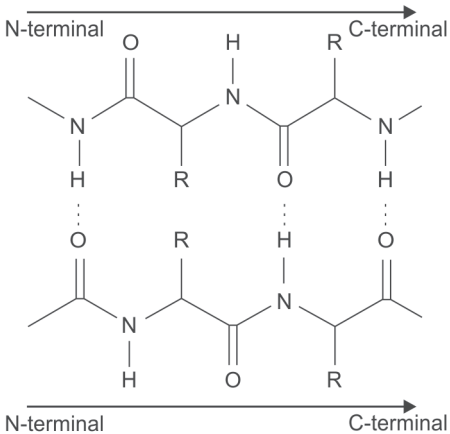


Fig. 7.2a: Parallel β-pleated sheet

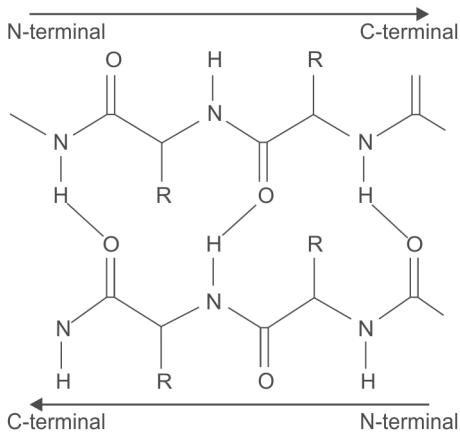


Fig. 7.2b: Antiparallel β-pleated sheet

4. Explain various bonds seen in protein structure.

Six kinds of bonds are seen in a protein structure. They are peptide bonds, hydrogen bonds, van der Waals interactions, hydrophobic interactions, ionic interactions and disulfide linkages.

Hydrogen bonds: It is formed by sharing of hydrogen between two electron donors. H is donated by: -OH, -NH, $-NH_2$; H-acceptors: COO^-, C = O, -S-S-.

Electrostatic (ionic) bonds: It is formed by attraction between two oppositely charged amino acids. Positively charged amino acids—lysine, arginine. Negatively charged amino acids—aspartic acid and glutamic acid.

Hydrophobic bonds: It is formed by interaction between the side chains of non-polar amino acids. They are seen in the core of proteins.

van der Waals forces: These are attraction-repulsion forces between all atoms due to oscillating dipoles.

Disulfide bonds: These are covalent linkages that may form when the thiol groups of two cysteine residues are oxidized to a disulfide: 2 R-SH → R-S-S-R.

Peptide bonds: It is a covalent bond formed between α-carboxyl group of one amino acid with α-amino group of the succeeding amino acid.

5. What is isoelectric pH? Mention its significance.

Definition: The pH at which a protein possesses equal number of positive and negative charges (net charge is zero) is called isoelectric pH with respect to that protein (Table 7.1).

Significance: At this pH, proteins have least solubility and they precipitate in solution.

Table 7.1: Isoelectric pH of different proteins

Proteins	Isoelectric pH
Pepsin	1.1
Casein	4.6
Albumin	4.7

6. What is denaturation of proteins?

Definition: Denaturation of proteins is an irreversible disruption of secondary, tertiary and quaternary structure of proteins leading to loss of their physical, chemical and biological properties.

Denaturing agents

- *Physical agents:* Heat, vigorous mixing, X-rays, ultraviolet (UV) radiation, etc.
- *Chemical agents:* Acids, alkali, organic solvents, salts of heavy metals, high concentration of urea, etc.

7. Name some biologically important peptides giving their significance.

Definition: Peptides with few amino acids (4–10) are generally termed as oligopeptides (Table 7.2).

Table 7.2: Biologically important peptides

Name	Property
Carnosine (β-alanyl-L-histidine) Anserine (N-methyl-carnosine)	These two peptides are found in muscle. They activate myosin adenosine triphosphatase (ATPase) activity
Glutathione (GSH tripeptide: γ-glutamyl-cysteinyl-glycine)	It is an important component of cellular antioxidant defense system. It participates as a cofactor in many reactions
	Two molecules of GSH can donate a pair of hydrogen to reduce a substrate $H_2O_2 + 2GSH \xrightarrow{\textit{Glutathione peroxidase}} 2H_2O + GS\text{-}SG$
Thyrotropin-releasing hormone (TRH) (contain 3 amino acids)	Secreted by hypothalamus. It stimulates the release of thyroid-stimulating hormone (TSH) from anterior pituitary
Vasopressin (antidiuretic hormone: contains 9 amino acids)	Secreted by posterior pituitary gland. It regulates water excretion by kidney
Enkephalin (pentapeptide)	Peptide found in brain, which inhibits the sense of pain
Angiotensin II	A peptide with 8 amino acids, which stimulates release of aldosterone from adrenal cortex
Bradykinin (contains 9 amino acids)	Vasodilator peptide

8. Enumerate different functions of proteins with suitable examples.

Function of proteins are shown in Table 7.3.

Table 7.3: Functions of proteins [MN: C TRENDS]

Functions	Examples
Structural proteins	Keratin of hair and nails, collagen (bones, cartilage and connective tissue)
Contractile proteins	Actin, myosin
Transport proteins	Hemoglobin, serum albumin, transferrin
Regulatory proteins	Hormones (insulin, glucagon, growth hormone)
Enzymes	Pepsin, trypsin, hexokinase
Defense proteins	Immunoglobulin, fibrinogen
Storage proteins	Ferritin, hemosiderin

9. Classify proteins based on nutritional value.

Based on nutritional value, proteins are classified as:

a. **Complete proteins (first class proteins):** These are rich in all essential amino acids. For example, egg albumin.

b. **Incomplete proteins:** They lack one or more essential amino acids. For example, pulse proteins are poor in methionine and cereal proteins are poor in lysine (the essential amino acid that is missing in a given protein is called limiting amino acid with respect to that protein).

10. Explain methods for separation of plasma proteins.

a. **Salt fractionation:** In concentrated salt solutions like ammonium sulfate, proteins precipitate because of decreased availability of water molecules that keep them in solution. This is utilized to isolate the protein of interest. For example, globulins are precipitated by 21%–28% ammonium sulfate and albumin is precipitated by saturated ammonium sulfate.

b. **Precipitation by organic solvents:** Solvents like methanol, ethanol, acetone, etc. are dehydrating agents, which remove water needed to keep the proteins in solution resulting in their precipitation.

c. **Gel filtration:** Columns packed with gels of various porosity are used to separate proteins depending on molecular weight, size and shape. Here, proteins with higher molecular weight pass through the column faster than smaller ones, which get trapped in the gel matrix.

d. **Electrophoresis:** It is separation of proteins based on migration of the charged molecule in an electric field.

- In alkaline pH, proteins have more negative charge and move towards anode; rate of migration is directly proportional to the charge on the protein and inversely proportional to molecular weight
- In a normal pattern, albumin moves fastest followed by α1, α2, β and γ–globulin. γ-globulins move very slowly and they remain near the point of application (Fig. 7.3).

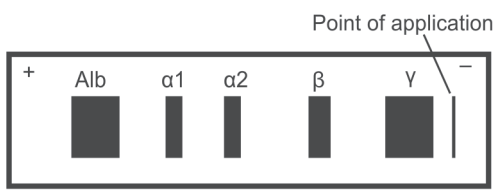


Fig. 7.3: Normal serum electrophoresis

- Relative percentage of various plasma proteins:
  Albumin: 55%–60%
  α1: 2%–4% (retinol binding globulin, cortisol binding globulin, thyroxine binding globulin)
  α2: 6%–12% (haptoglobin, ceruloplasmin)
  β: 12%–14% (transferrin, hemopexin)
  γ: 12%–24% (immunoglobulin).

11. Write short notes on albumin.

Albumin is a major plasma protein with molecular weight of 69,000 Daltons, having 585 amino acids.

Synthesis: It is synthesized in the liver (12 g/day) and has a half-life of 20 days.

Normal serum level: 3.5–5 g/dL.

Significance: Serum albumin level reflects liver function.

Functions of albumin: [MN: NOT B].

Nutritive function: It serves as a source of amino acids for tissue protein synthesis.

Osmotic function: Contributes to 80% of total plasma oncotic pressure (25 mm Hg), maintains blood volume and body fluid distribution.

Transport: Albumin transports many hydrophobic substances like bilirubin, heavy metals, calcium, copper, free fatty acids, drugs, thyroxine, etc.

Buffering action: Since it is present in high concentration, it shows maximum buffering capacity.

Histidine residues (pK = 6.1) present on albumin are responsible for its buffering action.

Hypoalbuminemia: Albumin level (< 2 g/dL) leads to enhanced fluid retention in tissue spaces leading to edema.

Causes of hypoalbuminemia [MN: BURP Him]

- **B**urns and trauma
- **U**ndernutrition (malnutrition)
- **R**enal diseases
- **P**ancreatitis—leads to malabsorption
- **H**epatic diseases.

12. Write short notes on insulin.

Synthesis: Insulin is a polypeptide hormone produced in pancreas by β-cells of islets of Langerhans. It is released from β-cells as proinsulin, which undergoes proteolytic cleavage and forms C-peptide and mature insulin (Fig. 7.4).

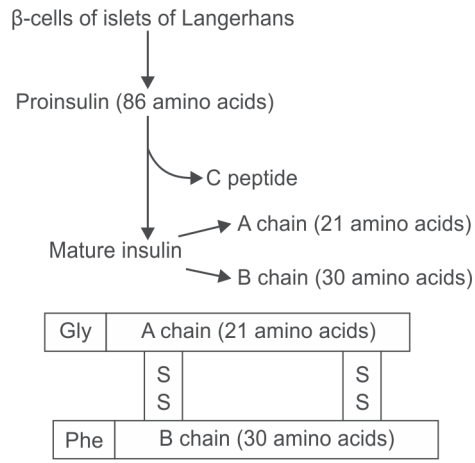


Fig. 7.4: Structure of insulin

Structure: Consists of two chains, A and B chain linked by two interchain disulfide bonds.

Function: Insulin decreases blood glucose by activating glycolysis, protein synthesis, lipogenesis, glycogenesis, ketogenesis and inhibiting gluconeogenesis, lipolysis, proteolysis and ketolysis.

13. Write short notes on immunoglobulins.

Definition: Immunoglobulins (Ig) are the defense proteins in plasma produced by B-lymphocytes in response to a foreign antigen. They bind to antigens and destroy them.

Classification [MN: GAMED]: Five different types of immunoglobulins depending on the type of heavy chain (Table 7.4).

Table 7.4: Types of immunoglobulins

Immunoglobulin	Heavy chain
IgG	Gamma (γ)
IgA	Alpha (α)
IgM	Mu (μ)
IgE	Epsilon (ε)
IgD	Delta (δ)

Functions of immunoglobulins are given in Table 7.5.

Table 7.5: Functions of immunoglobulins

Type	Properties	Functions
IgG	Major immunoglobulin present in highest amount in plasma (75%–80%).	Protects the body against various infections. It can cross placenta and provide immunity to fetus.
IgA	Occurs as a monomer or dimer held by J chain. Produced by secretory cells of respiratory, digestive and urinary tracts, lacrimal and salivary glands.	It provides local immunity.
IgM	Largest Ig composed of 5 Y-shaped units held by J chains.	It is the first antibody produced when bacteria/virus enters the body. It cannot cross the blood vessel wall.
IgE	Contains a single Y-shaped monomer. It is produced by plasma cells.	It binds tightly with mast cells releasing histamine and causing allergy. It increases during allergy.
IgD	Present on the surface of B cells.	Functions as B cell receptor.

General structure of immunoglobulin (Fig. 7.5)

Variable and constant region: Each chain (L or H) has an amino terminal (variable region) and carboxyl terminal (constant region).

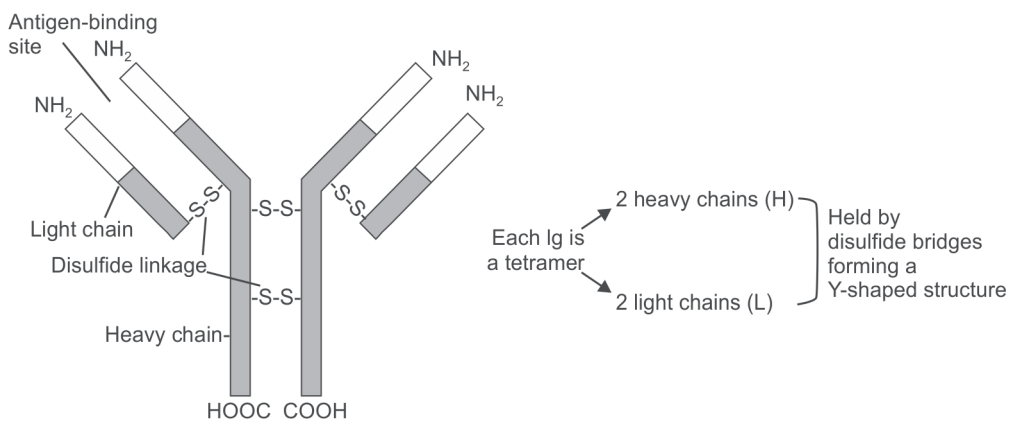


Fig. 7.5: Structure of immunoglobulin (Unshaded portion = variable region; shaded portion = constant region)

Antigen-binding site: Two antigen-binding sites on the two arms of the Y formed by amino terminal half of light chain and one fourth of heavy chain.

Fc segment: C-terminal half of the heavy chain forms the Fc segment.

Bonds which stabilize immunoglobulin structure: Interchain and intrachain disulfide bonds.

14. Write a note on structure of collagen.

Collagen: It is the most abundant fibrous protein forming structural component in human body.

Structure (triple helix): It has three polypeptides that are wound around each other to form a rope-like structure.

Bonds stabilize triple helix: Hydrogen bonds between adjacent polypeptides.

Amino acid sequence: Collagen is rich in glycine and proline. Glycine is found as every third amino acid and represented by Gly-X-Y, where X is frequently proline and Y is often hydroxyproline or hydroxylysine. Vitamin C is required for maturation of collagen.

Defects in formation and maturation of collagen

Scurvy: Deficiency of vitamin C (required for hydroxylation of proline and lysine) leads to poor maturation of collagen. Patient presents with bleeding of gums, poor wound healing and easy bruisability.

Ehlers-Danlos syndrome: Stretchy skin and loose joints with poor strength of connective tissue is due to a defect in collagen processing.

Osteogenesis imperfecta or brittle bone syndrome is a disorder with brittle bone, poor healing of wounds and twisted spine.

15. Explain the structure and functions of hemoglobin.

Types of hemoglobin (in adult blood)

- HbA (97%)—2 α and 2 β chains
- HbA_2 (3%)—2 α and 2 δ chains
- HbF—2 α and 2 γ chains.

Structure of adult hemoglobin (Fig 7.6): It is a tetramer protein made up of two types of polypeptide chains. Almost 80% of hemoglobin structure is α-helix, which is divided into eight segments (A to H). Heme group occupies a crevice lined by non-polar residues and two histidines (E-7 and F-8).

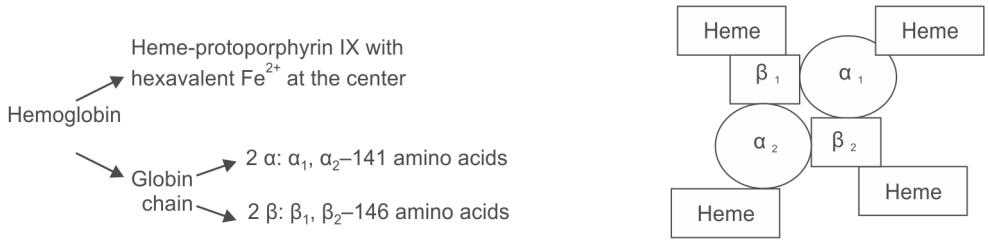


Fig. 7.6: Structure of hemoglobin

Bonds between two polypeptides in each of dimer ($\alpha_1\beta_1$) and ($\alpha_2\beta_2$): Interchain interactions, ionic bonds and hydrogen bonds.

Relative position of dimers results in two forms of Hb: T-form (taut or tense) and R-form (relaxed). T-form has low affinity for oxygen. R-form has high affinity for oxygen. Hemoglobin can bind four oxygen molecules, one each at four heme groups.

Factors affecting binding of O_2 to Hb: Partial pressure of O_2, pH, partial pressure of CO_2 and the presence of 2,3-bisphosphoglycerate.

Cooperativity: Binding of O_2 to one heme increases the affinity of other hemes for oxygen.

Functions of hemoglobin

1. Transport of oxygen from lungs to peripheral tissues and CO_2 from periphery to lungs.
2. Acts as buffer to maintain the pH of the blood.

16. Write briefly on oxygen transport by hemoglobin.

a. Binding of oxygen to hemoglobin:

Binding of first oxygen molecule to deoxyhemoglobin

↓

Movement of heme iron into plane of heme

↓

Rupture of salt bridges between subunits

↓

Transition of hemoglobin from low affinity T (taut) state to high affinity R (relaxed state)

↓

Rupture of remaining few salt bridges

Progressive increase in affinity of remaining heme of unoxygenated subunits for oxygen **(cooperativity)**

↓

Binding of oxygen to remaining subunits

b. Release of oxygen from hemoglobin:

Decrease in pH/increase in pCO_2 (peripheral tissues)

↓

Decrease affinity of hemoglobin for oxygen; hemoglobin stabilized in the T state (Bohr effect)

↓

Release of oxygen to peripheral tissues

17. What are hemoglobinopathies?

Hemoglobinopathies are group of disorders characterized by production of abnormal Hb molecules. For example, sickle cell disease, hemoglobin C disease, methemoglobinemia and thalassemia (Table 7.6).

Table 7.6: Hemoglobinopathies

Disease	Defect	Symptoms
Sickle cell anemia (homozygous)	Autosomal recessive disorder. Here, glutamate at 6th position of β-chain is replaced by non-polar valine (it moves slower than HbA towards anode in electrophoresis).	In deoxygenated state, HbS polymerizes into long fibrous aggregates → blocks capillaries → tissue anoxia → pain and death of cells.
Sickle cell trait (heterozygous)	Same as above.	Does not show symptoms.
Hemoglobin C disease	Lysine substituting glutamate at 6th position of β-globin chain (it moves slower than HbS in electrophoresis).	Mild disease, with chronic hemolytic anemia.

Contd...

Contd...

Disease	Defect	Symptoms
HbM	Replacements of α-58 histidine or β-92 histidine by tyrosine.	Oxidation of Fe^{2+} of heme to Fe^{3+} forms → fails to bind oxygen.
Thalassemias	Imbalance in the synthesis of globin chains. Caused by variety of gene mutations.	Hereditary hemolytic diseases.
α-thalassemias	Synthesis of α-chain is decreased or absent. one gene is defective → silent carrier; two genes are defective → α-thalassemia trait; three α-globin genes are defective → hemoglobin H disease.	Moderately severe hemolytic anemia Defect in all four α-globin gene causes fetal death.
β-thalassemias	β-globin chain is decreased or absent; one gene is defective → β-thalassemia minor; two genes are defective → β-thalassemia major.	Increased HbF (α_2, γ_2 and γ_4 occurs) Severe → require regular major blood transfusions.

Key Points

Acute phase proteins: Levels of certain plasma proteins are increased by several thousand folds in inflammatory and neoplastic conditions. They are called acute phase proteins, e.g. α-1-antitrypsin, haptoglobin, ceruloplasmin, C-reactive protein, etc.

Globulins: Different types of globulins present are α_1, α_2, β_1, β_2 and γ. The α- and β-globulins transport hormones, minerals, lipids, vitamins, etc. The γ-globulins provide immunity against infections.

Fibrinogen: It is an acute phase protein synthesized by liver; required for blood coagulation.

α_1-antitrypsin: It protects the elastic tissues of the lung from destructive action of elastase. Inherited deficiency of this protein is associated with emphysema and cirrhosis. It is a positive acute phase protein.

α_2-macroglobulin: It is an α_2-globulin and it inactivates all proteinases; its concentration is markedly increased in nephrotic syndrome.

C-reactive protein: It is synthesized in liver. Its levels are highly elevated in inflammation (acute phase protein). Normal plasma range is 0.5–1 mg/dL. Used in predicting the risk of cardiovascular disease.

Haptoglobin: It is an α_2-globulin capable of binding hemoglobin. Low level indicates hemolysis.

Ceruloplasmin: Normal serum level of ceruloplasmin is 25–50 mg/dL. 90% of serum copper is bound to this protein. It is elevated in infections and malignant condition. It has oxidase activity and is involved in iron metabolism. Decreased ceruloplasmin concentration is associated with Wilson disease.

Transferrin: Normal serum level of transferrin is 200–300 mg/dL. It transports iron. Increased in iron deficiency. Usually one third of its capacity is saturated with iron (TIBC—total iron binding capacity).

Multiple myeloma: Malignancy of a clone of plasma cells. Usually occurs above the age of 45. Here, large amount of immunoglobulin light chains are excreted in urine, which is called Bence Jones proteins. Serum electrophoresis shows a classical distinct band (M-band) between β and γ regions.

Liver diseases: Since albumin is synthesized in liver, its level decreases in chronic liver failure and cirrhosis. As the half-life of albumin is 20 days, acute liver diseases will not show any change in albumin concentration.

Renal diseases: Albumin may be excreted in urine because of defect in glomerular filtration (albuminuria). In nephrotic syndrome, large amount of albumin is lost in urine.

Microalbuminuria: Small amounts of albumin are lost in acute nephritis and urinary tract infection (30–300 mg/L). Microalbuminuria is an early predictor of future renal disease (normal range of albumin in urine is 0–30 mg/L).

Amyloidosis: Due to accumulation of misfolded proteins, this results in a neurodegenerative disorder, Alzheimer disease.

Prion diseases (Creutzfeldt-Jakob disease): These are a group of neurodegenerative disorders caused due to accumulation of prions (infectious misfolded proteins).

8 Digestion, Absorption and Metabolism of Proteins

1. Explain the digestion and absorption of proteins.

Protein digestion can be divided into gastric, pancreatic and intestinal phases.

Gastric phase: Food in stomach → (+) gastrin → (+) hydrochloric acid (HCl) secretion (Figs 8.1 and 8.2).

Rennin: Secreted from stomach of infant; helps in conversion of casein into calcium paracaseinate, which can be acted upon further by pepsin.

Pepsin: The chief cells of stomach secrete inactive pepsinogen, which is activated by HCl.

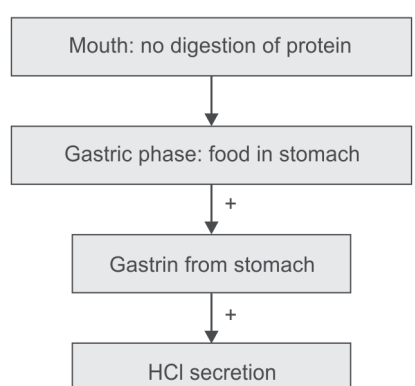


Fig. 8.1: Effect of food on acid secretion (+ = stimulates)

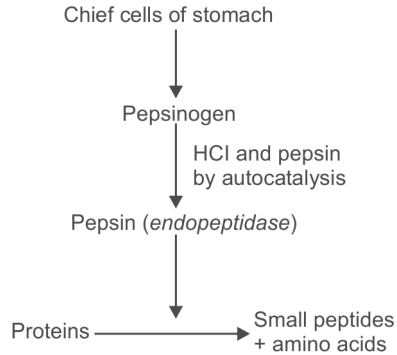


Fig. 8.2: Gastric phase of protein digestion

$$\text{Casein} \xrightarrow{\text{Rennin}} \text{Calcium paracaseinate} \xrightarrow{\text{Pepsin}} \text{Small peptides and amino acids}$$

Pancreatic phase (Fig. 8.3):

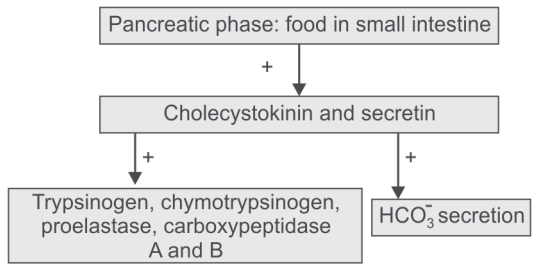


Fig. 8.3: Events leading to release of pancreatic enzymes (+ = stimulates secretion)

$$\text{Trypsinogen} \xrightarrow{\textit{Enterokinase}} \text{Trypsin} \longrightarrow \text{Cleaves peptides at carboxyl group of lysine and arginine (basic amino acids)}$$

$$\text{Chymotrypsinogen} \xrightarrow{\text{Trypsin}} \text{Chymotrypsin} \longrightarrow \text{Cleaves at carboxyl group of aromatic amino acids}$$

$$\text{Proelastase} \xrightarrow{\text{Trypsin}} \text{Elastase} \longrightarrow \text{Cleaves the carboxyl group of } \mathbf{G}\text{lycine, } \mathbf{A}\text{rginine and } \mathbf{S}\text{erine [MN: GAS]}$$

Procarboxypeptidase A and B $\xrightarrow{\text{Trypsin}}$ Carboxypeptidase A and B $\longrightarrow$ Cleave one amino acid at a time from carboxyl end of peptides

Intestinal phase: Digested products of gastric and pancreatic phase (oligopeptides, tripeptides and dipeptides) enter into intestinal phase of digestion. Aminopeptidase is an exopeptidase that cleaves one amino acid at a time from amino terminals of peptides. Dipeptidases and tripeptidases are endopeptidases. These enzymes convert peptides to amino acids.

$$\text{Dipeptides} \xrightarrow{\textit{Dipeptidase}} \text{Amino acids}$$

$$\text{Tripeptides} \xrightarrow{\textit{Tripeptidase}} \text{Amino acids}$$

$$\text{Oligopeptides} \xrightarrow{\textit{Aminopeptidase}} \text{Amino acids}$$

Absorption of amino acids: It occurs from upper small intestine (duodenum and proximal jejunum). Amino acids are absorbed along with sodium (secondary active transport, an energy dependent process (Fig. 8.4, p. 107). There are five transport systems for various types of amino acids (Table 8.1). Any defect in these transport system can cause disorders.

Table 8.1: Amino acid transport systems

Transport system	Amino acids transported	Disorders
Small and neutral AA	Ala, Ser, Thr	Hartnup disease
Large and neutral AA	ILe, Leu, Val, Tyr, Trp, Phe	Hartnup disease
Basic AA and cysteine [MN: COAL]	Cys, Orn, Arg, Lys	Cystinuria
Acidic AA	Glu and Asp	No disorder
Imino acid and Gly	Pro, Hydroxy Pro, Gly	Glycinuria

AA, amino acid; Ala, alanine; Ser, serine; Thr, threonine; ILe, isoleucine; Leu, leucine; Val, valine; Tyr, tyrosine; Trp, tryptophan; Phe, phenylalanine; Cys, cysteine; Orn, ornithine; Arg, arginine; Lys, lysine; Glu, glutamic acid; Asp, aspartic acid; Pro, proline; Hydroxy Pro, hydroxy proline; Gly, glycine.

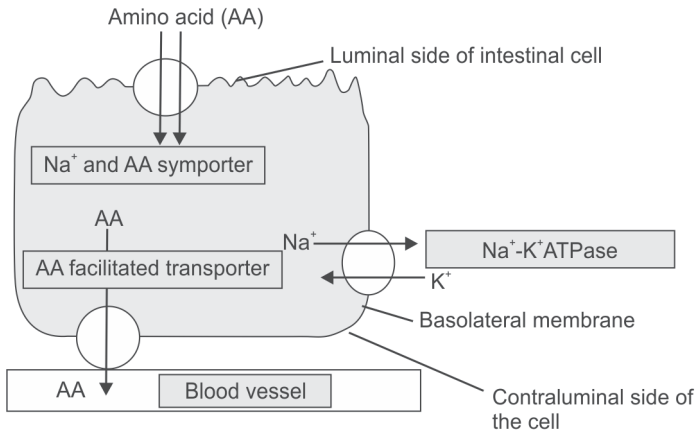


Fig. 8.4: Mechanism of absorption of amino acids (Na^+, sodium ion; AA, amino acid; K^+, potassium ion; ATPase, adenosine triphosphatase).

2. What is amino acid pool?

Amino acid pool is the total pool of amino acids available in blood at any given time for various anabolic and catabolic activities. It is influenced by the type of diet, nutritional status, metabolism, health condition, age, etc. (Fig. 8.5).

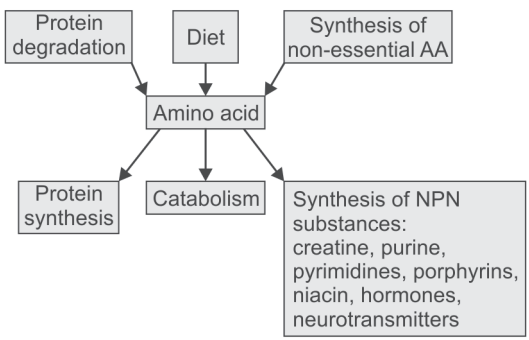


Fig. 8.5: Amino acid (AA) pool (NPN, non-protein nitrogenous)

3. What are transaminases? What are their significance?

Definition: These are enzymes that catalyze the reversible transfer of an amino group between one 'α' amino acid and an 'α' keto acid (Fig. 8.6).

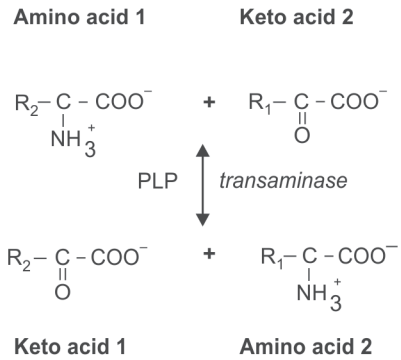


Fig. 8.6: Transamination (PLP, pyridoxal phosphate)

Features of transaminases

- Catalyze reversible reaction
- Require pyridoxal phosphate (PLP) as coenzyme
- Except lysine, threonine, proline and hydroxyproline, all amino acids undergo this reaction
- Transaminase levels will be increased in hepatitis, myocardial infarction, etc.—useful in diagnosis
- Transaminases funnel amino groups from excess dietary amino acids to those amino acids (e.g. glutamate) that can be deaminated.

Functions of transamination

- Degradation of surplus amino acids
- Synthesis of essential amino acids
- Gluconeogenesis.

4. What are deamination reactions? What is its significance?

Definition: These reactions cause removal of amino group from an amino acid.

Classification

a. *Oxidative deamination*

- Glutamate dehydrogenase (major pathway of oxidative deamination): Catalyzes removal of nitrogen group from amino acid pool (Fig. 8.7).

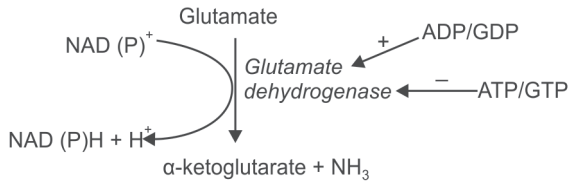
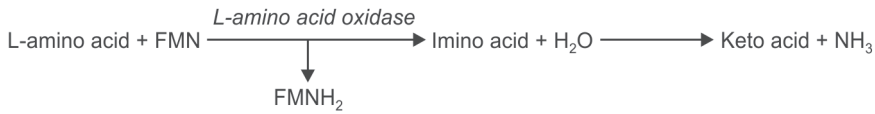


Fig. 8.7: Oxidative deamination (+ = stimulation; – = inhibition)

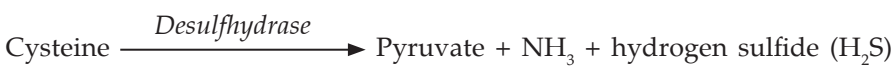
- L and D-amino acid oxidase (minor pathway): Catalyzes removal of nitrogen from amino acids in peroxisomes of liver cells.



b. *Non-oxidative deamination:* Following are the examples for non-oxidative deamination.

- Dehydration

- Desulfhydration



- Straight deaminations

5. Explain the formation and metabolism of ammonia. Add a note on regulation of urea cycle.

Reactions, which generate ammonia in the body:

Transamination: Glutamate ⟶ α-ketoglutarate + NH_3

Deamination (oxidative): Glutamate ⟶ α-ketoglutarate + NH_3

Dehydration: Serine ⟶ Pyruvate + NH_3

Desulfhydration: Cysteine ⟶ Pyruvate + NH_3

Direct deamination: Histidine ⟶ Urocanic acid + NH_3

Intestinal putrefaction: Urea ⟶ NH_3 (bacterial urease enzyme)

Purine and pyrimidine catabolism ⟶ NH_3

Transport: Ammonia is very toxic, so it is transported in blood as glutamate (by transamination of amino acids), glutamine (from brain to liver) and alanine (from muscle).

Fate of ammonia (Fig. 8.8)

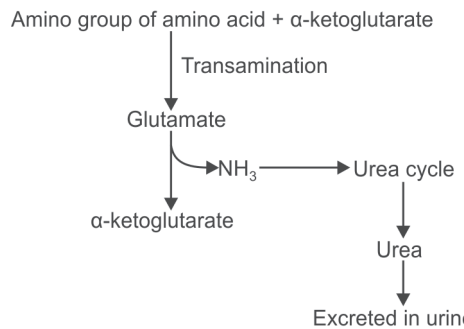


Fig. 8.8: Metabolism of amino group

Urea cycle: Converts the toxic ammonia into less toxic and more soluble urea, which is excreted in the urine (Fig. 8.9).

Requirements

- *Starting material:* CO_2 and NH_3
- *Site:* Liver
- *Subcellular site:* Partly mitochondrial and partly cytosolic
- *Nitrogen atom donors in cycle:* Ammonia and aspartate (amino group)
- *Energy (ATP):* Three ATPs are used.

Regulation of urea cycle: At the level of key enzyme carbamoyl phosphate synthetase-1 (CPS-1).

Short-term regulation: N-acetyl glutamate is positive allosteric effector of CPS-1.

Long-term regulation: By induction of enzymes of urea cycle at the gene level.

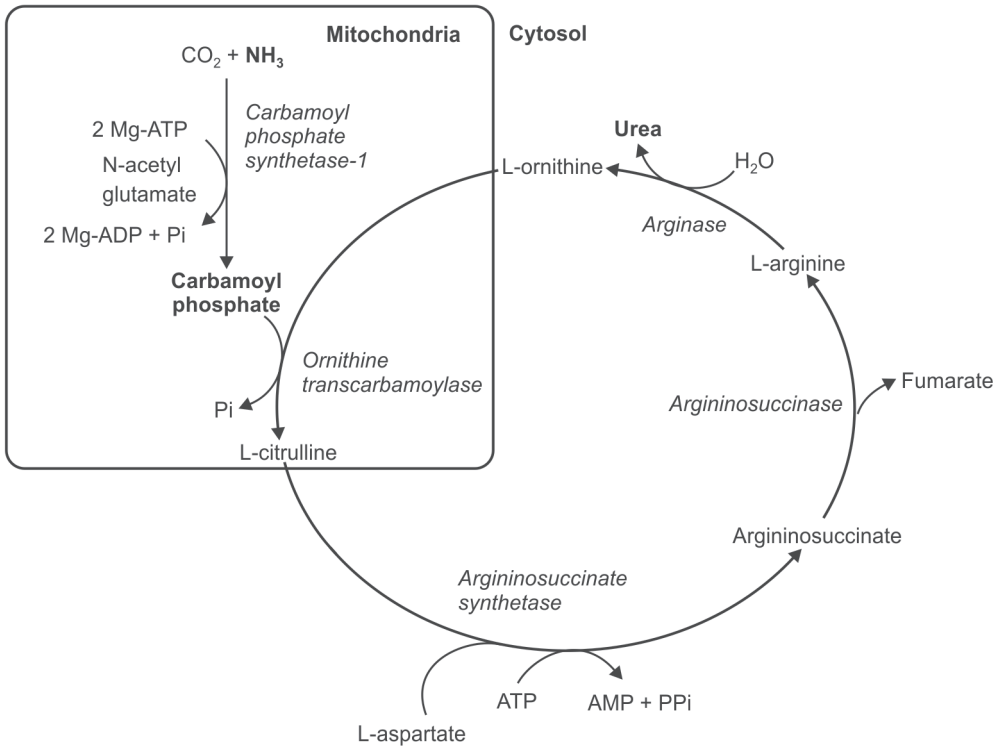


Fig. 8.9: Urea cycle (reactions in the box occur in mitochondria) (CO_2, carbon dioxide; NH_3, ammonia; Mg, magnesium; ATP, adenosine triphosphate; ADP, adenosine diphosphate; Pi, inorganic phosphate; AMP, adenosine monophosphate; H_2O, water).

6. Compare the enzymes carbamoyl phosphate synthetase I and II.

Comparison of the enzymes is shown in Table 8.2.

Table 8.2: Comparison of carbamoyl phosphate synthetase I and II

	Carbamoyl phosphate synthetase I	Carbamoyl phosphate synthetase II
Site	Mitochondria	Cytosol
Substrate	Ammonia	Amide group of glutamine
Pathway	Urea synthesis	Pyrimidine synthesis
Activator	N-acetylglutamate	Phosphoribosyl pyrophosphate (PRPP)
Inhibitor	–	Uridine triphosphate (UTP)

7. Explain urea cycle defects.

Urea cycle defects: are due to deficiency or lack of any enzyme in urea cycle (Table 8.3).

Table 8.3: Urea cycle disorders

Disorder	Enzyme deficiency	Features
Hyperammonemia type I	Carbamoyl phosphate synthetase I	Fatal or death occurs few days after birth
Hyperammonemia type II	Ornithine transcarbamoylase	X-linked; increased glutamine in blood and CSF*
Citrullinemia	Argininosuccinate synthetase	Autosomal recessive; increased citrulline excretion in urine; CSF citrulline levels are elevated
Argininosuccinic aciduria	Argininosuccinase	Elevated argininosuccinic acid levels in blood, CSF and urine
Hyperargininemia	Arginase	Elevated arginine levels in blood, CSF

*CSF, cerebrospinal fluid

General clinical features: Ammonia intoxication causes vomiting, mental retardation, convulsions, slurring of speech and blurring of vision.

Mechanism for toxicity

↑ **NH_3** → ↑conversion of α-ketoglutarate to glutamate/glutamine → ↓ activity of Krebs cycle and decreased ATP formation

↑ **Glutamate** → ↑ γ-aminobutyric acid (GABA) = an inhibitory neurotransmitter in brain

↑ **Glutamine** → ↑ osmotic effect → cerebral edema

Biochemical picture in urea cycle defects: ↑ glutamine, citrulline or arginine (blood and urine); ↑ ammonia, ↓ urea in blood and respiratory alkalosis.

Treatment of urea cycle defects

Hemodialysis: Removes ammonia from blood.

Low-protein diet: For less ammonia formation.

Arginine supplementation (in certain defects of urea cycle): Activates N-acetylglutamate synthase and gives ornithine, which is required for first reaction of urea cycle.

Citrulline supplementation (in certain defects of urea cycle): Helps to generate ornithine (as mentioned in above sentence).

Sodium benzoate and sodium phenylbutyrate: Covalently bind glycine (forming hippurate) and glutamine (forming phenylacetylglutamine) respectively, which are excreted.

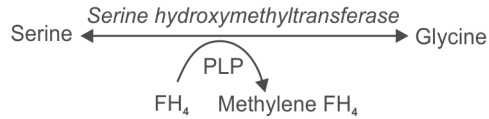
GLYCINE

8. Explain glycine metabolism (anabolism + catabolism).

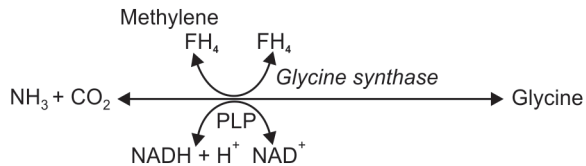
Glycine is the simplest aliphatic amino acid without asymmetric carbon atom and nutritionally non-essential.

Glycine biosynthesis: Glycine can be synthesized from serine, threonine, ammonia, glyoxylate **[MN: STAG]** and choline.

a. *Serine*

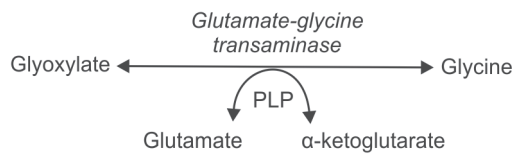


b. *Glycine synthase:* substrates—CO_2 and NH_3

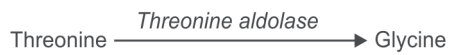


c. *Choline*

d. *Glyoxylate*

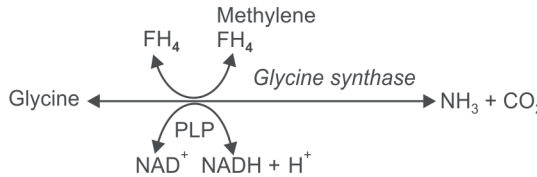


e. *Threonine*

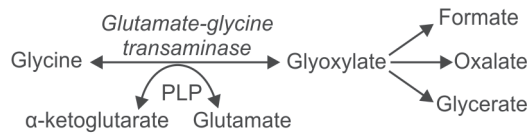


Degradation of glycine

a. *Reversal of glycine synthase reaction:* Glycine is broken down to NH_3 and CO_2.



b. *Glycine transaminase:* Converts glycine to glyoxylate.



9. Explain the metabolic disorders of glycine.

Metabolic disorders of glycine are shown in Table 8.4.

Table 8.4: Metabolic disorders of glycine

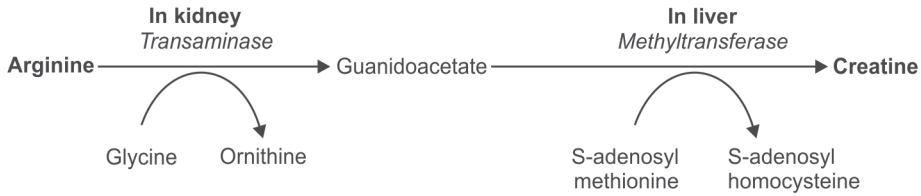
Disease	Defect	Clinical features
Primary hyperoxaluria	Defect in transamination of glyoxylate to glycine (type I). Defect in D-glyceric acid dehydrogenase. Defect in decarboxylation of glyoxylate to formate.	↑ oxalic acid in blood and urine. Renal stones—oxalate + calcium = calcium oxalate stones, which deposit in renal/extrarenal tissues.
Hyperglycinemia	Glycine cleavage system.	Elevated glycine in blood and urine. Mental retardation.
Glycinuria	Defect in reabsorption of glycine/proline in renal tubules.	Benign: Increased excretion of glycine and proline in urine.

10. Explain the formation of specialized products of glycine and their significance.

List of specialized products from glycine: Glutathione, **C**reatine, heme and purine ring (MN: **GLyCIN**; I = **I**ron containing heme; N = **N**itrogenous base purine).

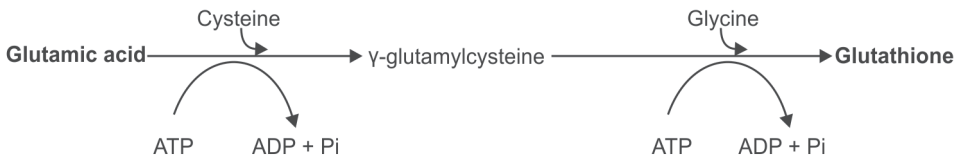
a. *Creatine*

- Phosphorylated (creatine phosphate) form is required for muscle contraction
- The amount of creatinine produced is related to muscle mass of a person and is constant
- Kidney function—serum creatinine and creatinine clearance is used to assess the kidney function.



b. *Glutathione*

- Serves as a reductant [maintains red blood cell (RBC) membrane integrity]
- Plays a role in the detoxification of drugs to render them more water soluble
- Helps in the transport of amino acids across cell membranes (the γ-glutamyl cycle)
- Serves as a cofactor for some enzymatic reactions.



c. *Heme synthesis*

- Glycine is starting material for heme synthesis.

Glycine + succinyl-CoA ⟶ Amino levulenic acid (ALA) ⟶ **Heme**

d. *Purine ring*

- Carbons 4, 5 and 7th nitrogen atoms in the purine ring are donated by glycine (Fig. 8.10).

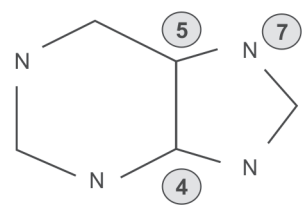


Fig. 8.10: Glycine contribution in purine ring

PHENYLALANINE

11. Explain the metabolism of phenylalanine. Substantiate why phenylalanine is both glucogenic and ketogenic.

- Metabolism of phenylalanine is same as that of tyrosine since phenylalanine is converted to tyrosine (refer Q No: 12)
- Phenylalanine is both glucogenic and ketogenic amino acid: because after getting converted into tyrosine, it is catabolized to **fumarate and acetyl-CoA**, which produce glucose and ketone bodies respectively (Fig. 8.11).

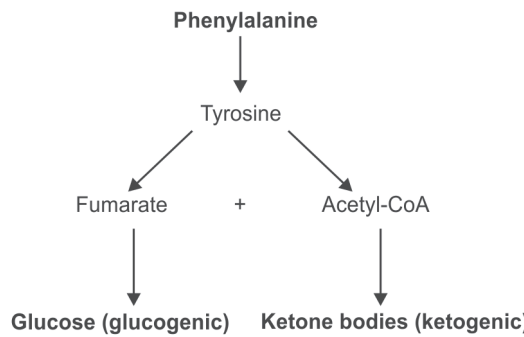
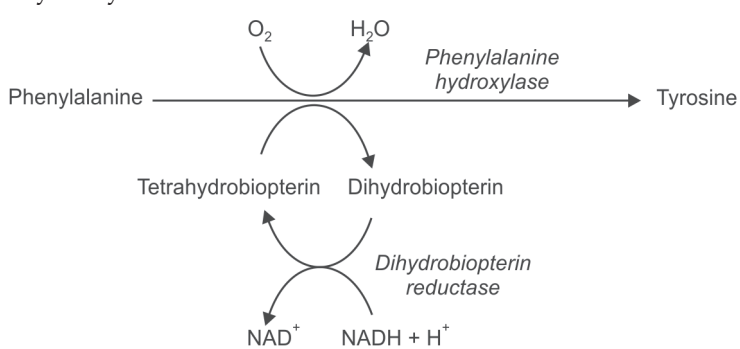


Fig. 8.11: Metabolism of phenylalanine

12. Discuss the metabolism of tyrosine. Add a note on disorders associated with tyrosine metabolism.

Synthesis: It is formed from essential amino acid phenylalanine in the presence of enzyme phenylalanine hydroxylase.



Catabolism: It generates fumarate (glucogenic) and acetyl-CoA (ketogenic) as shown in Figure 8.12.

