mitochondrial membrane is impermeable to small molecules, with the exception of oxygen, carbon-dioxide and water. All of the other substrates of mitochondrial metabolism, as well as its products, therefore have to be moved through the innermembrane with the help of special transporters. Embedded within the innermembrane of the mitochondria are various protein carriers, principally cytochromes.

The respiratory chain is organized in the form of multi-protein complexes. The inner space also contains ATP synthase and other enzymes. The inner membrane plays a major role in oxidative phosphorylation. The matrix of the mitochondrion is like a gel with ~50% protein. The matrix contains enzymes involved in the oxidation of pyruvate, amino acids, fatty acids, TCA cycle, as well as NAD+, FAD, ADP and Pi.

The energy liberated during the oxidation of carbohydrate and food is available in mitochondria as reducing equivalents (2H) that are collected by the respiratory chain for oxidation and coupled generation of ATP (Figure 8.2).

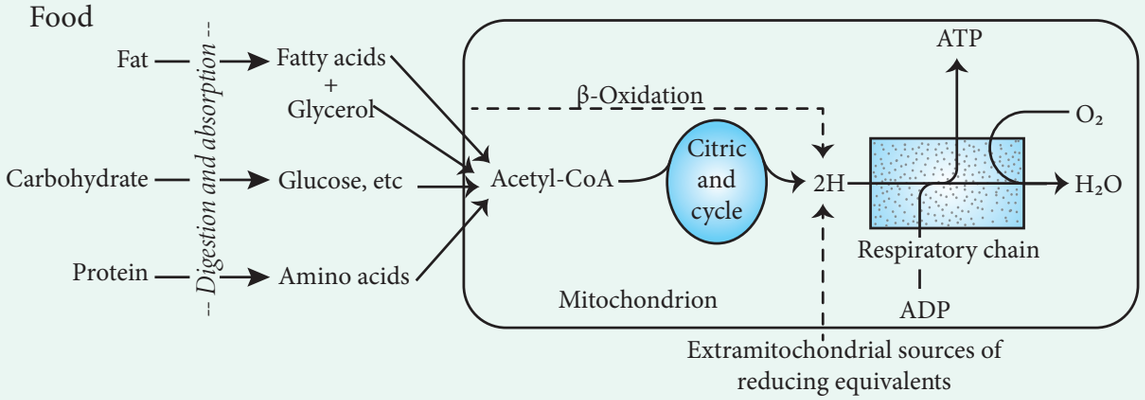


Fig.8.2. Digestion and absorption of food and production of energy

8.3.2 Components of the electron transport chain

The components of the ETC in eukaryotes are located in the inner mitochondrial membrane and organized into four large protein complexes (Figure 8.3). These complexes consist of several proteins and prosthetic groups. Apart from these, two other molecules, coenzyme Q and cytochrome c also play a significant role in the ETC. Complex I, also known as NADH-coenzyme Q oxidoreductase, or NADH dehydrogenase, catalyses the transfer of electrons from NADH to Coenzyme Q (CoQ or Ubiquinone UQ), because it is ubiquitous in biological systems). It contains one molecule of flavin mononucleotide (FMN) and 8 iron-sulfur clusters that participate in electron transfer. Complex II [Succinate Dehydrogenase or Succinate–Coenzyme Q Oxidoreductase] contains succinate dehydrogenase and three small hydrophobic subunits, and pass electrons from succinate to CoQ. Complex III is Coenzyme QH_2-cytochrome c oxidoreductase. It passes electrons from reduced CoQ to cytochrome c. It contains cytochromes b, c1 and one [2Fe-2S] cluster. Complex IV is cytochrome c oxidase. It catalyses the one-electron oxidation of four consecutive reduced cytochrome c molecules and four-electron reduction of

one oxygen molecule to yield 2 molecules of water. The flow of electrons through the complexes spanning I to IV results in pumping of protons from the matrix across the inner mitochondrial membrane into the intermembrane space. The proton-motive force that is generated powers ATP synthesis. Complex V catalyzes ATP synthesis.