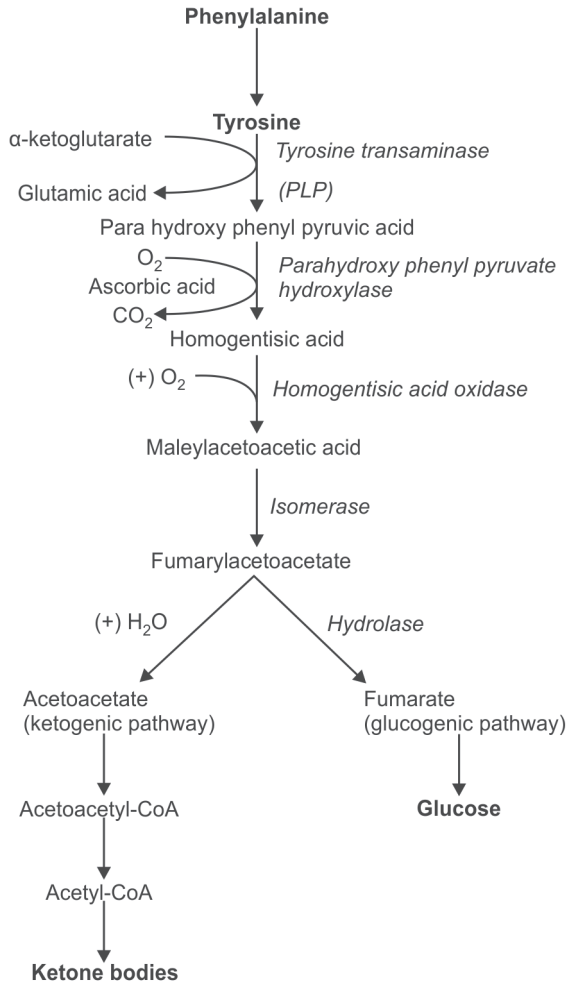


Fig. 8.12: Catabolism of tyrosine (PLP, pyridoxal phosphate)

13. Explain the formation of specialized products from tyrosine.

List of specialized products from tyrosine

- Dopamine, norepinephrine, epinephrine (catecholamines)

- Thyroid hormones—T_3 and T_4
- Melanin.

a. **Synthesis of catecholamines:** Dopamine, norepinephrine and epinephrine (Fig. 8.13).

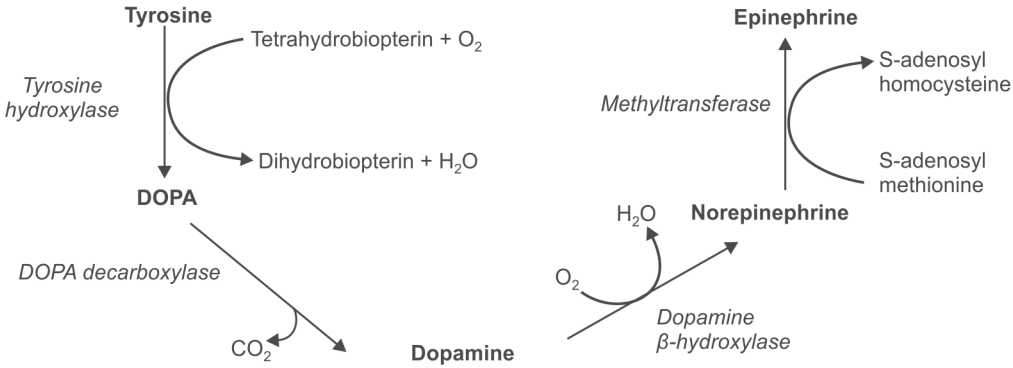
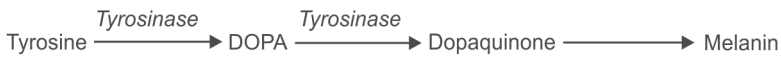


Fig. 8.13: Synthesis of catecholamines (DOPA, dihydroxyphenylalanine)

b. **Synthesis of melanin**



c. **Thyroid hormones:** Triiodothyronine (T_3), thyroxine (T_4) (Fig. 8.14).

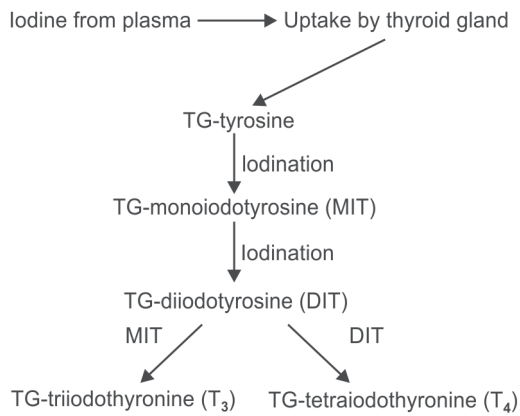


Fig. 8.14: Synthesis of thyroid hormones (TG, thyroglobulin)

14. Explain the causes and features of phenylketonuria.

Phenylketonuria (PKU): Is due to deficiency of phenylalanine hydroxylase (PAH) or dihydrobiopterin reductase. The types of PKU are explained in Table 8.5.

Table 8.5: Classification of phenylketonuria

Type	Defect	Clinical features and treatment
Type I phenylketonuria (PKU)	Lack of phenylalanine hydroxylase (PAH), also called classical PKU	Hyperphenylalaninemia (> 20 mg/dL): mental retardation, convulsions, mousy odor in urine Treatment: phenylalanine free diet
Type II	Deficiency of dihydrobiopterin reductase	Mild disease, diet therapy
Type III	Deficiency of dihydrobiopterin reductase	Mild disease, diet therapy
Type IV	Defective synthesis of dihydrobiopterin	Tetrahydrobiopterin therapy
Type V	–	Tetrahydrobiopterin therapy

Consequences of PKU (Fig. 8.15)

- *Cancer*—increased risk of skin cancers due to photosensitivity.

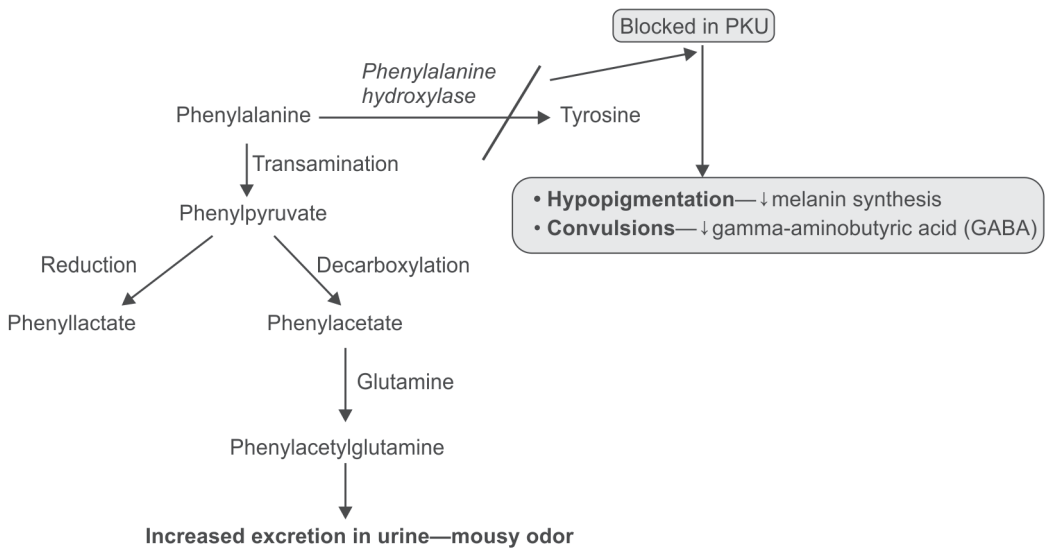


Fig. 8.15: Consequences of phenylketonuria

Biochemical tests for diagnosis and screening of PKU

Elevated blood phenylalanine may not be detectable until 3–4 days postpartum, so these tests are done 2–3 days after birth.

- Ferric chloride test in urine—blue-green color
- Guthrie test
- Prenatal screening—by deoxyribonucleic acid (DNA) analysis of amniotic cells.

Treatment of PKU

Phenylalanine free diet—to prevent early mental retardation.

15. Write a short note on alkaptonuria.

Cause: It is due to deficiency of homogentisic acid oxidase leading to excretion of homogentisic acid in urine.

Clinical features

- It is a relatively benign disorder
- *Dark urine:* On exposure to air, urine becomes dark
- *Arthritis:* Binding of homogentisic acid to cartilage induces inflammation of joints
- *Ochronosis:* Pigmentation of cartilage (often seen on the ear lobules).

Diagnosis

- Ferric chloride test—urine shows green color
- High performance liquid chromatography—to demonstrate elevated homogentisic acid.

16. Write short notes on tyrosinemia and albinism.

a. **Tyrosinemia** (Table 8.6).

Table 8.6: Types of tyrosinemia

Type	Defect	Clinical features
Tyrosinemia type I	Fumarylacetoacetate hydrolase	Hepatic failure, hypoglycemia
Tyrosinemia type II	Tyrosine transaminase	Photophobia, corneal erosion, keratosis

b. **Albinism:** It is due to deficiency of enzyme tyrosinase in the melanocytes leading to decreased melanin synthesis.

Clinical features

- Hypopigmentation of hair, skin and fundus.

TRYPTOPHAN

17. Explain how tryptophan is both glucogenic and ketogenic.

Catabolism: Tryptophan catabolism generates alanine and acetyl CoA, which form glucose and ketone bodies respectively (Fig. 8.16).

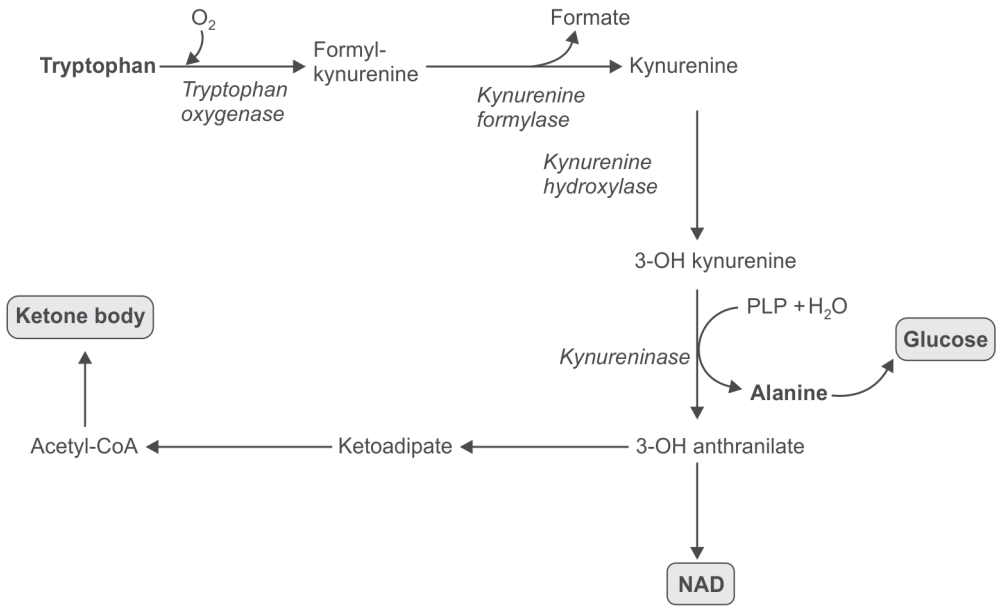


Fig. 8.16: Catabolism of tryptophan

18. Explain formation of specialized products from tryptophan.

List of specialized products from tryptophan:

a. Serotonin.
b. Melatonin.
c. Nicotinamide adenine dinucleotide (NAD).

a. **Serotonin (Fig. 8.17)**

Functions

- Neurotransmitter
- Sleep inducer
- Vasoconstriction and smooth muscle contraction
- Platelet aggregation.

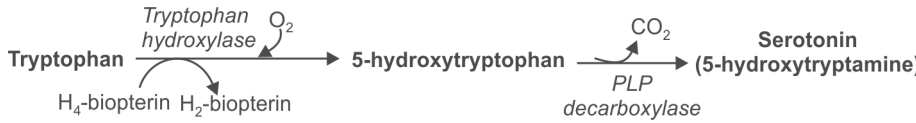


Fig. 8.17: Serotonin synthesis (H_2-biopterin, dihydrobiopterin; H_4-biopterin, tetrahydrobiopterin)

b. **Melatonin:** Secreted by pineal gland (Fig. 8.18).
Function: Neurotransmission and maintenance of circadian rhythm (sleep-wake cycle).

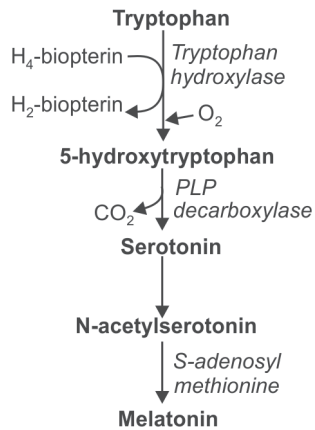


Fig. 8.18: Melatonin synthesis

c. **Nicotinamide (NAD):** Coenzyme for oxidation reduction reactions (refer vitamin niacin for more details).

19. Write a note on disorders of tryptophan metabolism.

Disorders of tryptophan metabolism are shown in Table 8.7.

Table 8.7: Disorders of tryptophan metabolism

Name	Defect	Clinical features
Hartnup disease	Defect in intestinal/renal absorption of tryptophan and other neutral amino acids—**v**aline, **a**lanine, **s**erine, **t**hreonine **[MN: VAST].**	Pellagra-like features—dementia, dermatitis, diarrhea, increased excretion of indican in urine and stool (Fig. 8.19a).
Carcinoid syndrome	Tumor of argentaffin tissue. Increased serotonin secretion.	Inceased excretion of 5-OH indole acetate (5 HIAA) in urine. Associated with flushing, hypertension, diarrhea, etc. (Fig. 8.19b).

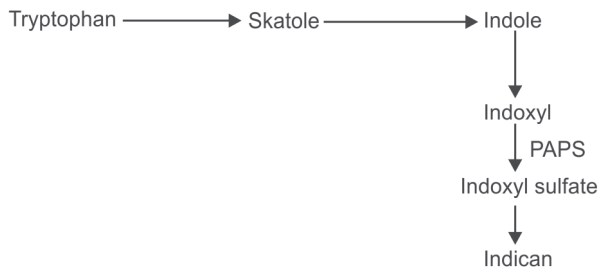


Fig. 8.19a: Tryptophan degradation in intestine (PAPS, phosphoadenosylphosphosulfate)

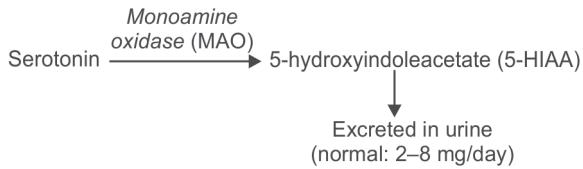


Fig. 8.19b: Degradation of serotonin

METHIONINE

20. Discuss the metabolism of methionine.

- Methionine is a glucogenic, essential amino acid
- **Products from methionine**: S-adenosylmethionine (SAM), cysteine and the products of transmethylation reactions.

Catabolism (Fig. 8.20)

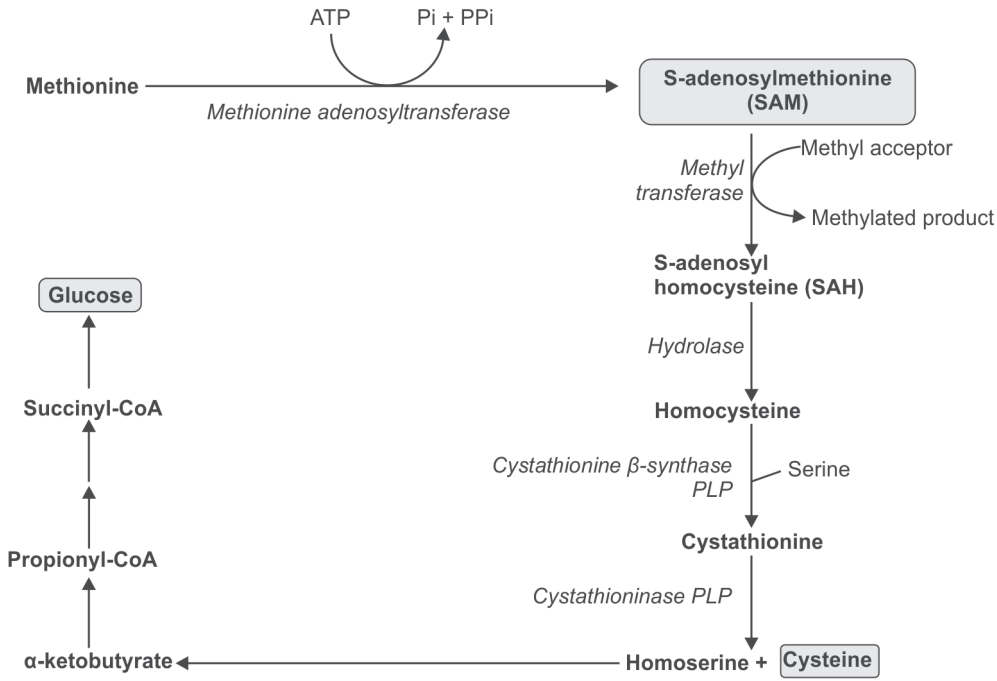
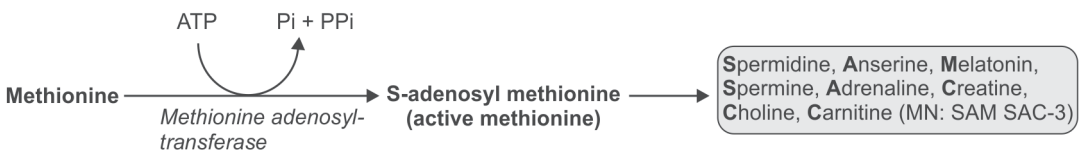


Fig. 8.20: Catabolism of methionine

21. Explain transmethylation reactions.

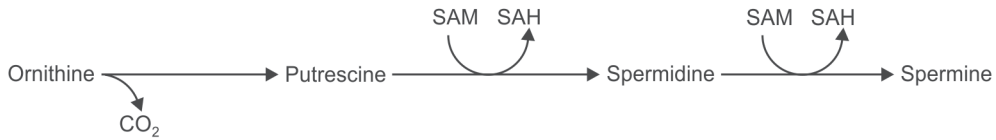
Methionine undergoes activation in the presence of ATP to produce SAM—methyl donor for the transmethylation reactions **[MN: SAM SAC].**



Examples for transmethylation

- Guanidoacetate + SAM ⟶ Creatine + S-adenosylhomocysteine (SAH)
- Norepinephrine + SAM ⟶ Epinephrine + SAH

- Nicotinic acid + SAM ⟶ N-methylnicotinamide + SAH
- N-acetyl serotonin + SAM ⟶ Melatonin + SAH
- Synthesis of polyamines from ornithine:



22. Name polyamines and mention their significance.

Polyamines: Putrescine, spermidine and spermine.

Synthesis: From ornithine and SAM.

Significance: Polyamines are essential for cell proliferation. In cancer, ornithine decarboxylase activity and polyamine excretion in urine are increased.

23. Write a note on disorders of methionine metabolism.

Disorders of methionine metabolism are shown in Table 8.8.

Table 8.8: Disorders of methionine metabolism

Name	Defect	Clinical features
Hypermethioninemia	Methionine adenosyltransferase	Increased plasma methionine. Benign condition
Cystathioninuria	Cystathionase	Mental retardation
Homocystinuria (autosomal recessive)	Cystathionine β-synthase	Dislocation of lens, osteoporosis, cardiovascular disease

24. Explain the causes and features of homocystinuria.

Homocystinuria: Occurs due to defect in metabolism of homocysteine (Table 8.9) leading to accumulation of homocysteine in blood (Fig. 8.21).

Table 8.9: Classification and clinical features of homocystinuria

Type	Defect	Clinical features
Type I	Cystathionine β-synthase deficiency	↑ homocysteine in blood and urine; mental retardation, osteoporosis, dislocation of lens, thrombosis. (Homocyteine interferes with collagen cross-linking; stimulates platelet aggregation and vascular thrombosis by activating Hageman factor)

Contd...

Contd...

Type	Defect	Clinical features
Type II	Methylene tetrahydrofolate (THF) reductase	↑ homocysteine in blood and urine + above features
Type III	Defect in synthesis of active methylcobalamin (vitamin B_{12})	↑ homocysteine in blood and urine + above features

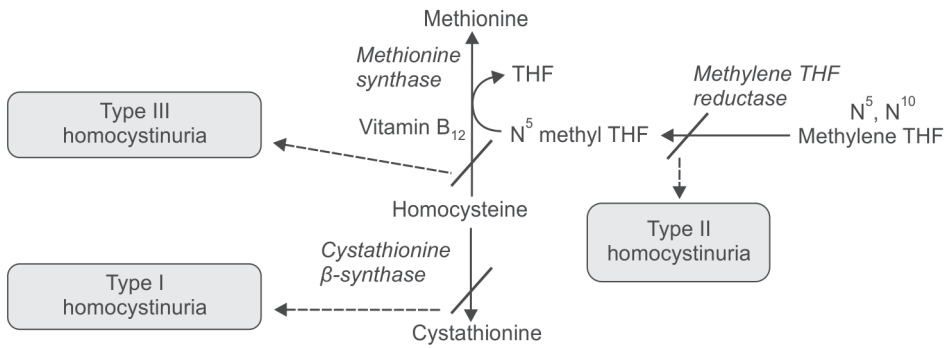


Fig. 8.21: Causes of hemocystinuria

Biochemical tests for diagnosis of homocystinuria: Sodium nitroprusside test—detects elevated homocysteine levels.

- Estimation of methionine levels in blood and urine
- Cystathionine β-synthase levels in fibroblast cultures.

CYSTEINE

It is a glucogenic and non-essential amino acid.

25. Explain the metabolism of cysteine (formation + degradation). Add a note on compounds formed from cysteine.

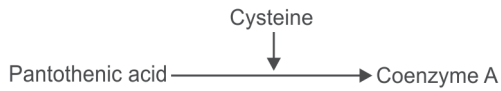
Formation: Cysteine is formed during metabolism of methionine (refer Fig. 8.20).

Degradation: Transaminase converts cysteine into mercaptopyruvate and then into pyruvate and hydrogen sulfide.

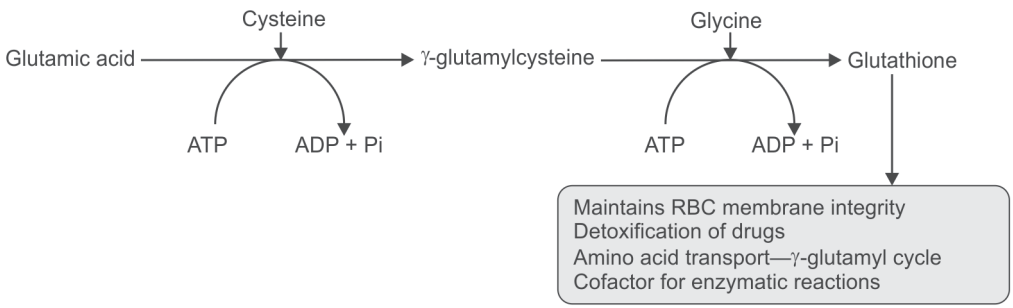
Specialized products from cysteine

- Coenzyme A
- 4-phosphopantetheine
- Glutathione
- Taurine
- 3-phosphoadenosyl-5-phosphosulfate (PAPS).

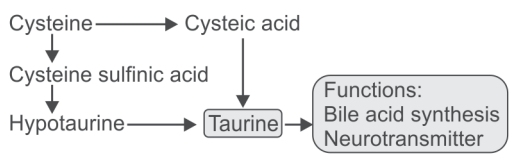
a. Formation of coenzyme A



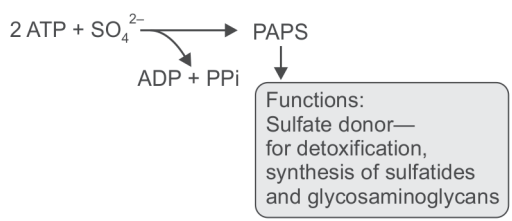
b. Glutathione



c. Taurine



d. 3-phosphoadenosyl-5-phosphosulfate (PAPS)



26. Explain the metabolic defects of cysteine metabolism.

Metabolic defects of cysteine metabolism are shown in Table 8.10.

Table 8.10: Metabolic disorders of cysteine metabolism

Disease	Defect	Clinical features
Cystinuria	Defect in cysteine transport system in kidney. Increased excretion of **c**ysteine, **o**rnithine, **a**rginine, **l**ysine [MN: COAL] in urine	Increased excretion of these amino acids in urine
Cystinosis	Defective carrier-mediated transport of cysteine	Cysteine gets deposited in soft tissues (kidney, cornea, reticuloendothelial system)

HISTIDINE

27. Explain catabolism and specialized products from histidine.

Catabolism of Histidine

Histidine is a glucogenic and semi-essential amino acid (Fig. 8.22).

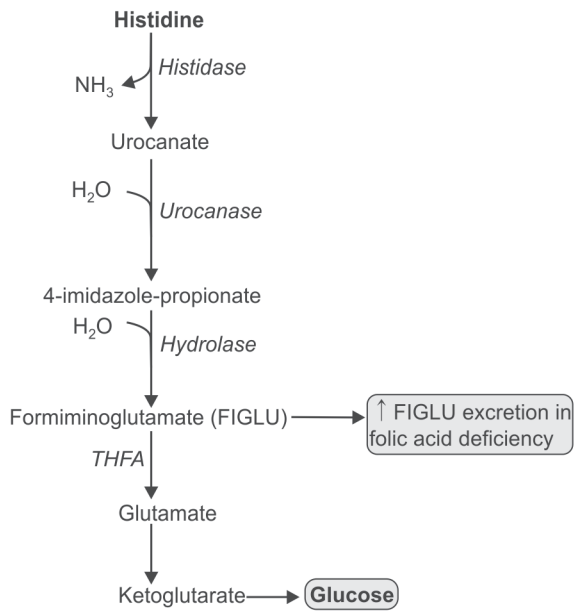
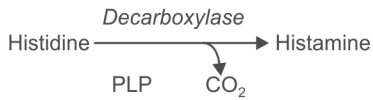


Fig. 8.22: Histidine catabolism (THFA, tetrahydrofolic acid)

Specialized products from histidine

a. **Histamine:** Formed from histidine by decarboxylation reaction (Fig. 8.23).
b. **Carnosine and anserine:** These are formed from histidine and are required for muscle contraction.



Effects of histamine:

H_1 receptor—mediator of allergy — Chlorpheniramine as antiallergic

H_2 receptor—secretion of HCl in stomach — Ranitidine in peptic ulcer

Drugs blocking histamine action

H_3 receptor—in brain-stimulant effects on central nervous system (CNS)

Fig. 8.23: Formation and effects of histamine

28. Explain the metabolic disorders of histidine

Metabolic disorders of histidine is shown in Table 8.11.

Table 8.11: Metabolic disorders of histidine

Disease	Defect	Clinical features
Histidinemia	Enzyme histidase	Mental retardation, increased urine and plasma histidine
Urocanic aciduria	Enzyme urocanase	Benign: Excretion of urocanic acid in urine
Folate deficiency	Folate deficiency	Formiminoglutamate (FIGLU) excretion, megaloblastic anemia

BRANCHED CHAIN AMINO ACIDS

29. Discuss the catabolism of branched chain amino acids. Add a note on Maple syrup urine disease (MSUD).

Catabolism: Refer Figure 8.24.

Maple syrup urine disease

Defect: Branched chain α-keto acid dehydrogenase deficiency.

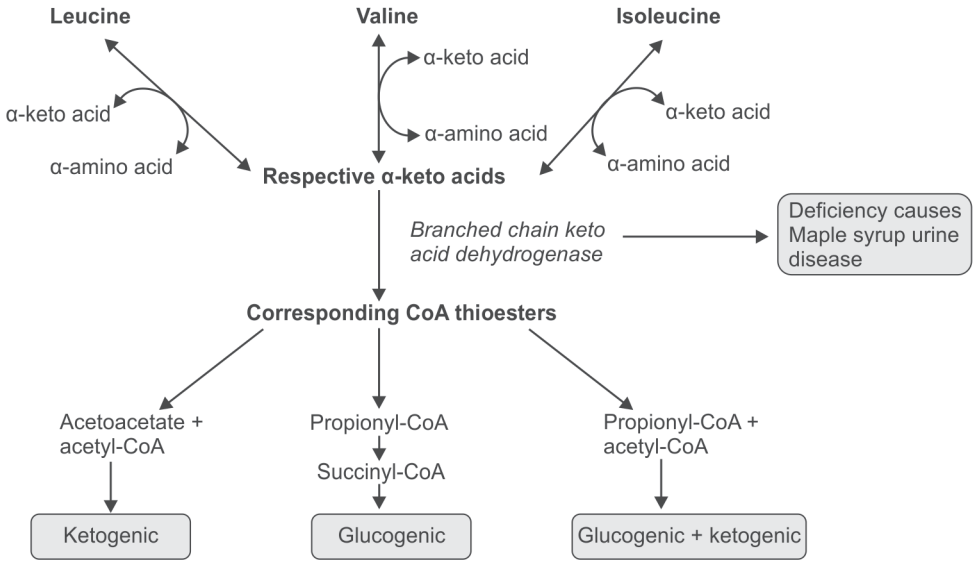


Fig. 8.24: Catabolism of branched chain amino acids; leucine (ketogenic); valine (glucogenic); isoleucine (glucogenic + ketogenic).

Clinical features

- Vomiting
- Mental retardation (due to defective formation of myelin in CNS)
- Neurological manifestations (due to impairment in transport and metabolism of other amino acids)
- Acidosis (due to accumulation of corresponding keto acids)
- Urine has odor of burnt sugar (Maple syrup).

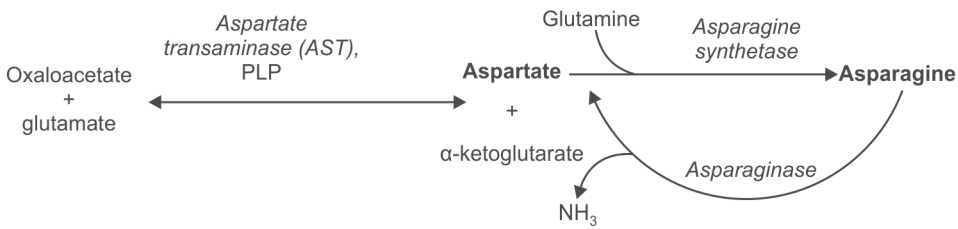
Biochemical tests for diagnosis and screening: Rothera's test is positive (urine), detection of amino acids by chromatography or by enzyme analysis.

Treatment: Early detection and restriction of branched chain amino acids in the diet.

ASPARTATE AND ASPARAGINE

30. Write the reaction by which aspartate and asparagine are formed. Name the compounds derived from aspartate and asparagine.

Synthesis: Aspartate is a non-essential, glucogenic amino acid synthesized from oxaloacetate.



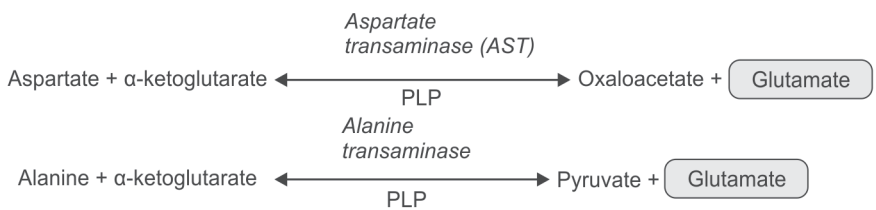
Compounds derived from aspartate and asparagine

- Urea synthesis
- N_1 of purine ring
- Formation of ATP from IMP
- N_1, C_4, C_5, C_6 of pyrimidine ring
- Glucose (glucogenic) and protein synthesis.

GLUTAMATE

31. Explain how glutamate is synthesized and catabolized? Add a note on metabolic functions of glutamate.

Synthesis



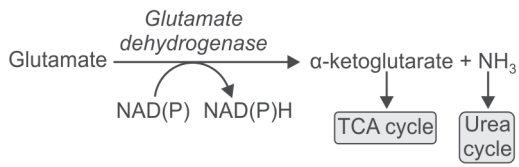
a. Glutamate is a non-essential amino acid synthesized from α-ketoglutarate by transamination reaction.
b. From histidine—refer histidine metabolism.

c. Glutamine $\xrightarrow{Glutaminase}$ Glutamate + NH_3

d. Glutamate is also formed during proline and arginine metabolism.

Catabolism

By glutamate dehydrogenase: Converted into α-ketoglutarate and enters into tricarboxylic acid (TCA) cycle.



Metabolic functions (significance) of glutamate

- Urea formation
- Glucose formation
- Formation of N-acetyl glutamate (NAG): This is a positive modifier of carbamoyl phosphate synthetase I in urea cycle
- For trapping ammonia in non-toxic form—as glutamine
- Vitamin K-dependent activation of clotting factors requires glutamate for activation by γ-carboxylation (refer vitamin K)
- It is an excitatory neurotransmitter in the brain
- It is required for formation of glutathione—intracellular antioxidant
- It is required for formation of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter.

32. What is the metabolic significance of glutamine?

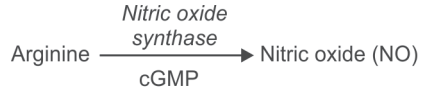
- Ammonia is trapped in this form
- N_3 and N_9 of purine, amino group of GMP and N_3 of pyrimidine comes from glutamine
- Maintenance of acid-base balance
- Takes part in conjugation reaction (phenylacetylglutamine)
- Required for synthesis of NAD and amino sugars.

33. Write a note on compounds formed from arginine.

List of compounds from arginine:

- Proteins—histones are rich in arginine

- Creatine (glycine + arginine + SAM)
- Ornithine—used for urea cycle, polyamine and glutamate synthesis
- Glucose—arginine is glucogenic amino acid
- Nitric oxide (NO).



Functions of nitric oxide:

- Smooth muscle relaxation (BP regulation)
- Increases excretion of Na^+ and H_2O
- Inhibits platelet aggregation
- Neurotransmitter
- Increases host defence
- Glyceryl trinitrate—in treatment of angina pectoris.

Key Points

Hartnup disease: An autosomal recessive disorder with impairment of neutral amino acid transport in renal tubules and small intestine leading to reduced intestinal absorption and increased renal loss of tryptophan. This causes decreased availability of tryptophan and frequently pellagra-like rashes.

Some small peptides can be absorbed through the gaps between the intestinal cells and by γ-glutamyl cycle. This kind of absorption of intact proteins may be beneficial [immunoglobulin A (IgA) by newborn from colostrum] or harmful (gluten in non-tropical sprue).

Phenylalanine

Phenylalanine: Aromatic, essential, neutral, polar, glucogenic + ketogenic amino acid.
Pheochromocytoma: It is a neuroendocrine tumor of adrenal medulla (chromaffin cells), which secretes excessive amount of catecholamines. It is diagnosed by estimation of urinary vanillylmandelic acid (VMA) (normal 2–8 mg/day). Patient should avoid foods like chocolate, coffee, banana, vanilla ice cream, citrus fruits before the test as they contain vanillin, which produces high VMA in urine.
Albinism: Defect in tyrosinase characterized by hypopigmentation.
Alkaptonuria: Defect in homogentisic acid oxidase, characterized by dark urine and arthritis.
Tyrosinemia: Defect in tyrosine transaminase.
α-methyl dopa: Is used in the treatment of hypertension. It inhibits DOPA decarboxylase and prevents the formation of epinephrine.
Carbidopa is administered along with levodopa in Parkinson's disease: It will prevent peripheral decarboxylation of levodopa → increased amount of levodopa reaches the brain.

Tryptophan

Tryptophan is—polar, **h**eterocyclic, **a**romatic, **n**eutral, essential amino acid [MN: PHAN]
Tryptophan degradation in intestine: By bacteria to form indole and skatole (Fig. 8.19a).

Methionine

Methionine: Is sulfur containing essential glucogenic amino acid.
Folate-trap: To regenerate methionine from homocysteine, vitamin B_{12} and tetrahydrofolate are essential. In vitamin B_{12} deficiency, conversion of homocysteine to methionine and regeneration of active folate is blocked.
Difluoromethylornithine (DFMO): Suicide inhibitor of ornithine decarboxylase; used in the treatment of *Pneumocystis carinii*, kala-azar and sleeping sickness.

Cysteine

Cysteine is a sulfur containing non-essential and glucogenic amino acid. Keratin is rich in cysteine.

Histidine

Histidine is an essential basic glucogenic amino acid. It has an imidazole group, which has pKa of 6.8 and has maximum buffering capacity at physiological pH.
FIGLU test: After giving histidine load, FIGLU excretion in urine is measured. It is an indicator of folate deficiency and can be used to differentiate megaloblastic anemia due to folate or B_{12} deficiency.

Branched Chain Amino Acids

Branched chain amino acids are transaminated and decarboxylated and finally converted into acetyl -CoA or propionyl-CoA.

Aspartate and Asparagine

Asparaginase catalyzes cleavage of asparagine to aspartate and ammonia. Even though most cells produce all the asparagine they need, some leukemia cells require exogenous asparagine. If asparaginase is given to these patients, it can deprive the neoplastic cells of the asparagine that is essential for their characteristic rapid growth. This can be exploited in **treatment of cancer.**

9 Biological Oxidation

1. Define standard free energy change (ΔG°) and energy coupling. Give some examples of reactions with negative ΔG°. Add a note on energy coupling.

Definition: Standard free energy change is the energy change of a reaction during conversion of 1 mole of each reactant to 1 mole of respective product at standard temperature and pressure (ΔG°). If the free energy change is negative, the reaction occurs spontaneously and there is loss of free energy (exergonic). If the free energy change is positive, energy is gained for the reaction to proceed (endergonic). Reactions having negative standard free energy are given in Table 9.1.

Table 9.1: Reactions having negative standard free energy

Compound	$\Delta G^{\circ\prime}$ (kcal/mol)
Phosphoenolpyruvate → Pyruvate	–14.8
1,3-bisphosphoglycerate → 3-phosphoglycerate + Pi	–11.8
Creatine phosphate → Creatine + Pi	–10.3
ATP → ADP + Pi	–7.3
Glucose-1-phosphate → Glucose + Pi	–5.0
Glucose-6-phosphate → Glucose + Pi	–3.3
Glycerol-3-phosphate → Glycerol + Pi	–2.2

Energy coupling: Free energy released during an exergonic reaction can be used for the completion of another endergonic reaction.

For example:

Phosphorylation of glucose is an endergonic reaction. For this reaction to occur, it is coupled with hydrolysis of ATP (exergonic reaction).

2. Define and give examples of high-energy compounds.

Definition: Compounds, which have a high standard free energy (ΔG°) of hydrolysis of –7 kcal/mol or more are called high-energy compounds. These compounds release a large quantity of free energy on hydrolysis (Table 9.2).

Table 9.2: High-energy compounds

Types of high-energy compound (~)	Examples
Phosphates	Adenosine triphosphate (ATP), adenosine diphosphate (ADP), guanosine triphosphate (GTP), uridine triphosphate (UTP) 1,3-bisphosphoglycerate Creatine phosphate Carbamoyl phosphate
Sulfur compounds	S-adenosylmethionine (SAM) Acetyl-CoA

3. Draw a neat diagram to show the flow of electrons in electron transport chain indicating ATP producing sites.

Electron transport chain: It is involved in the transport of electrons and generation of ATP from free energy released during the electron transport (oxidative phosphorylation). Coenzymes, which can donate/accept electrons along with specialized electron carriers, constitute the electron transport chain. The electrons are finally transported to oxygen to reduce it to H_2O (Fig. 9.1).

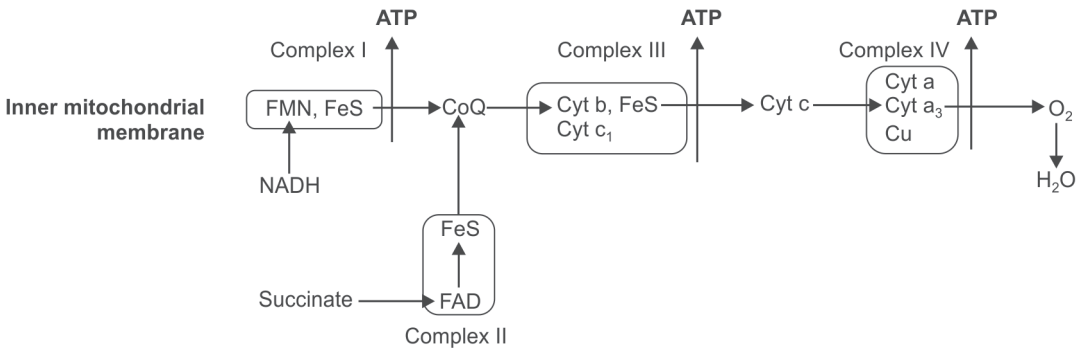


Fig. 9.1: Components of electron transport chain (FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; NADH, nicotinamide adenine dinucleotide; FeS, iron-sulfur; ATP, adenosine triphosphate)

Components of Electron Transport Chain

Location: Inner mitochondrial membrane

Protein complexes: I, II, III, IV

Complex I: NADH-Q oxidoreductase

Complex II: Succinate-Q reductase

Complex III: Cytochrome c oxidoreductase (Cyt b, Cyt c_1, iron-sulfur protein)

Complex IV: Cytochrome c oxidase (Cyt a, Cyt a_3 and Cu)

Mobile electron carriers: Coenzyme Q, cytochrome c.

Source of Electrons

- NADH: It is generated in the reactions catalyzed by pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, isocitrate dehydrogenase and malate dehydrogenase
- $FADH_2$: It is generated in the reactions catalyzed by succinate dehydrogenase, fatty acyl-CoA dehydrogenase.

4. Define chemiosmotic hypothesis with neat labeled diagram.

Chemiosmotic theory explains the process of oxidative phosphorylation. It states that free energy liberated during the transport of electrons by electron transport chain is utilized in translocating protons actively from matrix to intermembrane space of mitochondria. This generates a proton gradient resulting in reentry of protons into matrix from intermembrane space, through ATP synthase, leading to ATP synthesis (Fig. 9.2). Thus, oxidation is coupled to phosphorylation. ATP synthase complex is located in the inner mitochondrial membrane and consists of F_o and F_1 subcomplexes. The protons reenter mitochondrial matrix by passing through F_o subcomplex of ATP synthase, which results in conformational change in F_1 leading to synthesis of ATP.

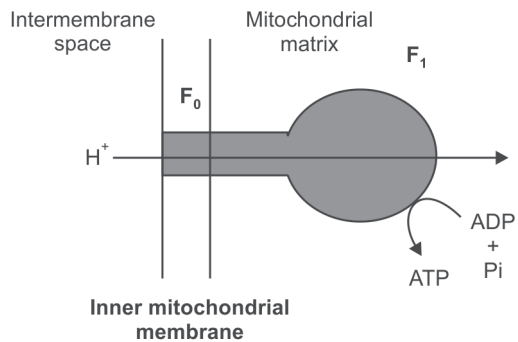


Fig. 9.2: Adenosine triphosphate (ATP) synthase complex

Sites of Oxidative Phosphorylation

- Site 1: NADH and CoQ
- Site 2: Cyt b and Cyt c_1
- Site 3: Cyt a, Cyt a_3 and molecular oxygen:
  - 2.5 ATP molecules are generated per molecule of NADH oxidized
  - 1.5 ATP molecules are generated per molecule of $FADH_2$ oxidized.

5. Explain the shuttle mechanisms. What is their significance?

Definition: Shuttles are transport mechanisms that help to transport reducing equivalents ($NADH + H^+$) generated in cytosol during glycolysis to the matrix of mitochondria, where they are processed to generate ATPs. Examples of shuttles:

i. Malate-aspartate shuttle (Fig. 9.3).

ii. Glycerophosphate shuttle (Fig. 9.4).

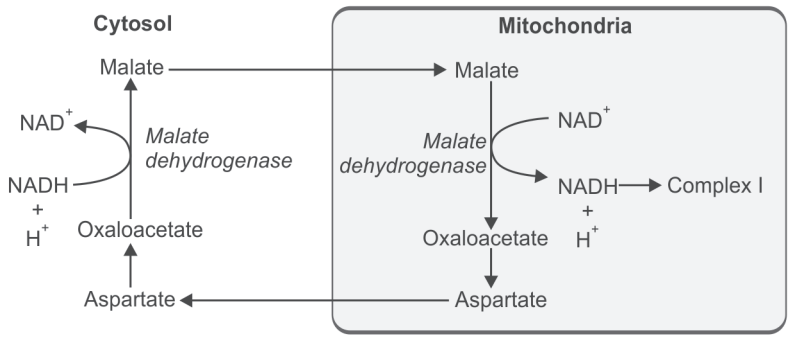


Fig. 9.3: Malate-aspartate shuttle

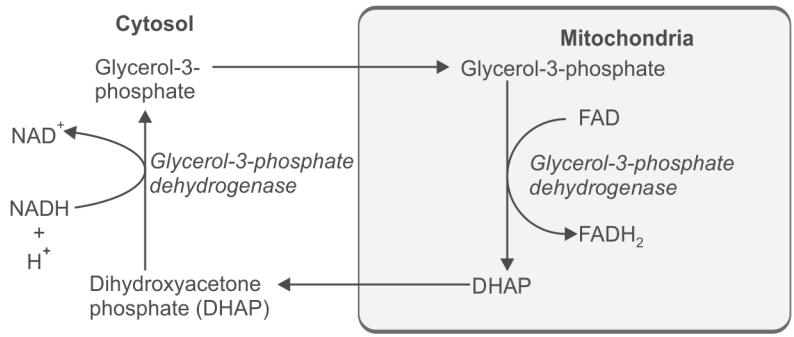


Fig. 9.4: Glycerophosphate shuttle

Significance of shuttle mechanism is shown in Figure 9.5.

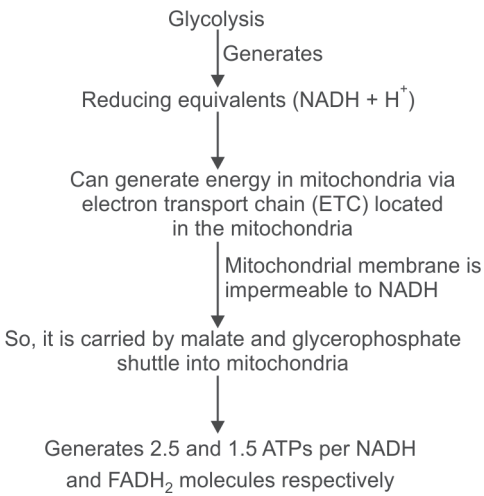


Fig. 9.5: Significance of shuttle mechanism

6. Enlist various inhibitors and uncouplers of electron transport chain indicating where they act.

Inhibitors of electron transport chain are given in Table 9.3. In the presence of uncouplers, oxidation occurs and energy released is not converted to ATP, but to heat.

Table 9.3: Inhibitors and uncouplers of electron transport chain

Inhibitors					Uncouplers
Complex I → CoQ	**Complex II → CoQ**	**Complex III b → c_1**	**Complex IV → O_2**	**ATP synthase**	Thyroxin* LCFA*
Amobarbital		Antimycin A	Cyanide	Oligomycin	Thermogenin*
Rotenone	Carboxin	BAL	Carbon monoxide	Atractyloside	Bilirubin*
Piericidin A	Malonate				2,4-dinitrophenol
			Hydrogen sulfide		Dinitrocresol
					Pentachlorophenol

BAL, British anti-Lewisite; LCFA, long-chain fatty acid; *Physiological uncouplers.

Key Points

British anti-Lewisite (BAL) or dimercaprol: It is used in the treatment of arsenic, mercury and lead and other heavy metal poisoning. In addition, it used for the treatment of Wilson's disease, a genetic disorder in which the body tends to retain copper.

Gibbs free energy (ΔG): It is that part of the total energy change in a system that is available for doing work.

First law of thermodynamics: During a reaction, total energy of a system remains constant; only one form of energy is converted into another form.

Second law of thermodynamics: In spontaneous reactions, total entropy of a system increases.

Entropy (S): It is the extent of disorder of the system.

Redox potential (E_0): It is a quantitative measure of tendency of oxidant to accept electrons or the tendency of reductant to lose electrons.

Enzymes catalyzing redox reactions: Oxidases, dehydrogenases, hydroperoxidases, oxygenase, superoxide dismutase.

Thermogenin: Physiological uncoupler found in brown adipose tissue. Thermogenin dissociates oxidation from phosphorylation. Thus, it dissipates energy as heat.

Leber hereditary optic neuropathy (LHON), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episode (MELAS): Diseases due to mutations in mitochondrial DNA with impaired ATP synthesis.

P/O ratio: Ratio between numbers of ADP converted to ATP per atom of oxygen. P/O ratio is 2.5 for oxidation of substrate producing NADH (pyruvate, malate). P/O ratio is 1.5 for oxidation of substrate producing $FADH_2$ (succinate, fatty acyl-CoA).

10 Vitamins

1. Define and classify vitamins with suitable examples.

Definition: Vitamins are organic substances, required in very small amounts, for optimum growth and health, but cannot be synthesized in our body (except vitamin D and niacin).

Classification

i. Fat-soluble vitamins.

ii. Water-soluble vitamins (Fig. 10.1).

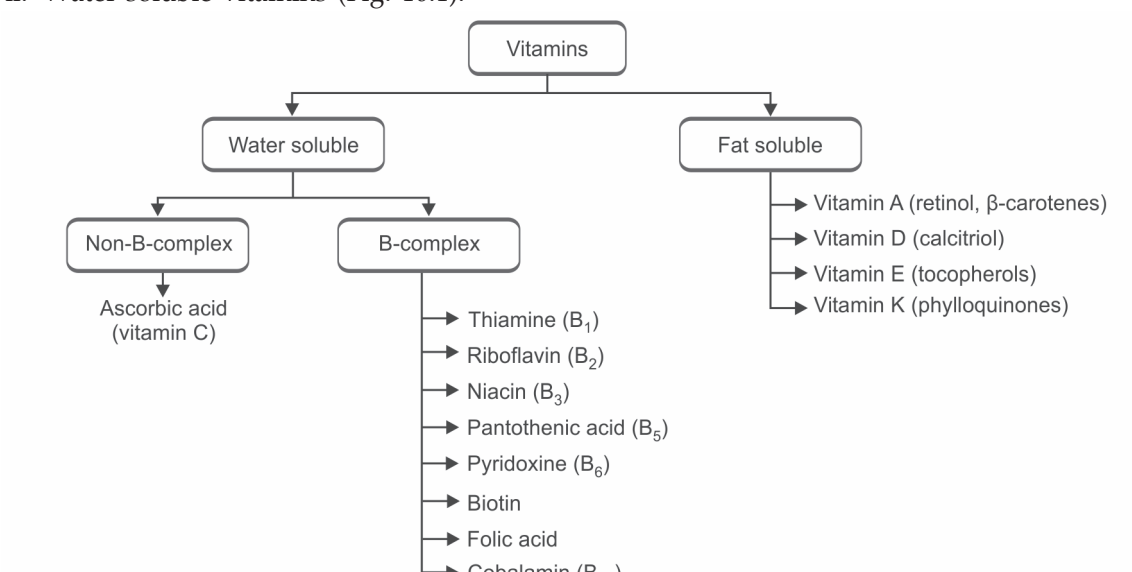


Fig. 10.1: Classification of vitamins with examples

FAT-SOLUBLE VITAMINS

Vitamin A

2. Discuss vitamin A: a. forms, b. sources, c. RDA, d. functions, e. deficiency and toxic manifestations.

Forms of vitamin A: Retinol, retinal, retinoic acid and beta carotene. The sources, RDA, functions, deficiency and toxic manifestations are mentioned in Tables 10.1 and 10.2.

Table 10.1: Sources, RDA* and functions of vitamin A

RDA	Sources	Functions
750 µg of vitamin A	Animal sources: liver, egg yolk, fish liver oil, milk Plant sources (carotene): carrot, mango, papaya, green vegetables	• Vision • Growth, development and tissue differentiation • For normal reproduction • Maintain integrity of epithelial cells • Synthesis of glycoproteins • Antioxidant

*RDA, recommended daily allowance

- **Visual cycle:** Retinol is transported from liver to retina. In the retina, all-trans-retinol is converted to 11-cis-retinol and then 11-cis-retinal, which combines with opsin to from rhodopsin (visual pigment of the rods). When light falls on rhodopsin, conformational changes occur resulting in the formation of metarhodopsin II, which activates transducin leading to generation of nerve impulse. Finally, all-trans-retinal and opsin are released. The all-trans-retinal is converted to 11-cis-retinal, which binds to opsin to regenerate rhodopsin and the cycle is completed (Fig. 10.2, p. 143).

Table 10.2: Deficiency symptoms and toxic manifestations of vitamin A

Deficiency symptoms	Toxic manifestations [MN: HALT]
Vision: Loss of sensitivity to green light, increased dark adaptation time, night blindness, xerophthalmia, Bitot's spots, keratomalacia, blindness	**H**eadache, **h**epatomegaly, **h**ypercalcemia **A**lopecia
Others: Follicular hyperkeratosis, increased susceptibility to infection, anemia	Hyper**l**ipidemia **T**eratogenicity

- **Night blindness (nyctalopia):** Vision is impaired in the dark.
- **Xerophthalmia:** Keratinization of conjunctiva and cornea → dryness of conjunctiva and cornea.
- **Bitot's spots:** Grayish white plaques in conjunctiva due to conjunctival thickening.
- **Keratomalacia:** It is softening of the cornea. Infections can occur with perforation and blindness.

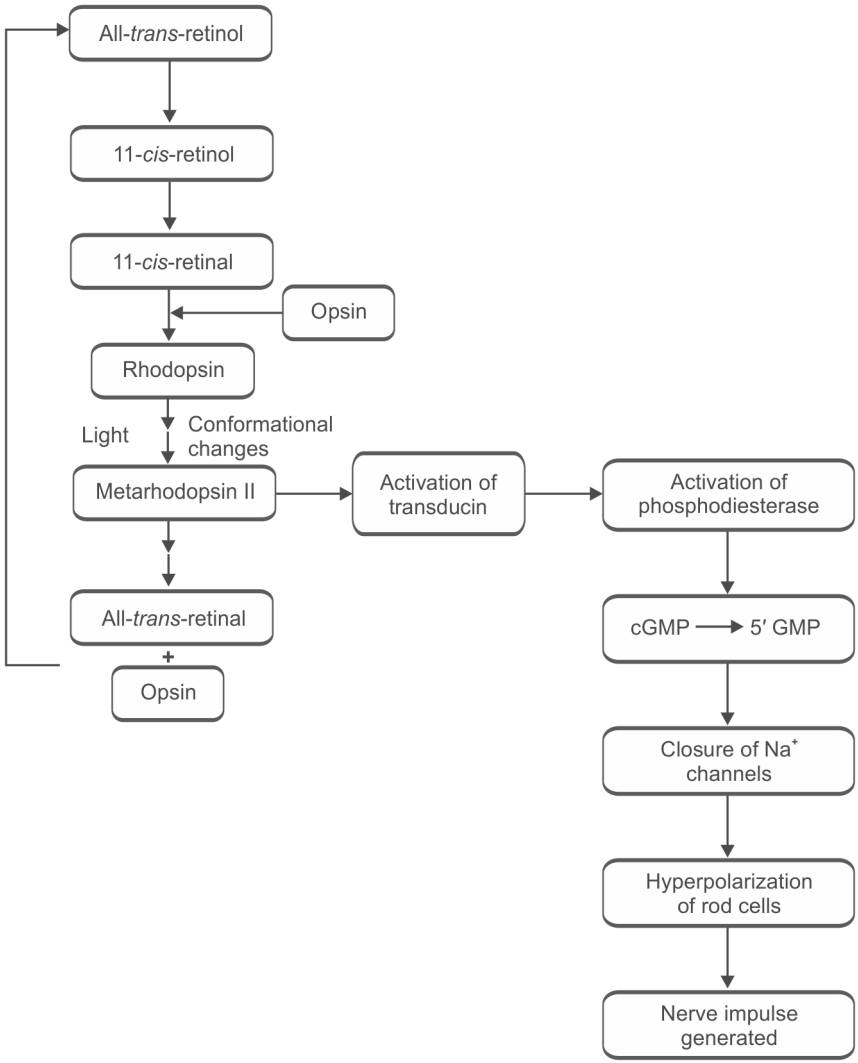


Fig. 10.2: Wald's visual cycle

Key Points

β-carotene dioxygenase: Cleaves β-carotene in the intestine to produce two retinal molecules.
Retinol: It is esterified with palmitic acid and stored as retinyl palmitate in the liver.
Retinol-binding protein (RBP): Transports retinol from liver to tissues. It is the earliest marker of protein energy malnutrition as its concentration in serum decreases early (half-life is only 10 hours).
Conopsin: Photosensitive protein present in cones. There are three types of conopsin—porphyropsin (red), iodopsin (green), cyanopsin (blue).

Vitamin D

3. Discuss vitamin D: a. sources, b. RDA, c. synthesis and functions of calcitriol, d. deficiency and toxic manifestations.

The sources, RDA and deficiency manifestations are mentioned in Tables 10.3 and 10.4.

Table 10.3: RDA* and sources of vitamin D

Vitamin D forms	RDA and sources
Cholecalciferol/ergocalciferol/ calcitriol	5–15 µg or 400 IU Fish liver oil, egg yolk

*RDA, recommended daily allowance

Metabolic Functions of Vitamin D

Maintains serum calcium and phosphorus levels by acting on bone, kidney and intestine.

- **Bone:** Causes mobilization of calcium and phosphate from the bone and promotes bone mineralization
- **Kidney:** Promotes reabsorption of calcium and phosphorus → decreases excretion of calcium and phosphorus
- **Intestine:** Increases absorption of calcium (via calbindin) and phosphorus.

Causes of Deficiency

- Inadequate dietary intake
- Lack of exposure to sunlight
- Impaired absorption
- Liver and kidney diseases.

Table 10.4: Deficiency manifestations of vitamin D

Deficiency diseases	Signs and symptoms
Rickets: Improper mineralization resulting in soft bones (occurs in children)	Bow legs, forward projection of the breast one (pigeon chest), beading in the ribs (rachitic rosary), asymmetrical or odd-shaped skull
Osteomalacia: Demineralization of previously formed bone leading to increased susceptibility to fractures (occurs in adults)	Increased tendency of bone fractures, bone pain and tenderness all over the body Scoliosis: Lateral bending of spine Kyphosis: Forward bending of spine

Toxic Manifestations of Vitamin D

Loss of appetite, nausea, thirst, calcium deposition in the heart, arteries and kidney (renal calculi).

4. Why is vitamin D called hormone?

- Synthesized inside the body, unlike a classical vitamin, which has to be supplied in the diet
- Synthesized as an inactive form and gets activated inside the body only when required (Fig. 10.3)
- Like any hormone, it has a half-life (8 hours)
- It acts on distant target organs

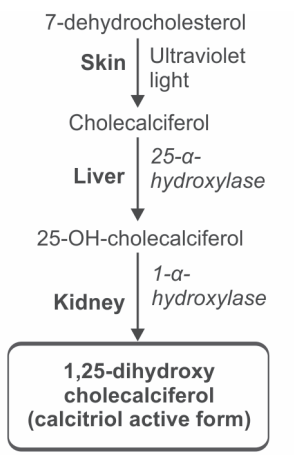


Fig. 10.3: Calcitriol synthesis

- It binds to a cytosolic receptor, which enters the nucleus and binds to a specific region on genes and controls it. This action mimics that of a steroid hormone
- It is self-regulated, i.e. when its action is not required, it is converted into an inactive metabolite 24,25-dihydroxycholecalciferol (Fig. 10.4).

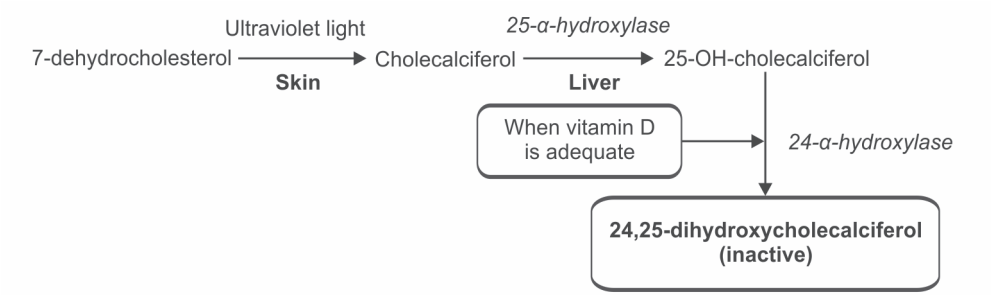


Fig. 10.4: Autoregulation of calcitriol

5. Why does a person with chronic liver or chronic renal disease suffer from manifestations of vitamin D deficiency?

In renal disease, 1-α-hydroxylation is impaired; in liver disease, 25-α-hydroxylation does not occur. Thus, synthesis of calcitriol is impaired and the patient may present with symptoms and signs of vitamin D deficiency.

Key Points

Transporter for vitamin D: Vitamin D_2 and D_3 are absorbed from the intestine and transported to the liver bound to a specific vitamin D-binding protein.

1-α-hydroxylase: It is present in the proximal convoluted tubules of the kidneys, bone and placenta.

Mechanism of action of calcitriol is similar to steroid hormones: Upon entering the nucleus of a cell, calcitriol functions as a steroid hormone and associates with the vitamin D receptor (VDR) and promotes its association with the retinoic acid X receptor (RXR), which binds to the gene and modulates transcription of calbindin in the intestine.

Hereditary rickets: It is an inherited form of the disease—the kidneys are unable to retain phosphate.

Fanconi syndrome: Disease of the proximal renal tubules of the kidney in which phosphate is passed into urine, instead of being reabsorbed.

Anticonvulsants like phenobarbital: Can cause hypocalcemia, as it induces a microsomal enzyme (cytochrome P450), which inactivates vitamin D.

Vitamin K

6. What are the forms, sources, RDA, functions and deficiency manifestations of vitamin K?

Forms of vitamin K: Phylloquinone (K_1), menaquinone (K_2) and menadione (K_3). Recommended daily allowance (RDA) of vitamin K is 70–140 μg. The sources, functions, causes and deficiency manifestations are mentioned in Tables 10.5 and 10.6.

Table 10.5: Sources and functions of vitamin K

Sources	Functions
Green vegetables: cabbage, cauliflower, spinach (K_1); formed by intestinal bacteria, egg yolk, meat, liver (K_2); water soluble, synthetic form (K_3)	• Cofactor for synthesis of γ-carboxyglutamic acid (Gla) (Fig. 10.5) • Formation of Gla is a post-translational modification required for activation of clotting factors II, VII, IX and X. Gla helps these proteins to trap calcium • Other Gla proteins: osteocalcin and matrix Gla-protein (MGP) of bone

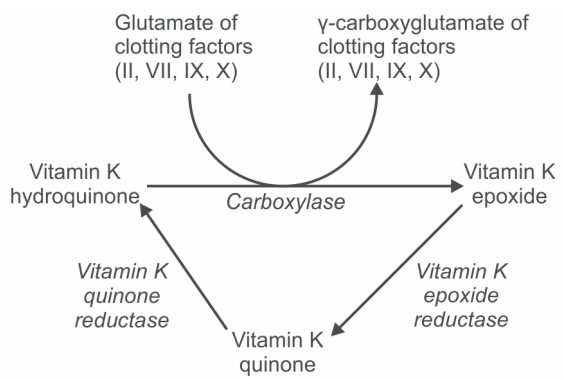


Fig. 10.5: Vitamin K cycle

Table 10.6: Causes and deficiency manifestations of vitamin K

Causes for deficiency	Manifestations
Prolonged antibiotic treatment: Kills intestinal flora (which forms vitamin K in adults) Obstructive jaundice: No bile salt enters into the intestine, which is necessary for absorption of fat-soluble vitamins Malabsorption syndrome Newborn (premature): Their gut is sterile and mother's milk is not a good source of vitamin K Anticonvulsant treatment: They interfere with absorption of vitamin K	• Easy bruising, ecchymosis, bleeding • Increased prothrombin time

Key Points

Differentiating features of vitamin K and vitamin C deficiency: In contrast to vitamin K deficiency, vitamin C deficiency will have increased bleeding time, but prothrombin time will be normal. Also, there will be gum hyperplasia, inflammation, skeletal deformity and poor wound healing.

Warfarin and dicumarol: Structural analogues of vitamin K, competitively inhibit the enzyme epoxide reductase and vitamin K quinone reductase. They are used as anticoagulants.

Toxic manifestations of vitamin K: In premature babies, menadione $\rightarrow\uparrow$ hemolysis $\rightarrow\uparrow$ serum unconjugated bilirubin $\rightarrow$ kernicterus.

Vitamin E

7. Mention the sources, RDA and functions of vitamin E.

The RDA, sources and functions of vitamin E are mentioned in Table 10.7.

Table 10.7: RDA*, sources and functions of vitamin E

Active form	RDA and sources	Functions
α-tocopherol	RDA: 8–10 mg Wheat germ oil (richest source), oils from nuts and seeds	• **Antioxidant:** Prevents peroxidation of polyunsaturated fatty acids and plasma lipoproteins • **Prevention of atherosclerosis, cancer and aging:** by reducing oxidative stress • May have a role in cell signaling

*RDA, recommended daily allowance

Key Points

Vitamin E deficiency: Deficiency in humans is very rare. The major symptoms are increased red blood cell (RBC) fragility due to peroxidation.

WATER-SOLUBLE VITAMINS

Thiamine (Vitamin B_1)

8. What are the sources, RDA, functions and deficiency manifestations of thiamine?

The sources, functions and deficiency manifestations are mentioned in Table 10.8 and Box 10.1.

Table 10.8: Sources, RDA and functions of thiamine

Coenzyme form	Sources and RDA	Functions
Thiamine pyrophosphate (TPP)	Unrefined grains, legumes (e.g. beans), nuts and yeast RDA: 1.0–1.5 mg (directly proportional to amount of carbohydrates in the diet)	• Coenzyme for pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched chain α-ketoacid dehydrogenase and transketolase • Nerve conduction

Box 10.1: Deficiency manifestations of thiamine

Deficiency disease: (Fig. 10.6)

Dry beriberi (mainly neurological features)

- Peripheral neuropathy—diminished sensation and weakness in the legs and arms
- Muscle pain and tenderness, and difficulty in rising from a squatting position

Wet (cardiac) beriberi

- Edema, difficulty in breathing and ultimately congestive heart failure

Wernicke's encephalopathy and Korsakoff's psychosis: Seen in alcoholics and manifests as:

- Abnormal eye movements
- Abnormal gait
- Dementia
- Psychosis

Infantile beriberi: Occurs in children born to mother with thiamine deficiency. It presents with tachycardia, convulsions, etc.

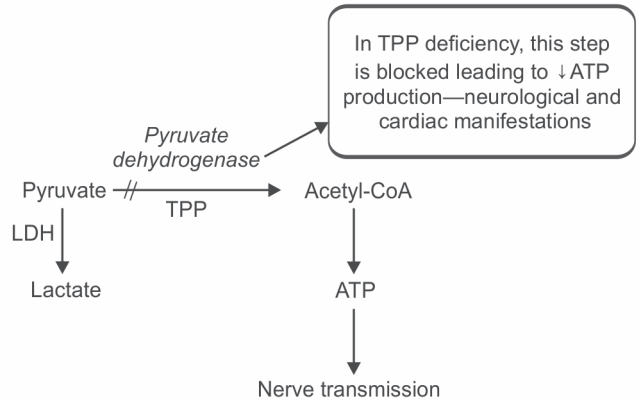


Fig. 10.6: Consequences of thiamine deficiency (TPP, thiamine pyrophosphate; ATP, adenosine triphosphate; LDH, lactate dehydrogenase; // = blocked)

Key Points

Requirement of B_1 increases when a person is placed on high-carbohydrate diet: This is due to increase in TPP-mediated reactions (e.g. pyruvate to acetyl-CoA, α-ketoglutarate to succinyl-CoA, etc.) in carbohydrate metabolism.

Antithiamine factors: Certain raw freshwater fish, raw shellfish, ferns (they contain thiaminases, which destroys thiamine).

Thiamine pyrophosphotransferase: Converts thiamine to its active form TPP in the brain and liver.

Transketolase activity: in RBCs is used to measure the thiamine status in an individual.

Riboflavin (Vitamin B_2)

9. What are the sources, RDA and functions of riboflavin?

The sources, RDA and functions are mentioned in Table 10.9.

Table 10.9: Sources, RDA and functions of riboflavin

Coenzyme forms	RDA and sources	Functions
Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)	RDA: 1.2–1.7 mg Milk and dairy products, egg, whole cereals, green leafy vegetables	Flavin coenzymes are involved in mitochondrial respiratory chain, fatty acid and amino acid oxidation, and citric acid cycle. They play a role in the metabolism of drugs and toxins (cytochrome P450) **FMN-dependent enzymes** • L-amino acid oxidase • NADH dehydrogenase (electron transport chain) **FAD-dependent enzymes** • Pyruvate dehydrogenase • α-ketoglutarate dehydrogenase • Glycerol-3-phosphate dehydrogenase (shuttle transport in electron transport chain) • Acyl-CoA dehydrogenase • Xanthine oxidase

Key Points

Deficiency manifestations of riboflavin: Glossitis, angular stomatitis, cheilosis.

Niacin (Vitamin B_3)

10. What are the sources, RDA, functions and deficiency manifestations of niacin?

The sources, functions and deficiency manifestations are mentioned in Tables 10.10 and 10.11.

Table 10.10: Sources, RDA and functions of niacin

Coenzyme form	RDA and sources	Functions
Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$)	RDA: 10–15 mg Yeast, meat, poultry, fish, cereals, legumes	Coenzyme in oxidation-reduction (redox) reactions • NAD^+ is involved in the catabolism of carbohydrates, fats, proteins and alcohol to produce energy • For example: α-ketoglutarate $\rightarrow$ succinyl-CoA; Succinate $\rightarrow$ fumarate • $NADP^+$ functions more often in biosynthetic (anabolic) reactions, such as in the synthesis of fatty acids and cholesterol • NAD^+ is source of ADP-ribose for ADP-ribosylation of proteins

Table 10.11: Causes and deficiency manifestations of niacin

Causes for deficiency	Manifestations of niacin deficiency
Inadequate dietary intake, Hartnup disease, carcinoid syndrome, pyridoxine (B_6) deficiency	**Pellagra (4D):** **D**ermatitis, **D**iarrhea, **D**ementia, **D**eath **Dermatitis:** Scaly, dark-pigmented rash, develops symmetrically in areas exposed to sunlight **Diarrhea:** With blood and mucus in stools **Dementia:** Loss of memory, inability to concentrate, irritability

Key Points

Carcinoid syndrome and pellagra: Normally 1%–2% of tryptophan is converted to serotonin in argentaffin cells of gastrointestinal tract (GIT) and the rest is converted to niacin. But in carcinoid syndrome (tumor of argentaffin cells), more than 60% of tryptophan is diverted for serotonin, so less is available for niacin synthesis leading to pellagra.

Hartnup disease and pellagra: Tryptophan and other neutral amino acids are not absorbed from intestine and also from kidneys, thus depleting amino acid tryptophan. This in turn leads to niacin deficiency (pellagra).

Pyridoxine and pellagra: Synthesis of nicotinamide from tryptophan is pyridoxal phosphate (PLP) dependent; so in its deficiency, this pathway will not proceed and may lead to pellagra.

Pharmacologic doses of nicotinic acid have been known to reduce serum cholesterol.

Pyridoxine (Vitamin B_6)

11. What are the sources, RDA and functions of pyridoxine?

The RDA, sources and functions are mentioned in Table 10.12.

Table 10.12: RDA, sources and functions of pyridoxine

Coenzyme form	RDA and sources	Functions
Pyridoxal phosphate (PLP)	RDA: 1.4–2.0 mg Whole grain cereals, eggs, legumes, milk	PLP acts as a coenzyme for: • Transamination: Aspartate + α-ketoglutarate $\rightarrow$ glutamate + oxaloacetate • Deamination: Serine $\rightarrow$ pyruvate • Transsulfuration: Formation of cysteine from methionine • Cystathionine synthase, cystathionase, kynureninase • Heme synthesis: Aminolevulinic acid (ALA) synthase • Decarboxylation reactions of amino acids • Ceramide synthesis (palmitoyl-CoA + serine) • Glycogenolysis

Key Points

Tuberculosis: The antitubercular drug isoniazid, competes with pyridoxine kinase and blocks the formation of PLP. Thus, long-term treatment with isoniazid can result in pyridoxine deficiency, which manifests as peripheral neuropathy; so pyridoxine is given along with isoniazid in tuberculosis patients.

Treatment of microcytic anemia: Along with iron and other supplements, pyridoxine is given, which helps in heme synthesis [aminolevulinic acid (ALA) synthase reaction].

In infants, pyridoxine deficiency can predispose to seizures: This may be due to decreased formation of γ-aminobutyric acid (GABA) from glutamic acid (decarboxylation reaction). GABA is an inhibitory neurotransmitter, so its decreased concentration can lead to seizures.

Deficiency manifestations of B_6: Irritability, depression, confusion, glossitis, stomatitis.

Cycloserine, penicillamine: Form complexes with vitamin B_6, creating a functional deficiency.

Parkinsonism treated with levodopa: Administration of pyridoxine will facilitate the peripheral decarboxylation of levodopa to dopamine; treatment becomes less effective as less of levodopa is available to cross into the brain.

Dietary requirement of B_6 increases when a person is placed on high-protein diet: Pyridoxine has significant role in protein metabolism (transamination, decarboxylation, deamination and condensation reactions). So, its requirement is directly proportional to protein intake.

Folic Acid (Vitamin B_9)

12. What are the sources, RDA, functions and deficiency manifestations of folic acid?

The RDA, sources, functions and deficiency manifestations are mentioned in Tables 10.13 and 10.14.

Table 10.13: RDA, sources and functions of folic acid

Coenzyme form	RDA and sources	Functions
Tetrahydrofolic acid (THFA)	RDA: 200 µg Green leafy vegetables (foliage), legumes and fortified cereals	• Carry and transfer various forms of one carbon units during biosynthetic reactions; one carbon donors are **G**lycine, **H**istidine, **O**dd chain fatty acids, **S**erine, **T**ryptophan **[MN: GHOST]** • Required for biosynthesis of serine, methionine, glycine, choline, purine nucleotides and thymidylate (dTMP) • Conversion of homocysteine to methionine

Table 10.14: Causes and deficiency manifestations of folate

Causes of deficiency	Manifestations of folate deficiency
Dietary deficiency: alcoholics and overcooking of food **Impaired absorption:** tropical sprue, celiac disease **Increased demand:** pregnancy, lactation **Anticancer drugs:** methotrexate	↓ DNA synthesis during erythrocyte maturation → ↓ DNA replication + uninhibited protein synthesis → bigger than normal RBCs **(megaloblasts)** **Homocystinemia:** Folic acid is required (with B_{12} and B_6) for synthesis of methionine from homocysteine. So, folic acid deficiency leads to homocystinemia **Neural tube defect:** Since folate is required for nucleic acid synthesis, lack of this vitamin leads to neural tube defects in newborn. Hence, every pregnant woman is administered folic acid in the first trimester **Predisposition to cancer**

13. Mention folate antagonists and their uses.

The folate antagonists and their uses is given in Table 10.15.

Table 10.15: Folate antagonists

Names	Uses
Methotrexate	Inhibits dihydrofolate reductase. Used as an anticancer drug
Trimethoprim	Inhibits bacterial dihydrofolate reductase. Used as an antimicrobial agent
Pyrimethamine	Antimalarial agent
Sulfonamide	Antibacterial agent

Key Points

Folinic acid (leucovorin or citrovorum factor): It is administered before giving methotrexate to prevent its systemic toxicity; methotrexate blocks the conversion of dihydrofolate to THFA. Folinic acid (N_5 formyl-THFA) is the active coenzyme form and minimizes toxic effect of methotrexate on normal cells; this is leucovorin rescue.

Formiminoglutamate (FIGLU) excretion test: It is done to detect folic acid deficiency. In folic acid deficiency, increased excretion of FIGLU is observed after a load, due to impaired conversion of FIGLU to glutamate.

Cobalamin (Vitamin B_{12})

14. What are the sources, RDA, functions and deficiency manifestations of cobalamin?

The RDA, sources, functions and deficiency manifestations of cobalamin (vitamin B_{12}) are given in Tables 10.16 and 10.17.

Table 10.16: RDA, sources and functions of cobalamin

Coenzyme form	RDA and sources	Functions
5-deoxyadenosylcobalamin, Methylcobalamin	RDA: 1 μg Only bacteria can synthesize vitamin B_{12} Animal products such as meat, eggs, fish No plant source	• **Methylcobalamin:** Required for enzyme methionine synthase (homocysteine → methionine) • **5-deoxyadenosylcobalamin:** Required for enzyme methylmalonyl-CoA mutase (propionyl-CoA → succinyl-CoA)

Table 10.17: Causes and deficiency manifestations of cobalamin

Causes of deficiency	Deficiency manifestations
Decreased intake: Strict vegetarians and alcoholics **Impaired absorption:** Due to lack of intrinsic factor (pernicious anemia); atrophic gastritis **Increased demand:** Pregnancy	**Megaloblastic anemia:** Due to folate trap, folate is unavailable to participate in DNA synthesis resulting in formation of megaloblasts leading to anemia **Homocysteinemia:** Vitamin B_{12} is required (with folic acid and B_6) for synthesis of methionine from homocysteine ↓ Vitamin B_{12} → ↑ homocysteine → ↑ risk of cardiovascular disease **Methylmalonic acidemia:** Due to block in the conversion of methyl malonic acid to succinyl-CoA **Neurological symptoms:** Demyelination due to vitamin B_{12} deficiency—peripheral neuropathy, ataxia, both sensory and motor fibers are affected

Key Points

Absorption of B_{12}: Vitamin B_{12} binds to intrinsic factor (IF), a glycoprotein secreted by parietal cells of stomach. Receptors on the surface of the ileum take up the IF-B_{12} complex in the presence of calcium. It is bound to transcobalamin II and transported to the liver.

Vitamin B_{12}: Only water-soluble vitamin stored in the liver (sufficient for upto 6 years).

Subacute combined degeneration of spinal cord (SACD): Group of neurologic symptoms—peripheral neuropathy, tingling, numbness, weakness, mental disturbances and ataxia seen in cobalamin deficiency.

Folate trap: In vitamin B_{12} deficiency, conversion of methyltetrahydrofolate (methyl-FH_4) to tetrahydrofolate (FH_4) is affected and results in accumulation of methyl TH_4. Thus, deficiency of vitamin B_{12} can cause secondary folate deficiency.

Cyanocobalamin, mehylcobalamin (oral) and hydroxocobalamin (parenteral/injectable): Are commercially available forms of cobalamin.

Schilling test: This is a test used to detect pernicious anemia (or to detect cause of megaloblastic anemia). The patient is first given a saturating dose of vitamin B_{12} by injection and then given radiolabeled B_{12} orally. If after sometime, there is no appearance of radioactivity in urine, it means oral B_{12} is not absorbed. Then, oral radiolabeled vitamin B_{12} is given along with intrinsic factor and urine radioactivity is checked after some time. If it appears in urine, it indicates the person has pernicious anemia. If there is still no radioactivity in urine, the person has malabsorption syndrome.

Biotin (Vitamin B_7)

15. What are the sources, RDA, functions and deficiency manifestations of biotin?

The RDA, sources, functions and deficiency manifestations of biotin (vitamin B_7) is given in Table 10.18.

Table 10.18: RDA, sources, functions and deficiency manifestations of biotin

RDA and sources	Functions	Deficiency manifestations
RDA: 30–100 μg Egg yolk, liver, yeast, intestinal flora	Required for carboxylation reactions in fatty acid synthesis and gluconeogenesis • Acetyl-CoA carboxylase • Pyruvate carboxylase • Propionyl-CoA carboxylase	Dermatitis, hair loss (deficiency is rare)

Key Points

Biotinidase: Shown to catalyze the biotinylation of histones, suggesting that biotin may play a role in DNA replication and transcription.

Consumption of raw egg white for a prolonged period may cause biotin deficiency as it contains a protein avidin, which strongly binds biotin and prevents its absorption.

Pantothenic Acid (Vitamin B_5)

16. What are the sources, RDA and functions of pantothenic acid?

The sources, RDA, functions and deficiency manifestations of pantothenic acid (vitamin B_5) are given in Table 10.19.

Table 10.19: RDA, sources, functions and deficiency manifestations of pantothenic acid

Coenzyme form	RDA and Sources	Functions	Deficiency manifestations
Coenzyme A (CoASH)	RDA: 10 mg Liver, yeast, egg yolk, intestinal flora	• Tricarboxylic acid (TCA) cycle and gluconeogenesis • For synthesis of fatty acid, cholesterol and steroid hormones • Acetylcholine, melatonin synthesis • Heme synthesis • Ketone body synthesis and utilization	Deficiency is rare • **Burning foot syndrome:** Numbness and tingling of hands and feet

Ascorbic Acid (Vitamin C)

17. What are the sources, RDA, functions and deficiency manifestations of vitamin C?

The RDA, sources and functions of ascorbic acid (vitamin C) is given in Table 10.20.

Table 10.20: RDA, sources and functions of vitamin C

RDA and sources	Functions
RDA: 75 mg Amla, guava, citrus fruits, green vegetables	• Hydroxylation of proline and lysine residues in collagen • Antioxidant: protects proteins, lipids, carbohydrates and nucleic acids from damage by free radicals • Synthesis of norepinephrine, carnitine • Involved in the conversion of cholesterol to bile acid • Role in tryptophan and tyrosine metabolism

Scurvy

Deficiency of vitamin C leads to scurvy: There is poor hydroxylation of proline and lysine residues of collagen.

Symptoms: Bleeding and easy bruising, hair and tooth loss, joint pain and swelling, poor wound healing, etc.

Treatment: Vitamin C supplementation.

Key Points

High doses of vitamin C predisposes to renal stone: Vitamin C is metabolized to oxalic acid and it is capable of precipitating calcium as calcium oxalate in the urine.

Vitamin C cannot be synthesized in humans: Because they lack the enzyme, L-gulonolactone oxidase.

Lipoic acid: Acts as coenzyme for oxidation-reduction reactions (pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) and has antioxidant functions.

Choline: Lipotropic factor and prevents fatty liver.

11 Minerals

MACROMINERALS

1. Classify minerals. Add a note on the sources, requirements and metabolic functions of macrominerals.

The classification of minerals is given in Table 11.1.

Table 11.1: Classification of minerals

Macrominerals	Microminerals (trace elements)	Toxic minerals
Daily requirement > 100 mg, e.g. calcium, magnesium, sodium, potassium, phosphorus, chloride and sulfur	Daily requirement < 100 mg, e.g. iron, iodine, copper, zinc, manganese, selenium, fluoride	Aluminum, lead, cadmium, mercury

The sources, requirements and metabolic functions of macrominerals are given in Table 11.2.

Table 11.2: Sources, requirements and metabolic functions of macrominerals

Mineral and serum levels	Food sources	Daily requirement	Metabolic functions
Calcium 9–11 mg%	Milk and dairy products, cereals, fish, egg, cabbage	Adults: 500–800 mg Children and lactating mother: 1,000–1,300 mg	• Muscle contraction • Secretion of hormones • Bone and teeth formation • Second messenger • Nerve transmission • Activation of enzymes • Blood coagulation
Phosphorus Adults: 2.5–4.5 mg/dL Children: 4–6 mg/dL	Milk, cereals, meat, fish, nuts	800–1,200 mg	• Formation of bone and teeth • Acid-base regulation—acts as a buffer • Energy storage and transfer • Regulation of enzyme activity • Part of nucleic acids

Contd...

Contd...

Mineral and serum levels	Food sources	Daily requirements	Metabolic functions
Magnesium 1.8–2.2 mg/dL	Unrefined grains, nuts, milk, green leafy vegetables	300–400 mg	• Cofactor for enzymes hexokinase and fructokinase • Muscle and nerve function
Sodium 135–145 mEq/L	Salt, nuts, whole grains, butter, legumes	1–5 g	• Osmotic pressure and water balance; regulates plasma volume • Acid-base balance • Cell membrane permeability • Muscle and nerve function
Chloride 95–105 mEq/L	Salt, leafy vegetables	1.5 g	• Acid-base balance, fluid and electrolyte balance • Acid secretion in the stomach
Potassium 3.5–5 mEq/L	Banana, tender coconut water, apple, dates, legumes, meat	2–5 g	• Major cation in the intracellular fluid • Maintenance of intracellular osmotic pressure • Normal muscle and nerve function • Acid secretion in the stomach is by H^+-K^+-ATPase

2. Enlist the factors affecting calcium absorption.

The factors affecting calcium absorption are given in Table 11.3.

Table 11.3: Factors affecting calcium absorption

Facilitators of calcium absorption [MN: CLAAP]	Inhibitors of calcium absorption
Calcitriol	Phytic acid
Lysine	Oxalates
Acidic pH	Fatty acids
Arginine	Phosphates
Parathyroid hormone (PTH)	

3. Explain factors regulating serum calcium levels.

Calcium balance is regulated by calcitriol, calcitonin and parathyroid hormone (PTH) through their actions on kidney, bone and intestine (Table 11.4).

Table 11.4: Regulation of serum calcium			
Organ	**Calcitriol (↑ plasma calcium)**	**PTH (↑ plasma calcium)**	**Calcitonin (↓ plasma calcium)**
Intestine	↑ absorption of calcium and phosphate	↑ absorption of calcium (mediated by calcitriol)	–
Kidney	↑ reabsorption of calcium and phosphate	↑ reabsorption of calcium and ↑ excretion of phosphate	↑ excretion of phosphate
Bone	↑ bone resorption ↑ bone mineralization	↑ bone resorption (↑ osteoclast activity)	↓ bone resorption

↑, increase; ↓, decrease

4. Enlist the causes of hypercalcemia and hypocalcemia.

Hypercalcemia: Serum calcium > 11 mg/dL.

Hypocalcemia: Serum calcium < 8.5 mg/dL.

The causes of hypercalcemia and hypocalcemia are given in Table 11.5.

Table 11.5: Causes of hypercalcemia and hypocalcemia	
Hypercalcemia [MN: Hyper PTH]	**Hypocalcemia [MN: Hypo PARR]**
Hyperparathyroidism	**Hypo**parathyroidism
Multiple myeloma	**P**seudohypoparathyroidism
Milk-alkali syndrome	**A**cute pancreatitis
Paget's disease	Dietary deficiency
Thiazide diuretics	**R**enal tubular acidosis
Hypervitaminosis D	**R**enal failure

5. Enlist the causes of hyperphosphatemia and hypophosphatemia.

The causes of hyperphosphatemia and hypophosphatemia are given in Table 11.6.

Table 11.6: Causes of hyperphosphatemia and hypophosphatemia	
Hyperphosphatemia	**Hypophosphatemia**
Renal failure	Malnutrition
Hypoparathyroidism	Hyperparathyroidism
High doses of calcitriol	Fanconi syndrome
	Aluminum-containing antacids

6. Enlist the causes and clinical manifestations of hypernatremia and hyponatremia.

Hypernatremia: ↑ sodium level in blood > 145 mEq/L.

Hyponatremia: ↓ sodium level in blood < 135 mEq/L.

The causes and clinical manifestations of hyponatremia and hypernatremia are given in Table 11.7.

Table 11.7: Causes and clinical manifestations of hypernatremia and hyponatremia

Causes of hyponatremia [MN: SIADH]	**Causes of hypernatremia**
Sweating	Dehydration
Inappropriate ADH secretion (SIADH)	Diabetes insipidus
Addison's disease	Steroids
Diuretics, **Di**arrhea	Cushing's disease
Heart failure	Primary hyperaldosteronism
Clinical manifestations of hyponatremia	**Clinical manifestations of hypernatremia**
Drowsiness	Nausea
Confusion	Vomiting
Decrease in BP	Thirst
Tremors	Restlessness
Coma	Confusion

7. What are the causes and effects of hypokalemia?

The causes and effects of hypokalemia (serum K^+ < 3.5 mEq/L) are given in Table 11.8.

Table 11.8: Causes and effects of hypokalemia

Causes	**Effects**
Diarrhea Conn's syndrome Insulin therapy of diabetic ketoacidosis Alkalosis Diuretics—thiazides, loop diuretics	Muscle weakness and cramps, abnormal heart rhythm—arrhythmias, paralytic ileus, depressed reflexes

8. What are the causes and effects of hyperkalemia?

The causes and effects of hyperkalemia (serum K^+ > 5 mEq/L) are given in Table 11.9.

Table 11.9: Causes and effects of hyperkalemia

Causes	Effects
Renal failure Addison's disease Potassium-sparing diuretics Hemolysis Tissue damage* Metabolic acidosis*	Bradycardia, cardiac arrhythmias, cardiac arrest in diastole

*Due to redistribution of potassium to extracellular fluid.

Key points

Acidosis and hypercalcemia: Acidosis causes release of calcium bound to albumin leading to an increase in plasma ionizable calcium. Reverse occurs in alkalosis.
Toxicity of magnesium: Diarrhea, lethargy, CNS depression, cardiac arrhythmia.
Calcium toxicity: Loss of appetite, nausea, vomiting, constipation and renal stones.
Tetany: Caused due to extensive spasm of skeletal muscle in persons with hypocalcemia.

MICROMINERALS

9. Enlist the sources, daily requirements and metabolic functions of microminerals.

The sources, daily requirements and metabolic functions of microminerals are given in Table 11.10.

Table 11.10: Sources, daily requirements and metabolic functions of microminerals

Mineral	RDA and sources	Metabolic functions
Iron	Recommended daily allowance (RDA) Men: 10 mg Women: 20 mg Pregnancy: 40 mg Liver, meat, poultry, fish, leafy vegetables, dairy products, dry fruits, jaggery	• Oxygen transport and storage • Electron transport and energy metabolism • Component of enzymes: Xanthine oxidase, cytochrome P450, tryptophan pyrrolase, ribonucleotide reductase
Copper	RDA: 2–3 mg Meat, shellfish, cereals	• Oxidation-reduction reactions: Cytochrome c oxidase, lysyl oxidase, dopamine-β-monooxygenase, monoamine oxidase, tyrosinase, extracellular superoxide dismutase • Scavenging of free radicals: ceruloplasmin • Iron absorption

Contd...

Contd...

Mineral	RDA and sources	Metabolic functions
Iodine	RDA: adults: 100–150 μg Pregnant women: 200 μg Seafood, iodized salt	• Formation of thyroid hormones-thyroxine (T_4) and triiodothyronine (T_3)
Zinc	RDA: 10–20 mg Shellfish, meat, nuts and legumes	• Cofactor for superoxide dismutase (cytosolic), carbonic anhydrase, carboxypeptidase A, DNA and RNA polymerase, alcohol dehydrogenase • Required for secretion and storage of insulin • Protein structure and regulation of gene expression: zinc finger motif • Maintain the taste: Gusten, a protein containing zinc, helps in taste sensation • Has a role in apoptosis, hair growth, sperm maturation and wound healing
Manganese	RDA: 5 mg Nuts, tea leaves	• Cofactor for superoxide dismutase (of mitochondria), pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK)
Fluoride	RDA: 1 ppm in drinking water; Marine fish, fluoridated toothpastes	• It forms fluorapatite layer on the tooth enamel and protects the tooth against decay
Selenium	RDA: 50–100 μg Meat, seafood	• Enzymes requiring selenium: Glutathione peroxidase, iodothyronine deiodinase • Antioxidant

10. Enumerate iron absorption and factors affecting it.

The absorption of iron is shown in Figure 11.1 and factors affecting iron absorption are given in Table 11.11.

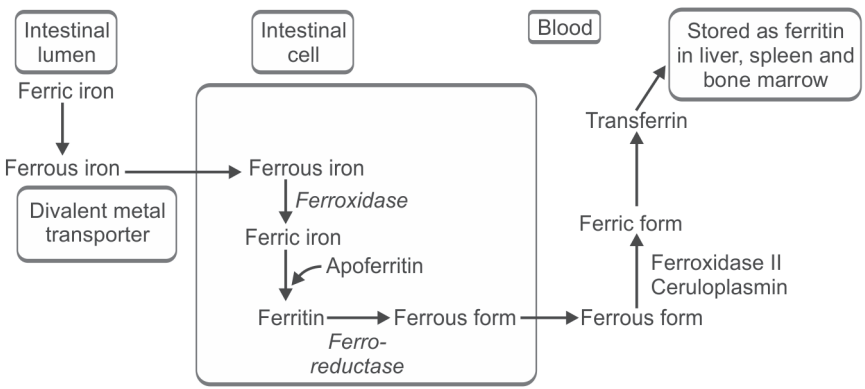


Fig. 11.1: Iron absorption

Regulation of Iron Absorption

i. **Mucosal block theory:** Iron metabolism is regulated at the level of absorption. If there is excess of ferritin in mucosal cells, iron absorption is blocked. If the ferritin content in the mucosal cell is less, more iron is absorbed. This mechanism of regulation of iron absorption, when iron is in excess in mucosal cells is called mucosal block theory.

ii. **Anemia:** There is increased iron absorption in anemia.

Table 11.11: Factors affecting iron absorption

Enhancers of iron absorption* (facilitate conversion of ferric to ferrous form)	Inhibitors of iron absorption* (form insoluble complexes with iron)
Vitamin C	Phytic acid
Citric acid	Calcium
Acidic pH	Polyphenols
Lactic acid	Phosphates, oxalates, antacids

*Only non-heme iron in the diet is influenced by factors mentioned.

11. What are the causes and manifestations of iron deficiency?

Causes

Nutritional deficiency, menstruation, repeated pregnancy, chronic blood loss (piles), hookworm infestation.

Manifestations

- Fatigue, tachycardia and palpitations. In severe iron deficiency, brittle and spoon-shaped nails, sores at the corners of the mouth and atrophy of taste buds can occur
- Difficulty in swallowing due to the formation of webs of tissue in the throat and esophagus (Plummer-Vinson syndrome)
- Pica: A behavioral disturbance characterized by the consumption of non-food items
- Peripheral smear shows: Microcytic, hypochromic anemia.

12. What are the causes of iron overload?

- **Hemochromatosis:** An increase in total body iron (> 15 g) with tissue damage. Iron overload could be hereditary or secondary

  Hereditary hemochromatosis: It is due to gene mutation. There is increased absorption of iron from the small intestine

Secondary iron overload is due to ineffective erythropoiesis, repeated blood transfusions, excess of iron intake (bantu siderosis), etc. This may predispose to bronze diabetes (skin pigmentation along with diabetes mellitus due to pancreatic damage).

Key Points

Goiter: Iodine deficiency in adults leads to enlargement of thyroid glands (goiter) and hypothyroidism.

Congenital hypothyroidism (cretinism): Due to iodine deficiency in pregnant mother causing irreversible mental retardation in newborn (thyroid hormone is required for the myelination of the CNS).

Menkes disease: It is due to defect in transport of copper from intestinal cell to blood. It is characterized by mental retardation, impaired growth and kinky hair.

Wilson's disease (hepatolenticular degeneration): It is due to defect in transport of copper and secretion of ceruloplasmin from the liver. There is accumulation of copper in liver, basal ganglia, cerebral cortex, cornea (Kayser-Fleischer ring) and kidney.

Acrodermatitis enteropathica: Genetic disorder resulting from impaired uptake and transport of zinc; patient presents with perioral, genital, anal dermatitis, hair loss, growth retardation, diarrhea and decreased cell-mediated immunity.

Keshan disease: Seen among young women and children in a selenium deficient region of China. It is characterized by the sudden onset of cardiac insufficiency.

Kashin-Beck disease: It is due to selenium deficiency characterized by the degeneration of the articular cartilage between joints.

Dental fluorosis: It is a result of excess fluoride intake prior to the eruption of the first permanent teeth characterized by small opaque white flecks or spots on the enamel of the teeth. Severe dental fluorosis results in marked staining and pitting of the teeth.

Skeletal fluorosis: It is a toxic manifestation of fluoride excess characterized by increased bone mass. This may progress to calcification of ligaments, immobility, muscle wasting and neurological problems.

Iron is stored in reticuloendothelial system (RES): Bone marrow, liver and intestinal mucosal cells as ferritin.

Copper: Has a role in iron metabolism.

Antacids: H_2 receptor antagonists and proton pump inhibitors may impair iron absorption.

Goitrogens: Some foods (cabbage, cauliflower) contain substances that interfere with iodine utilization or thyroid hormone production. They are called as goitrogens.

Molybdenum: Required for action of enzyme xanthine oxidase.

Cobalt: It is constituent of vitamin B_{12} and is also used in treatment of cancer (radioactive cobalt).

12 Nutrition

1. Define calorific value of food with examples.

Definition: Calorific value is defined as the amount of energy obtained from 1 g of foodstuff (Table 12.1).

Table 12.1: Calorific values of different foods

Name of the foodstuff	Calorific value (cal/g)
Carbohydrates	4
Proteins	4
Lipids	9
Alcohol	7

2. Define respiratory quotient with examples.

Definition: Respiratory quotient (RQ) is defined as the ratio of volume of carbon dioxide generated to the oxygen used up during a given time (Table 12.2).

Table 12.2: Respiratory quotient of different foods

Type of food	Respiratory quotient
Carbohydrates	1
Proteins	0.8
Fats	0.7

3. Define basal metabolic rate (BMR). What is the unit of expression of BMR? Add a note on factors affecting the same.

Definition: Basal metabolic rate (BMR) may be defined as the energy required by an awake individual in resting, postabsorptive state (12 hours after last meal).

Average BMR is 24 kcal/kg/day or 34 kcal/m^2/h.

Factors Affecting BMR

- *Age:* BMR of children is much higher than adults
- *Sex:* Women normally have lower BMR than men
- *Surface area:* BMR is directly proportional to the body surface area
- *Climate:* In colder climates, the BMR is high and in tropical climates it is proportionately low
- *Fever:* During febrile states, BMR is high
- *Hormones:* Thyroid hormones increase BMR.

4. Define specific dynamic action (thermogenic effect of food) with examples.

Definition: Specific dynamic action (SDA) may be defined as the extra heat produced other than the energy normally generated from a particular amount of food. This 'extra heat' is derived from energy reserves of the body. It is used for the metabolic interconversions of food in the liver before it can be used by the body (Table 12.3).

Table 12.3: SDA of different foods

Type of diet	Specific dynamic action (SDA)
Proteins	30%
Carbohydrates	15%
Fats	5%
Mixed diet	10%

5. What are dietary fibers? Why are they important?

Definition: Dietary fibers are non-digestible/non-absorbable carbohydrates in diet. Average intake of these should be 15–25 g/day. For example, cellulose, hemicellulose, lignin, pectin, etc.

Functions of Dietary Fibers

- Increase peristalsis and prevent constipation
- Increase bile acid excretion
- Increase cholesterol excretion
- Prevent colon cancer
- Improve glucose tolerance
- Act as an antioxidant.

Adverse Effects of Fiber

Consumption of large quantities of fiber can:
- Affect the absorption of certain nutrients
- Can cause flatulence and discomfort due to fermentation of some fibers by intestinal bacteria.

Sources of Dietary Fiber

The sources of dietary fibers are fruits, leafy vegetables, whole wheat legumes, rice bran, etc.

6. Define glycemic index with examples. What is its significance?

Definition: It is a measure of increase in blood glucose after consuming 50 grams of food as compared to that seen after consuming 50 grams of glucose [glucose tolerance test (GTT)].

$$\frac{\text{Area under GTT curve after 50 g of test meal}}{\text{Area under GTT curve after 50 g of glucose}} \times 100$$

Significance: Diabetics should consume food with low glycemic index. Foods like ice cream, milk, legumes, peas, beans, peanuts have low index; potato, bread, rice, fruits have high glycemic index.

7. Write a note on nitrogen balance.

Definition: It is a state when a person's daily intake of nitrogen is equal to its daily excretion.

Classification

I = Intake, U = Urinary, F = Fecal, S = Sweat concentration of nitrogen.

i. **Negative nitrogen balance:** It occurs when a person's excretion of nitrogen exceeds his daily intake and is often associated with muscle wasting ($I < U + F + S$). For example,
   - Kwashiorkor and marasmus
   - Corticosteroid therapy
   - Cancer and uncontrolled diabetes.

ii. **Positive nitrogen balance:** It occurs when a person's nitrogen intake is more than excretion ($I > U + F + S$). It is often associated with muscle growth. For example,
   - Growing children
   - Pregnant woman
   - Recovery from illness.

8. Write briefly on parameters used to assess nutritional value of proteins.

Parameters used to assess nutritional value of proteins are given in Table 12.4.

Table 12.4: Parameters to assess the nutritional value of proteins

Parameter	Definition	Significance
Biological value (BV)	Percentage of absorbed protein retained in the body $\frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$	Index of quality of protein BV of egg—94 BV of milk—84
Net protein utilization (NPU)	Percentage of ingested nitrogen retained in the body $\frac{\text{Nitrogen retained}}{\text{Nitrogen ingested}} \times 100$	Index of quality of protein NPU of egg—91 NPU of milk—75
Protein efficiency ratio (PER)	It is the gain in body weight in grams per gram of protein ingested $\frac{\text{Gain in body weight in gram}}{\text{Protein intake in gram}} \times 100$	Index of quality of protein PER of egg—4.5 PER of milk—3.0
Chemical score (CS)	Gives an idea about essential amino acid (AA) content of a protein $\frac{\text{mg of AA per gram of test protein}}{\text{mg of same AA per gram of reference protein}} \times 100$	Index of quality of protein CS of egg—100 CS of milk—65

9. What is balanced diet? What are the factors to be considered, while prescribing a balanced diet?

Definition: It is a diet, which contains different types of food in an amount that meets the daily requirement for calories and nutrients for optimal growth and development.

Factors to be Considered, While Formulating the Balanced Diet

- Measure body weight
- Obtain BMR and add the extra requirement depending on physical activity
- Calculate total calorie requirement
- Add specific dynamic action—10% of total requirement
- Provide carbohydrate:protein:fat = 60:20:20.

Calculation of Energy Requirement Depending on Physical Activity

- Calculate BMR
  + 30% of BMR for sedentary work
  + 40% of BMR for moderate work
  + 50% of BMR for heavy work
- Then add 10% of the above total as SDA
- Make it to nearest multiple of 50.

Example 1: Calculate the energy requirement (BMR = 34–37 kcal/m^2/h) of a male sedentary worker with a body surface area of 1.7 m^2.

Energy requirement at basal level = 34 × 1.7 × 24 = 1,387.2 kcal
For sedentary work, 30% of above value = 416.16
Total = 1,387.2 + 416.16 = 1,803.36 kcal
SDA = 10% of the total calorie requirement = 180.34 kcal
Total energy requirement = 1,803.36 + 180.34 = 1,983.70 kcal/day = 2,000 kcal/day.

Example 2: Calculate the energy requirement of a 55 kg male doing moderate work (BMR = 24 kcal/kg/day).

Energy requirement at basal level = 24 × 55 = 1,320 kcal/day.
For moderate activity, add 40% of above value = 528 kcal
Total = 1,320 + 528 = 1,848 kcal/day
SDA = 10% of the total calorie requirement = 184 kcal
Total energy requirement = 1,848 + 184 = 2,032 kcal/day = 2,050 kcal/day.

10. What is food guide pyramid?

Definition: It is a schematic representation of five food groups that provide all the necessary nutrients in recommended quantities. Foods that are at the base of pyramid can be taken more than those at the top (Fig. 12.1). The five basic food groups are:

i. Grains.
ii. Vegetables.
iii. Fruits.
iv. Meat and dairy products.
v. Sugar and fat.

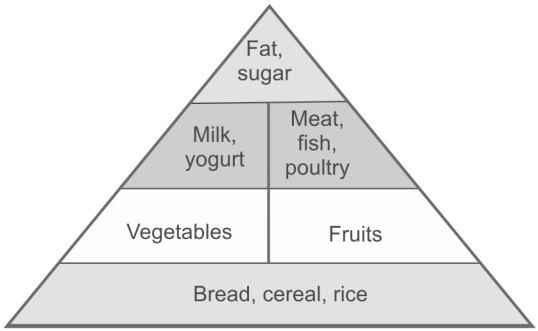


Fig. 12.1: Food guide pyramid

11. Define and classify protein energy malnutrition. Enlist the features of kwashiorkor and marasmus.

Definition: Imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth and maintenance of specific functions.

The extreme forms of protein energy malnutrition (PEM) are kwashiorkor and marasmus (Table 12.5).

Table 12.5: Features of marasmus and kwashiorkor

Sl No	Features	Kwashiorkor	Marasmus
1.	Age group affected	Older children in 2nd or 3rd year of life	Infants below 1 year of age
2.	Defect	Protein deficiency	Decreased calorie intake
3.	Muscle wasting	Muscle wasting is masked by edema	Emaciated child, with gross wasting of muscle and subcutaneous tissue
4.	Mental changes	Child is listless, apathetic and lethargic	Irritable
5.	Edema	Present	Absent
6.	Appetite	Decreased	Good
7.	Serum albumin	Markedly decreased	Normal or mildly reduced serum proteins

12. Write briefly about obesity.

Definition: It is a disorder characterized by accumulation of excess body fat. Overeating and reduced physical activity can lead to sustained deposition of fat.

Classification Based on BMI

Body mass index: It is defined as a ratio between weight in (kg) and square of the height in meters (W/H^2). It is used for identification and grading of obesity.

- Pre-obese: 25.00–29.99
- Obese class I: 30.00–34.99
- Obese class II: 35.00–39.99
- Obese class III: $\geq$ 40.

Causes of obesity

- Excess food intake: High-fat diet
- Sedentary lifestyle
- Genetic factors: Leptin (controls body fat) has got a role in development of obesity
- Environmental and endocrine factors.

Complications of obesity: Hypertension, diabetes mellitus, coronary artery disease, etc.

13. Write a note on total parenteral nutrition (TPN).

Definition: Administration of nutrients parenterally (bypass gastrointestinal tract) to meet the nutritional requirement of a patient. TPN is given to prevent the risk of malnutrition and its effects, like infection, weakness and immobility, which predispose to various diseases and may delay recovery from the illness. The nutrients administered include carbohydrates, proteins, lipids, electrolytes, vitamins and minerals.

Indications for TPN

- Preoperative nutrition to improve the outcome of surgery in severely malnourished patients
- Critical illness, cancer cachexia
- Liver failure, renal failure
- Acute pancreatitis, inflammatory bowel disease
- HIV, hyperemesis gravidarum.

Complications of TPN

- Acid-base imbalance
- Fluid overload
- Electrolyte imbalance

- Cardiovascular failure
- Hyperosmolar non-ketotic coma
- Infection.

Key Points

Lactulose (fructose + galactose): It is a synthetic sugar used in the treatment of constipation (osmotic action) and hepatic encephalopathy.

Sucralose, aspartame, saccharin, neotame: These are artificial sweeteners used in food industry.

Trans-fatty acids: Are chemically classified as unsaturated fatty acids, but they increase the risk of atherosclerosis. They are formed during the hydrogenation of vegetable oils.

Polyunsaturated fatty acids (linoleic acid, linolenic acid, arachidonic acid): Protect against atherosclerosis and coronary artery disease.

Role of taurine in infant nutrition: Taurine has an important role in fat absorption in preterm and possibly term infants (taurine-conjugated bile acids).

Mutual supplementation of proteins: To overcome limiting amino acids in a given type of food, mixed diets are given so that deficiency of amino acid in one food will be supplemented from others. For example, rice (lysine and threonine are limiting amino acids) + dal (lacking sulfur containing amino acids).

Metabolic syndrome (syndrome X): It is a disorder characterized by abdominal obesity, glucose intolerance, insulin resistance, hyperinsulinemia, dyslipidemia and hypertension.

Anorexia nervosa: It is a disorder associated with severe weight loss due to reduced intake of food (fear of obesity).

Bulimia nervosa: Eating disorder characterized by episodes of overeating followed by induced vomiting.

13 Nucleic Acid Chemistry

CHEMISTRY OF NUCLEOTIDES

1. Explain purines and pyrimidines with suitable examples.

Purines and pyrimidines are heterocyclic compounds containing nitrogen, which form the structure of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Figs 13.1, 13.2 and Table 13.1).

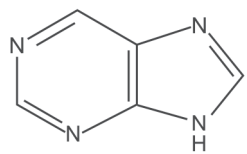


Fig. 13.1: General structure of purine

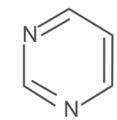


Fig. 13.2: General structure of pyrimidine

Table 13.1: Purines and pyrimidines

Base	Purines	Pyrimidines
Major bases in nucleic acids	Adenine (A) Guanine (G)	Cytosine (C) Uracil (U) Thymine (T)
Minor bases in nucleic acids	7-methyl guanine Dimethyl adenine	5-methylcytosine 5-hydroxymethylcytosine
Metabolic intermediates and analogues	Hypoxanthine, xanthine, uric acid, caffeine, theophylline, allopurinol	5-fluorouracil

2. What are nucleosides?

Definition: Nucleosides are glycosides formed by combination of nitrogenous base with pentose sugar (Fig. 13.3 and Table 13.2).

Composition: Base (purine or pyrimidine) + pentose sugar (ribose or deoxyribose). Ribonucleoside has ribose and deoxyribonucleoside has deoxyribose.

For example: Adenine + ribose → Adenosine
Guanine + ribose → Guanosine
Uracil + ribose → Uridine
Cytosine + ribose → Cytidine
Thymine + ribose → Thymidine
Adenine + deoxyribose → Deoxyadenosine

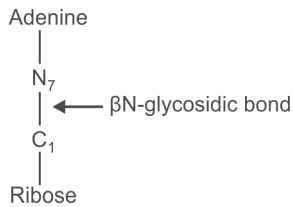


Fig. 13.3: Structure of nucleoside

Table 13.2: Nucleosides

Name	Examples
Nucleosides in DNA*	• Deoxyadenosine, deoxyguanosine, deoxycytidine, deoxythymidine
Nucleosides in RNA†	• Adenosine, guanosine, cytidine, uridine
Other nucleosides	• Pseudouridine, thymidine, S-adenosyl methionine, 5-deoxyadenosyl cobalamin

*DNA, deoxyribonucleic acid; †RNA, ribonucleic acid.

3. What are nucleotides? Give some examples.

Definition: Nucleotides are phosphoric acid esters of nucleosides (nucleoside + phosphate). They form the basic units of DNA and RNA (Fig. 13.4 and Table 13.3).

Composition: Base (purine or pyrimidine) + pentose sugar (ribose or deoxyribose) + phosphate.

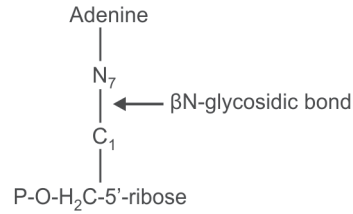


Fig. 13.4: Structure of nucleotide (AMP)

Table 13.3: Examples and functions of nucleotides

Examples	Functions
Adenosine triphosphate (ATP) and guanosine triphosphate (GTP)	• High-energy compounds
Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP)	• Second messenger
3'-phosphoadenosine-5'-phosphosulfate (PAPS)	• Sulfate donor
Nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), CoASH	• Coenzymes
Uridine diphosphate (UDP)-glucose	• Glycogen and UDP-glucuronic acid synthesis
UDP-glucuronic acid	• Detoxification
Cytidine diphosphate (CDP)-choline	• Synthesis of lecithin and sphingomyelin

Deoxynucleotides: Nucleotides with 2′-deoxyribose. For example, deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP).

STRUCTURE AND FUNCTIONS OF NUCLEIC ACIDS

4. Describe Watson-Crick model of DNA with a figure. What are the differences between DNA and RNA?

i. In 1953, Watson and Crick proposed the DNA structure (Fig. 13.5).

ii. DNA consists of two polydeoxyribonucleotide strands coiled around the same axis to form a right-handed helix. DNA is composed of deoxyribonucleotides (deoxyribose + phosphate in diester linkage + bases like A, T, C, G).

iii. Polydeoxyribonucleotide strand is formed by phosphodiester bond between 3′-OH group of one sugar and 5'-OH group of another sugar.

iv. One strand is oriented in 5′ to 3′ direction; the other is oriented in 3′ to 5′ direction (antiparallel).

v. The two strands are complementary to each other and are held together by hydrogen bonds between the bases.

vi. Each strand acts as a template for synthesis of daughter DNA strand.

vii. Base pairing rule: Adenine pairs with thymine by two hydrogen bonds; guanine pairs with cytosine by three hydrogen bonds. Four deoxyribonucleotides are deoxyadenylate, deoxyguanylate, deoxycytidylate and thymidylate.

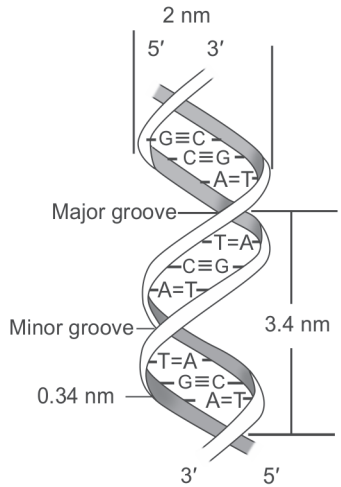


Fig. 13.5: Structure of DNA (G, guanine; C, cytosine; A, adenine; T, thymine)

viii. Chargaff's rule: It states that, total amount of purines are equal to total amount of pyrimidines in a DNA double helix (A + G = T + C).

ix. The backbone of DNA is made up of alternating deoxyribose and phosphate groups (hydrophilic). The hydrophobic nitrogenous bases are towards the core of double helix. The bases are arranged perpendicular to the axis of helix.

x. The spatial relationship between two strands creates two types of grooves (major and minor grooves). These grooves are the sites of interaction of DNA regulatory proteins.

xi. The diameter of the helix is 2 nm (20 Å).

xii. Each turn of the helix has 10 base pairs with a pitch of 3.4 nm (34 Å) and the bases are 0.34 nm (3.4 Å) apart from each other along the helix.

Types of DNA

- B-form—DNA usually seen in human cells
- A-form—right-handed helix with 11 base pairs per turn
- Z-form—left-handed helix with 12 base pairs per turn.

Differences between DNA and RNA are given in Table 13.4.

Table 13.4: Differences between DNA and RNA

DNA	RNA
Found in nucleus	Found in nucleus and cytoplasm
Bases are A, T, G and C	Bases are A, U, G and C
Deoxyribose is the sugar component	Ribose is the sugar component
Double stranded	Single stranded (usually)
Genetic information is transcribed to form different types of RNA	Genetic information is translated to form proteins

5. Describe the structure and functions of different types of RNAs.

Ribonucleic acid (RNA) is a single-stranded polymer of ribonucleotides linked by phosphodiester bond between 3′-OH of a preceding nucleotide and 5′-OH of next nucleotide.

Ribonucleotides contain three major components:

i. Ribose sugar.
ii. Nitrogenous bases—purines (adenine and guanine) or pyrimidines (cytosine and uracil).
iii. Phosphate.

Types

Messenger RNA

- Single-stranded polyribonucleotide strand formed by transcription. It carries genetic information from DNA for protein synthesis [**heterogeneous RNA (hnRNA),** the precursor form of messenger RNA (mRNA), is processed to form mRNA]
- The mRNAs differ in size and sequences depending upon the protein that has to be synthesized (heterogeneous)
- The coding region of mRNA is sandwiched between initiator codon (AUG) and terminator codons (UGA, UAA, UAG)

- **5′ cap:** The eukaryotic mRNA is capped at the 5′ end by 7-methyl guanosine triphosphate, which protects it from hydrolysis by 5′ exonuclease; it also helps in initiation of protein synthesis
- Poly(A) tail: 3′ terminal contains a polymer of adenylate residues, which stabilizes the mRNA
- The mRNA is complementary to template strand of DNA.

Transfer RNA (Fig. 13.6)

i. Transfer RNA (tRNA) functions as an adapter, which brings a specific amino acid from cytosol to the site of protein synthesis.
ii. It is small in size (75 nucleotides).
iii. Although there are 20 amino acids, around 32 tRNAs are found in humans.
iv. Intrastrand hydrogen bonds present in tRNA gives it a clover leaf shape.
v. A highly conserved sequence CCA is present towards the 3′ end (acceptor arm). The last nucleotide, adenine at the 3′ end is involved in binding covalently to a specific amino acid.
vi. Three loops present in tRNA are:
   - **D arm:** Formed by 2 or 3 dihydrouridine residues
   - **Anticodon arm:** It has a triplet codon (complementary to the codon on mRNA molecule) that specifically interacts with the codon on mRNA
   - **TΨC arm:** T, Ψ and C stands for thymine, pseudouridine and cytosine.

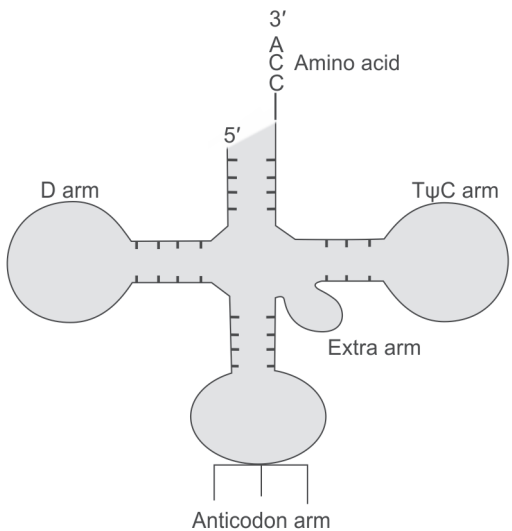


Fig. 13.6: Structure of tRNA

Ribosomal RNA

i. Ribosomal RNAs (rRNAs) are the most abundant forms of RNA, which are associated with ribosomes.

ii. They have catalytic activity (like enzymes). They have a role in translation. Ribosomal subunits are given in the Table 13.5.

Table 13.5: Subunits of ribosome

Subunits	Prokaryotes	Eukaryotes
Larger subunit	50S	60S
Smaller subunit	30S	40S

Key Points

Nucleoside: Base (purine or pyrimidine) + pentose sugar (ribose or deoxyribose).

Nucleotide: Base (purine or pyrimidine) + pentose sugar (ribose or deoxyribose) + phosphate.

Purine analogues: Allopurinol and 6-mercaptopurine used in the treatment of gout and cancer respectively.

Pyrimidine analogues: 5'-fluorouracil (thymidylate synthase inhibitor) used in the treatment of cancer.

Nucleoside analogues: Arabinosylcytosine and 5'-iododeoxyuridine are used in the treatment of cancer and herpetic keratitis respectively.

Base pairing rule: Adenine-thymine is linked by two hydrogen bonds and guanine-cytosine are held together by three hydrogen bonds.

Chargaff's rule: Total amount of purines are equal to total amount of pyrimidines in double helix **(A + G = T + C)**.

Melting temperature (Tm) for DNA: At 90°C, half of double-stranded DNA denatures into single-stranded DNA.

Small nuclear RNA (snRNA): The snRNAs U_1, U_2, U_4, U_5, U_6 are required for splicing of heterogeneous nuclear RNA.

Unusual bases and nucleosides in the tRNA: Thymidine, dihydrouracil, hypoxanthine, pseudouridine.

14 Nucleic Acid Metabolism

1. Describe the sources of carbon and nitrogen atoms of purine nucleotide. Write the pathway for de novo purine synthesis. Add a note on its regulation and inhibitors.

Definition: Purine synthesis involves sequential addition of carbon and nitrogen atoms to ribose 5′-phosphate to generate nine-membered ring (Fig. 14.1). Sources of carbon and nitrogen atoms of purine nucleotide are given in the Table 14.1.

Site: Liver.

Subcellular site: Cytosol.

Starting material: Ribose 5′-phosphate.

End product: Inosine monophosphate (IMP).

Table 14.1: Sources of different atoms of purine ring

Atoms	Sources
N_1	Aspartate
C_2, C_8	N_{10} tetrahydrofolate
N_3, N_9	Glutamine
C_4, C_5, N_7	Glycine
C_6	CO_2

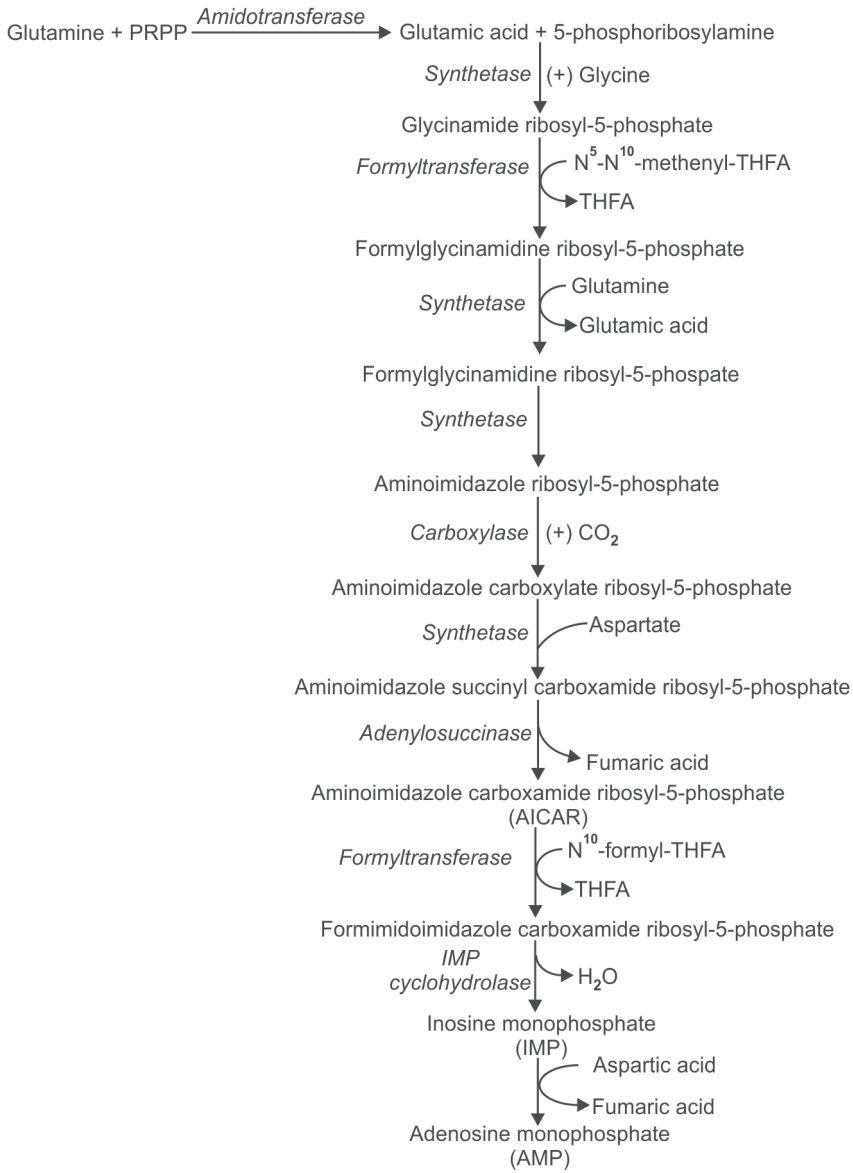


Fig. 14.1: Synthesis of purine ring

Regulators and inhibitors of purine synthesis are explained in Tables 14.2 and 14.3 respectively.

Table 14.2: Regulation of purine synthesis

Enzyme	Inhibitor	Stimulator
PRPP* synthetase	AMP‡, GMP§	–
Amidotransferase	GMP, AMP	–
Adenylosuccinate synthetase	AMP	GTP‖
IMP† dehydrogenase	GMP	–

*PRPP, phosphoribosyl pyrophosphate; †IMP, inosine monophosphate; ‡AMP, adenosine monophosphate; §GMP, guanosine monophosphate; ‖GTP, guanosine triphosphate.

Table 14.3: Inhibitors of purine synthesis

Inhibitor	Mechanism of action
Sulfonamides	PABA* analogues block folate synthesis in microbes
6-mercaptopurine	Inhibits formation of AMP and GMP from IMP
Mycophenolic acid	Inhibits IMP† dehydrogenase (used to prevent graft rejection)
Methotrexate	Inhibits dihydrofolate reductase, reduces availability of active folate and blocks purine synthesis; it is used as an anticancer agent

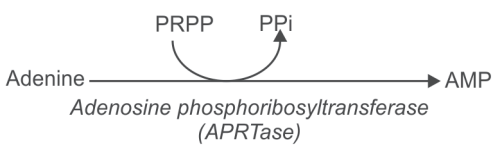
*PABA, para-aminobenzoic acid; †IMP, inosine monophosphate.

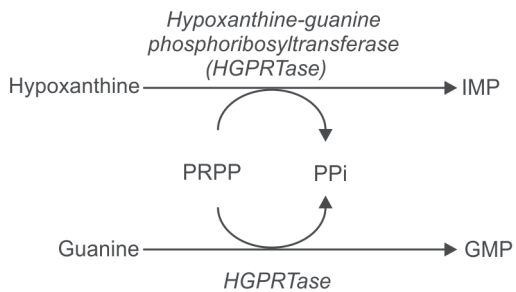
2. How are purine bases salvaged? Add a note on Lesch-Nyhan syndrome.

Definition: It involves conversion of purines, purine ribonucleosides and purine deoxyribonucleosides to corresponding mononucleotides.

Reactions:

i. Phosphoribosylation of free purines.





ii. **Phosphorylation of purine ribonucleoside**, e.g.

$$\text{Adenosine + ATP} \xrightarrow{\textit{Adenosine kinase}} \text{AMP + ADP}$$

Significance: This pathway is the source of nucleotides for erythrocytes and brain. As they lack amidotransferase, they cannot synthesize purines.

Lesch-Nyhan syndrome

Inheritance: X-linked.

Defect: Complete/partial absence of hypoxanthine-guanine phosphoribosyltransferase (HGPRTase).

Consequences

- Poor salvage of hypoxanthine and guanine leads to excessive uric acid formation
- ↓ HGPRTase → ↑ phosphoribosyl pyrophosphate (PRPP), ↓ GMP and ↓ IMP → ↑ de novo synthesis of purines; improper utilization + ↑ production of purines → ↑ degradation of purines → ↑ uric acid.

Clinical Features

- Hyperuricemia and gouty arthritis
- Urate stones
- Self-mutilation
- Mental retardation.

Treatment

Allopurinol (refer Question 3).

3. How is uric acid formed in the body? Add a note on gout.

Uric acid: It is the end product of purine catabolism (excreted in urine) (Fig. 14.2, p. 185).

Starting material: Purines.

End product: Uric acid.

Gout: It is an acute inflammatory condition caused by increased production and deposition of monosodium urate crystals in the joints and soft tissues.

Causes of Primary Gout [MN: GASP]

- **G**lucose-6-phosphatase deficiency (von Gierke disease)
- **A**midotransferase not responding to feedback inhibition

- **S**alvage pathway defect (Lesch-Nyhan syndrome)
- **P**RPP synthetase not under regulation.

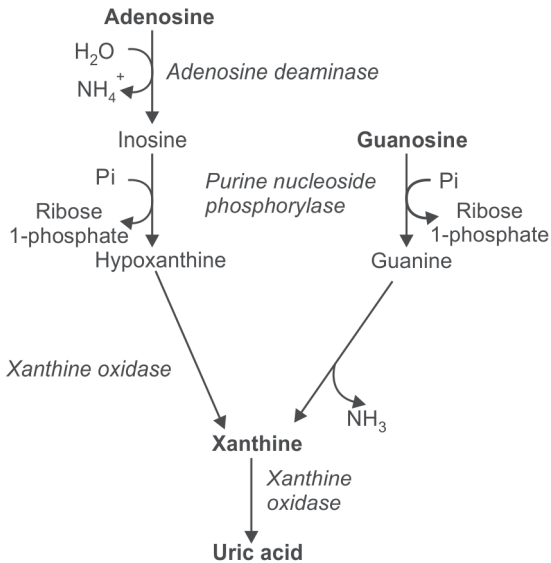


Fig. 14.2: Steps of uric acid synthesis

Causes of Secondary Gout [MN: CRP]

- **C**ancer
- **R**enal failure
- **P**soriasis.

Clinical Features

Red, tender, swollen joint in feet and hands; ↑ uric acid → sodium urate crystals deposited in the joints → joint inflammation → gouty arthritis.

Treatment

- Allopurinol: Inhibits xanthine oxidase → ↓ uric acid production
- Probenecid → ↑ excretion of uric acid in the urine
- Non-steroidal anti-inflammatory drugs, steroids and colchicine: ↓ pain and inflammation.

4. Describe the causes and features of hyperuricemia. How is it treated?

Definition: ↑ levels of uric acid in the blood (normal level: 4–7 mg/dL).

Causes, features and treatment (refer Question 3).

5. Describe the synthesis of pyrimidine ring. Add a note on its regulation and associated disorders.

Definition: Sequential addition of nitrogen and carbon atoms to form a six-membered pyrimidine ring (Table 14.4 and Fig. 14.3, p. 187).

14.4: Sources of different atoms of pyrimidine ring

Atoms	Sources
N_3, C_2	Carbamoyl phosphate
C_4, C_5, C_6, N_1	Aspartic acid

Site: Cytosol and mitochondria.

Disorders of pyrimidine metabolism

Orotic aciduria: Refer Question 7.

6. Write a note on severe combined immunodeficiency (SCID).

Inheritance: Autosomal recessive.

Cause: Adenosine deaminase and purine nucleoside phosphorylase deficiency → ↑ dATP and dGTP → (–) ribonucleotide reductase → ↓ DNA synthesis → decreased proliferation of B and T lymphocytes → severe immune deficiency [(–) = inhibition].

Clinical features: Repeated life-threatening infections.

Treatment: Bone marrow transplantation and gene therapy.

7. Write briefly about orotic aciduria.

Definition: ↑ excretion of orotic acid in the urine.

Inheritance: Autosomal recessive.

Types and causes

- **Type I orotic aciduria:** Deficiency of enzyme orotate phosphoribosyltransferase and orotidylate decarboxylase
- **Type II orotic aciduria:** Deficiency of orotidylate decarboxylase.

Clinical features: ↑ orotic acid in urine, megaloblastic anemia and failure to thrive.

Treatment: Oral uridine/cytidine → (–) enzymes in the pyrimidine synthesis pathway.

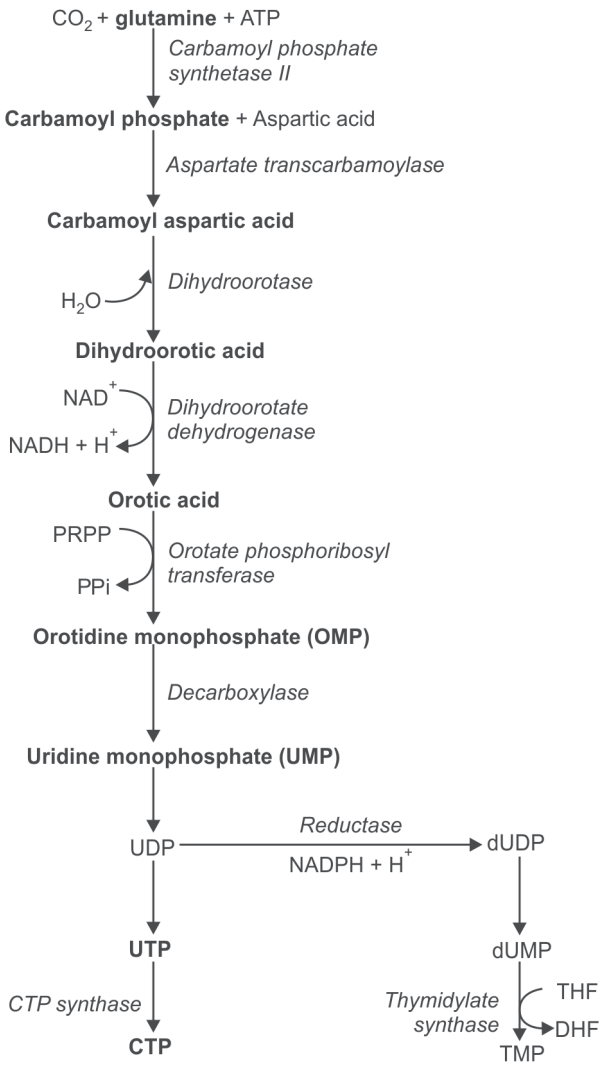


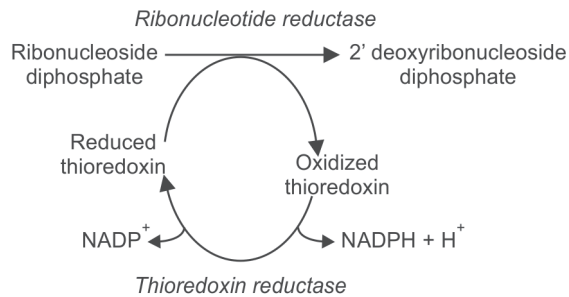
Fig. 14.3: Pyrimidine synthesis (UMP, UDP, UTP, uridine mono, di and triphosphate respectively; CTP, cytidine triphosphate; TMP, thymidine monophosphate; dUMP, dUDP, deoxyuridine mono and diphosphate respectively)

- **Urea cycle disorder:** Ornithine transcarbamoylase deficiency (type II hyperammonemia) → ↑ carbamoyl phosphate → enters cytosol → ↑ orotic acid production
- **Allopurinol:** Competes with orotate phosphoribosyltransferase to inhibit the phosphoribosylation of orotate, which may lead to orotic aciduria.

8. Explain the synthesis of deoxyribonucleotides.

Purine and pyrimidine ribonucleotides undergo reduction at the 2′ carbon to give rise to respective deoxyribonucleotides in the presence of enzyme ribonucleotide reductase complex.

- Site: Actively dividing cells
- Requirements: Thioredoxin, thioredoxin reductase, NADPH
- General reaction:



Specific Reactions

CDP → 2′dCDP → 2′dCTP
UDP → 2′dUDP → 2′dTTP
GDP → 2′dGDP → 2′dGTP
ADP → 2′dADP → 2′dATP

Key Points

Folic acid: It is required for purine synthesis. Folate deficiency → impaired DNA synthesis → megaloblastic anemia and neural tube defects.

Methotrexate: (–) dihydrofolate reductase → used as an anticancer agent.

5′-fluorouracil: (–) thymidylate synthase → anticancer agent.

Lesch-Nyhan syndrome: X-linked recessive disorder with defect in hypoxanthine-guanine phosphoribosyltransferase (HGPRTase).

Normal plasma uric acid level: 3–7 mg/dL.

Gout: It is an acute inflammatory condition caused by increased production and deposition of monosodium urate crystals in the joints and soft tissues.

Allopurinol (competitive and also suicide inhibitor): Inhibits xanthine oxidase, thereby decreases the production of uric acid.

Severe combined immunodeficiency (SCID): Autosomal recessive disorder caused due to deficiency of adenosine deaminase and purine nucleoside phosphorylase, leading to dysfunctional T and B lymphocytes.

Carbamoyl phosphate synthetase II (CPS II): It is a cytosolic enzyme required for pyrimidine synthesis.

Orotic aciduria: Autosomal recessive disorder $\rightarrow \uparrow$ orotic acid in urine; due to deficiency of enzyme orotate phosphoribosyltransferase and orotidylate decarboxylase.

15 Molecular Biology-I

REPLICATION

1. Write an account on replication. Add a note on xeroderma pigmentosum.

Definition: It is the process in which two identical copies of daughter deoxyribonucleic acid (DNA) molecules are formed from parent DNA during cell division.

Replication is semiconservative: Each daughter DNA gets one strand from parent DNA. The other strand is newly synthesized.

Requirements for DNA replication

i. **Double-stranded DNA.**

ii. **Deoxyadenosine triphosphate (dATP), deoxyguanosine triphosphate (dGTP), deoxythymidine triphosphate (dTTP) and deoxycytidine triphosphate (dCTP).**

iii. **Enzymes:**

- **DNA helicases:** Unwind the double helix using energy (ATP)
- **DNA topoisomerase I (nuclease and ligase activity):** Relieves the supercoiling of DNA by cutting and resealing **one** strand of DNA
- **DNA topoisomerase II (nuclease and ligase activity):** Relieves the supercoiling of DNA by breaking and resealing **both** the strands of DNA
- **DNA polymerase:** It synthesizes a complementary strand of DNA from single strand of DNA (template) by polymerization of deoxynucleotides
  - *DNA polymerase I:* Required for proofreading and fills the gap between Okazaki fragments of lagging strand; in proofreading, copying errors are identified and corrected (5′-3′ polymerase activity, 3′-5′ and 5′-3′ exonuclease activity)
  - *DNA polymerase II:* Required for proofreading and DNA repair

  - *DNA polymerase III*: It is the main replication enzyme, also required for proofreading (5′-3′ polymerase activity and 3′-5′ exonuclease activity).
- **DNA ligase:** Seals the nick in single strand as well as in Okazaki fragments.

iv. **Proteins:**
- **dnaA:** Binds to AT-rich region → unwinding of DNA
- **Single-stranded DNA-binding proteins (SSB proteins):** Prevent annealing of separated DNA strands
- **Primase:** Initiates synthesis of short segment of RNA (RNA primer).

Steps in DNA Replication

i. **Identification of site of origin of replication (ori) by specific proteins:** Origin of replication is a unique nucleotide sequence from where DNA replication begins. In prokaryotes, there is a single ori, which consists of AT-rich region. In eukaryotes, multiple origins of replication exist.

ii. **Unwinding of double-stranded DNA to form 2 single-stranded DNA (ssDNA):** dnaA protein binds to AT-rich region in the origin of replication → local melting/unwinding of dsDNA → short segment of ssDNA formed (required for initiation of DNA synthesis). Single strand binding (SSB) proteins attach to each strand and prevent their annealing (maintain DNA in single strand form). Separation of strands of DNA is required as DNA polymerase binds to single strand of DNA.

iii. **Formation of replication fork, synthesis of primer and initiation of DNA synthesis (Fig 15.1):** Unwinding of DNA causes formation of replication fork → DNA helicase binds to the fork → unwinding of adjacent double-stranded region → primase binds to DNA at 3′ end of each strand → synthesis of short segment of RNA (RNA primer) → DNA polymerase using RNA primer, initiates synthesis of daughter strand (complementary to template) in 5′ → 3′ direction. Both parent strands are simultaneously replicated in the 5' → 3' direction. The replication forks, thus, advance in opposite direction from their origin. This process results in the formation of 'replication bubbles'.

Direction of DNA synthesis

a. **Leading strand:** DNA is synthesized continuously in 5′ → 3′ direction towards the replication fork (Fig. 15.1) and it needs only one RNA primer.

b. **Lagging strand:** It is synthesized in short stretches in 5′ → 3′ direction, but away from replication fork. These short stretches of discontinuous DNA are called Okazaki fragments. It requires many RNA primers.

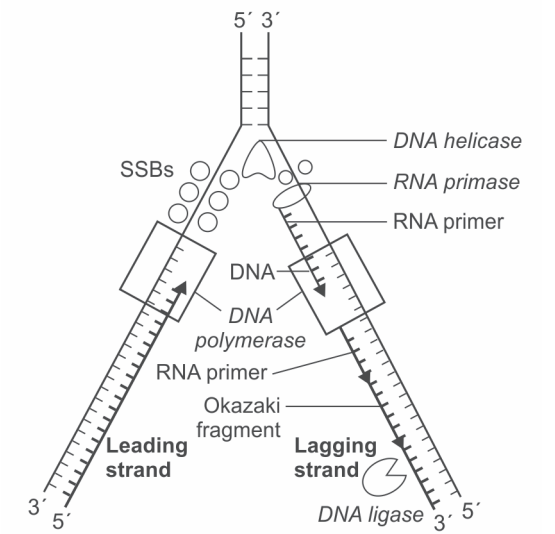


Fig. 15.1: Replication fork (DNA, deoxyribonucleic acid; SSBs, single-stranded DNA-binding proteins; RNA, ribonucleic acid).

Unwinding of DNA during replication creates supercoils, which are relieved by topoisomerase I and II.

iv. **Elongation**

a. It is the process where there is sequential addition of deoxynucleotides via phosphodiester bonds. First phosphodiester bond is formed between 3′–OH group of RNA primer and 5′ phosphate group of first entering deoxynucleotide. DNA polymerase (DNA pol III) elongates new DNA strand by adding deoxynucleotides (dATP, dGTP, dTTP, dCTP) one at a time, to the 3′ end of growing chain, complementary to bases in the template strand.

b. Proofreading of newly formed DNA: Any errors due to mismatched nucleotides during replication are immediately repaired to prevent lethal mutations. This is mainly done by DNA polymerase III and DNA polymerase I.

c. Excision of RNA primer.

- *DNA polymerase I,* which removes the RNA primer (5′-3′ exonuclease activity) and synthesizes DNA that replaces RNA using 5′-3′ polymerase activity; it also proofreads the new chain using 3′-5′ exonuclease activity

- *DNA ligase:* Catalyzes the formation of phosphodiester bond between DNA synthesized by DNA polymerase III and that formed by DNA polymerase I using energy (ATP).

v. In eukaryotes, an additional step occurs. The newly synthesized dsDNA reforms chromatin structure.

Xeroderma Pigmentosum

Xeroderma pigmentosum is an autosomal recessive disorder with defective nucleotide excision repair due to deficiency of enzyme **ultraviolet (UV) specific endonuclease**. In this condition, exposure to UV light can cause mutations in DNA (pyrimidine dimers), which may lead to cancer.

2. What are Okazaki fragments?

Okazaki fragments are short segments of single-stranded DNA that are synthesized discontinuously on lagging strand during DNA replication (refer Fig. 15.1). DNA polymerase I removes the RNA primers (5′-3′ exonuclease activity) between these fragments and synthesize DNA that replaces RNA (5′-3′ polymerase activity). DNA ligase finally joins these fragments (refer Elongation, p. 192).

3. Write a note on inhibitors of replication.

Inhibitors of replication, mechanism of action and therapeutic uses are given in Table 15.1.

Table 15.1: Inhibitors of replication

Inhibitor	Mechanism of action	Therapeutic use
Ciprofloxacin, novobiocin, nalidixic acid	DNA gyrase inhibition	Antibiotics
Etoposide, doxorubicin	Inhibit topoisomerase II	Anticancer agents
Cytosine arabinoside	Prevents chain elongation	Anticancer agent
Adenine arabinoside	Prevents chain elongation	Antiviral drug

Key Points

DNA replication occurs in **S (synthetic) phase** of cell cycle.
Proofreading: It is mainly done by DNA polymerase III and DNA polymerase I.
RNA primer (5–12 nucleotides long): Required for DNA synthesis; produced by primase.
Postreplicative modifications of DNA: These include methylation of DNA and mismatch repair.
Huntington's disease: Autosomal dominant disease with motor and cognitive dysfunction. This is due to expansion of trinucleotide repeats (CAG), which codes for glutamate in huntingtin protein (altering its function).

Xeroderma pigmentosum: It is an autosomal recessive disorder with defective nucleotide excision repair due to deficiency of enzyme **UV-specific endonuclease**.

Fanconi anemia: Due to defective repair of interstrand DNA cross-links presenting with microcephaly, mental retardation, anemia and leukopenia.

Telomeres: It is the 3' end of mammalian DNA with 5'-TTAGGG-3' repeats. The number of these repeats decreases with each cell division and indicates normal aging of cells. In germ cells, the enzyme telomerase maintains the length of telomere.

Cancer and telomerase: Telomerase activity is found to be high in cancer cells, which make them immortal and replicate indefinitely. Telomerase inhibitors are under development as potential anti-cancer agents.

TRANSCRIPTION

4. Write briefly about promoter regions.

Promoter region: These are highly conserved regions of DNA both in pro- and eu-karyotes, which are recognized by RNA polymerases to initiate the process of transcription.

Prokaryotic Promoters (Fig. 15.2)

a. **Pribnow box (TATA box):** Stretch of 6 nucleotides (5′-TATAAT-3′) located about 10 nucleotides upstream from transcription start site. This has low melting temperature due to lack of GC pairs. This will result in unwinding of DNA.

b. **–35 sequence:** It is a sequence of 8 nucleotides (5′-TGTTGACA-3′) located approximately 35 bp upstream from transcription start site.

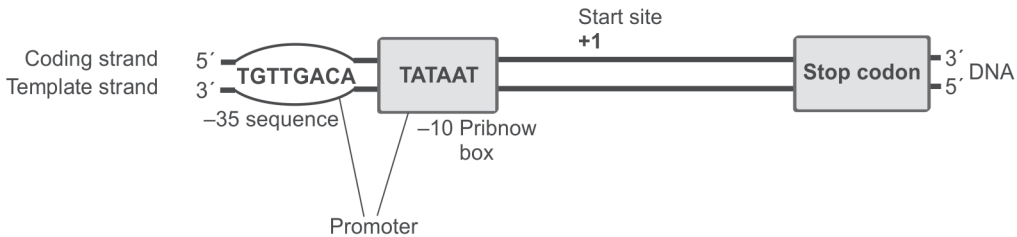


Fig. 15.2: Prokaryotic promoter regions

Eukaryotic Promoters

About 25 nucleotides upstream of the transcription start site, similar to prokaryotes, a TATA or Hogness box is present. Another consensus sequence CAAT box is present about 70–80 nucleotides upstream of transcription start site.

All these promoters help in deciding the origin of transcription by facilitating effective binding of RNA polymerase.

5. Describe transcription process. Add a note on inhibitors of transcription.

Definition: Transcription is the process of synthesizing RNA from a DNA template. It takes place in the 5' → 3' direction. The newly synthesized mRNA [primary transcript, heterogeneous nuclear RNA (hnRNA)] is identical to the other DNA strand—the coding strand (except having uracil in place of thymine).

Requirements

- **DNA to be copied**
- **RNA polymerase, RNAP (holoenzyme):** Core enzyme (2α, 1β, 1β') + σ (sigma) factor. It is the enzyme that synthesizes mRNA
- **Termination factor—ρ (rho):** For termination of transcription
- **ATP**
- **Helicase:** Unwinding of DNA during transcription
- **Topoisomerase I and II:** Remove supercoiling.

Steps in Transcription

Steps in transcription include initiation, elongation and termination.

i. **Binding of RNAP to the template strand of DNA and formation of preinitiation complex:** RNA polymerase (holoenzyme) binds to promoter region (-35 sequence) of DNA. It then moves on and binds to TATA box → causes local unwinding of DNA.

ii. **Initiation of chain synthesis:** The first nucleotide of RNA binds to nucleotide binding site of 'β' subunit of RNA polymerase to form 5′ end of RNA. RNA polymerase moves to next base on the template strand. A corresponding nucleotide binds to RNA polymerase and phosphodiester bond is formed between the two nucleotides (Fig. 15.3).

iii. **Clearance of promoter:** Nucleotides continue to be added. Once RNA has 10–20 nucleotides, RNA polymerase leaves the promoter site (promoter clearance) and moves along template strand.

iv. **Elongation:** After promoter clearance, elongation phase starts. New nucleotides are added to the nascent mRNA complementary to the template strand. RNA polymerase uses ribonucleotides ATP, GTP, CTP and UTP. For addition of each ribonucleotide, energy equivalent to two ATP is used (Table 15.2). Elongation complex containing RNA polymerase moves along DNA template → unwinding of DNA downstream by RNAP.

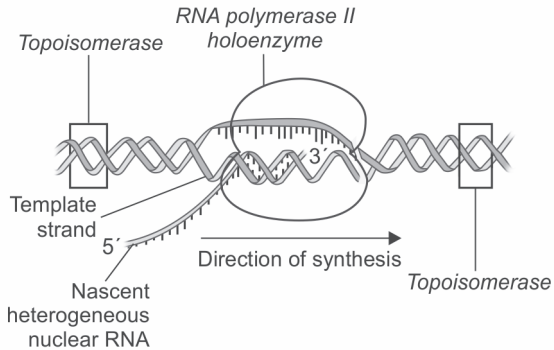


Fig. 15.3: Transcription

Unwinding causes supercoils, which is relieved by topoisomerases.

Table 15.2: DNA template is copied in the following manner

DNA template	Corresponding base on RNA strand
G	C
C	G
T	A
A	U

v. Termination: Process of transcription continues until termination signal sequence is reached on the template strand of DNA.

Rho-dependent termination: Rho is a specific protein, which recognizes the termination signal → dissociation of RNAP from DNA and release of newly synthesized mRNA.

Rho-independent termination: Newly synthesized RNA forms hairpin structure. It also has series of Us near the 3' end, which helps in release of newly synthesized mRNA.

The inhibitors of transcription are given in Table 15.3.

Table 15.3: Inhibitors of transcription

Drug	Mechanism of action	Application
Rifampicin	Inhibits 'β' subunit of prokaryotic RNA polymerase	Treatment of tuberculosis
Actinomycin D	Binds to DNA template and interferes with movement of RNA polymerase	Anticancer agent
α-amanitin	Inactivates RNA polymerase II	Mushroom poison

6. Write a note on histones.

- Histones are proteins, rich in lysine and arginine; they are associated with chromatin
- They form ionic bonds with DNA
- They are required for packaging of DNA into nucleosomes
- They play an important role in regulation of gene expression
- They are of five types namely H1, H2A, H2B, H3 and H4
- Histone acetylation/deacetylation can alter their interaction with DNA. This makes the chromatin more/less accessible for transcription, respectively.

7. Write briefly on reverse transcriptase.

Definition: Reverse transcriptase is the RNA-dependent DNA polymerase. It synthesizes DNA from RNA. It is present in retroviruses where it synthesizes viral DNA from viral RNA (Fig. 15.4).

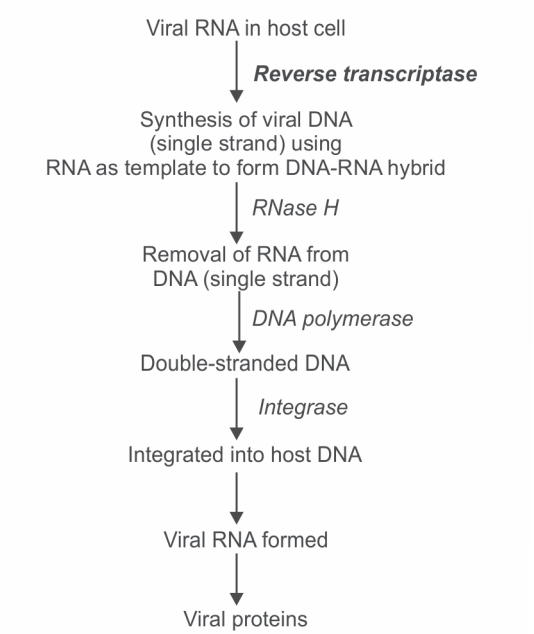


Fig. 15.4: Synthesis of viral proteins by reverse transcriptase

Applications

- Preparing complementary DNA (cDNA) library
- RT-PCR (reverse transcriptase-polymerase chain reaction)
- Reverse transcriptase inhibitors: zidovudine, lamivudine, etc. are used in the treatment of HIV infection.

8. Write a note on post-transcriptional modification.

Definition: Process by which newly synthesized RNA (primary transcript) is modified to mature mRNAs, rRNAs and tRNAs. This type of modification is mainly seen in eukaryotic mRNA, pro- and eukaryotic rRNA and tRNA.

Post-transcriptional Modifications of mRNA

a. **5′ capping:** The primary RNA transcript is known as hnRNA. 7-methylguanosine triphosphate is attached to the 5′ end of this RNA (capping). S-adenosyl methionine is the methyl donor. 5′ capping helps in stabilization of mRNA and initiation of translation.

b. **Poly-A tail:** A chain of 20–250 adenine nucleotides are attached to the 3′ end of mRNA. Poly-A tail helps in stabilization of RNA and translation.

c. **Removal of introns:** The primary transcript has exons (coding region) and introns (non-coding regions). Small nuclear RNAs (snRNAs) in association with some proteins form small nuclear ribonucleoprotein particles (SnRNPs) → bind to RNA to form spliceosomes, which remove the introns and join the exons (Fig. 15.5).

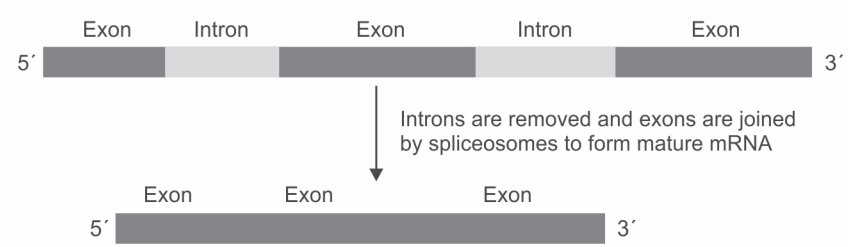


Fig. 15.5: Removal of introns of mRNA

Post-transcriptional Modifications of tRNA (Pro- and Eu-karyotes)

- CCA tail added to 3′ end
- Some bases are modified by reduction, methylation, deamination, etc.

Post-transcriptional Modifications of rRNA (Prokaryotes)

- 23S rRNA + 5S rRNA + proteins → 50S subunit
- 16S rRNA + proteins → 30S subunit; 30S + 50S → 70S ribosome.

Post-transcriptional Modifications of rRNA (Eukaryotes)

- 28S rRNA + 5.85S rRNA + 5S rRNA + proteins → 60S subunit
- 18S rRNA + proteins → 40S subunit; 40S + 60S → 80S ribosome.

Key Points

RNA polymerase has no proofreading activity, so transcription is more error prone compared to replication.
Mushroom poisoning (α-amanitin): Inhibits RNA polymerase II and prevents elongation.
Spliceosome: It removes the introns and joins the exons to form mature mRNA.
Reverse transcriptase: It is the RNA-dependent DNA polymerase present in retroviruses, where they copy the viral RNA genome into DNA.

TRANSLATION

9. Describe the process of translation in a. Prokaryotes b. Eukaryotes.

Definition: Translation is the process by which mRNA is translated to produce a polypeptide sequence, also known as a protein (Fig. 15.6).

Fig. 15.6: Synthesis of proteins

Requirements for Translation

a. **Amino acids:** For synthesis of polypeptide chain.

b. **Transfer RNA:** At least one tRNA per amino acid.

c. **Aminoacyl-tRNA synthetase:** Required for attachment of amino acids to the specific tRNAs. This requires energy (two ATP molecules).

d. **Messenger RNA (mRNA):** Has codons, which dictate synthesis of polypeptide chain.

e. **Ribosomes (50S, 30S in prokaryotes; 40S, 60S in eukaryotes):** Located in the cytosol as free form or associated with endoplasmic reticulum.
   - **A, P and E** sites on ribosome (p. 201) for binding tRNA
     - **A-site:** For binding of incoming aminoacyl-tRNA as specified by codon at that site
     - **P-site:** Has peptidyl tRNA (tRNA with newly synthesized chain of amino acids) attached to it
     - **E-site:** It is occupied by empty tRNA.

f. **Initiation, elongation and termination factors.**

g. **Energy from:**
   - 2 ATP for binding of amino acids to specific tRNA
   - 2 GTP for binding of aminoacyl-tRNA to A-site and translocation.

h. **Binding between codon on mRNA and specific anticodon of tRNA:** As per base pairing rule (Fig. 15.7).

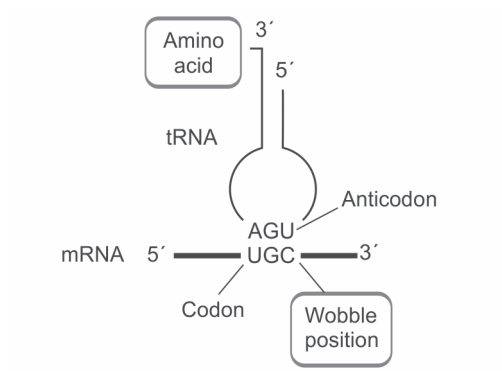


Fig. 15.7: Codon-anticodon interaction

Steps of Translation in Prokaryotes

i. **Initiation:** Mechanism by which ribosome recognizes nucleotide sequence on mRNA to initiate the translation.

- **Shine-Dalgarno sequence (purine-rich region 5′-UAAAGGAGG-3′):** Located 6–10 bases upstream from AUG codon on the mRNA; this facilitates the binding of 30S ribosome to mRNA
- **Initiation codon AUG (Fig. 15.8):** Located on mRNA; it is recognized by initiator tRNA with N-formylmethionine.

$$\text{tRNA-methionine} + N_{10}\text{ formyl THF} \xrightarrow{\textit{Transformylase}} \text{tRNA-N-formylmethionine (fMet-tRNA)} + \text{THF}$$

a. **Formation of 30S initiation complex:** 30S ribosome + mRNA + aminoacyl-tRNA specified by start codon + initiation factors (IF 1, 2, 3) → 30S initiation complex is formed (Fig. 15.8).

b. **Formation of 70S initiation complex (Fig. 15.9):**

$$\text{50S ribosome} + \text{30S initiation complex} + \text{GTP} \xrightarrow{\text{IF 1, 2, 3}} \text{70S initiation complex}$$

The fMet-tRNA is at the P-site.

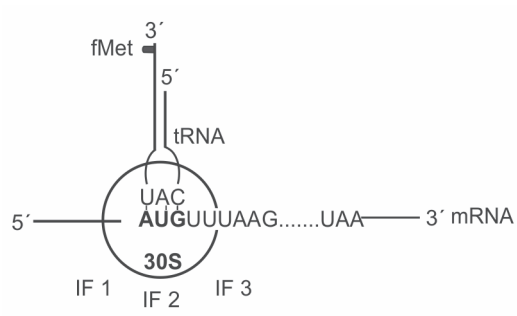


Fig. 15.8: 30S initiation complex

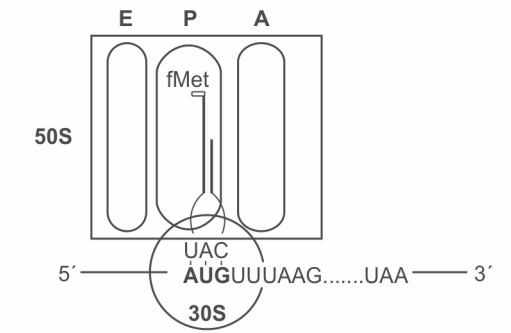


Fig. 15.9: 70S initiation complex

ii. **Elongation:**

a. **Binding of appropriate tRNA to empty A-site:** Elongation factors (EF-Tu, EF-Ts), in the presence of GTP, will help in binding of appropriate tRNA with an amino acid to next codon in the empty A-site (Fig. 15.10).

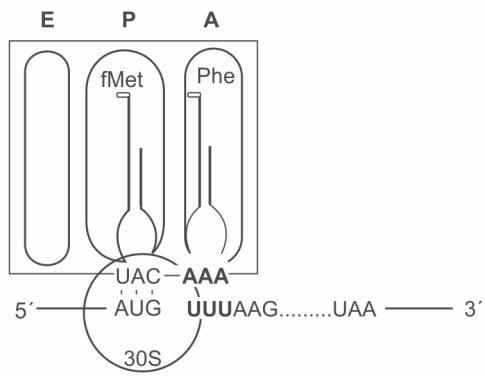


Fig. 15.10: Binding of tRNA to A-site

b. **Peptide bond formation:** Peptidyl transferase transfers the amino acid/peptide from the P-site on to amino acid at the A-site and catalyzes peptide bond formation between the amino acids. The tRNA at the P-site now does not have an amino acid (empty tRNA).

c. **Translocation:** Ribosome moves a distance of three nucleotides along mRNA in the 5′-3′ direction in the presence of GTP and EF-G. Thus, the empty tRNA, which was at the P-site, now lies at E-site; peptidyl tRNA at A-site is now at P-site; A-site is empty (Fig. 15.11).

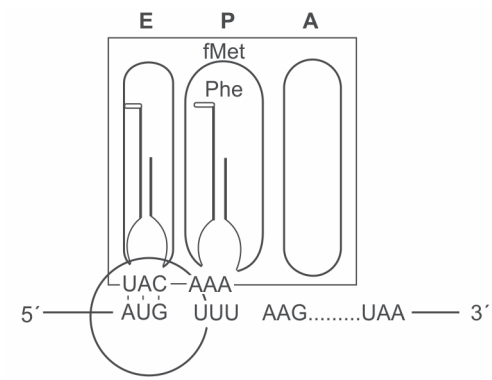


Fig. 15.11: Translocation

A new aminoacyl-tRNA binds to corresponding codon on mRNA at A-site. These steps for formation of required peptide are repeated until termination codon of mRNA is at A-site.

iii. **Termination:** When termination codon (UAA, UGA, UAG) of mRNA is at A-site, it is recognized by release factor (RF). RF binds to this site and releases newly formed peptide in the presence of GTP. The ribosome dissociates into its subunits.

Steps of Translation in Eukaryotes

i. **Formation of aminoacyl-tRNA:**

$$\text{Amino acid} + \text{ATP} + \text{tRNA} \xrightarrow{\textit{Aminoacyl-tRNA synthetase}} \text{Aminoacyl-tRNA} + \text{AMP}$$

ii. **Initiation of protein synthesis:**

a. *Dissociation of ribosome* → 40S subunit + 60S subunit.

b. *Formation of 43S preinitiation complex:*

GTP + initiation factor 2 + Met-tRNA (methionine is the first amino acid to be incorporated in protein synthesis)

↓

Complex formed

↓

Binds to 40S ribosomal subunit

↓

43S preinitiation complex formed

c. *Formation of 48S initiation complex:*

mRNA binds to 43S preinitiation complex

↓

48S initiation complex

The initiation (start) codon AUG on mRNA is identified by Kozak sequence.

The anticodon of Met-tRNA binds to initiation codon AUG on mRNA.

d. *Formation of 80S initiation complex:*

48S initiation complex binds with 60S ribosomal subunit

↓

80S initiation complex formed

Met-tRNA is attached to P-site of ribosome. The A and E sites are free.

iii. **Elongation:** Addition of amino acids to the new growing peptide chain.
   a. Binding of new, appropriate aminoacyl-tRNA to codon at acceptor A-site.
   b. Formation of peptide bond by peptidyl transferase between new amino acid of tRNA at A-site and amino acid attached to tRNA at P-site → transfer of amino acid/growing peptide chain from P-site to amino acid at A-site. Thus, tRNA at P-site now does not have any amino acid.
   c. Translocation:

   Ribosome moves along mRNA by 3 nucleotides (one codon) in the 5′ → 3′ direction

   ↓

   Movement of tRNA from P to E site from which it is released
   Movement of peptidyl-tRNA from A to P site.
   A-site is empty—a new aminoacyl-tRNA binds with codon of mRNA at this site.

iv. **Termination:**

Process of addition of amino acids continues till the termination (stop) codon on mRNA is reached at the A-site. There is no tRNA with anticodon complementary to the stop codon at A-site.

Releasing factor recognizes stop codon at A-site

↓

Binds to A-site

↓

Causes release of newly formed peptide chain, tRNA and mRNA from ribosomes

↓

Dissociation of 80S ribosome → 60S + 40S subunits

10. Describe the properties of genetic code. Add a note on Wobble hypothesis.

- Genetic code is a sequence of 3 nucleotides (triplet code) in mRNA coding for an amino acid
- There are totally 64 different codons (4^3) from 4 nucleotides in different combinations
- AUG: Start codon, codes for methionine
- UAA, UAG, UGA: Stop codons, do not code for any amino acid—cause termination of peptide synthesis.

Properties of Genetic Code

- **Universal:** Same codons code for same amino acids in all living organisms
- **Specific:** The specific codon always code for the same amino acid

- **Non-overlapping and comma less:** Codons are read in a continuous manner without any punctuation
- **It is degenerate (redundant):** An amino acid may have more than one codon coding for it, since there are 61 codons for 20 amino acids, e.g. arginine has six codons.

Wobble Hypothesis

Pairing between the bases at third position (last nucleotide) of codon on mRNA and first position of anticodon of tRNA is non-traditional (not always as per Watson-Crick rule). So, a single tRNA anticodon can bind to more than one codon (refer Fig. 15.7). For example, the codons for glycine GGU, GGC and GGA pair with a single anticodon CCI of tRNA. The base I at third position of anticodon can pair with either U, C or A of mRNA codon (Table 15.4). This is 'Wobble' [*Note:* Pairing between bases at first and second position of codon and that of anticodon (second and third position) is as per Watson-Crick rule].

Table 15.4: Non-traditional base pairing between codon and anticodon

mRNA codon **(5' → 3')** for glycine	GG**U**	GG**C**	GG**A**
tRNA anticodon **(3' → 5')** for glycine	CC**I**	CC**I**	CC**I**

11. Write a note on inhibitors of translation.

Inhibitors of translation and clinical significance are given in Table 15.5.

Table 15.5: Inhibitors of translation

Inhibitor	Site of action	Clinical significance
Streptomycin	Binds to 30S subunit and interferes with initiation	Antibacterial agent
Tetracycline	Inhibits binding of aminoacyl-tRNA to m-RNA ribosome complex	Antibacterial agent
Chloramphenicol	Inhibits peptidyl transferase	Antibacterial agent
Erythromycin	Inhibits translocation	Antibacterial agent
Diphtheria toxin	Inactivates elongation factor (eEF-2) and prevents translocation	Causes diphtheria
Puromycin	Structural analogue of tyrosinyl-tRNA and binds to A-site causing premature release of polypeptide chain	Antibiotic used in cell cultures

12. Write a short note on post-translational modification.

Definition: Process by which newly synthesized polypeptide chain undergoes modifications to produce biologically active protein.

i. **Trimming:** Proteins are synthesized as functionally inactive large precursors. They undergo trimming by proteases to produce functionally active protein. For example,

Proinsulin ⟶ Insulin

Pepsinogen ⟶ Pepsin

Trypsinogen ⟶ Trypsin

ii. **Covalent modification:** Proteins may be activated or inactivated by covalent attachment of variety of chemical groups.

a. **Phosphorylation:** Hydroxyl group of serine, threonine and tyrosine residues in protein can undergo phosphorylation.

$$\underset{\text{(inactive)}}{\text{Glycogen phosphorylase}} \xrightarrow{\textit{Phosphorylase kinase}} \underset{\text{(active)}}{\text{Glycogen phosphorylase (P)}}$$

b. **Glycosylation:** Attachment of carbohydrate to hydroxyl groups of serine, threonine, hydroxylysine (O-linked) and asparagine (N-linked).

Collagen synthesis: Hydroxylysine undergo glycosylation.

c. **Hydroxylation:** Proline and lysine undergo hydroxylation during collagen synthesis.

d. **Carboxylation:** Clotting factors undergo γ-carboxylation of glutamic acid residues in the presence of vitamin K during coagulation.

e. **Farnesylation:** For anchoring of proteins to membranes.

Key Points

Starting codon: AUG (methionine).

UAA, UAG, UGA: Stop codons, do not code for any amino acid.

Selenocysteine: It is considered as 21st amino acid, which is coded by UGA stop codon.

Protein farnesyltransferase inhibitors: Tipifarnib; anticancer agent used in the treatment of acute myeloid leukemia.

Protein targeting: Is sorting of synthesized protein into required location.

16 Molecular Biology-II

DNA REPAIR AND MUTATIONS

1. Explain causes and types of DNA damage.

Causes of DNA Damage

- Mismatch of bases during replication
- Spontaneous deamination: Changes the cytosine (C) to → uracil (U), adenine (A) → hypoxanthine (H) and guanine (G) → xanthine (X)
- Physical agents: Ultraviolet (UV) light, X-ray, ionizing radiation
- Chemical agents (mutagens): For example, anticancer drugs like methotrexate, cyclophosphamide, etc.

Types of DNA Damage

- **Single base alteration:** Nucleotide deletion/insertion, loss of amino groups from C, A, G, alkylation of base, base analogue incorporation
- **Two base alteration:** Thymine dimer formation by cross-linkage between bases of same strand (induced by UV radiation)
- **DNA single/double strand breaks:** Induced by ionizing radiation/reactive oxygen species.

2. Explain various DNA repair mechanisms and add a note on xeroderma pigmentosum.

In order to maintain the integrity of the genome, DNA undergoes repair.

Nucleotide Excision Repair (NER)

For example, repair of pyrimidine dimers and base adducts (Fig. 16.1). UV-specific endonuclease recognizes and cleaves a piece of DNA on both ends of the dimer → DNA polymerase and DNA ligase together will repair and replace the gap with proper nucleotides. Defects in this type of repair mechanism can lead to xeroderma pigmentosum, Cockayne's syndrome, ataxia-telangiectasia, etc.

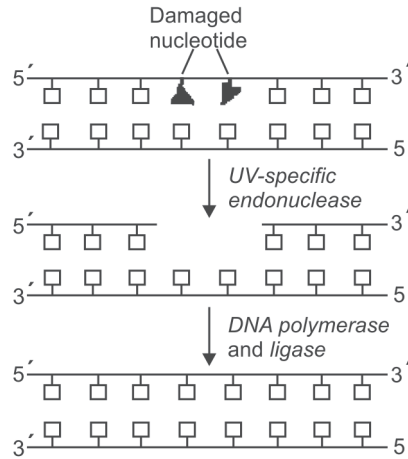


Fig. 16.1: Nucleotide excision repair

Xeroderma Pigmentosum

Refer to Chapter 15, question number 1.

Mismatch Repair

i. Replication errors (escaped from proofreading) → mismatch of one to several bases (e.g. instead of thymine, cytosine may be incorporated opposite to adenine).

   Base mismatch on the daughter strand is recognized by endonuclease → cleavage of strand near the defect → mismatched bases are removed from the daughter strand by exonucleases → DNA polymerase I and DNA ligase repair and replace the gap with appropriate nucleotides (Fig. 16.2).

ii. Defect in mismatch repair: May lead to hereditary non-polyposis colon cancer.

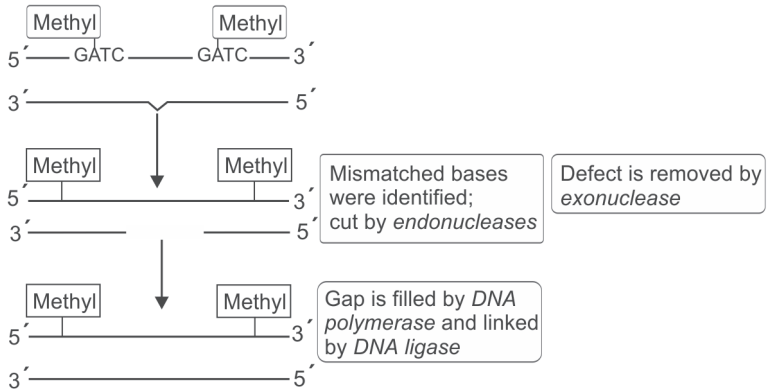


Fig. 16.2: Mismatch repair

Base Excision Repair

Cytosine, adenine and guanine can undergo spontaneous deamination to form uracil, hypoxanthine and xanthine respectively. Abnormal bases are recognized by DNA glycosylases → hydrolytically remove the abnormal base from their sugar → apyrimidinic or apurinic site (AP site) → AP endonucleases → produce break in the strand → deoxyribose phosphate lyase → removes sugar phosphate residue → DNA polymerase I and ligase → regeneration of strand (Fig. 16.3).

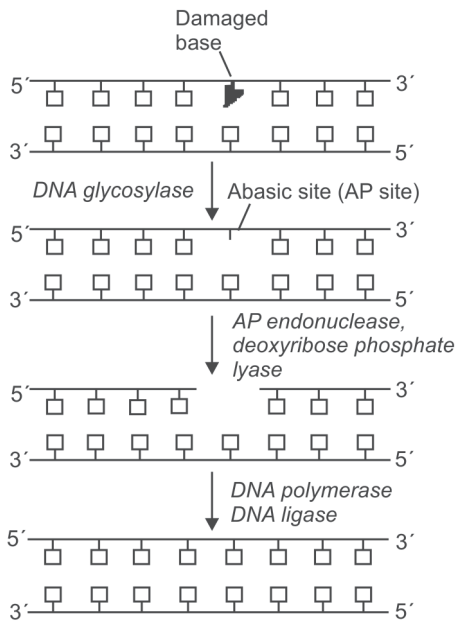


Fig. 16.3: Base excision repair

Double-strand Break Repair

Double-strand break repair is caused by ionizing radiation, free radicals and chemotherapy.

Repair Mechanism

- Non-homologous end joining (NHEJ): Two non-homologous ends of the broken DNA strand are brought together by proteins with some loss of DNA

- Homologous recombination: Two non-homologous ends of the broken DNA strand are brought together by proteins without loss of DNA.

3. Define and classify mutations with examples.

Definition: A mutation is a change in the sequence of nucleotides of DNA. It may or may not alter the amino acid sequence of the protein encoded by that gene.

Chromosomal Mutations

- **Philadelphia chromosome:** Chromosomes 9–22 translocation → activation of c-Abl; seen in 80% of chronic myeloid leukemia cases
- **Burkitt's lymphoma:** 8–14 translocation → c-Myc activation
- **Non-Hodgkin's lymphoma:** 14–18 translocation → activation of bcl-2 → suppression of apoptosis.

Gene Mutation

- Point mutation
- Frameshift mutation
- Others.

Point mutation: Alteration in a single base is point mutation. This is of two types depending on the type of base replaced.

a. *Transition:* Purine replaced by purine or pyrimidine replaced by pyrimidine.
   For example, A ↔ G or C ↔ T.
b. *Transversion:* Purine replacing pyrimidine or pyrimidine replacing purine.
   For example, A ↔ T or C ↔ G or A ↔ C or T ↔ G.

Effects of Point Mutation

a. **Silent mutation:** New codon formed after mutation codes for the same amino acid as original codon. There is no change in the amino acid sequence and property of protein.
b. **Missense mutation:** New codon formed after mutation codes for a different amino acid. This alters the amino acid sequence and may alter the function of protein. The effects could be acceptable (function of protein not altered), partially acceptable (protein function is partially affected) and non-acceptable (non-functional protein formed—could be fatal) (Table 16.1).

c. **Nonsense mutation:** New codon formed after mutation codes for terminator codon leading to premature termination of protein synthesis (Fig. 16.4).

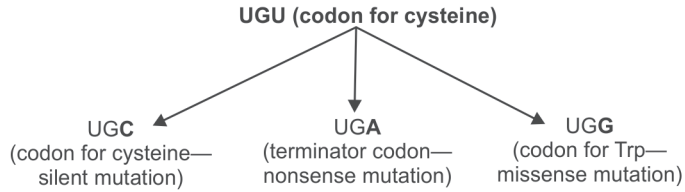


Fig. 16.4: Various consequences of point mutations

Table 16.1: Effects of missense mutations (A, B = 'α' helical segments of globin chain)

Effects	Normal hemoglobin	Mutated hemoglobin
Acceptable	B 61: AAA (lysine)	B 61: AAU (asparagine): Hb Hikari
Partially acceptable	B 6: GAG (glutamic acid)	B 6: GUG (valine): Hb S (sickle cell disease)
Non-acceptable	A 58: CAC (histidine)	A 58: UAC (tyrosine): Hb M

Frameshift mutations: It occurs due to insertion or deletion of nucleotides. It has more serious consequences than point mutation as entire frame (sequence of codons for amino acids) changes from the point of insertion or deletion. It can lead to formation of a non-functional protein, premature termination of protein synthesis (Fig. 16.5, p. 212).

The amino acid sequence, distal to insertion or deletion of nucleotide, changes. For example, frameshift mutation has been demonstrated in diseases like cystic fibrosis, thalassemia, etc.

Other Mutations

- **Huntington's disease: CAG** trinucleotide sequence is repeated many times → insertion of many extra glutamine residues in the huntingtin protein → unstable proteins → accumulation of protein aggregates
- **Fragile X syndrome and myotonic dystrophy:** Trinucleotide expansion occurs in the untranslated portion of a gene → ↓ in the amount of protein produced
- **Splice site mutation** can affect removal of introns and protein formation. For example, β-thalassemia (incorrect splicing of β-globin mRNA) and systemic lupus erythematosus (SLE) (autoimmune antibodies against snRNP).

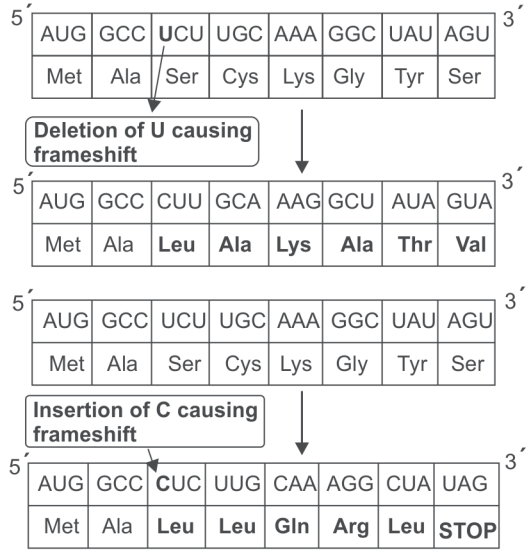


Fig. 16.5: Frameshift mutation

REGULATION OF GENE EXPRESSION

4. Explain Lac operon model of gene expression.

Lac Operon Model

Operon: In prokaryotes, genes that code for proteins in a metabolic pathway are arranged in a linear fashion along with a regulatory region (Fig. 16.6) [**MN: RPOS** = **R**egulation of **P**rokaryotic **O**peron **S**ystem].

In prokaryotic operon, genes are arranged in the following order from 5′ to 3′ on the chromosome.

- **R**egulatory gene: Forms mRNA → forms a repressor protein → which binds operator gene → prevents translation of the protein (constitutive)
- **P**romoter gene: RNA polymerase binds to this region
- **O**perator gene: Site where repressor protein binds
- **S**tructural gene: Responsible for formation of proteins.

Lactose Operon or Lac Operon (Fig. 16.6)

Lac operon has three structural genes coding for different enzymes required for lactose metabolism:

- ***Lac Z* for β-galactosidase:** Hydrolyzes lactose
- ***Lac Y* for permease:** Helps in movement of lactose into cell
- ***Lac A* for thiogalactoside transacetylase:** Function unknown.

There is an operator, promoter, CAP-binding site and a regulatory gene (Lac I).

a. **When *Escherichia coli* is exposed to glucose only,** repressor protein is formed → binds operator gene → prevents the movement of RNA polymerase (RNAP) bound to promoter → no proteins (enzymes) formed (repression).

b. **In the presence of lactose and absence of glucose (derepression).**

- Small amounts of lactose enter *E. coli* → converted to allolactose (inducer) → binds to repressor proteins and prevents its interaction with operator gene → no obstruction for movement of RNAP → protein (enzyme) synthesis occurs
- Absence of glucose → (+) adenylyl cyclase → ↑ cyclic adenosine monophosphate (cAMP) → cAMP-catabolite activator protein (CAP) complex → binds to CAP site → ↑ ability of bound RNAP to initiate transcription of *lac Z, lac Y* and *lac A* genes to produce β-galactosidase, permease and thiogalactoside transacetylase, respectively. These enzymes help *E. coli* to metabolize lactose to produce energy.

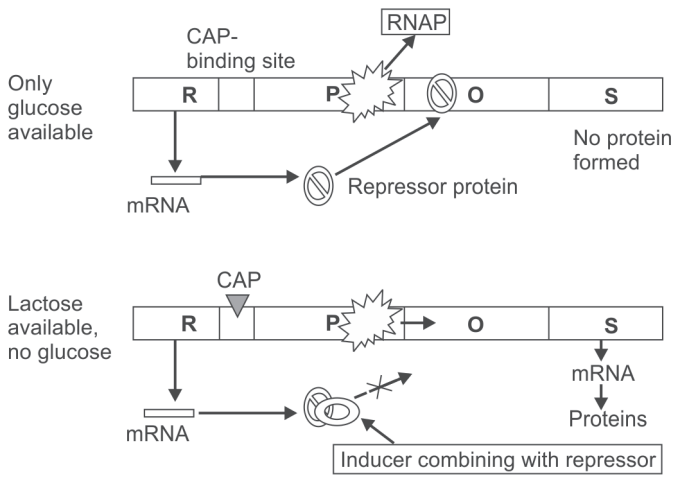


Fig. 16.6: Regulation of **p**rokaryotic Lac **o**peron **s**ystem **[MN: RPOS]** (RNAP, ribonucleic acid polymerase)

c. When both glucose and lactose are available:
Operator site is free from inhibition as allolactose binds with repressor protein.

Glucose → ↓ cAMP → no CAP-cAMP complex formation → RNAP cannot function effectively → no protein (enzymes) formed → lactose cannot be utilized. Hence, glucose is used first even though operator site is free. After all glucose is utilized by bacteria → ↑ cAMP → cAMP-CAP complex formation → (+) RNAP → protein (enzyme) formation.

5. Briefly explain eukaryotic gene regulation.

Unlike prokaryotes, eukaryotic gene regulation is much complex. It can occur by six different mechanisms.

a. *Gene amplification:* This occurs when a constant stimulus exists for a long time, e.g. dihydrofolate reductase gene gets amplified (increase in the number of *DHFR* genes) in a person on antifolate drugs (for cancer).

b. *Gene switching:* When a person is exposed to a foreign antigen for the first time, immunoglobulin M (IgM) is produced. Later, by gene switching for the same antigen, IgG is produced.

c. *Rearrangement of genes in DNA:* Depending on the nature of antigens exposed, many types of antibodies can be produced from a limited number of J, V and C segments. This is by rearrangement of genes.

d. *Control of transcription:* This is done by cis- and trans-acting elements on DNA like enhancers/silencers, hormones binding to hormone response elements, various proteins binding to DNA, histone modifications, etc.

e. *Post-transcriptional regulation:* Post-transcriptional modifications like 5′ capping, poly A tail, splicing, etc. can regulate the stability of RNA. A transcribed monocistronic RNA can form different proteins by RNA editing (alteration of a base in mRNA), e.g. apolipoproteins Apo B_{100} and Apo B_{48} are formed from the same RNA.

f. *Translational regulation:* This is not a major mechanism in eukaryotes. For example, heme can block its own synthesis by inhibiting the production of enzymes responsible for it.

Key Point

Cistron: Smallest unit of gene expression. It codes for one peptide or a subunit of protein.

CANCER GENETICS

6. Explain cell cycle.

Cell cycle consists of mitosis phase and interphase. Interphase is the time between end of previous mitosis and commencement of next (Fig. 16.7).

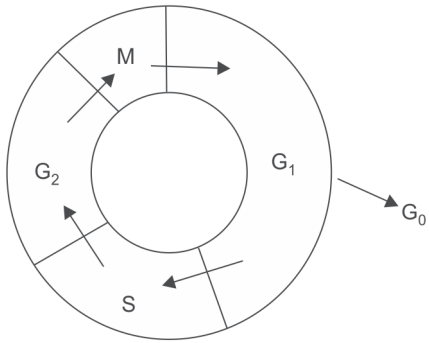


Fig. 16.7: Cell cycle

Interphase

Interphase consists of (G—gap; S—synthetic):

a. G_1 phase.
b. S phase.
c. G_2 phase.

G_1 Phase

Longest phase and lasts for 10–12 hours. It is also called the growth phase. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication.

S Phase

S phase is called synthetic phase (DNA synthesis) and usually lasts for 6–8 hours. Whenever there is a mitogenic signal, DNA starts replicating and each chromatid forms its duplicate sister chromatid.

G_2 Phase

G_2 phase usually lasts for 4–6 hours. As DNA becomes tetraploid; cell volume, cytoplasm and protein synthesis increases; cell prepares itself for division.

Mitosis Phase

Mitosis is the shortest phase of cell cycle lasting for about 1 hour. Chromosomes condense, nuclear membrane dissolves, chromosomes pair and mitotic spindles are formed. Then chromosomes separate and cytoplasmic division follows. Daughter cells may enter into G_0 or G_1 phase.

Cells may enter into G_0 phase (resting phase)—this may occur due to lack of nutrients, growth factor or contact. Some cells, after division, may enter into G_0 phase for a long time/ lifelong. This occurs in those cells, which will not multiply rapidly like neurons, cardiac muscles, skeletal muscles, etc. Cells can re-enter G_1 from G_0 phase.

Regulation

Check point is at G_1/S transition, G_2 phase and G_2/M transitions depending on the availability of growth factors, mitogenic signals, cyclins and cyclin-dependent protein kinases. Cyclin-dependent protein kinases phosphorylate specific proteins. This regulates the metabolic activities of the cell.

7. Explain oncogenes and proto-oncogenes with examples.

- **Oncogenes:** These are cancer causing genes (Table 16.2)
- **Proto-oncogenes:** These genes are found in normal cells and code for growth stimulating proteins.

  Oncogenes were first discovered in viruses. They are named based on the type of cancer they cause and their respective proto-oncogenes are named after oncogenes with a prefix c (c = cellular).

  For example, Rous **s**a**rc**oma virus contains **src** oncogene, which produces sarcoma. Normal human cells also contain closely similar gene ***c-src*.**

Table 16.2: Oncogenes and proto-oncogenes

Proto-oncogene	Protein formed	Oncogene
c-sis	B-chain of PDGF*	*sis*
c-erbB	Receptor for EGF†	*erbB*

Contd...

Contd...

Proto-oncogene	Protein formed	Oncogene
c-myc	Nuclear TF‡	*myc*
c-ras	GTPase	*ras*
c-abl	Tyrosine kinase (signal transducer)	*abl*

*PDGF, platelet-derived growth factor; †EGF, epidermal growth factor; ‡TF, transcription factor.

8. Explain the mechanisms of conversion of proto-oncogene to oncogene.

There are different mechanisms by which proto-oncogene can become an oncogene.

i. **Chromosomal translocation:** A part of one chromosome is removed and attached to another chromosome. For example, in Burkitt's lymphoma, 8–14 translocations occurs leading to activation of inactive proto-oncogene *(c-myc)* on chromosome 14.

ii. **Gene amplification:** An increase in the number of a particular gene. For example, gene amplification of dihydrofolate reductase *(DHFR)* gene in cancer makes it resistant to the anticancer drug.

iii. **Viral insertion:** The terminals of retroviruses have enhancers. Here, integration of a retrovirus (with enhancers) close to a proto-oncogene activates the proto-oncogene to oncogene. For example, a leukemia virus gets integrated near *c-myc* gene leading to its activation.

iv. **Point mutation:** For example, point mutation in *ras* oncogene results in decreased GTPase activity that can lead to pancreatic, colon and lung cancer.

9. Define and classify tumor markers with suitable examples.

Definition: Tumor marker is a substance produced by tumor tissues that can be detected and estimated in body fluids to detect presence of tumor/cancer.

General Uses of Tumor Markers

- Screening of cancer
- Diagnosis of cancer
- To decide prognosis of cancer
- To monitor cancer treatment
- To detect cancer recurrence.

For example, Bence Jones protein (light chain of immunoglobulin produced in excess by the plasma cells) present in urine of patients with multiple myeloma was the first

reported tumor marker. This precipitates between 45°C and 60°C; beyond 80°C, it starts to dissolve.

Types of Tumor Markers

Depending on their nature, tumor markers are divided into oncofetal products: α-fetoprotein (AFP); carcinoembryonic antigen (CEA); enzymes—alkaline phosphatase (ALP), acid phosphatase; hormones—β-human chorionic gonadotropin (β-HCG), calcitonin and others (Table 16.3).

Table 16.3: Tumor markers

Tumor marker	Associated cancer	Family (type)
α-fetoprotein (AFP)	Hepatoma, germ cell tumor	Oncofetal antigen
Carcinoembryonic antigen (CEA)	Colorectal, ovarian cancer	Oncofetal antigen
Alkaline phosphatase (ALP)	Bone cancer	Enzymes
Prostate-specific antigen (PSA)	Prostate cancer	Enzymes
Acid phosphatase	Prostate cancer	Enzymes
'β'-human chorionic gonadotropin (β-HCG)	Choriocarcinoma	Hormones
Calcitonin	Carcinoma of thyroid	Hormones
Vanillylmandelic acid (VMA)	Pheochromocytoma	Hormone metabolite
Hydroxyindoleacetic acid (HIAA)	Carcinoid syndrome	Hormone metabolite
Bence-Jones protein	Multiple myeloma	Others
Cancer antigen (CA)-125	Ovarian cancer	Others

Note: Some of the tumor markers may be elevated in non-cancerous conditions.

10. Define and classify carcinogens.

Definition: Carcinogen is a substance capable of inducing cancer in a normal person.

Classification

- Physical
- Chemical
- Biological
- Hormonal.

Physical Agents

For example,

X-ray, UV ray → Free radicals → Pyrimidine dimer → Cancer

Chemical Agents

Indirect: Chemical (procarcinogen) is metabolically activated to a carcinogen, which causes DNA damage. For example, tobacco smoke, smoked food, coal tar, etc.
Aflatoxin: It is produced by a fungus *Aspergillus* (in improperly stored nuts, wheat and rice). This toxin is converted into a carcinogen by CYP450 microsomal enzymes in our body.
Direct: Chemical interacts directly with DNA to cause damage. For example, alkylating agents like chlorambucil, cyclophosphamide, etc.

Chemical carcinogens can produce cancer at:

- **Site of exposure,** e.g. tobacco—buccal cancer
- **Site of metabolism,** e.g. aflatoxin—hepatoma
- **Site of elimination,** e.g. aromatic drugs—bladder cancer.

Biological Agents

For example, viruses:

a. DNA viruses: **[MN: HEAP]** = **H**epatitis B/**E**pstein-Barr/**A**denoma/**P**olyoma.
b. RNA viruses: **[MN: RAIL]** = **R**ous sarcoma/**AI**DS/**L**eukemia (sarcoma).

Hormonal Agents: ***[MN: CEA]***

a. **C**ontraceptives can cause breast cancer.
b. **E**strogen can cause endometrial cancer.
c. **A**nabolic steroids can cause liver cancer.

Key Points

Cancer may be caused by inactivation (by mutation) of tumor suppressor gene. For example, *RB* inactivation causes retinoblastoma; p53 inactivation causes breast cancer, lung cancer, etc.

Apoptosis: Programmed cell death in response to external and internal injury.

p53 gene: Codes for a nuclear protein. It is a transcriptional regulator. Whenever there is DNA damage, it causes cell cycle arrest. If there is extensive DNA damage, it causes apoptosis.

Retinoblastoma *(RB)* gene: The protein formed from this is a nuclear regulator of cell cycle. It inhibits E2F transcription factor. Inactivation of this gene causes retinoblastoma.

Cancer cells show different morphology and growth behavior. They have round shape, grow densely due to loss of contact inhibition, loss of anchorage dependence and have ↓ requirement of growth factors. Cancer is characterized by:

1. **S**pread to other tissues (metastasis).
2. **I**nvasion of local tissue.
3. **L**oss of control on cell division.
4. **L**oss of contact inhibition.

[MN: SPILL]

MOLECULAR BIOLOGY TECHNIQUES

11. Explain DNA cloning (recombinant DNA technology).

Definition: It is a laboratory method by which genetic material from different sources is joined together. The hybrid genetic material is introduced into a replicating cell for amplification (production of many copies) of genetic material of interest.

Requirements: Restriction endonucleases, vectors and host cell (*E. coli).*

Restriction endonucleases (REs): These are endonucleotidases, which recognize a palindrome stretch of DNA sequences (restriction site) and cleaves it. The fragments produced are restriction fragments. As shown below, **palindrome** is a sequence, which reads the same on complementary strands when read from 5′ to 3′ direction.

5′—**GAATTC**—3′
3′—**CTTAAG**—5′

Vector: It is a DNA molecule to which DNA of interest is incorporated. It should be capable of replication in a host cell and should have at least one restriction site and one marker gene. For example, plasmids, phages (bacterial viruses), cosmids, etc.

Plasmids: These are small, circular, extrachromosomal, duplex DNA found in some bacteria, which carry genes for antibiotic resistance. These can replicate using host replication system. A few kilobase pair length of foreign DNA can be inserted into them.

Steps in Recombinant DNA Technique

i. Human DNA of interest is isolated.
ii. A restriction endonuclease (RE) cleaves it.
iii. Vector DNA (plasmid carrying gene for antibiotic resistance) is cut with same RE; the vector and human DNA fragments are joined by ligase to get recombinant DNA (hybrid DNA).

iv. Mix recombinant DNA + suspension of bacterial cells. This leads to entry of recombinant DNA into bacteria (transformation) (Fig. 16.8, p. 222).

v. These bacteria are grown in a media containing antibiotics. Cells that have taken up plasmid (containing DNA of interest) will survive as plasmid provides antibiotic resistance to bacteria.

vi. Surviving cells multiply to form a colony of cells called clone. These bacteria are lysed to get many copies of recombinant plasmids. These plasmids are then lysed with the same restriction enzymes to get copies of DNA of interest.

Uses of Recombinant DNA Technology [MN: PART]

- **P**renatal diagnosis of genetic disorders
- **A**bundant production of proteins (insulin, growth hormone, clotting factors)
- **R**ape suspect identification (medicolegal)
- **T**ransgenesis (Dolly), gene therapy.

12. Explain the types of DNA libraries.

Definition: It is a collection of restriction fragments of DNA of an organism.

- **Genomic library:** It is a collection of restriction fragments of entire genome of an organism
- **Complementary deoxyribonucleic acid (cDNA) library:** It is a collection of DNA produced from mRNAs of an organism.

13. Explain gene therapy with its applications.

Definition: Gene therapy is a technique involving insertion of a gene for the missing enzyme/protein resulting in production of correct protein.

Types (Depending on Cell Type)

i. **Somatic cell gene therapy:** Non-reproductive cells are used to insert normal functional gene.

ii. **Germ cell gene therapy:** Germ cells are taken and corrected by introducing normal functional gene.

Classification Depending on Method of Gene Therapy

i. Ex vivo **gene therapy:** Cells obtained from the patient → missing gene introduced into the cell → corrected cells are reintroduced into the patient. This was tried in severe combined immunodeficiency (SCID) caused due to deficiency of enzyme adenosine deaminase (ADA) (Fig. 16.9, p. 223).

ii. In vivo **gene therapy has been tried:** The normal gene is introduced into the cells directly in vivo.

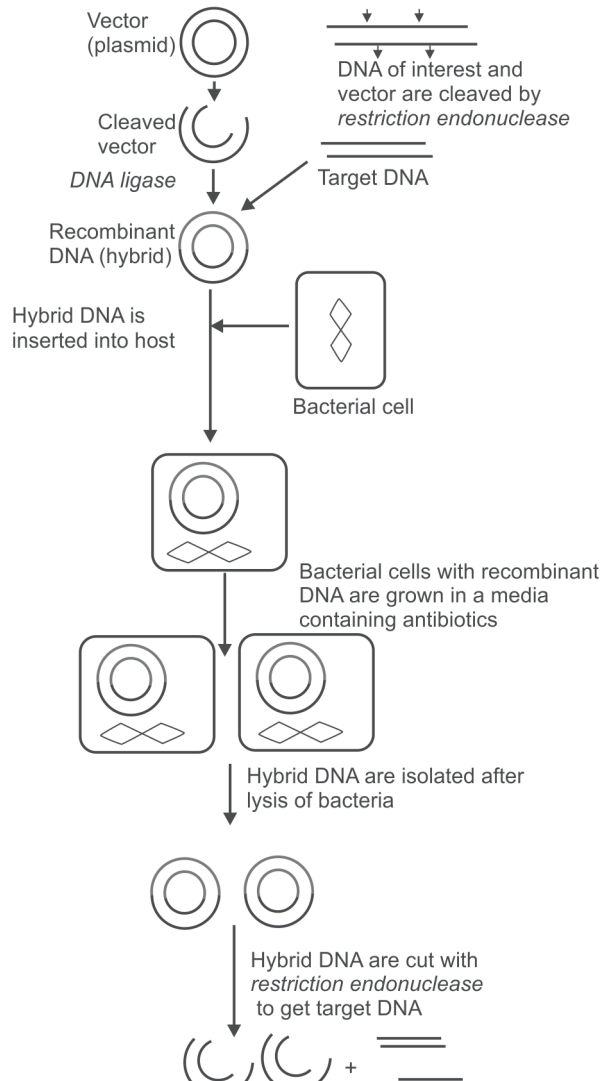


Fig. 16.8: Steps in recombinant DNA technique

Steps in Ex Vivo Gene Therapy (Fig. 16.9)

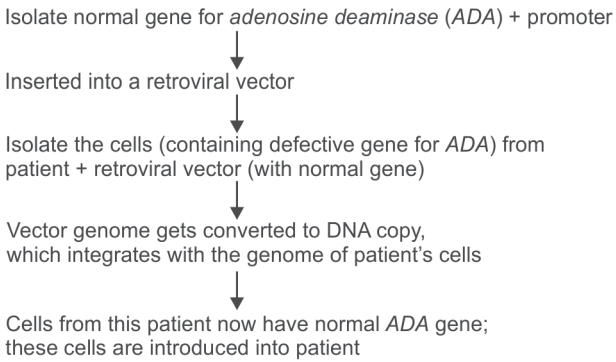


Fig. 16.9: Steps in gene therapy

Diseases treated by gene therapy: Hemophilia A, cystic fibrosis, familial hypercholesterolemia, α-1 antitrypsin deficiency (emphysema).

14. Explain Southern blotting (steps) and its applications.

Definition: Process by which DNA of interest is identified in a cell.

Applications of Southern Blotting [MN: RFLP]

- **R**ape suspect identification (medicolegal, paternity disputes)
- **F**ingerprinting DNA
- **L**earn (identify) heterozygous carrier/defective gene (detect mutations—insertion/deletion of nucleotides, point mutation)
- **P**renatal screening of inherited disorders, sickle cell anemia.

Steps in Southern Blotting (Fig. 16.10)

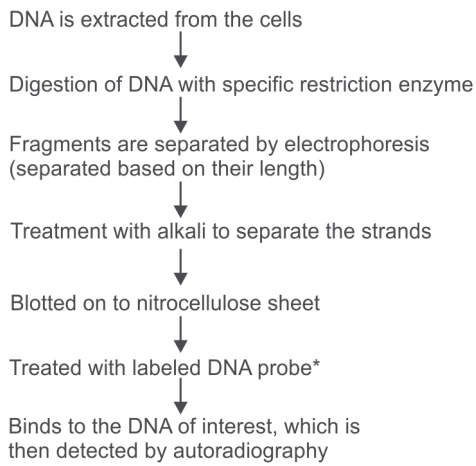


Fig. 16.10: Steps in Southern blotting (*DNA probe: short segment of single-stranded DNA labeled with radioisotope, complementary to target DNA)

15. Explain restriction fragment length polymorphism (RFLP) and its applications.

Definition: Polymorphism is a variation in DNA sequence in a population. Often, they occur in intervening sequences or non-coding segments of DNA. RFLP is an inherited variation in the length of restriction fragments between individuals. RFLP could be due to single base changes or due to the presence of variable numbers of tandem repeats **(VNTR).** Tandem repeats are short DNA sequences repeated several times at various regions in genome. Number of repeat sequence varies from one to another. Accordingly, when sliced with a restriction endonuclease, the fragment length may vary. It is identified by Southern blotting.

Applications of RFLP [MN: RFLP]

- **R**ape suspect identification, medicolegal, paternity disputes
- **F**ingerprinting DNA
- **L**earn (identify) heterozygous carrier/defective gene
- **P**renatal screening of inherited disorders, sickle cell anemia.

Restriction Fragment Length Polymorphism Analysis of Sickle Cell Anemia

Mutation abolishes recognition site of a restriction endonuclease, **MstII.** Normal DNA produces **1.15 kb** fragment, while β^s gene (sickle cell gene) generates **1.35 kb** fragment. This can be detected by prenatal screening using fetal DNA.

16. Explain northern blotting and its applications.

Definition: Technique by which **RNA** of interest is identified. Mixture of mRNAs → electrophoresis → blot on a membrane → add radiolabeled probe → binds to target mRNA → band formed → identified by autoradiography. There is no restriction digestion (as RNA is short as compared to DNA) and no alkali denaturation (as RNA is single stranded).

Applications of Northern Blotting

- Detection/quantification of mRNA in tissues
- Detection of size variation in RNA from different tissues
- To study expression of mRNA in different tissues during development and during various diseases.

17. Explain western blotting and its applications.

Definition: Western blotting is a technique to identify a protein of interest. Proteins are separated by electrophoresis (on polyacrylamide gel) → blotted on to nitrocellulose sheet → labeled antibody added → reacts with antigen → produces a band. This is useful in detection and quantification of minute amounts of protein.

Applications: Confirmatory test for AIDS, hepatitis.

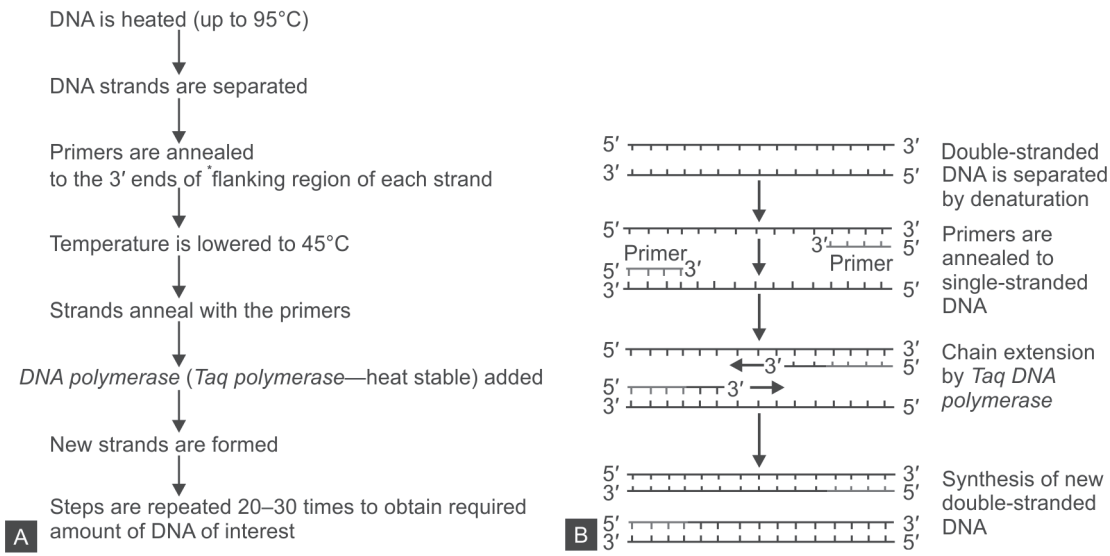
18. Explain enzyme-linked immunosorbent assay (ELISA).

Refer Chapter 19, Radioisotopes.

19. Explain polymerase chain reaction (PCR) and its applications.

Definition: Polymerase chain reaction is a method to amplify a selected sequence of DNA. It can produce millions of copies of a selected DNA sequence in few hours. It has high sensitivity and speed. There is an increase in the number of copies of DNA of interest with each cycle.

Steps in PCR (Figs 16.11A and B)



Figs 16.11A and B: Steps in PCR (*flanking region: nucleotide sequence adjacent to target sequence in the DNA)

Applications [MN: PCR]

- **P**renatal diagnosis of inherited diseases, e.g. cystic fibrosis
- **C**ancer detection—detect mutant gene
- **R**ape cases (forensics), analyze materials from crime scene
- **I**nfectious diseases—detect HIV when small number of cells is infected.

20. Explain hybridoma technology and its applications.

Definition: Hybridoma technology is a method by which monoclonal antibodies are produced from a single clone of plasma cells. Monoclonal antibodies are directed against one epitope of an antigen.

Steps in Hybridoma Technology (Fig. 16.12)

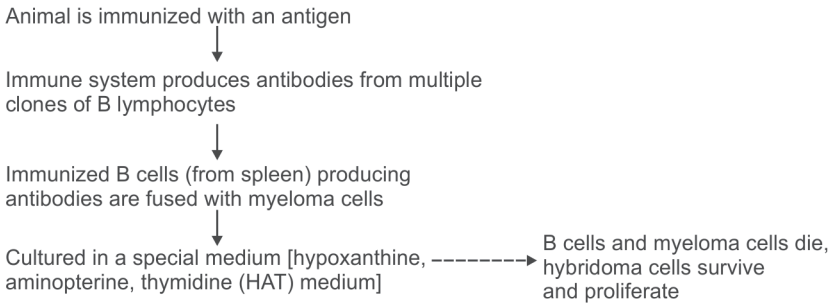


Fig. 16.12: Hybridoma technology

B cells from spleen produce antibody, but are not immortal. Myeloma cells grow permanently, but do not produce antibodies. Fusion of these cells → hybridoma cells → produce antibody and are immortal.

Applications [MN: HAT]

Monoclonal antibodies produced by this technique can be used for:

- **H**ormone detection
- **A**ntigen detection: Protein, infections
- **T**umor marker detection
- Drug delivery for treatment of various diseases.

Key Points

Antisense therapy: Based on the principle that microRNA (short RNA of 21–23 nucleotides) is used for silencing (gene silencing) of undesired mRNA (prevents translation). This therapy has been tried in acute leukemia and human immunodeficiency virus (HIV) infection. A short half-life and lack of specificity of antisense RNA molecules are the limitations.

RNA interference: Small double-stranded RNA, when artificially introduced into a cell, can silence mRNA. The presence of double-stranded RNA in a eukaryotic cell can trigger a process known as RNA interference or RNAi (also known as RNA silencing or RNA inactivation).

Small nucleolar RNA: Involved in formation of ribosomes from precursor 28S, 18S and 5.8S; also involved in telomere synthesis.

X-inactive specific transcript (XIST) RNA: Responsible for inactivation of one of X chromosome in females.

DNA probe: A fragment of DNA labeled with ^{32}P used to identify a target DNA fragment.

Restriction endonucleases: Recognize and cleave specific sequence in a DNA. Used in recombinant DNA technology, e.g. EcoR1.

Endonucleases: Cleave internal phosphodiester bonds.

Exonucleases: Hydrolyze terminal nucleotides.

Vectors: Carriers of DNA fragments to be inserted into the host cell. For example, plasmids, bacteriophages, cosmids, yeast artificial chromosomes.

DNA sequencing: Detection of base sequence in a DNA, which is done by Sanger method.

Molecular chaperones: They are proteins that facilitate the folding of proteins, e.g. heat shock proteins.

Protein targeting: Directing a synthesized protein into its exact location.

Transgenic animal: It is an animal with inserted foreign gene in its genome. This is useful in the production of proteins of interest.

ACQUIRED IMMUNODEFICIENCY SYNDROME

21. Explain acquired immunodeficiency syndrome (AIDS) and the tests available for its diagnosis.

i. Acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus having RNA genome with reverse transcriptase. Its core is surrounded by membrane, which is studded with spike-like assembly of gp41 and gp120 viral proteins, which helps it to infect the host cells. It infects $CD4^+$ T cells, macrophages and dendritic cells.

ii. **Transmission:** Blood transfusion, semen, vaginal secretion or breast milk, contaminated needles, mother to infant during pregnancy, childbirth, etc.

Phases of Infection

i. **Window period:** After the virus infection, initially for 2–12 weeks no antibodies will be detected in the host. This is called 'window period' (seronegative period), but during this phase, virus capsid antigen p24 may be detected in host.

ii. **Intermediate phase (seropositive phase):** Person looks normal, but can transmit the disease to others; antibodies can be detected by ELISA; this period lasts for 5–10 years.

iii. **AIDS:** In this phase, $CD4^+$ T cells count falls ($< 400/mm^3$), which leads to decreased immunity. Patient usually dies within 2 years in this phase without treatment.

Laboratory Tests

- **Enzyme-linked immunosorbent assay (ELISA):** Antibodies to viral gp120 is detected by this method; it is a screening test and may show false negative response
- **Western blot:** Six different viral components are detected with this test and is a confirmative test
- **Polymerase chain reaction (PCR):** This is the most sensitive test and can detect infection in the seronegative phase as it detects the viral genome
- **Treatment:** Anti-HIV drugs
  - Reverse transcriptase inhibitors: Azidothymidine (AZT), delavirdine, nevirapine
  - Inhibitors of protease: Saquinavir, ritonavir
  - Fusion inhibitors: Enfuvirtide.

22. Write briefly about fluorescent in situ hybridization (FISH) technique.

Definition: Fluorescent in situ hybridization (FISH) is a technique that can be used to detect and localize specific DNA sequences on chromosomes (Fig. 16.13).

Requirements

- Fluorescent probes—fragments of DNA/RNA labeled with fluorescent molecule. They bind complementarily to specific sequence of the chromosomes. Probe is constructed by cloning techniques
- Target DNA of interest
- Fluorescence microscope—to detect the site of binding of fluorescent probe.

Applications: [MN: CLAM] for detection of:

i. **C**hromosomal aberrations (translocations).
ii. **L**ocalization of a specific gene on chromosome.
iii. **A**mplification of genes in cancer can be detected.
iv. **m**RNA expression.

Steps of FISH (Fig. 16.13)

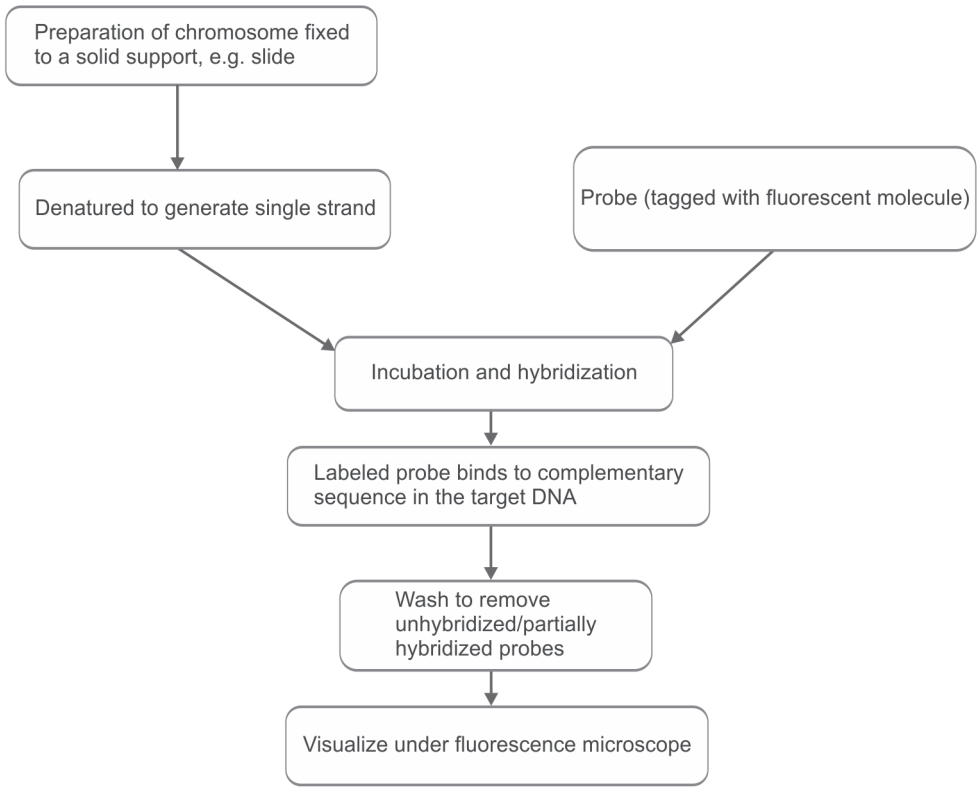


Fig. 16.13: Fluorescent in situ hybridization technique

23. Explain the principle and applications of microarray technique.

A DNA microarray consists of thousands of immobilized single-stranded DNA fragments of known nucleotide sequence organized on a small solid support like slide/membrane. By this technique, thousands of genes can be studied at a given time. Analysis of activity (active/inactive) of genes will help in understanding the normal functions of a cell and how functions are affected following a change in the gene. With the help of microarray technology, different diseases can be studied. An important area for the application of this technique is cancer (Fig. 16.14). Cancers can be classified based on the gene activity in the tumor cells. Treatment can then be developed to target directly a specific type of cancer.

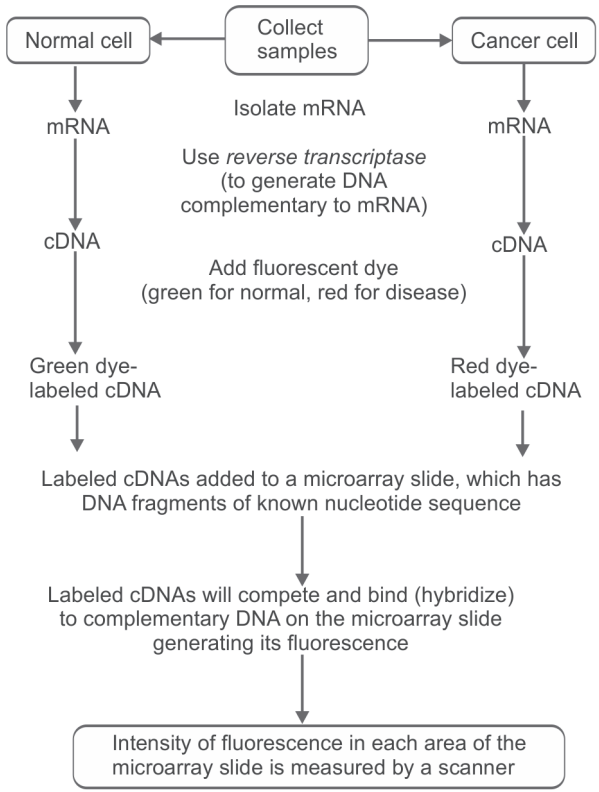


Fig. 16.14: Microarray technique

Highly active gene → produces more mRNAs → more cDNA bind/hybridize to DNA on microarray slide → generate bright fluorescence.

Inactive gene → no mRNAs → no cDNAs → no hybridization on the slide → no fluorescence.

i. Green spot: More mRNA produced from normal cell → the gene is highly expressed in normal cells.
ii. Red spot: More mRNA produced from cancer cell → the gene is highly expressed in cancer cells.
iii. Yellow spot: Both normal and cancer cells produce same amount of mRNA → the particular gene is equally expressed in both types of cells.
iv. Black/dark spot: None of the cells produced the particular mRNA.

Applications

- To analyze changes in gene expression (by collecting mRNA molecules from a particular cell)
- To observe mutations in the gene (genotyping)
- To observe genomic gains and losses
- Gene discovery
- Disease diagnosis: Classify the types of cancer on the basis of the patterns of gene activity in the tumor cells
- Pharmacogenomics: To study the correlations between therapeutic responses and the genetic profiles of the patients
- Toxicogenomics: Check the toxin-induced changes in the cell.

24. Write briefly on human genome project (HGP).

The project of DNA sequencing was initiated in the United States. The International Human Genome Sequencing Consortium and a private enterprise, Celera Genomics undertook the project of sequencing of human genome. It was an international scientific project with a primary goal of determining the sequence of human genome, identifying and mapping approximately 25,000 genes. The sequence of the entire human genome (with the exception of few gaps) was completed in 2003.

Goals were to

i. Identify all the 20,000–25,000 genes in human DNA.
ii. Determine the sequences of chemical base pairs that make up human DNA.
iii. Store this information in databases; develop tools for analysis of data.
iv. Address the ethical, legal and social issues (ELSI) that may arise from the project.

Techniques: Used for sequencing included mapping and shotgun sequencing.

Implications

- Isolation of almost any gene and study of its structure and function is possible
- Genes, which were previously unknown, were detected as a result of HGP
- Improvements in procedures for detecting disease causing genes—development in genetic, immunologic and microbiological diagnostics
- Gene therapy
- Use of genetic information for drug development and treatment (pharmacogenomics)
- Use in forensic medicine, e.g. help to identify crime suspect
- Assessment of damage following exposure to carcinogens, radiation, etc.

17 Acid-Base Balance and Disorders

1. Write briefly on buffer systems in the body. Add a note on Henderson-Hasselbalch equation.

Definition: Buffer is a solution of a mixture of weak acid with its conjugate base, or a weak base with its conjugate acid, which resists change in pH on addition of alkali or acid (Table 17.1).

Table 17.1: Buffer systems

Buffer systems	Base/acid	pKa
Bicarbonate	$\frac{HCO_3^-}{H_2CO_3}$	6.1
Phosphate	$\frac{HPO_4^{2-}}{H_2PO_4^-}$	6.8
Hemoglobin	$\frac{Hb}{HHb}$	
Protein	$\frac{Protein}{H\ protein}$	

pKa: Negative logarithm of dissociation constant K. Buffers are very effective within ±1 pH unit of their pKa.

Functions of buffer: Maintenance of intracellular and extracellular pH.

Henderson-Hasselbalch equation

$$pH = pKa + \log \frac{(Base)}{(Acid)}$$

Where,

- pH: Negative logarithm of hydrogen ion concentration
- pKa: Negative logarithm of dissociation constant.

Significance of Henderson-Hasselbach equation

- To determine acid-base status of an individual
- To measure any one of the parameter in the equation, if others are known
- Preparation of buffers.

2. What is the normal pH of blood? Define and classify acid-base disorders.

Normal pH of arterial blood is 7.35–7.45.

Classification of acid-base disorders

a. *Acidosis:* When arterial pH drops below 7.35.
   - Metabolic acidosis
   - Respiratory acidosis.

b. *Alkalosis:* When arterial blood pH rises above 7.45.
   - Metabolic alkalosis
   - Respiratory alkalosis.

c. *Mixed:* Presents with either acidosis or alkalosis in both systems together, e.g. metabolic acidosis with respiratory acidosis.

3. What is anion gap?

Anion gap: It is a measure of unmeasured anions (e.g. lactic acid, ketone bodies, sulfate, phosphate, etc.) in blood.

Normal range: 12 ± 5 mmol/L.

Formula: $[Na^+ + K^+] - [Cl^- + HCO_3^-]$.

Increased anion gap: It is seen in lactic acidosis, diabetic ketoacidosis.

4. What is metabolic acidosis? Mention some causes for it.

Definition: Metabolic acidosis is a decrease in pH due to a primary decrease in bicarbonate ($HCO_3^- < 22$ mEq/L) concentration in blood.

Serum bicarbonate (normal range): 22–27 mEq/L.

Causes

- Increased acid production—lactic acidosis, ketoacidosis
- Decreased acid excretion—renal failure
- Bicarbonate is used up for buffering of accumulated acids
- Increased bicarbonate loss—diarrhea, gastroenteritis.

Classification

a. *High anion gap metabolic acidosis:* Due to increased production/decreased excretion of unmeasured anions.

Causes [MN: U SLID]: **U**remia, **s**alicylate toxicity, **l**actic acidosis, **i**schemia, **d**iabetes mellitus (uncontrolled).

b. *Normal anion gap metabolic acidosis:* There is metabolic acidosis, but anion gap is normal.

Causes: Diarrhea (severe), renal tubular acidosis, intestinal fistula.

Biochemical findings in metabolic acidosis are given in Table 17.2.

Table 17.2: Biochemical findings in metabolic acidosis (uncompensated)

Parameter	Finding
pH	Decreased
pCO_2	Normal
Bicarbonate	Decreased

5. What is respiratory acidosis? Mention the causes.

Definition: Decrease in pH due to primary increase in pCO_2 (normal range: 35–45 mm Hg). Respiratory acidosis occurs when pCO_2 value is above 45 mm Hg. CO_2 retention results in increased carbonic acid in blood. Biochemical findings in respiratory acidosis is given in Table 17.3.

Table 17.3: Biochemical findings in respiratory acidosis (uncompensated)

Parameter	Finding
pH	Decreased
pCO_2	Increased
Bicarbonate	Normal

Causes [MN: COPD]

- **C**ardiac arrest
- **O**bstructive airway disease
- **P**aralysis of respiratory muscles due to polio or central nervous system (CNS) injury
- **D**rugs: Opiates causing respiratory depression.

6. Define and mention the causes for metabolic and respiratory alkalosis.

The causes and features of alkalosis is mentioned in Table 17.4.

Table 17.4: Causes and features of alkalosis

	Respiratory alkalosis	Metabolic alkalosis
Definition	Increase in pH due to primarily a decrease in CO_2; $\downarrow CO_2 \rightarrow \downarrow$ carbonic acid	Increase in pH due to primarily an $\uparrow$ in HCO_3^-; this is due to $\uparrow$ reabsorption of HCO_3^- or $\uparrow$ loss of H^+
Causes	**H**yperventilation, **a**nemia, **s**alicylate poisoning **[MN: HAS]**	Gastric **A**spiration, vomiting **I**atrogenic—overuse of antacids **D**iuretics **S**teroid excess (Cushing's syndrome) **[MN: AIDS]**
Features		
pH	Increased	Increased
pCO_2	Decreased	Normal
Bicarbonate	Normal	Increased

7. How is pH (hydrogen ion concentration) regulated in our body?

Concentration of hydrogen ion is regulated sequentially by:

- Blood buffer systems: act within seconds
- Respiratory center in the brainstem: acts within 1–3 minutes
- Renal mechanisms: require hours to days.

Buffers in Body Fluids

They can buffer excess acids or bases, but they cannot eliminate them from the body. Three major chemical buffer systems in our body are:

a. **Bicarbonate (HCO_3^-/H_2CO_3) buffer system (base to acid ratio 20:1):** Important extracellular buffer.

b. **Phosphate buffer system ($HPO_4^{2-}/H_2PO_4^-$):** This system is an effective buffer in urine and intracellular fluid. At physiologic pH, its base to acid ratio remains at 4:1.

c. **Protein buffer system (protein/H protein):** Histidine residue in proteins has pKa value of 6.1, which is close to normal pH and hence is most effective in buffering.

Respiratory System

There is a reversible equilibrium between:

- Dissolved carbon dioxide (H_2CO_3) and water
- Carbonic acid, hydrogen and bicarbonate ions.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

Mechanism of Regulation

- Decrease in plasma pH → stimulation of chemoreceptors in respiratory center → deeper and more rapid breathing → expels more carbon dioxide (hyperventilation) → ↓ H_2CO_3 → ↑ pH
- When ↑ plasma pH → slower, shallow breathing → ↑ H_2CO_3 → ↑ H^+ → ↓ pH.

  Where more carbon dioxide (CO_2) is formed, there is an increase in hydrogen ion (H^+) and bicarbonate (HCO_3^-). H^+ is bound by Hb to form HHb. Once this reaches lungs, H^+ attached to Hb is replaced by O_2 forming HbO_2. HCO_3^- reacts with H^+ to form H_2CO_3. H_2CO_3 dissociates to CO_2 and H_2O. CO_2 is expelled from the lungs. This mechanism effectively converts the carbonic acid to CO_2, which can be eliminated from the lungs.

Renal Mechanisms of Acid-base Balance

Acid-base balance occurs by:

a. Reabsorption of filtered bicarbonate.
b. Excretion of hydrogen ions with simultaneous regeneration of bicarbonate (in proximal convoluted tubule).
c. Excretion of titratable acid as sodium dihydrogen phosphate (distal convoluted tubule).
d. Excretion of ammonium ions in distal tubule.
   i. Reabsorption of filtered bicarbonate (Fig. 17.1)

   H^+ generated in tubular cell → lumen → reacts with HCO_3^- → H_2CO_3 → $H_2O + CO_2$. CO_2 diffuses into the tubular cell → reacts with H_2O → H_2CO_3 → $H^+ + HCO_3^-$. Bicarbonate is absorbed into plasma.

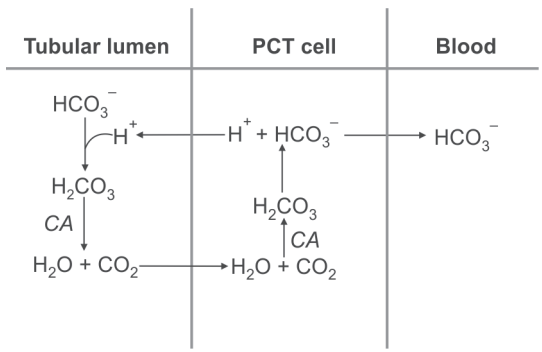


Fig. 17.1: Reabsorption of filtered bicarbonate (CA, carbonic anhydrase)

ii. Excretion of hydrogen ions with simultaneous regeneration of bicarbonate and excretion of titratable acid as sodium dihydrogen phosphate (Fig. 17.2).

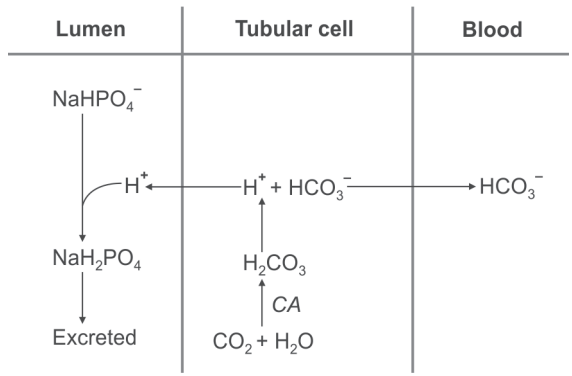


Fig. 17.2: Excretion of H^+ with simultaneous regeneration of bicarbonate and excretion of titratable acid as sodium dihydrogen phosphate (CA, carbonic anhydrase).

iii. Excretion of ammonium ions (Fig. 17.3)

Filtered H^+ combines with NH_3 to form ammonium ion (NH_4) and is eliminated.

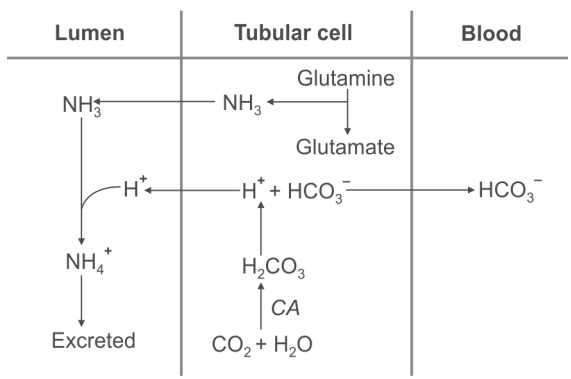


Fig. 17.3: Excretion of ammonium ions (CA, carbonic anhydrase)

8. What is the relation between serum potassium and acid-base disorders?

- **In metabolic acidosis:** As there are more H^+ ions in plasma, body tries to buffer it by exchanging H^+ with intracellular potassium ions leading to hyperkalemia, which manifests as severe bradycardia, cardiac arrhythmias and cardiac arrest
- **In metabolic alkalosis:** Shift of extracellular K^+ into the cells in exchange for H^+, leading to hypokalemia. Also, the kidneys retain H^+ in exchange for potassium, which also leads to hypokalemia. Hypokalemia → tachycardia → cardiac arrhythmias.

Key Points

Compensatory changes

Compensation comes from the complementary system (other system) to aid the inadequacy of one system. For example, the respiratory system will attempt to correct metabolic acid-base imbalances. The kidneys will work to correct acid-base imbalance caused by respiratory disease.

Respiratory compensation

a. ***In metabolic acidosis:*** Increased rate of respiration removes carbon dioxide from the blood; pCO_2 falls bringing back the pH to normal.
b. ***In metabolic alkalosis:*** As a compensatory mechanism, there is slow, shallow breathing, allowing carbon dioxide to accumulate in blood, which increases carbonic acid in blood and brings down the pH to normal level.

Renal compensation

a. ***In respiratory acidosis:*** Kidneys try to retain bicarbonate to offset the acidosis.
b. ***In respiratory alkalosis:*** Kidneys eliminate bicarbonate from the body.

Calcium in acid-base disorders

a. *In acidosis,* because of increased H^+ in serum, plasma proteins try to buffer them, which may displace some protein bound calcium, so plasma calcium increases relatively in acidosis.
b. *In alkalosis,* because of high pH, proteins are negatively charged; they bind to positively charged Ca^{2+}. This may lead to relative hypocalcemia.

General instructions for identifying an acid-base disorder

Look at the pH value in the arterial blood gas (ABG) analysis report.

Is there an acid-base disorder present?

- If pH is less than 7.35, then it is acidosis; if pH is more than 7.45, then alkalosis
- If pH is within normal range then,
  - Either acid-base disorder is not likely to be present (if other parameters of ABG analysis are normal) or
  - It is a fully compensated acid-base disorder (if other parameters of ABG are abnormal).

Look at pCO_2 and HCO_3^- values to identify the primary change.

Initial change is the abnormal value that correlates with the abnormal pH.

- If pH is low (acidosis); primary change is increased pCO_2 or decreased HCO_3^-
  - Low pH + increased pCO_2 = respiratory acidosis
  - Low pH + Low HCO_3^- = metabolic acidosis.
- If pH is increased (alkalosis); primary change is decreased pCO_2 or increased HCO_3^-
  - Increased pH + decreased pCO_2 = respiratory alkalosis
  - Increased pH + increased HCO_3^- = metabolic alkalosis.

Once the initial change (primary event) is identified, then the other abnormal parameter is the compensatory response (Table 17.5); if its direction of change is the same as primary event then it is simple acid-base disorder. Compensatory response indicates that the disorder is compensated.

Table 17.5: Primary and compensatory event

Acid-base disorder	Initial chemical change	Compensatory response
Respiratory acidosis	↑ pCO_2	↑ HCO_3^-
Respiratory alkalosis	↓ pCO_2	↓ HCO_3^-
Metabolic acidosis	↓ HCO_3^-	↓ pCO_2
Metabolic alkalosis	↑ HCO_3^-	↑ pCO_2

Note: Both the primary event and compensatory response occur in the same direction, i.e. either both increase or both decrease.

Problem Solving Exercises

1. **A 35-year-old man was admitted to hospital with an asthmatic attack. His ABG report showed: pO_2 70 mm Hg, pCO_2 30 mm Hg, pH 7.50, HCO_3^-: 23 mEq/L. Comment on this.**
   a. **Look at pH:** It is increased—alkalosis.
   b. **Look at pCO_2 and HCO_3^-:** Low pCO_2 is the primary change, hence it is respiratory alkalosis. As HCO_3^- is within normal range, it is uncompensated respiratory alkalosis.
2. **A 65-year-old chronic smoker was admitted with increasing breathlessness. History revealed he is a patient of COPD. His ABG showed following: pO_2 45 mm Hg, pCO_2 53 mm Hg, pH 7.3, HCO_3^-: 35 mmol/L.**
   a. **Look at pH:** Slightly decreased.
   b. **Look at pCO_2 and HCO_3^-:** pCO_2 is high and HCO_3^- is also high. The primary response is increase in pCO_2, so it is respiratory acidosis.
   c. HCO_3^- is increased as compensatory mechanism to bring back the pH to normal (direction of change is same).
   d. **Impression:** Respiratory acidosis (partially compensated as pH is not normal).

3. **A 50-year-old man came to the outpatient clinic with a history of intermittent profuse vomiting and loss of weight. He had tachycardia and hypotension. History revealed he is a case of pyloric stenosis. ABG report showed: blood pH 7.55, pCO_2 48 mm Hg, HCO_3^- 35 mmol/L, hyponatremia, hypokalemia. His urine pH was 3.5.**
   a. **Look at pH:** Increased, so alkalosis.
   b. **Look at pCO_2 and HCO_3^-:** HCO_3^- is high and pCO_2 is slightly increased. The primary response is increase in HCO_3^- (loss of hydrogen ions due to vomiting) and increase in pCO_2 is compensatory mechanism (direction of change is same as primary event).
   c. **Impression:** Metabolic alkalosis (partially compensated).
   d. **Comment:** This patient had depletion of chloride due to vomiting, which limited reabsorption of sodium in the thick ascending limb of the loop of Henle. So, sodium is exchanged for hydrogen and potassium ions in the distal nephron leading to hypokalemia and acidic urine inspite of alkalosis. This is called paradoxical acid urine.
4. **During resuscitation of a 60-year-old man from a cardiorespiratory arrest, the blood gas analysis revealed: pH 7.00 (7.37–7.44), pCO_2 52 (35–45 mm Hg), bicarbonate 11 (23–30 mEq/L), pO_2 91 mm Hg (during treatment with 48% oxygen), lactic acid 7 mmol/L (0.7–1.8**).
   a. **Look at pH:** Decreased, so acidosis.
   b. **Look at pCO_2 and HCO_3^-:** Bicarbonate is low and pCO_2 slightly increased. Both indicate primary event.
   c. pCO_2 is increased and bicarbonate is decreased (opposite direction)—it indicates mixed acid-base disorder.
   d. **Impression:** Metabolic acidosis with respiratory acidosis (lack of ventilation).

18 Organ Function Tests

LIVER FUNCTION TESTS

1. Write a short note on liver function tests.

Liver function tests: They are tests done to assess the functional capacity of liver (Tables 18.1 to 18.3).

Functions of liver

- *Metabolism:* Carbohydrates, lipids and proteins
- *Excretion:* Bilirubin, bile acids and bile salts
- *Synthesis:* Albumin, α- and β-globulins, clotting factors, cholesterol, lipoprotein
- *Storage:* Glycogen, vitamins (A, D, B_{12}), etc.
- *Detoxification and drug metabolism.*

Liver function tests are used to

- Detect and diagnose liver disease
- Evaluate the severity of liver disease
- Monitor response to therapy
- Assess prognosis of liver disease.

Table 18.1: Liver function tests

Class	Tests
Tests based on **excretory** function	Estimation of serum/urine bilirubin, bromsulfthalein
Tests based on **serum enzymes (indicator of liver damage/cholestasis)**	Estimation of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT)
Tests based on **synthetic** functions	Total proteins, serum albumin, globulin, albumin globulin ratio, prothrombin time
Tests based on **detoxification**	Hippuric acid test, blood ammonia

Table 18.2: Important liver function tests

Tests	Normal range	Methods	Clinical utility
Total bilirubin	0.2–0.8 mg/dL	van den Bergh reaction	Helps in diagnosis of jaundice
Direct bilirubin	0.1–0.2 mg/dL	van den Bergh reaction	↑ in hepatic and obstructive jaundice
Indirect bilirubin	0.2–0.6 mg/dL	Total bilirubin – direct bilirubin	↑ in hemolytic jaundice
ALT	5–40 U/L	Enzymatic method	↑ in liver damage (e.g. hepatitis)
AST	5–40 U/L	Enzymatic method	↑ in liver damage (e.g. hepatitis)
ALP	40–140 U/L	Enzymatic method	↑ in obstructive jaundice
Total protein	6–8 g/dL	Biuret	↓ in cirrhosis of liver
Albumin	3.5–5 g/dL	Biuret	↓ in cirrhosis of liver
Globulin	2–3.5 mg/dL	Total protein – albumin	↑ in multiple myeloma, ↓ in HIV infection

HIV, human immunodeficiency virus; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; ↑ = increased; ↓ = decreased.

Table 18.3: Other tests with uses

Tests	Normal range	Clinical utility
γ-glutamyl transferase (GGT)	10–50 U/L	↑ in alcoholic hepatitis and obstructive jaundice
Prothrombin time	< 14 second	↑ in hepatocellular disease
Plasma ammonia	25–94 μg/dL	↑ in severe hepatocellular disease
Alfa-fetoprotein (AFP)	< 15 ng/mL	↑ in germ cell tumor, ↑ in maternal serum in neural tube defect in fetus

↑ = increased; ↓ = decreased

2. Explain the principle and applications of van den Bergh reaction.

van den Bergh reaction: It is a test done to detect the presence of bilirubin in blood and the type of jaundice (Table 18.4).

Principle: It is based on the formation of a purple-colored azobilirubin, when serum containing bilirubin is allowed to react with freshly prepared diazo reagent.

Ehrlich's Diazo reagent: It is prepared by mixing 10 mL of sulfanilic acid in HCl + 0.8 mL of sodium nitrite in water.

Response obtained: Three types.

Direct positive: Conjugated bilirubin, if present in serum, reacts directly with diazo reagent to give a purple color immediately.

Indirect positive: Unconjugated bilirubin in serum reacts with diazo reagent only on addition of methanol to give a purple color.

Biphasic reaction: It is observed when both conjugated and unconjugated bilirubin is increased in serum. Initially, a purple color is formed immediately (direct positive) on addition of reagent. On addition of methanol, the color intensifies (indirect positive).

Normal levels in blood

- Total bilirubin: 0.2–0.8 mg/dL
- Unconjugated bilirubin: 0.2–0.6 mg/dL
- Conjugated bilirubin: 0.1–0.2 mg/dL.

Table 18.4: Pattern of van den Bergh reaction in different types of jaundice

Types of jaundice	Causes	Types of bilirubin in blood
Prehepatic	Rh incompatibility Sickle cell anemia	Unconjugated ↑ (indirect positive)
Hepatic	Viral hepatitis—A, B, C Toxic hepatitis—alcohol	Conjugated ↑ Unconjugated ↑ (biphasic reaction)
Posthepatic	Gallstones Pancreatic tumor	Conjugated ↑ (direct positive)

↑ = increased

3. Explain the principle and applications of Ehrlich's test.

Principle: Urobilinogen in urine reacts with Ehrlich's reagent to form a red color, which intensifies on addition of sodium acetate (Table 18.5).

Table 18.5: Ehrlich's test in different types of jaundice

Conditions	Urine urobilinogen
Normal range	0–4 mg/24 hour
Prehepatic	↑
Hepatic	↓ if there is intrahepatic obstruction
Obstructive jaundice	Absent

↑ = increased; ↓ = decreased

4. Write a note on hippuric acid test.

Indication: To assess the detoxifying capacity of liver.

Principle: Benzoic acid (sodium benzoate), administered orally or intravenously, combines with glycine to form hippuric acid. Amount of hippuric acid excreted in urine in a fixed time is determined. Thus, hippuric acid determines the conjugating capacity of the liver.

Types

- *Oral hippuric acid test:* Normally, at least 3 g of hippuric acid should be excreted at the end of 4 hours after ingestion of 6 g of sodium benzoate
- *Intravenous hippuric acid test:* It is done only if absorption is impaired or patient has nausea and vomiting.

  Interpretation: Decreased excretion means decreased conjugating capacity of the liver (due to damage to hepatocytes).

5. Explain the biochemical findings in blood, urine and feces in different types of jaundice.

Refer Chapter 22, question number 3.

Key Points

van den Bergh test: This is done to detect serum bilirubin. Direct reaction detects the conjugated bilirubin and indirect reaction detects unconjugated bilirubin.

Hemolytic jaundice (hemolysis): Elevated unconjugated bilirubin + absent urinary bilirubin + increased urobilinogen in urine.

Hepatic jaundice (viral hepatitis): Elevated conjugated and unconjugated bilirubin + elevated aspartate transaminase (AST) and alanine transaminase (ALT).

Obstructive jaundice (gallstones): Elevated conjugated bilirubin + increased urinary bilirubin + absent urine urobilinogen.

γ-glutamyl transferase: It is used to detect alcoholic liver disease and obstructive jaundice.

Alkaline phosphatase: This is a canalicular enzyme; elevated in obstructive jaundice.

Hay's sulfur test: For detection of bile salts in the urine.

Fouchet's test: For detection of bile pigments in urine.

Ehrlich's aldehyde test: For detection of urobilinogen in urine.

RENAL FUNCTION TESTS

6. Describe the renal function tests.

Renal function tests: Group of tests done to assess the functional capacity of kidneys.

Functions of kidney

- Elimination of metabolic end products and foreign compounds (drugs)
- Maintenance of water and electrolyte balance
- Maintenance of acid-base balance
- Endocrine functions—production of calcitriol, renin and erythropoietin.

Renal function tests are used to (Tables 18.6 and 18.7)

- Identify renal dysfunction
- Diagnose renal disease
- Monitor progression of renal disease
- Monitor response to treatment.

Table 18.6: Renal function tests

Serum urea	Creatinine clearance (glomerular function)
Serum creatinine	Concentration/dilution test; acidification test (tubular function)
Serum uric acid	Serum electrolytes—Na^+, K^+, Cl^- HCO_3^-
Urine examination—physical, chemical characteristics; microscopy	Serum calcium and phosphorus

Table 18.7: Renal function tests with clinical utility

Tests	Normal value	Clinical utility
Blood urea	10–40 mg/dL	↑ in acute and chronic renal failure, obstruction in the lower urinary tract
Serum creatinine	0.6–1.4 mg/dL	↑ in acute and chronic renal failure
Serum uric acid	3–7 mg/dL	↑ in acute and chronic renal failure
Serum electrolytes Na^+ K^+ Cl^- HCO_3^-	 133–146 mEq/L 3.5–5 mEq/L 96–106 mEq/L 22–26 mEq/L	To assess the acid-base status and electrolyte balance in renal disease and to monitor treatment
Serum calcium	9–11 mg/dL	↓ in chronic renal failure
Serum phosphorus	2–4.5 mg/dL	↑ in chronic renal failure
Concentration and dilution tests		To diagnose tubular damage: abnormal response seen in chronic renal failure
Routine urine examination		
Physical characteristics Volume Specific gravity	 1.5 L 1.015–1.025	 Altered in renal failure Altered in renal failure
Microscopy	Absent	Bacterial infections—pus cells present Hematuria—RBC present
Protein	Absent	Present in nephrotic syndrome, acute glomerulonephritis
Sugar	Absent	Present in diabetes mellitus

↑ = increased; ↓ = decreased

7. Define renal clearance. Write the formula for renal clearance. Name renal clearance tests with normal values.

Renal clearance: It is defined as the volume of plasma from which a measured amount of substance can be completely eliminated into urine per unit time. Renal clearance depends on renal blood flow and glomerular filtration rate (Table 18.8).

$$\text{Clearance (mL/min)} = \frac{UV}{P}$$

Where, U = concentration of cleared substance in urine (mg/dL).
V = volume of urine (mL/min).
P = plasma concentration of the substance (mg/dL).

Use: To measure the glomerular filtration rate (GFR). Normal GFR is 125 mL/min.

Table 18.8: Different renal clearance tests with normal values

Tests	Normal value	Significance
Urea clearance	75 mL/min	Not suitable since urea is secreted as well as reabsorbed. Value obtained is lower than glomerular filtration rate (GFR).
Creatinine clearance	90–120 mL/min	Advantage: Creatinine is an endogenous substance, it is not much affected by non-renal factors. Hence, it is a better indicator of renal function than urea.
Inulin clearance	125 mL/min	Advantage: Gold standard. It is neither reabsorbed nor secreted by renal tubules. Measures exact GFR. Disadvantage: Exogenous, can cause allergy in some people.

8. Write short notes on creatinine clearance.

Creatinine clearance: It is defined as volume of plasma from which a measured amount of creatinine could be completely eliminated into urine per unit time.

Advantages of creatinine clearance over other clearance tests

- Creatinine is an endogenous substance and easy to measure
- Creatinine excretion is constant
- Serum creatinine levels are not much affected by non-renal factors.

$$\text{Creatinine clearance (mL/min)} = \frac{UV}{P}$$

Where, U = concentration of creatinine in urine (mg/dL).

V = volume of urine (mL/min).

P = plasma concentration of creatinine (mg/dL).

Use: To measure the glomerular filtration rate. Creatinine clearance is decreased in renal failure.

Key Points

Normal specific gravity of urine (1.012–1.024): Specific gravity is increased in diabetes mellitus, acute glomerulonephritis and decreased in diabetes insipidus.

Creatinine coefficient: It is defined as mg of creatinine excreted/kg/day. Normal range is 15–25.

Microalbuminuria: Excretion of albumin in urine in the range of 30–300 mg/24 hours. It is an early indicator of diabetic nephropathy.

Proteinuria: Causes are nephrotic syndrome, acute glomerulonephritis and multiple myeloma.

Normal serum creatinine: 0.6–1.4 mg/dL.

Normal blood urea: 10–40 mg/dL.

Normal glomerular filtration rate (GFR): 125 mL/min.

Creatinine clearance (120 mL/min): It is defined as volume of plasma from which a measured amount of creatinine could be completely eliminated into urine per unit time. Formula for creatinine clearance (mL/min) = UV/P.

Cockcroft and Gault formula: Used to measure creatinine clearance.

$$\text{Creatinine clearance} = \frac{(140 - \text{Age in year}) \times \text{weight (Kg)}}{\text{Creatinine (mg/dL)} \times 72}$$

NPN: Non-protein nitrogen (NPN) includes urea, creatinine and uric acid. They are increased in renal dysfunction.

BUN: Blood urea nitrogen (BUN). It is a measure of the amount of urea nitrogen in blood. Normal value is 7–21 mg/dL. Urea nitrogen concentration can be converted to serum urea concentration (mg/dL) by multiplying by 2.14.

Abnormal constituents of urine: Blood (acute glomerulonephritis), glucose (diabetes mellitus), ketone bodies (diabetic ketoacidosis), bile salts and pigments (obstructive jaundice); some of the abnormal constituents can be detected by dipstick strips.

THYROID FUNCTION TESTS

9. How do you biochemically investigate the thyroid function?

Thyroid function tests: Group of tests done to evaluate the functional capacity of thyroid gland (Tables 18.9 and 18.10).

Thyroid function tests are used to

- Detect and diagnose thyroid disease
- Monitor response to treatment of thyroid disorders
- Evaluate the prognosis of thyroid cancer.

Table 18.9: Thyroid function tests

Routinely done tests	Special tests
Triiodothyronine (T_3)	Free T_3, free T_4
Thyroxine (T_4)	Anti-thyroperoxidase antibodies
Thyroid-stimulating hormone (TSH)	Anti-thyroglobulin antibodies Iodine uptake studies Thyroid scan Stimulation and suppression tests

Table 18.10: Blood picture in thyroid function tests

Condition	Clinical features	T_3	T_4	TSH
Normal (euthyroid)	Normal	1.8–3 nmol/L	65–150 nmol/L	0.5–5 μIU/mL
Hyperthyroidism	Heat intolerance, increased pulse rate, diarrhea, tremors, etc.	High	High	Low
Hypothyroidism	**Cretinism (children)** Growth and mental retardation	Low	Low	High
	Myxedema (adults) Cold intolerance, hoarseness of voice, hypercholesterolemia, etc.	Low	Low	High

Key Points

T_3, T_4, TSH: Routinely done thyroid function tests.
Deficiency of thyroxine causes myxedema and cretinism.
Excess of thyroxine: Seen in Graves' disease.
Goiter: Characterized by swelling of thyroid gland in response to increased stimulation by TSH, whose secretion is increased due to reduced synthesis of thyroid hormones caused by deficiency of iodine.

GASTRIC FUNCTION TESTS

10. Write a note on functions of gastric juice.

Functions: The functions of gastric juice are given in Table 18.11.

Table 18.11: Constituents and functions of gastric juice

Components	Functions
Hydrochloric acid (HCl)	• Maintains acidic pH for activation of pepsinogen; germicidal • Denatures proteins, thus helps in protein digestion
Pepsin	• Digestion of protein into smaller components • Activation of pepsinogen
Rennin (in infants)	• Digestion of milk proteins in infants
Mucin	• Protects gastric mucosa against corrosion by acids
Intrinsic factor	• Absorption of vitamin B_{12}

11. Write briefly about gastric function tests.

Gastric function tests: These are the tests done to assess gastric function by gastric juice analysis (Table 18.12).

Indications

- Diagnosis of achlorhydria, peptic ulcer
- Diagnosis of Zollinger-Ellison syndrome
- To determine completeness of surgical vagotomy
- Diagnosis of pernicious anemia.

Table 18.12: Gastric function tests with their uses

Tests	Significance
Free acidity	Absent in gastric carcinoma ↑ in gastrinoma
Total acidity	↑ in pyloric obstruction
Starch	Present: Due to delayed gastric emptying resulting from pyloric obstruction in gastric carcinoma
Lactic acid	Present: Due to delayed gastric emptying resulting from pyloric obstruction in gastric carcinoma
Blood	Present: Gastric ulcer, carcinoma
Bile salts and bile pigments	Present: Regurgitation due to duodenal obstruction

Contd...

Contd...

Tests	Significance
Pentagastrin test	Zollinger-Ellison syndrome: Increased basal secretion Pernicious anemia: No acid secretion
Augmented histamine test	Pernicious anemia: No increase in free acidity
Hollander test (insulin-induced secretion of gastric acid)	To check completeness of vagotomy

12. Write a note on free and total acidity of gastric juice.

- Normal volume of fasting gastric juice is about 20–50 mL
- Increase in volume is seen in
  - Hypersecretion—duodenal ulcer
  - Delayed emptying—pyloric stenosis or regurgitation.

Free acidity: It is due to HCl in gastric juice.

- Achlorhydria: Free acidity is low
- Peptic ulcer: Free acidity is high.

Total acidity: About 10–40 mEq/L; it is due to free HCl + lactic acid + other organic acids.

Combined acidity: Total acidity–free acidity. This is due to presence of acids other than HCl. Combined acidity is increased in pyloric stenosis due to acids like lactic acid and butyric acids generated from bacterial fermentation of retained food products.

13. Explain Schilling test and its applications.

Schilling test: It is done to check the absorption of vitamin B_{12}. Radiolabeled vitamin B_{12} is ingested orally with and without intrinsic factor.

Pernicious anemia: Radiolabeled vitamin B_{12} starts appearing in urine only when given along with intrinsic factor.

Ileal (malabsorption) disease: Radiolabeled B_{12} will not appear in urine even after giving intrinsic factor.

14. Write a short note on fractional test meal.

Steps in fractional test meal

- Collection of gastric contents using Ryle's tube after overnight fasting, volume is noted
- Ingestion of test meal and time is noted
- Removal of 5–6 mL of gastric contents at every 15 min interval for 2–3 hours; basal, total and free acidity is measured.

Interpretation

Normal response: Free acidity levels are low initially, then steadily increase reaching a maximum at 60–90 min, thereafter the values return to basal levels in about 120–150 min. Total acidity follows similar pattern, but the values are 8–12 units higher than free acidity.

Abnormal response

- ***Hyperchlorhydria:*** High free acidity values at fasting, reaches peak level and values remain high throughout the test, e.g. duodenal ulcer, gastric ulcer
- ***Achlorhydria:*** Free acidity levels remain low or absent throughout the test, e.g. carcinoma stomach.

PANCREATIC FUNCTION TESTS

15. Write briefly how to biochemically assess pancreatic functions.

Pancreatic function tests: These are done to assess the functional capacity of pancreas (Table 18.13).

Indications

- To diagnose acute and chronic pancreatitis
- To diagnose cystic fibrosis
- Differential diagnosis of malabsorption syndromes.

Tests

- *Serum amylase:* Normal: 80–240 U/L
- *Serum lipase:* Normal: 5–80 U/L.

Table 18.13: Pancreatic function tests

Condition	Causes	Serum amylase	Lipase
Acute pancreatitis	Gallstone, alcohol consumption	Elevated	Elevated
Chronic pancreatitis	Gallstone, alcohol consumption	N/slight ↑	N/slight ↑
Cystic fibrosis	Mutation in *CFTR* gene	N/slight ↑	N/slight ↑

CFTR, cystic fibrosis transmembrane conductance regulator; N, normal.

Key Points

Serum amylase and lipase: Levels are elevated in acute pancreatitis.
Serum lipase: It is more specific for acute pancreatitis than amylase (amylase is also secreted from salivary glands).
Serum amylase levels: These are also elevated in mumps.

BIOCHEMICAL TESTS FOR CARDIAC DISEASES

16. Briefly discuss the biochemical tests for cardiac diseases.

Indications (Table 18.14)

- To diagnose acute myocardial infarction (AMI)
- To differentiate myocardial infarction from non-cardiac chest pain
- To monitor therapy
- To assess the severity of myocardial injury
- To diagnose cardiac failure—brain natriuretic peptide (BNP).

Non-enzyme markers—troponin T and troponin I, BNP, lipid profile.

Enzyme markers—CK, CK-MB isoform, AST, LDH, LDH_1/LDH_2 ratio.

Table 18.14: Cardiac function tests

Tests	Normal levels	Time of increase after MI	Significance
Total CK	15–100 IU/L	6–8 h	Acute MI—non-specific
CK-MB isoform	< 5% total CK	2–4 h	Acute MI—specific
Troponin T	< 0.1 mg/L	3–4 h	Acute MI—specific
Troponin I	< 1.5 mg/L	4–6 h	Acute MI—specific
Total LDH	100–200 IU/L	12–24 h	Acute MI—non-specific
LDH_1/LDH_2 ratio	< 1	12–24 h	Flipped ratio—acute MI
AST	4–40 IU/L	8 h	Acute MI
Brain natriuretic peptide (BNP)	40–125 pg/mL		Congestive cardiac failure

CK, creatinine kinase; LDH, lactate dehydrogenase; MI, myocardial infarction.

Key Points

Routinely done tests (cardiac specific markers) for diagnosis of acute myocardial infarction: CK-MB, troponin T and troponin I.

LDH flip: Normally LDH_1/LDH_2 ratio is less than 1. In acute myocardial infarction, LDH_1 activity increases due to myocardial injury and ratio is reversed.

Brain natriuretic peptide: It is a useful marker in diagnosis of cardiac failure.

Lipid profile (total cholesterol, triglycerides, LDL and HDL cholesterol): Levels are measured for early detection of risk in development of atherosclerosis and coronary artery diseases.

AST, LDH and CK: These are non-specific for detection of myocardial injury since their levels are also elevated in liver injury, hemolytic anemia and muscle dystrophies, respectively.

Total LDH or LDH_1: Useful for late detection (48 hour after chest pain) of myocardial infarction.

Myoglobin: It can also be used for diagnosis of AMI immediately (elevated early) after myocardial infarction. Elevated myoglobin may be non-specific, since it is present in skeletal muscle.

19 Radioisotopes

1. Define isotopes with examples.

Definition: Elements with the same atomic number (left subscript to the chemical symbol), but different mass number (left superscript to the chemical symbol). Behave similarly in chemical reactions (Table 19.1).

May be stable (nuclear composition does not change with time) or unstable (undergoes spontaneous decay).

Table 19.1: Some examples for isotopes

Stable	Unstable
${}^{2}_{1}H$ (deuterium), ${}^{1}_{1}H$	${}^{3}_{1}H$ (tritium)
${}^{12}_{6}C$ ${}^{13}_{6}C$	${}^{14}_{6}C$
${}^{127}_{53}I$	${}^{125}_{53}I$ ${}^{131}_{53}I$

2. Define radioactivity. What are the units of measurement of radioactivity?

Definition: It is a property of unstable isotopes to undergo spontaneous degradation of nucleus with emission of particles or rays to attain a stable conformation.

Units of radioactivity

a. The international system (SI) unit of radioactivity: Becqueral (Bq) = decay/second (dps).
b. Older unit of radioactivity: Curie (Ci) = 3.7×10^{10} dps.

3. Define radioactive decay and half-life.

Radioactive decay: It is a process by which an unstable (radioactive) nucleus can become stable by emitting particles and energy. The radiation emitted can either be 'α' particles, 'β' particles or γ-rays. Radionuclides are isotopes that can undergo radioactive decay.

Half-life of radioactive compound: Half-life is the time taken for a radioisotope to reach half of its original radioactivity (Table 19.2).

Table 19.2: Examples for half-life of radioactive elements

Radionuclide	Half-life
Iodine-131	8 days
Cobalt-60	5.26 years
Cesium-137	30 years
Carbon-14	5,730 years
Uranium-235	703,800,000 years

4. What are the forms of radiation? Give examples.

Non-ionizing radiation: It contains low energy electromagnetic waves. For example, ultraviolet, visible light, infrared, microwave and radio wave.

Ionizing radiation: This includes high speed particles and high energy electromagnetic waves. Their energy is high enough to remove orbital electrons from atoms. They can cause DNA damage, mutation and cancer. Their ability to destroy deoxyribonucleic acid (DNA) has been exploited to kill cancer cells.

Forms of Radiation

a. **Alpha (α) decay (2 protons + 2 neutrons):** After emission of 'α' particles, there is a decrease in atomic number by two and mass number by four (equivalent to helium nucleus). They have maximum ionizing and minimum penetration power (stopped by a sheet of paper). For example,

$$^{226}_{88}\text{Radium} \longrightarrow {}^{222}_{86}\text{Radon} \longrightarrow {}^{218}_{84}\text{Polonium} \longrightarrow {}^{214}_{82}\text{Lead}$$

b. **Beta (β) radiation:** When neutron splits, it forms proton (+), β (–) particle (electron) and neutrino. There is an increase in atomic number by one, but no change in mass number. 'β' radiation is negatively charged with negligible mass and has more penetration. It is used widely in clinical medicine for diagnosis and treatment.

For example,

$$^{14}_{6}\text{C} \longrightarrow {}^{14}_{7}\text{N} + \text{Neutrino} + \beta^{(-)}$$

c. **Gamma (γ) radiation and X-rays:** γ-rays are emitted, after emission of 'β' particle, when neutron is converted to proton or during electron capture by proton to form neutron. γ radiation are electromagnetic waves, have no charge or mass. It has maximum penetrating power. For example,

$$^{131}_{53}\text{I} \longrightarrow {}^{131}_{54}\text{Xenon} \longrightarrow {}^{131}_{54}\text{Xenon + Gamma}$$

5. What are the applications of radioisotopes?

Research

- Tracer technique: ^{14}C can be used to study metabolic pathways
- ^{131}I can be used to study half-life of immunoglobulins
- ^{131}I-labeled albumin can be used to study blood volume.

Diagnosis

- ^{131}I for thyroid scan: 'Cold nodule' (less/no uptake of radioactive isotope)
- ^{51}Cr to study red blood cell (RBC) life
- ^{90}Sr (strontium): Scan to detect osteoblastoma
- ^{125}I-labeled antigen is used in radioimmunoassay (RIA).

Treatment

Radiotherapy

a. Liquid form of radioactive isotope is administered ('β' particles are emitted). For example, ^{131}I is administered orally in thyroid carcinoma.

b. 'γ' radiation can be used to treat cancer. It can be directly applied on the tissue (brachytherapy) or as needle. For example, cesium (^{137}Cs) is useful for cervical carcinoma.

c. Teletherapy: Source kept at a distance in a thick-walled metal container with small aperture and radiation is focused on cancer tissue. γ-rays are used for this. For example, $^{60}Co/^{137}Cs$ for deep-seated cancers.

6. Write short notes on radioimmunoassay (RIA).

Principle

There is a competition between isotope-labeled antigen (fixed amount) and unlabeled antigen (in patient's sample) for binding to a constant number of antibodies or binding sites. As the amount of unlabeled antigen increases, more of it binds to the antibody by displacing the labeled antigen (Fig. 19.1).

Procedure

Radioactive isotope ^{125}I is often used.

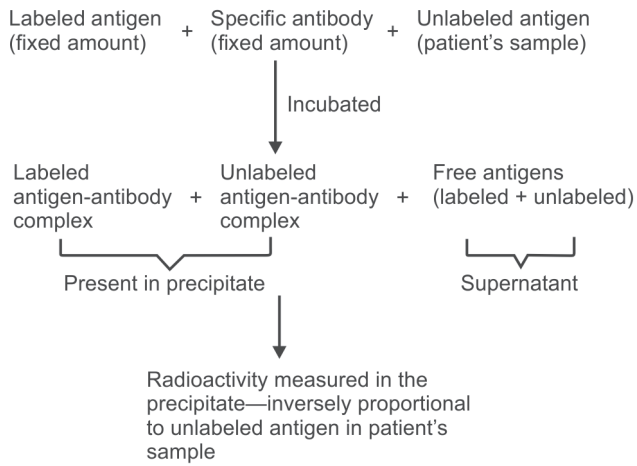


Fig. 19.1: Radioimmunoassay

Interpretation

If patient's serum has no antigen or very low antigen concentration, then more labeled antigens are bound to antibodies. At increasing concentrations of unlabeled antigens in patient's serum, more amount of radioactive (labeled) antigen is displaced from the antibody molecules. So, the precipitate will have relatively less radioactivity as concentration of labeled antigen-antibody complex in it decreases. Hence, the radioactivity of the precipitate is inversely proportional to the unlabeled antigen concentration in serum (Fig. 19.2).

Advantages

Very small quantities (microgram or picogram) of substances can be analyzed, only microcuries of radioactivity are used and radiation hazard is minimal.

Disadvantages

Half-life of ^{125}I is only 60 days; it can be carried out only in laboratories approved by authorities. Also, radioactive materials are expensive.

Uses: To detect and quantify antigens, hormones, tumor markers, etc.

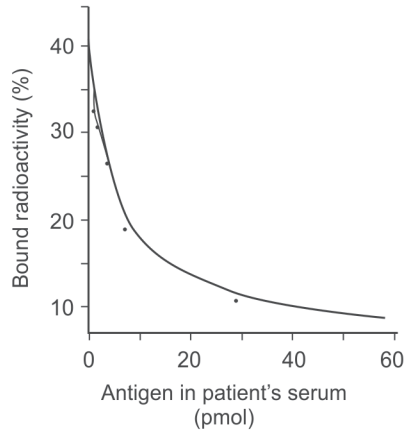


Fig. 19.2: Bound radioactivity decreases as unlabeled antigen concentration in patient's serum increases

7. Write short notes on enzyme-linked immunosorbent assay (ELISA).

ELISA is a widely used method for measuring the concentration of a particular molecule (e.g. hormone, drug, antigen, etc.) in biological fluids like serum, urine, etc. (Fig. 19.3).

Procedure of ELISA

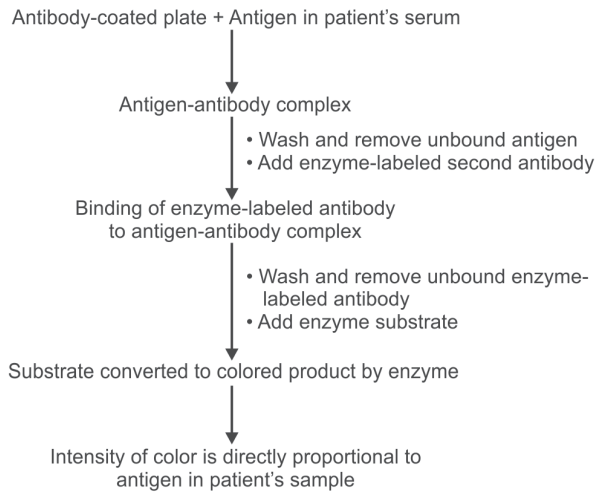


Fig. 19.3: Enzyme-linked immunosorbent assay

Applications

- To detect antigens, e.g. hepatitis B antigen
- To detect antibodies, e.g. HIV antibody
- To detect hormones, e.g. T_4, TSH, HCG, etc.

Advantages

No radiation hazard as no radiolabeled material is used. Simple, less time consuming and less expensive than RIA. Small quantities of hormones/antigen/antibodies can be detected.

8. Write briefly on effects of radiation on our body (radiation hazard).

Radiotherapy can cause some unwanted effects on the body. This damage depends on the system or organ exposed and dose of radiation.

a. **Skin:** Epilation (hair loss), dermatitis, hypopigmentation, loss of elasticity.
b. **Mucous membrane:** Nausea, vomiting, ulceration, bleeding, adhesion, fibrosis.
c. **Blood cells:** Thrombocytopenia, leukopenia, anemia.
d. **Reproductive organs:** Sterility, genetic alterations in offspring.
e. **Can produce cancer.**

Key Points

Radiosensitivity: Success of treatment depends upon how sensitive the tumor is to radiation.

Highly sensitive: Lymphoma, Hodgkin disease and neuroblastoma.

Moderately sensitive: Oral, cervical, breast cancer.

Poorly sensitive: Osteoblastoma, malignant melanoma.

Precautions to reduce hazards due to radiation

- Use lead shields, lead rubber gloves and aprons
- Radioactive substances should be properly stored and disposed.

Gray and rad: Units of absorbed dose of radiation by a tissue.

20 Metabolism of Xenobiotics (Detoxification)

1. Define xenobiotic.

Xenobiotic is a compound that is foreign to the body. It may include drugs, chemical carcinogens, polychlorinated biphenyls (PCBs) and insecticides.

2. Explain the process of detoxification with examples.

Detoxification: It is a process by which toxic compounds are converted into less toxic and easily excretable forms.

Sites of Xenobiotic Metabolism

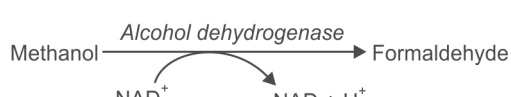
i. **Liver:** It is a major site for detoxification.
ii. **Non-hepatic sites:** Intestinal mucosa, kidney, lung, gastrointestinal (GI) tract and placenta.

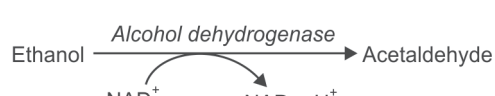
Xenobiotics are Detoxified in Two Phases

Phase 1

Attachment of new functional groups or transformation of existing functional groups by hydrolysis, oxidation, reduction, etc.

i. **Oxidation:** It is the removal of hydrogen or addition of oxygen. Oxidation is carried out by CYP450 system, alcohol dehydrogenase, aldehyde dehydrogenase and monoamine oxidase**.** For example,





ii. **Reduction:** It is the removal of oxygen and addition of hydrogen or electrons.
For example,

Picric acid ⟶ Picramic acid

Disulfiram ⟶ Dithiocarbamic acid

iii. **Hydrolysis:** It is the breakdown of compounds by addition of water.
For example,

Acetylsalicylic acid (Aspirin) + H_2O ⟶ Salicylic acid + Acetic acid

Atropine + H_2O ⟶ Tropine + Tropic acid

Phase 2

Conjugation: The existing functional group of a compound is conjugated by glucuronidation, acetylation, methylation, attachment of an amino acid or other mechanisms. Most of the conjugated compounds are water soluble and can be easily excreted.

i. **Glucuronidation:** The compound is conjugated with glucuronic acid.
For example,

Bilirubin + 2 UDP-glucuronic acid ⟶ Bilirubin diglucuronide

ii. **Conjugation with glycine.**
For example,

Benzoyl-CoA + Glycine ⟶ Hippuric acid

iii. **Acetylation.**
For example,

Sulfanilamide + Acetyl-CoA ⟶ Acetylsulfanilamide

iv. **Methylation.**
For example,

Nicotinamide + S-adenosylmethionine ⟶ Methylnicotinamide

v. **Sulfation.**
For example,

Phenol + Phosphoadenosylphosphosulfate (PAPS) ⟶ Phenylsulfate + PAP

3. How are the following compounds detoxified?

i. Indole.
ii. Aspirin.
iii. Paracetamol.
iv. Cyanide.
v. Phenobarbital.
vi. Isoniazid.

i. **Indole:** Undergoes deamination and decarboxylation to produce indolepyruvate and finally indoleacetate.

ii. **Aspirin:** It is detoxified by hydrolysis.

iii. **Paracetamol (acetaminophen):** It is detoxified by both glucuronidation and sulfation. Paracetamol can also be detoxified by CYP2E1 to produce N-acetyl-p-benzoquinoneimine (NAPQI), which conjugates with glutathione (GSH) to form inactive compound.

iv. **Cyanide:** Conjugates with thiosulfate to form less toxic thiocyanate.

v. **Phenobarbital:** Detoxified by hydroxylation by CYP450 group of monooxygenases in the liver followed by glucuronidation.

vi. **Isoniazid (antitubercular drug):** Undergoes acetylation. Genetic variations in the enzymes (acetyl transferases) lead to slow and fast acetylators.

4. Explain the role of glutathione in detoxification mechanism.

i. Glutathione (γ-glutamyl-cysteinylglycine) is a tripeptide consisting of glutamic acid, cysteine and glycine.

ii. Carcinogens (R) are conjugated with GSH, a reaction catalyzed by glutathione S-transferase to form glutathione conjugate, which is further metabolized and excreted as mercapturic acid (Fig. 20.1).

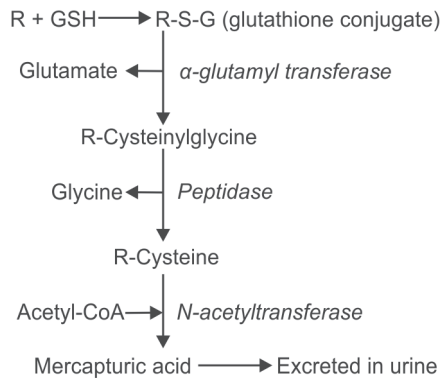


Fig. 20.1: Detoxification by glutathione

Key Points

N-acetyl-p-benzoquinoneimine (NAPQI): It is a metabolite (minor) of paracetamol. It is detoxified by conjugation with glutathione. In paracetamol overdose, excess NAPQI is generated, which depletes the hepatic glutathione stores and then causes damage to liver.

Most isoforms of CYP450 are induced by certain drugs: For example, administration of phenobarbital causes hypertrophy of smooth ER 3–4 folds in 4–5 days. This can result in increased metabolism of drugs.

Warfarin: Used to prevent blood clotting, is metabolized by CYP2C9, which is induced by phenobarbital. Thus, when warfarin is coadministered with phenobarbital, warfarin is rapidly metabolized.

Ethanol induces CYP2E1: Which increases the risk for carcinogenicity in alcoholics.

Certain isoforms of CYP450 (CYP1A1): Are involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) and are also responsible for carcinogenesis produced by these agents. For example, in the lung, it is involved in the conversion of inactive PAH inhaled by smoking to active carcinogens by hydroxylation.

Certain CYP450 enzymes exhibit genetic polymorphism: Some of them show low catalytic activity. This explains variations in drug responses noted among patients.

Activity of CYP450 is also affected by tissue or organ disease (e.g. cirrhosis of liver).

21 Miscellaneous

HORMONES

1. Define and classify hormones.

Definition: Hormones are chemical messengers, released from ductless glands into the bloodstream and regulate the activity of other tissues.

Classification

Based on Chemical Nature

i. **Peptide or protein hormones:** For example, insulin, glucagon, antidiuretic hormone, oxytocin.
ii. **Steroid hormones:** For example, glucocorticoids, mineralocorticoids, sex hormones.
iii. **Amino acid derivatives:** For example, epinephrine, norepinephrine, thyroxine (T_4), triiodothyronine (T_3).

Based on Mechanism of Action

i. **Group I hormones:** For example, glucocorticoids, estrogens, progesterone, mineralocorticoids, calcitriol, thyroxine.
   a. Action is mediated via **intracellular receptors**.
ii. **Group II hormones:** Action is mediated via **cell surface receptors** and second messengers. Hydrophilic in nature. They are divided into three categories depending on second messengers:
   a. Hormones using cyclic adenosine monophosphate **(cAMP)** as second messenger. For example, adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), parathormone (PTH), adrenaline.

b. Hormones using **phosphatidylinositol/calcium** as second messenger. For example, thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), gastrin, cholecystokinin (CCK).
c. Hormones using cyclic guanosine monophosphate **(cGMP) as second messenger**. For example, atrial natriuretic peptide (ANP).

2. Explain the general mechanism of action of group I hormones.

The general mechanism of action of group I hormones is shown in Figure 21.1.

Hormones (e.g. glucocorticoids) pass through the plasma membrane

↓

Bind to their specific, intracellular receptors in the cytoplasm of target cells

↓

Form hormone-receptor complex

↓

Undergoes conformational change

↓

Activated hormone-cytoplasmic receptor complex moves into the nucleus and binds with high affinity to a specific DNA sequence called hormone response element (HRE)

↓

Production of messenger ribonucleic acids (mRNAs)

↓

Production of specific protein, which influence metabolic processes

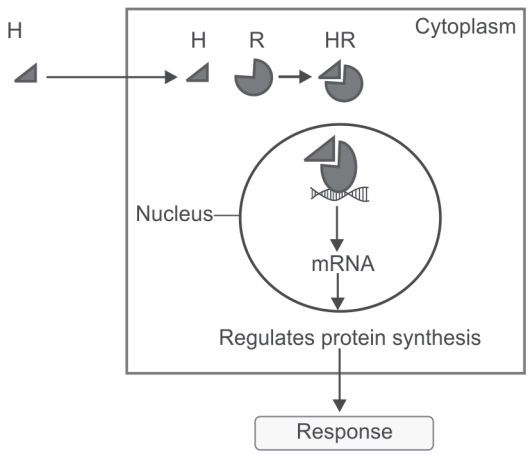


Fig. 21.1: Mechanism of action of group I hormones, e.g. glucocorticoids (H, hormone; R, receptor)

Example of effect of glucocorticoids: Induction of synthesis of gluconeogenic enzymes.

- Thyroid hormones directly enter nucleus → bind to their receptor, which is already bound to the response element on DNA → conformational changes in the receptor → gene transcription
- The hormone action, mediated through intracellular receptors is not immediate, since sufficient time is needed for transcription and translation.

3. Explain the mechanism of action of any one group II hormone (glucagon).

- Group II hormones bind to cell surface receptors resulting in the generation of certain molecules, namely **second messengers**
- Group II hormones are divided into three categories depending on second messengers (Refer question 1).

Mechanism

The mechanism of action of glucagon is shown in Figures 21.2 and 21.3.
Glucagon binds to G protein-coupled receptors and acts via cAMP as second messenger.
G protein is GTP-binding membrane protein. It consists of three subunits—α, β, γ.

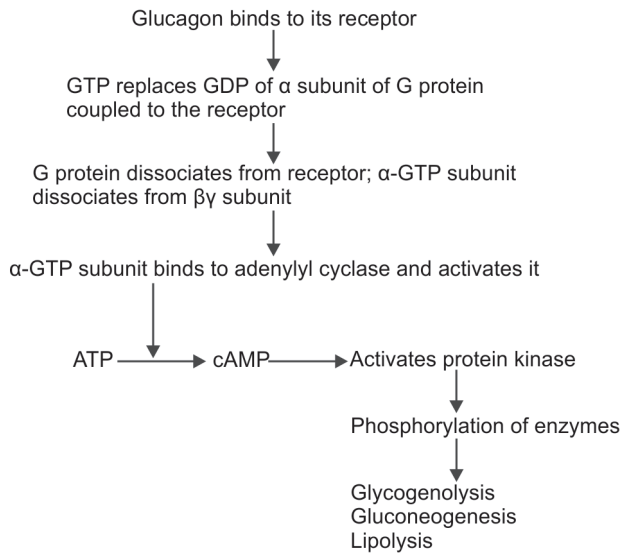


Fig. 21.2: Steps of glucagon action

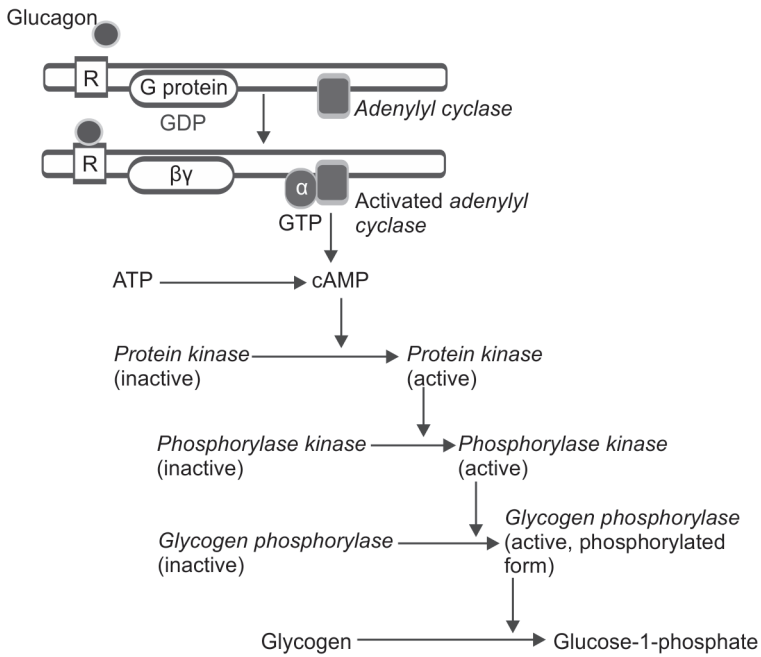


Fig. 21.3: Mechanism of action of glucagon (R, receptor; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GDP, guanosine diphosphate)

FEED-FAST CYCLE

4. Explain the metabolic changes in the well-fed state.

Well-fed State (Fig. 21.4 and Table 21.1)

- Increase in levels of blood glucose, amino acids and chylomicrons
- Increased insulin secretion. Glucose uptake into tissues from blood is promoted by insulin (except brain, RBCs)
- Glucose used as fuel by all tissues—brain utilizes glucose as fuel
- Energy stored as glycogen (liver, muscle) and triacylglycerol (adipose tissue)
- Amino acids from blood are taken up by tissues—protein synthesis occurs.

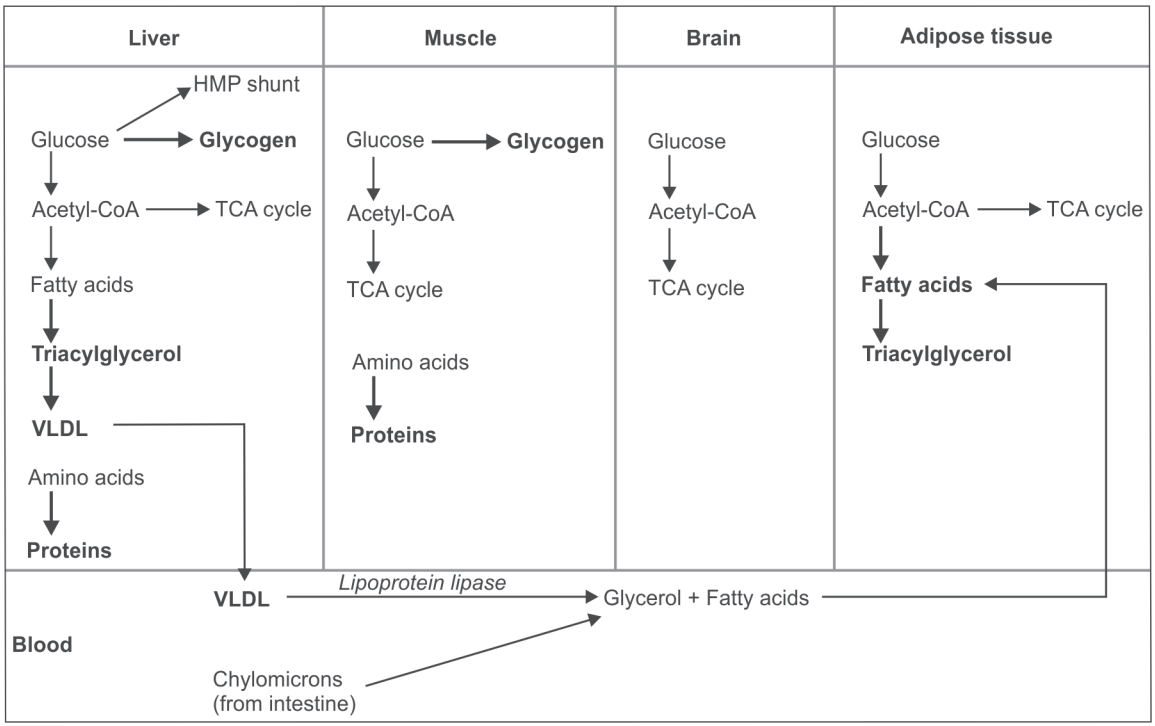


Fig. 21.4: Metabolic changes in various organ systems during well-fed state (HMP, hexose monophosphate; TCA, tricarboxylic acid; VLDL, very-low-density lipoprotein)

5. Explain the metabolic changes seen during fasting.

Starvation results from:

- Inability to obtain food
- Desire to lose weight
- Trauma, surgery, neoplasms, burns.

Fasting state (Fig. 21.5 and Table 21.1)

- Decreased blood glucose, amino acids and triacylglycerol
- Increased secretion of epinephrine, glucagon and cortisol; decreased insulin secretion
- Liver maintains blood glucose levels by glycogenolysis and gluconeogenesis
- ↑ protein breakdown → amino acids → hepatic gluconeogenesis

- Fatty acids (from hydrolysis of triacylglycerol in adipose tissue) is taken up by liver and muscle
- Increased ketone body formation in the liver → used as fuel by other tissues
- Brain uses glucose as fuel. On prolonged fasting, it utilizes ketone bodies.

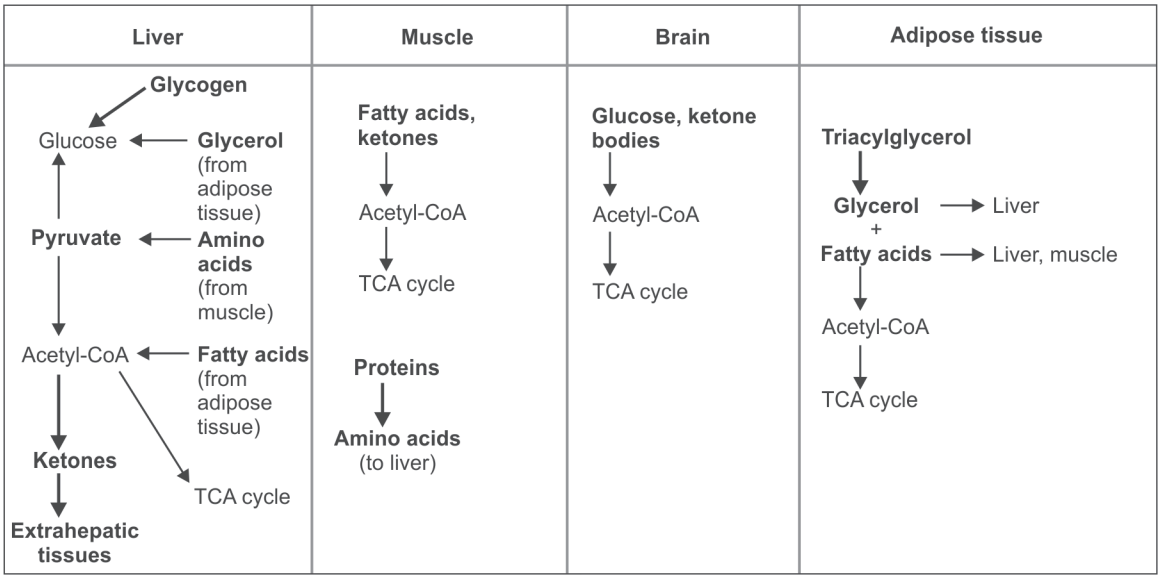


Fig. 21.5: Metabolic changes in various organ systems during starvation (TCA, tricarboxylic acid)

Table 21.1: Events during fast-feed cycle

Organ	Fed state	Fasting state
Liver	↑ glycogen synthesis	↑ glycogen breakdown
	↑ glycolysis	↑ gluconeogenesis
	↑ fatty acid synthesis	↑ utilization of fatty acids
	↑ triacylglycerol formation	↑ formation of ketone bodies
	↑ VLDL synthesis	
Adipose tissue	↑ entry of glucose into adipose tissue ↑ synthesis of triacylglycerol (↑ lipogenesis)	Hydrolysis of triacylglycerol to fatty acids (used as fuel by tissues) and glycerol (utilized in hepatic gluconeogenesis)

Contd...

Contd...

Organ	Fed state	Fasting state
Skeletal muscle	↑ entry of glucose	
	↑ synthesis of glycogen	↑ utilization of fatty acids, ketones as fuel
	↑ protein synthesis	↑ protein breakdown to amino acids → hepatic gluconeogenesis
Brain	Glucose used as fuel	Glucose and ketones used as fuel

6. Mention the different sources and fate of acetyl-CoA.

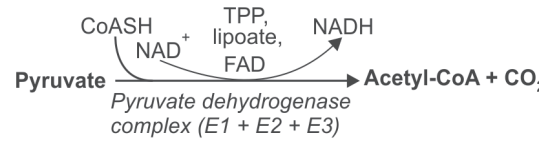
Sources and fate of acetyl-CoA is given in Table 21.2.

Table 21.2: Sources and fate of acetyl-CoA

Sources of acetyl-CoA	Fate of acetyl-CoA
Glycolysis	Cholesterol synthesis
β-oxidation of fatty acids	Fatty acid synthesis
Catabolism of ketogenic amino acids	Ketone body synthesis
Ketone bodies	Oxidation in tricarboxylic acid (TCA) cycle

7. Discuss the significance of multienzyme complexes with two examples.

Multienzyme complex is a protein consisting of more than one catalytic domains formed by structurally distinct and ordered collection of enzymes, often catalyzing successive steps in a metabolic pathway.



i. **Pyruvate dehydrogenase complex:** It is a complex assembly of three enzymes—pyruvate dehydrogenase (E_1), dihydrolipoyl transacetylase (E_2) and dihydrolipoyl dehydrogenase (E_3) and five coenzymes (lipoic acid, NAD^+, FAD, TPP and pantothenic acid).

This complex coordinates the conversion of pyruvate to acetyl-CoA with liberation of CO_2.

ii. **Fatty acid synthase complex**: It is a dimer with each unit having seven enzymes and one acyl carrier protein (ACP) (Fig. 21.6).

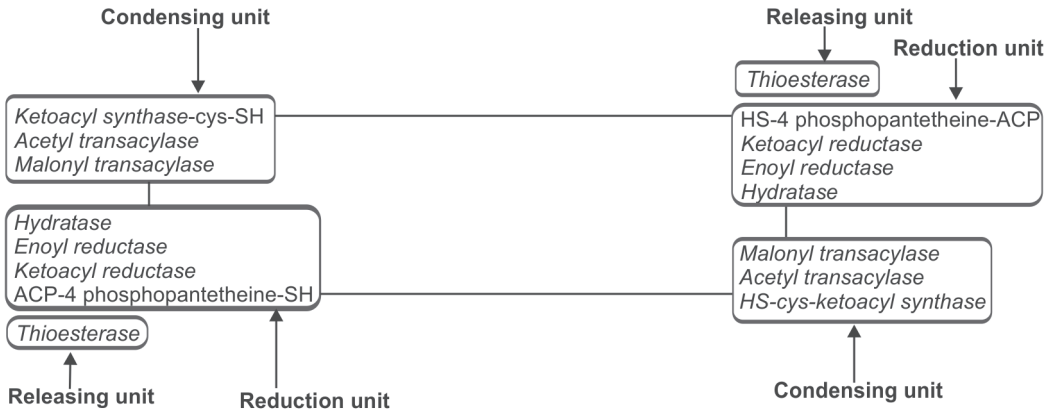


Fig. 21.6: Fatty acid synthase complex

For the synthesis of fatty acid, condensing unit of one monomer and reduction and releasing unit of other monomer coordinate to synthesize fatty acid. Thus, only a dimer is active.

Key Points

Enzymes active in dephosphorylated state: Glycogen synthase, phosphofructokinase-2, pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase.

Enzymes inactive in dephosphorylated state: Glycogen phosphorylase, fructose bisphosphate phosphatase-2 and hormone-sensitive lipase.

Adipocytes lack glycerol kinase: Glycerol-3-phosphate for triacylglycerol synthesis is provided by glycolysis.

Brain uses glucose and ketone bodies: Brain uses glucose (well-fed condition) and ketone bodies (starvation) as major sources of energy.

FREE RADICAL METABOLISM

8. Define free radicals. How are they produced? Add a note on effects of free radicals on our body.

Definition: Free radicals are highly reactive molecular species that contain one or more unpaired electrons in the outermost shell. They can potentially damage proteins, carbohydrates, fats and nucleic acids.

Generation of Free Radicals

They are produced endogenously:

- During metabolic reactions
- In electron transport chain
- During inflammation and infections
- By ionizing radiation.

Examples

Oxygen radicals also known as reactive oxygen species (ROS) include:

- Hydroxyl radical ($OH^{\bullet}$)
- Nitric oxide ($NO^{\bullet}$)
- Superoxide ($O_2^{\bullet}$)
- Lipid peroxide radical ($ROO^{\bullet}$)
- H_2O_2 is also considered as ROS, as it is highly reactive.
  Other free radicals—methyl radical.

Effects of Free Radicals on Body

Free radicals damage DNA, proteins and lipids. They modify proteins and lipids in low-density lipoprotein (LDL). Free radicals can cause peroxidation of lipids in membranes.

Free radicals are implicated in tissue damage that occur during:

- Autoimmune disease
- Myocardial infarction: Oxidation of LDL leading to atherosclerosis
- Cataract formation
- Aging
- Carcinogenesis: Mutation of genetic material.

9. What is lipid peroxidation?

Definition: It is the process by which unsaturated fatty acids interact with reactive oxygen species (compounds having unpaired electrons in their outermost shell) leading to oxidative degradation of lipid. It can be divided into three stages: initiation, propagation and termination.

Steps in Lipid Peroxidation

The three steps are as follows:

i. **Initiation:** Hydroxyl radicals and singlet oxygen can react with polyunsaturated fatty acids (PUFA) forming lipid peroxyl radicals.

$$PUFA\text{-}H + X^{\bullet} \longrightarrow PUFA^{\bullet} + X\text{-}H$$

$$PUFA^{\bullet} + O_2 \longrightarrow PUFA^{\bullet}\ OO^{\bullet}$$

ii. **Propagation:** Peroxyl radical formed is highly reactive and is able to propagate the chain reaction by reacting with another molecule of PUFA.

$$PUFA\text{-}OO^{\bullet} + PUFA\text{-}H \longrightarrow PUFA\text{-}OOH \text{ (hydroperoxide)} + PUFA$$

The lipid hydroperoxides produced (PUFA-OOH) can undergo reductive cleavage by reduced metals, such as Fe^{2+}.

$$Fe^{2+} + PUFA\text{-}OOH \longrightarrow Fe^{3+} + OH^{\bullet} + PUFA\text{-}O^{\bullet}$$

iii. **Termination:** This involves reaction of two radicals to form an inactive compound resulting in the termination of the reaction.

$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow RO - OR + O_2$$

$$ROO^{\bullet} + R^{\bullet} \longrightarrow RO - OR$$

10. How are free radicals metabolized?

Substances, which protect against damage by reactive oxygen species, are antioxidants. Antioxidants prevent lipid peroxidation by converting lipid hydroperoxide radicals into stable compounds.

i. **Endogenous antioxidants (preventive antioxidants):** They include enzyme systems in the body, which neutralize free radicals. For example, catalase, peroxidase, superoxide dismutase (SOD), etc.

$$O_2 \longrightarrow O_2^{\bullet} \xrightarrow{SOD} H_2O_2 \xrightarrow{Catalase} H_2O + O_2$$

ii. **Chain-breaking antioxidants:** For example, β-carotene, vitamin C, vitamin E, glutathione. Glutathione is a tripeptide present in all the cells of our body and is one of the main antioxidant in our blood. Selenium acts as a cofactor for glutathione peroxidase and can complement the requirements of exogenous antioxidant vitamins.

TECHNIQUES

11. Write a note on electrophoresis.

Definition: Electrophoresis is the migration of charged particles under the influence of electric current**.** All biologically important molecules possess ionizable groups and exist as charged species at any given pH, either as cations or anions**.** On application of electrical field (direct current), charged particles move towards the electrode of opposite charge (Fig. 21.7).

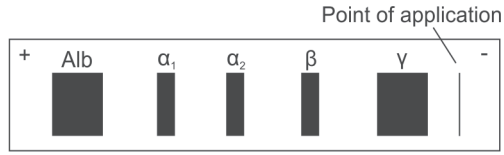


Fig. 21.7: Normal pattern of serum electrophoresis

Albumin: 55%–65%.

α_1 : 2%–4% (retinol-binding protein, T_4-binding globulin, cortisol-binding globulin).

α_2 : 6%–12% (haptoglobin, ceruloplasmin and prothrombin).

β : 12%–14% (transferrin, hemopexin).

γ : 12%–22% [immunoglobulin G, E, M (IgG, IgE, IgM)].

Applications

Separation of:

- Plasma proteins
- Lipoproteins
- Hemoglobin.

— To identify diseases states

12. Write a note on principle and application of chromatography.

Definition: Chromatography is separation of the analyte in the mixture based on differential partitioning between the mobile and stationary phases.

Classification

- Paper chromatography
- Column chromatography
- Thin layer chromatography (TLC)
- High-performance liquid chromatography (HPLC).

Paper Chromatography

Involves placing a small amount of sample solution onto a strip of chromatography paper placed in a jar containing a shallow layer of solvent. As the solvent rises/descends through the paper, it meets the sample mixture, which starts to travel up/down the paper with the solvent. This paper is made of cellulose, a polar substance; compounds within the mixture travel farther if they are non-polar, while polar substances get bound to the cellulose paper and therefore do not travel as far. Separation takes 14–16 hours. The ratio of fronts (Rf value) is calculated by:

$$Rf = \frac{\text{Distance moved by analyte}}{\text{Distance moved by solvent}}$$

TLC: Separation of biomolecules requires 2–4 hours.
HPLC: Separation is faster (within minutes) and better.

Application

- Separation of biomolecules (carbohydrates, amino acids, etc.).

13. Write the principle and applications of colorimetry.

Definition: Colorimetry is a technique used for quantitative estimation of color. Substances to be estimated colorimetrically should either be colored or capable of forming colored compounds on addition of reagents.

Principle

- **Beer's law:** The amount of light absorbed is directly proportional to the concentration of colored substance
- **Lambert's law:** The absorbance is directly proportional to the path length of liquid through which light passes.
  A = εcl (A = absorbance, ε = extinction coefficient, c = concentration, l = path length).

Limitations

- Less sensitive at very low concentration of substance to be estimated
- Works within the range of Beer's law
- Volatile and thermally unstable compounds cannot be estimated.

Applications

Quantitative analysis is of compounds in pure state or in biological samples.

ELECTROLYTES

14. List important anions and cations of our body.

Important anions and cations are given in Table 21.3.

Table 21.3: Important anions and cations in our body	
Important cations	**Important anions**
Sodium (Na^+)	Chloride (Cl^-)
Potassium (K^+)	Bicarbonate (HCO_3^-)
Calcium (Ca^{2+})	Phosphate ($H_2PO_4^-$ and HPO_4^{2-})
Magnesium (Mg^{2+})	Sulfate (SO_4^{2-})

15. What is Donnan membrane equilibrium?

Definition: Donnan membrane equilibrium states that, in any given compartment of the cell, the total number of anions should be equal to total number of cations (Table 21.4). If one compartment has non-diffusible anions before equilibrium, then at equilibrium it will have more diffusible cations. The products of diffusible electrolytes on both sides of membrane after equilibrium will be the same (Table 21.5). The total number of a particular ion before and after equilibrium is reached will be the same.

Table 21.4: Distribution of anions and cations in various body fluids		
Solutes	**Plasma (mEq/L)**	**Intracellular fluid (mEq/L)**
Cations		
Sodium	140	12
Potassium	4	160
Calcium	5	–
Magnesium	2	34
Total	**151**	**206**
Anions		
Chloride	104	2
Bicarbonate	24	10
Sulfate	1	–
Phosphate	2	140
Protein	15	54
Other anions	5	–
Total	**151**	**206**

Table 21.5: Distribution of diffusible electrolytes before and after equilibrium across semipermeable membrane

	Compartment I	Compartment II
Before equilibrium	••• •• ΔΔ ΔΔ Δ Positive charge = 5; negative charge = 5	••••• ••••• ○○○○○ ○○○○○ Positive charge = 10; negative charge = 10
After equilibrium	○○○○ ΔΔΔΔΔ ••••• •••• Positive charge = 9; negative charge = 9	○ ○○○○○ • ••••• Positive charge = 6; negative charge = 6
Product of diffusible electrolytes (sodium x chloride)	9 x 4 = 36	6 x 6 = 36

Δ = protein (negative charge); ○ = chloride ion (Cl^-); • = sodium ion (Na^+); (- -) = Semipermeable membrane

16. Enlist the functions of sodium and potassium.

Refer Chapter 11, Question 1.

17. Enlist the causes and clinical manifestations of sodium imbalance.

Refer Chapter 11, Question 6.

18. Enlist the causes and clinical manifestations of potassium imbalance.

Refer Chapter 11, Question 7.

19. Explain the mechanism of water and electrolyte balance.

Water and electrolyte balance is maintained by renin-angiotensin-aldosterone system and antidiuretic hormone (Figs 21.8 and 21.9).

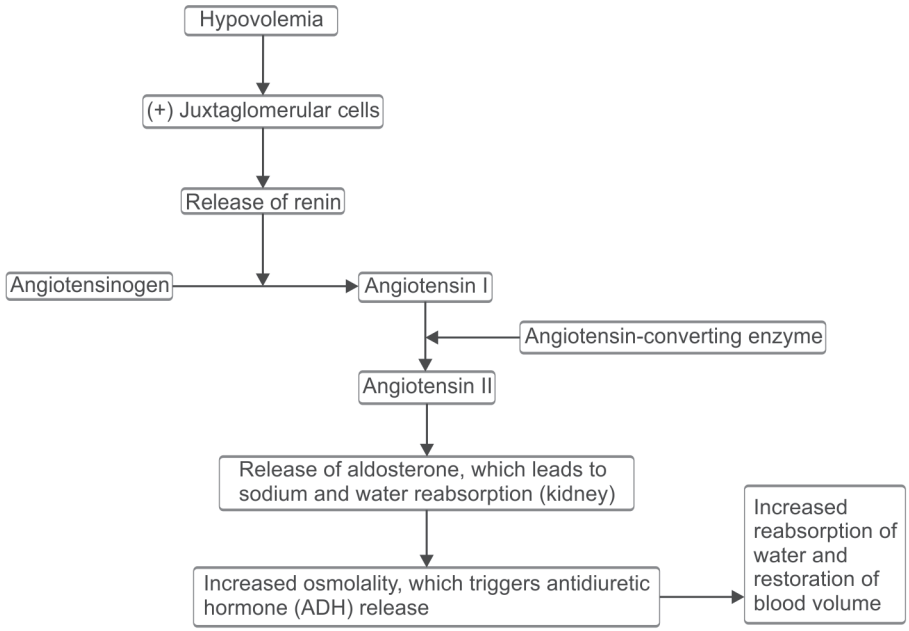


Fig. 21.8: Regulation of blood volume during hypovolemia (+ = stimulation)

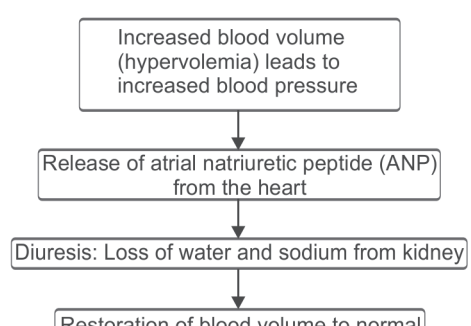


Fig. 21.9: Regulation of blood volume during hypervolemia

Key Points

Flame photometry: This technique is used for determination of sodium and potassium. Here, sample is fed into non-luminous flame in the form of fine spray. Sodium gives yellow and potassium gives violet color on ignition in flame, which is proportional to their respective concentrations.

Ion-selective electrodes: Used to measure sodium, potassium, chloride ions.

- **Sodium:** Accounts for 90%–95% of all solutes in the extracellular fluid (ECF). Contributes 280 mOsm of the total 300 mOsm of ECF. Reference range for serum sodium: 136–145 mEq/L
- **Potassium:** It is an important cation in the ICF. Reference range for the serum potassium is 3.5–5 mEq/L
- **Chloride:** It is a major ECF anion. Reference range for serum chloride: 94–111 mEq/L. CSF: 120–130 mEq/L.

Electrolyte concentration: Expressed in milliequivalents per liter (mEq/L). It is a measure of the number of electrical charges in 1 liter of solution. For single charged ions, 1 mEq = 1 mOsm. For bivalent ions, 1 mEq = 1/2 mOsm.

Osmotic concentration (Osm/L): Proportional to the number of osmotic particles formed, which is calculated as moles x n (n = of particles in solution). For example, 1 mole of NaCl (n = 2) forms a two osmolar solution in 1 L. 1 mole of $CaCl_2$ (n = 3) forms a 3 osmolar solution in 1 L. For physiological concentrations, mOsm units are most appropriate (1 mOsm = 10^{-3} osmoles/L). Plasma osmolarity measures ECF osmolarity. Plasma is dominated by [Na^+] and the associated anions. Under normal conditions, ECF osmolarity can be roughly estimated as: 2 [Na^+] = 270–290 mOsm/L.

22 Hemoglobin Metabolism

1. How is bilirubin formed and excreted?

Life span of mature red blood cells (RBCs) in bloodstream is around 60–120 days. In a 70 kg man, approximately 6 g/day of hemoglobin is liberated from breakdown of RBCs (Fig. 22.1) in the reticuloendothelial system. This hemoglobin is catabolized to form bilirubin. Bilirubin is further conjugated and excreted through bile.

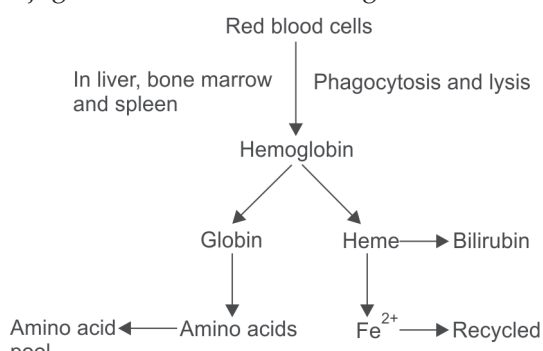


Fig. 22.1: Heme degradation

Steps in Bilirubin Metabolism (Fig. 22.2)

i. Breakdown of heme into bilirubin in reticuloendothelial cells.
ii. Transport of bilirubin (unconjugated) bound to albumin to liver.
iii. Uptake of bilirubin into liver cells.
iv. Conjugation of bilirubin with glucuronide by uridine diphosphate (UDP)-glucuronyl transferase.

v. Secretion of bilirubin into bile → intestine.
vi. Conversion of bilirubin to urobilinogen followed by reabsorption and excretion.

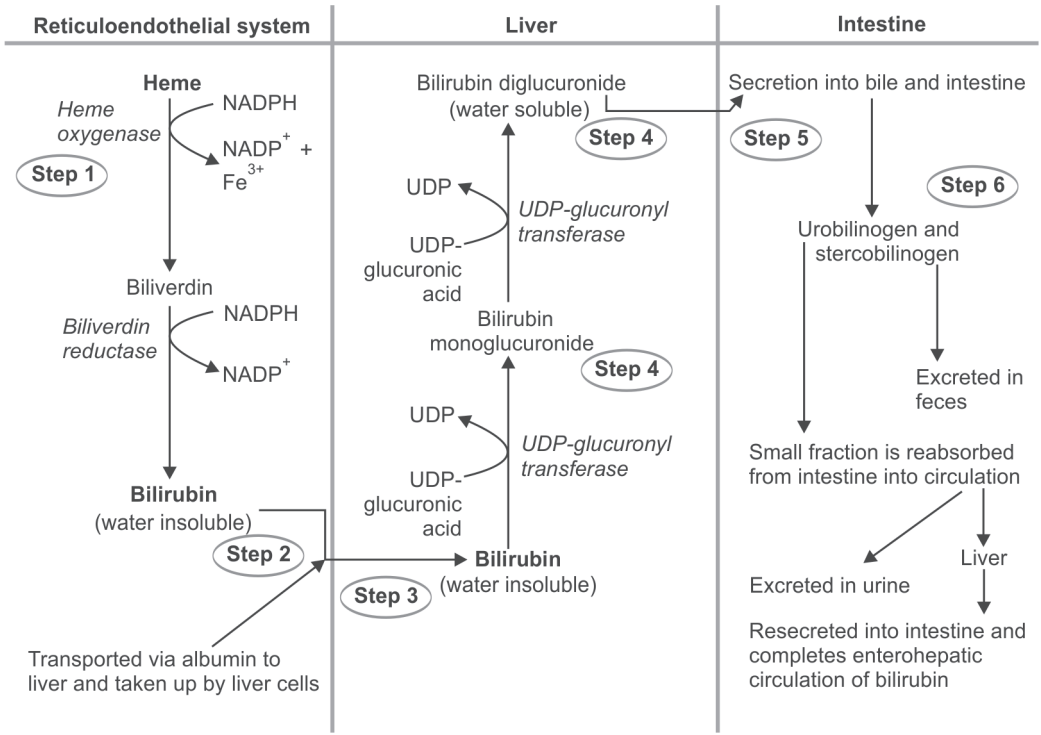


Fig. 22.2: Bilirubin metabolism (NADP, nicotinamide adenine dinucleotide phosphate; UDP, uridine diphosphate)

2. Write the principle and applications of van den Bergh reaction.

Refer question number 2 in Chapter 18.

3. Define jaundice. How can it be classified? Describe the biochemical investigations to differentiate different types of jaundice.

Definition: Jaundice is defined as yellowish discoloration of skin, nail beds and sclera. It is caused by deposition of bilirubin, secondary to increased bilirubin levels in the blood. When bilirubin concentration is more than 1 mg/dL, the condition is called hyperbilirubinemia. At a concentration of more than 2 mg/dL, bilirubin diffuses into tissues, which then becomes yellow, leading to jaundice or icterus.

Normal Levels in Blood

- **Total bilirubin:** 0.2–0.8 mg/dL
- **Unconjugated bilirubin:** 0.2–0.6 mg/dL
- **Conjugated bilirubin:** 0.1–0.2 mg/dL.

Classification

Jaundice is classified into three major types:

i. **Prehepatic (hemolytic):** Due to excessive hemolysis, bilirubin production exceeds the capacity of liver to conjugate it.
ii. **Hepatic:** Impaired uptake, conjugation or excretion of bilirubin.
iii. **Posthepatic (obstructive):** Caused by an obstruction in the biliary tract (Table 22.1).

Table 22.1: Classification and findings in jaundice

Type of jaundice	Causes	Serum bilirubin	Urine and feces	Serum ALT and AST	Serum ALP
Prehepatic [MN: MARS]	**M**alaria **A**utoimmune hemolytic anemia **R**h incompatibility **S**ickle cell anemia	↑ unconjugated bilirubin	• ↑ urobilinogen • Bilirubin negative • ↑ stercobilinogen	Normal or slight ↑	Normal or slight ↑
Hepatic	Hepatitis	↑ conjugated and ↑ unconjugated bilirubin	• Bilirubin present (if microobstruction) • ↓ urobilinogen (if microobstruction)	Markedly elevated	Normal or slight ↑
Post-hepatic	Gallstones Pancreatic tumor	↑ conjugated bilirubin	• Urobilinogen absent	Normal or slight ↑	Markedly elevated
	Cholangiocarcinoma		• Bilirubin present • Clay-colored stool		

↑ = increased; ↓ = decreased

4. Write a note on congenital hyperbilirubinemia.

Definition: A group of hereditary disorders of bilirubin metabolism due to defect in uptake, conjugation or secretion of bilirubin (Table 22.2).

Table 22.2: Congenital hyperbilirubinemia

Condition	Defects	Clinical features
Crigler-Najjar syndrome Type I	Severe deficiency of UDP-glucuronyl transferase	↑↑↑ serum unconjugated bilirubin Profound jaundice–does not respond to phenobarbital; often fatal
Crigler-Najjar syndrome Type II	Mild deficiency of UDP-glucuronyl transferase	↑ serum unconjugated bilirubin; mild jaundice; responsive to phenobarbital therapy
Gilbert syndrome	Reduced activity of glucuronyl transferase	↑ serum unconjugated bilirubin; mild jaundice
Dubin-Johnson syndrome	Abnormality in secretion of conjugated bilirubin into biliary system	↑ serum conjugated bilirubin; moderate jaundice
Rotor syndrome	Cause not known	↑ serum conjugated bilirubin; mild jaundice

↑ = increased; ↑↑↑ = markedly increased

5. Describe heme synthesis. Add a note on its regulation.

Requirements

- **Starting material:** Glycine and succinyl-coenzyme A (CoA), Fe^{2+}
- **End product:** Heme
- **Site:** RBC precursors (85%), liver (15%)
- **Subcellular site:** Mitochondria and cytosol
- **Enzymes**
- **Coenzyme:** Pyridoxal phosphate (PLP).

Regulation of Heme Synthesis

δ-aminolevulinic acid (ALA) synthase is **inhibited** by **heme and hematin (Fe^{3+} form).**

Heme

- Represses the synthesis of δ-ALA synthase by regulating gene transcription (negative regulation)
- Diminishes the transport of δ-ALA synthase from cytoplasm to mitochondria.

Drugs: ALA synthase is upregulated by a large number of drugs including barbiturates, testosterone and oral contraceptives. These drugs are metabolized by the ***microsomal***

cytochrome P450 monooxygenase system, which use up heme and decrease heme concentration, which in turn stimulates enzyme synthesis (derepression).

Glucose: Prevents induction of ALA synthase.

Steps in Heme Synthesis (Fig. 22.3)

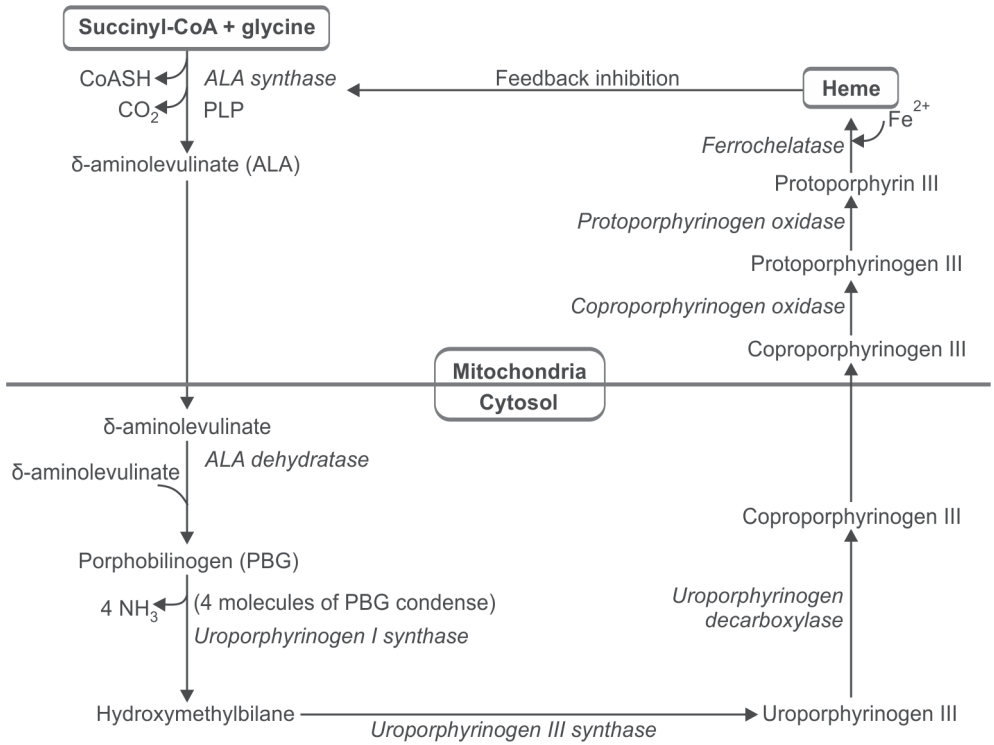


Fig. 22.3: Heme synthesis (ferrochelatase and ALA dehydratase are inhibited by lead)

6. What are porphyrias? How are they classified?

- Porphyrias are a group of disorders caused by deficiencies in enzymes of the heme biosynthetic pathway
- The majority of the porphyrias are inherited in an autosomal dominant manner except **congenital erythropoietic porphyria,** which is autosomal recessive

- Affected individuals have an accumulation of heme precursors that are **toxic at high concentrations and can lead to abdominal pain or photosensitivity (due to porphyrins)**
- Attacks of the disease are triggered by certain drugs, chemicals, foods and by exposure to sunlight (UV radiation)
- Drugs (phenobarbital, steroids) are metabolized by the microsomal cytochrome P450 monooxygenase system, which in turn induces δ-ALA synthase, resulting in increased levels of potentially harmful heme precursors prior to metabolic block
- Treatment involves administration of **hematin,** which provides negative feedback inhibition of ALA synthase leading to decreased formation of heme precursors.

Classification (Table 22.3)

i. **Erythropoietic:** Enzyme deficiency occurs in RBCs, e.g. congenital erythropoietic porphyria, protoporphyria.
ii. **Hepatic:** Enzyme defect occurs in liver, e.g. acute intermittent porphyria, porphyria cutanea tarda, hereditary coproporphyria, variegate porphyria.

Table 22.3: Types of porphyria

Types	Enzyme defects	Clinical features	Key findings
X-linked sideroblastic anemia (erythropoietic)	ALA synthase	Anemia	↓ RBC count ↓ Hb%
ALA dehydratase deficiency (hepatic)	ALA dehydratase	Abdominal pain, neuropsychiatric symptoms	↑ Urine ALA (δ-amino-levulinic acid)
Acute intermittent porphyria (hepatic)	Uroporphyrinogen I synthase	Abdominal pain, neuropsychiatric symptoms	↑ Urinary ALA and porphobilinogen (PBG)
Congenital erythropoietic porphyria (erythropoietic)	Uroporphyrinogen III synthase	Photosensitivity	Uroporphyrin (+) and PBG (−) in urine
Porphyria cutanea tarda (hepatic)	Uroporphyrinogen decarboxylase	Photosensitivity	Uroporphyrin (+) and PBG (−) in urine
Hereditary coproporphyria (hepatic)	Coproporphyrinogen oxidase	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary PBG and coproporphyrin (+); fecal coproporphyrin (+)
Variegate porphyria (hepatic)	Protoporphyrinogen oxidase	Photosensitivity, abdominal pain, neuropsychiatric symptoms	Urinary PBG and coproporphyrin (+)
Protoporphyria (erythropoietic)	Ferrochelatase	Photosensitivity	Fecal and red cell protoporphyrin (+)

(+), present; (−), absent

7. Write a note on acute intermittent porphyria.

Definition: It is a hepatic, autosomal dominant porphyria. It is caused by a deficiency in uroporphyrinogen I synthase, which is involved in the conversion of porphobilinogen (PBG) to hydroxymethylbilane.

Clinical Features

- Patients have neuropsychiatric symptoms and abdominal pain (neurovisceral) due to irritation of abdominal nerves and central nervous system by toxic products like δ-ALA and PBG
- Symptoms become more severe after administration of drugs like barbiturates, which induce cytochrome P450 and use up heme; this in turn stimulate key enzyme δ-ALA synthase and results in production of toxic concentrations of products like PBG and ALA
- PBG and δ-ALA accumulate in plasma and are excreted in urine.

Treatment

- Symptomatic—analgesics
- Avoiding triggering factors, e.g. drugs like phenobarbitone
- Hematin administration to suppress heme synthesis.

8. Write briefly on porphobilinogen.

Porphobilinogen is an intermediate compound in the heme synthetic pathway (Fig. 22.4).

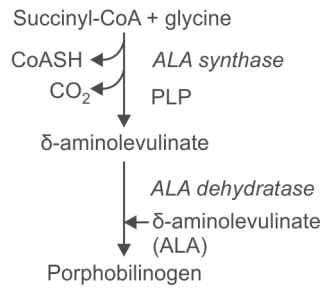


Fig. 22.4: Synthesis of porphobilinogen (PLP, pyridoxal phosphate)

- **Fate:** Four molecules of porphobilinogen condense in the presence of enzyme uroporphyrinogen I synthase to form hydroxymethylbilane
- **Acute intermittent porphyria:** It is due to deficiency of uroporphyrinogen I synthase. In this condition, porphobilinogen is not metabolized leading to its accumulation in tissues.

This in turn can cause irritation of abdominal nerves and nerve endings in central nervous system causing abdominal pain and neuropsychiatric manifestations.

9. Write short notes on neonatal jaundice.

Neonatal jaundice is seen in newborn infants, due to accelerated hemolysis and an immature hepatic system for bilirubin uptake, conjugation and secretion. It is commonly seen in premature infants and resolves within first 10 days of birth.

Causes

- Accelerated hemolysis
- Impaired glucuronyl transferase synthesis due to immaturity of liver
- Substrate UDP-glucuronic acid synthesis also may be inadequate.

Clinical Features

- Yellowish discoloration of skin and sclera due to elevated unconjugated bilirubin
- High levels (> 20 mg/dL) of unconjugated bilirubin are toxic to the newborn—due to its hydrophobicity, it can cross the blood-brain barrier and cause kernicterus, which causes mental retardation
- Mostly self-limiting; if serum bilirubin concentration exceeds 15 mg/dL for more than 15 days, it is usually pathological and needs to be further investigated.

Treatment

- If bilirubin levels are judged to be too high, then phototherapy is used to convert it to a water-soluble, non-toxic form (maleimide and geometric isomers)
- Phenobarbital is useful in treatment—inducer of glucuronyl transferase
- If necessary, exchange blood transfusion—used to remove excess bilirubin.

10. Write briefly on thalassemias.

Definition: Thalassemias are a group of hereditary hemolytic diseases in which an imbalance occurs in the synthesis of globin chains. They are the most common single gene diseases in humans.

Classification

a. **α-thalassemia**—synthesis of α-chain of hemoglobin is decreased (Table 22.4).
b. **β-thalassemia**—synthesis of β-chain of hemoglobin is decreased (Table 22.5).

Table 22.4: Classification and clinical features of α-thalassemia

Type	Genotype	Clinical features
Normal	αα/αα	Normal
Silent carrier	1 gene loss (-/α α/α)	No signs and symptoms
α-thalassemia minor	2 genes loss (-/α -/α)	Mild anemia
HbH disease (Bart)	3 genes loss (-/- -/α)	Mild to moderately severe anemia
		Due to absence of α-chain, γ-chain forms tetramer, which is called Bart hemoglobin. These tetramers have very high O_2 affinity and fail to deliver O_2 to tissues.
Hydrops fetalis	All 4 genes loss (-/- -/-)	Abortion or fetal death

α-thalassemia

These are defects in which synthesis of α-globin chains are decreased or absent. Normally, each individual has four copies of 'α' genes (two on each chromosome 16).

β-thalassemia

- Synthesis of β-globin chains is decreased or absent, whereas α-globin chain is normal; normally, each individual has two copies of β-globin gene (one on each chromosome 11)
- α-globin chains cannot form stable tetramers and therefore, precipitate causing premature death of cells initially destined to become mature red cells
- Accumulation of $\alpha_2\gamma_2$ (fetal hemoglobin, HbF) and γ_4 (Hb Barts) also occurs.

Table 22.5: Classification and clinical features of β-thalassemia

Type	Genotype	Clinical features
Normal	(β/β)	Normal
β-thalassemia minor	1 gene loss (β/β°)	Mild anemia
β-thalassemia major	2 genes defective (β°/β°)	β-globin gene is not expressed until late gestation; so physical manifestations of β-thalassemias appear only after birth. This leads to severe anemia requiring regular blood transfusions. Repeated transfusion though life saving, can cause iron overload (hemosiderosis) and death around 15–25 years of life.

11. Describe the structure of hemoglobin and methemoglobin. Mention any two functions of hemoglobin.

Structures of heme and hemoglobin are given in Figures 22.5 and 22.6 (refer Chapter 7, question number 15).

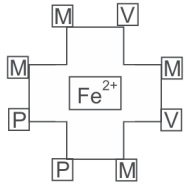


Fig. 22.5: Structure of heme (M, methyl; V, vinyl; P, propionyl)

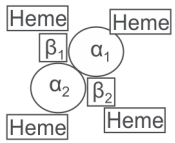


Fig. 22.6: Structure of hemoglobin

12. Explain the causes and features of methemoglobinemia.

Oxidation of ferrous (Fe^{2+}) component of heme to ferric (Fe^{3+}) state forms methemoglobin, which has poor affinity for oxygen → decreased oxygen transport.

Causes of Methemoglobinemia

- Drugs (nitrates) or reactive oxygen species (ROS) can transform hemoglobin to methemoglobin
- Inherited defect in NADH Cyt b5 reductase, which is responsible for the conversion of methemoglobin (Fe^{3+}) back to hemoglobin (Fe^{2+})
- Point mutation: Replacement of proximal/distal histidine of globin chains with tyrosine.

Clinical Features

- Symptoms are related to tissue hypoxia—anxiety, headache, dyspnea, etc.
- Chocolate cyanosis—dark-colored blood as a result of increase in methemoglobin.

13. Write a short note on sickle cell anemia.

Sickle Cell Disease (HbS Disease)

A genetic disorder of blood caused by a single nucleotide alteration in the β-globin gene (point mutation). In the mutant β-chain, there will be substitution of **glutamic acid by valine at 6th position of β-chain;** this will reduce the negative charge on the sickle cell hemoglobin, hence it moves slower than HbA in electrophoresis.

Classification

- **Homozygous (sickle cell disease):** Inheritance of two mutant genes from each parent that code for synthesis of β-globin chains of globin molecules, e.g. HbSS

- **Heterozygous (sickle cell trait):** Inheritance of one normal and one sickle cell gene from parents. Such heterozygotes contain both HbS and HbA.

Consequences of HbS

- Replacement of hydrophilic glutamic acid by hydrophobic valine on the surface creates a sticky patch at low oxygen tension and leads to polymerization of hemoglobin inside RBCs; this stiffens and distorts the RBCs producing sickled RBCs
- Such sickled cells frequently block the flow of blood in narrow capillaries, this leads to localized anoxia in the tissue causing pain and eventually death of cell in the vicinity of blockage
- Sickling is enhanced by increased **pCO_2, decreased pH and increased concentration of 2,3-bisphosphoglycerate (BPG)**.

Diagnosis: Hb Electrophoresis

Replacement of glutamate by valine in two β-chains reduces the negative charge on HbS—it moves slower than HbA at alkaline pH during hemoglobin electrophoresis.

Complications of Sickle Cell Disease

- **Anemia:** Due to accelerated hemolysis
- **Aplastic crisis:** Temporary lack of production of RBCs and other cells in bone marrow
- **Bone necrosis:** Degradation of bone tissue, which can lead to fracture in neck of femur
- **Hand and foot syndrome:** Painful swelling in the hands and feet
- **Severe infections:** Sepsis, meningitis and pneumonia; the risk of infection increases because the spleen does not function properly
- **Splenic sequestration crisis:** The spleen is the organ that filters blood; rapid enlargement of spleen can result due to entrapment of sickled cells and this condition can be life-threatening
- **Stroke:** Occurs when sickled cells block blood vessels within the brain.

Treatment of Sickle Cell Anemia

- Adequate hydration
- Analgesics
- Antibiotic therapy, if infection present
- Repeated transfusion
- Hydroxyurea—decreases the frequency of painful crises and reduces mortality.

Partial Advantage of Sickle Cell Trait

- Frequency of sickle cell anemia is high in black Africans
- Heterozygote state makes them less susceptible to falciparum malaria. The malaria parasite spends obligatory part of its life cycle in RBCs. In patients with sickle cell trait, RBCs with HbS have a shorter life span than normal RBCs. Thus, the parasite cannot complete the intracellular stage of development. This provides selective advantage to heterozygotes living in these regions where malaria is major cause of death.

14. Write briefly on anemia.

Definition: Anemia is characterized by a decrease in the normal number of RBCs to below normal levels or less than normal quantity of hemoglobin in the blood. This reduces the oxygen-carrying capacity of blood resulting in various signs and symptoms.

Consequences of Anemia (Fig. 22.7)

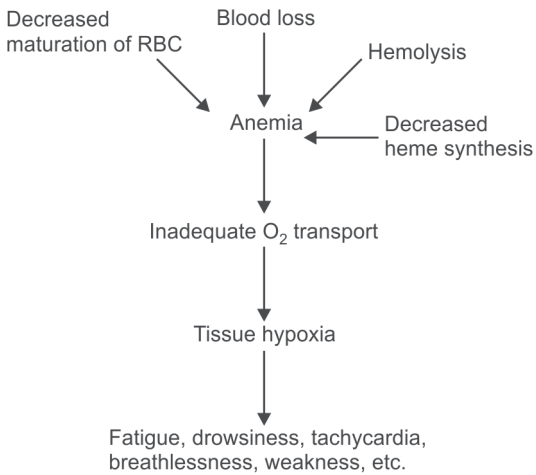


Fig. 22.7: Consequences of anemia

Classification of anemia is given in Table 22.6.

Table 22.6: Classification of anemia

Type	Causes	Consequences
Microcytic hypochromic anemia	• Deficiency of iron, copper, pyridoxine, pantothenic acid and vitamin C; thalassemia, lead poisoning • Chronic blood loss	• Decreased hemoglobin synthesis • Leads to iron deficiency
Macrocytic anemia	• Folic acid, vitamin B_{12} deficiency	• Decreased maturation of RBCs
Normocytic normochromic anemia	• Sickle cell disease, red cell metabolic defects, red cell membrane defects, niacin deficiency, riboflavin deficiency, G6PD deficiency	• Increased hemolysis
	• Acute bleeding	• Leads to loss of RBCs

Key Points

Heme-containing proteins: Hemoglobin, myoglobin, cytochromes, catalase, tryptophan pyrrolase.

Starting material for heme synthesis: Glycine + succinyl-CoA.

Number of glycine required to synthesize one molecule of heme: Eight.

Rate limiting enzyme in heme synthesis: α-aminolevulinate (ALA) synthase.

Mature RBCs cannot synthesize heme: Due to lack of mitochondria.

Lead poisoning: Lead inhibits ALA dehydratase and ferrochelatase.

Congenital erythropoietic porphyria: Only porphyria with autosomal recessive inheritance.

Acute intermittent porphyria: Is due to deficiency of uroporphyrinogen I synthase.

Precipitating factor for porphyrias: Barbiturates, alcohol consumption.

Enterohepatic circulation of bilirubin and bile salts: Small fraction of bilirubin and bile salts reabsorbed from intestine into circulation and resecreted into intestine.

Hemolytic jaundice (hemolysis): Elevated unconjugated bilirubin + absent urinary bilirubin + increased urobilinogen in urine.

Hepatic jaundice (viral hepatitis): Elevated conjugated and unconjugated bilirubin + elevated aspartate transaminase (AST) and alanine transaminase (ALT).

Obstructive jaundice (gallstones): Elevated conjugated bilirubin + increased urinary bilirubin excretion + urine urobilinogen absent.

Crigler-Najjar syndrome (type I): Due to complete absence of conjugating enzyme-UDP glucuronyl transferase.

Neonatal jaundice: Caused by inability of liver to conjugate bilirubin due to immaturity of conjugating system.

van den Bergh test: Is done to detect bilirubin in serum. Direct reaction detects the conjugated bilirubin and indirect reaction (adding methanol) measures total bilirubin.

Kernicterus: In newborns, when unconjugated bilirubin level gets elevated to > 20 mg/dL, it crosses the blood-brain barrier and damages the brain cells (mental retardation).

Adult hemoglobin: Is made of 2α and 2β chains (fetal Hb-2α and 2γ chains).

Myoglobin (heart and skeletal muscle): It is a hemoprotein with single polypeptide chain, which is structurally similar to individual polypeptide chains of hemoglobin. Myoglobin acts as a reservoir for oxygen.

α-thalassemia (4 genes for α-chain synthesis): Impaired synthesis of all α-chains results in HbH and hydrops fetalis, which is a severe form of α-thalassemia.

β-thalassemia (2 genes for β-chain synthesis): β-chain synthesis is impaired, β-thalassemia major is severe form in this group.

Manifestations of β-thalassemia appear only after birth: Because β-globin chain is not expressed until late gestation (fetal Hb-2α and 2γ chains).

Sickle cell disease (HbS disease): A point mutation in β-chain, which leads to substitution of glutamic acid by valine at 6th position. As a result of this, negative charge on the hemoglobin is reduced, which introduces sticky patch in Hb structure and its polymerization (sickling of RBCs) in venous blood.

Sickle cell trait individuals are less susceptible to *Plasmodium falciparum* malaria infection: Due to reduced life span of RBCs in such individuals, parasite cannot complete its life cycle.

HbC disease: Substitution of glutamic acid by lysine at 6th position of β-chain, leading to mild anemia.

HbSC disease: Compound heterozygote state with some β-chains having mutation found in HbS and others have mutations found in HbC.

Methemoglobinemia: Characterized by elevated methemoglobin (hemoglobin with iron in the ferric form), which may be due to nitrates, free radicals or congenital deficiency of enzyme NADH-cytochrome b5 reductase and substitution of proximal/distal histidine with tyrosine. Methemoglobin has low affinity for oxygen and causes cyanosis.

Hemoglobinopathy: Defect in the primary sequence of globin chain.

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