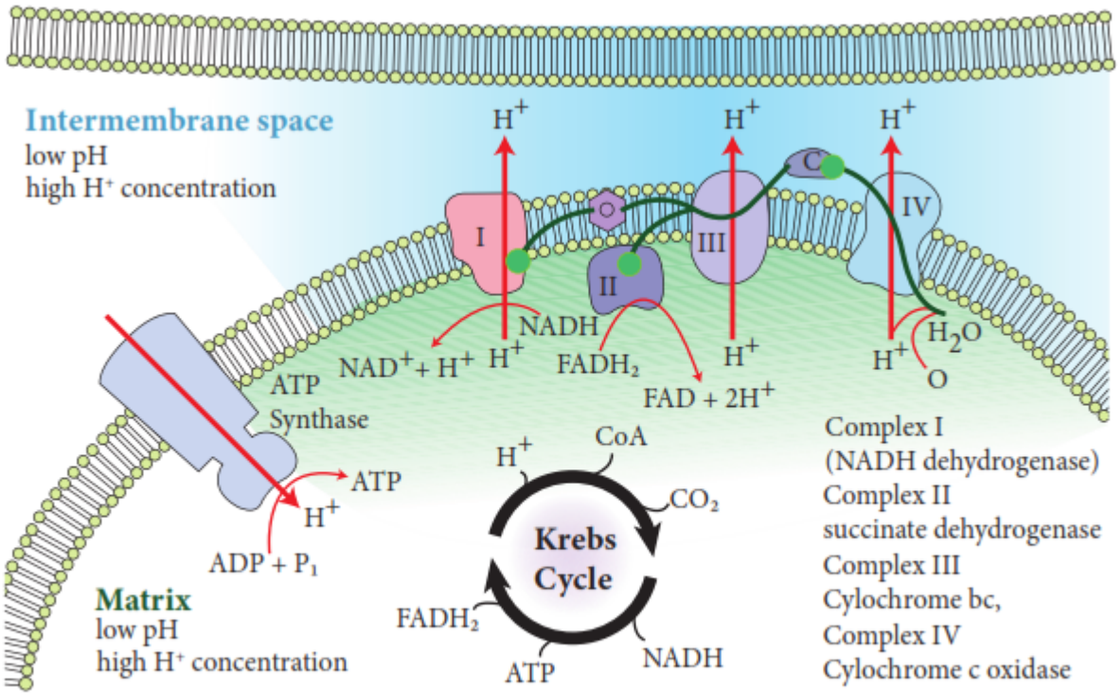


Fig.8.3. Overview of electron transport chain

A pair of electrons flowing from Complex I to Complex IV yields 3ATP, while a pair of electrons moving from Complex II to Complex IV yields 2ATP.

8.3.3 Reactions of Electron transport chain

Except coenzyme Q, all members of the respiratory chain are proteins. These proteins function as enzymes, for example, dehydrogenases, or they may contain iron as part of an iron-sulfur center. Also, they may contain iron co-ordinated to a porphyrin ring as in the cytochromes, or they may contain copper, as in cytochrome a + a3 complex.

Formation of NADH: NAD^+ is reduced to NADH by dehydrogenases. which remove two hydrogen atoms from the substrate. The major sources of NADH include several reactions of the citric acid cycle, fatty acid oxidation etc. Both electrons and one proton are transferred to the NAD^+ forming NADH and a free proton H^+.

NADH dehydrogenase: The NADH and H^+ formed are transferred to NADH dehydrogenase. This complex I, is the large protein complex comprises of 46 polypeptides. is buried in the mitochondrial inner membrane and has a bound molecule of flavin mononucleotide (FMN). NADH dehydrogenase reduces FMN to $FMNH_2$. The sequential transfer of two electrons to the iron sulfur center composed of iron atoms complexed with an equal number of sulfide ions releases four protons into the intermembrane space (Fig.8.4). NADH dehydrogenase

complex functions to accept high energy electrons from NADH. It acts as a proton pump that uses the movement of electrons to move hydrogen ions into the innermembrane space. These clusters are essential for the transfer of hydrogen to the next segment of the chain, Coenzyme Q.

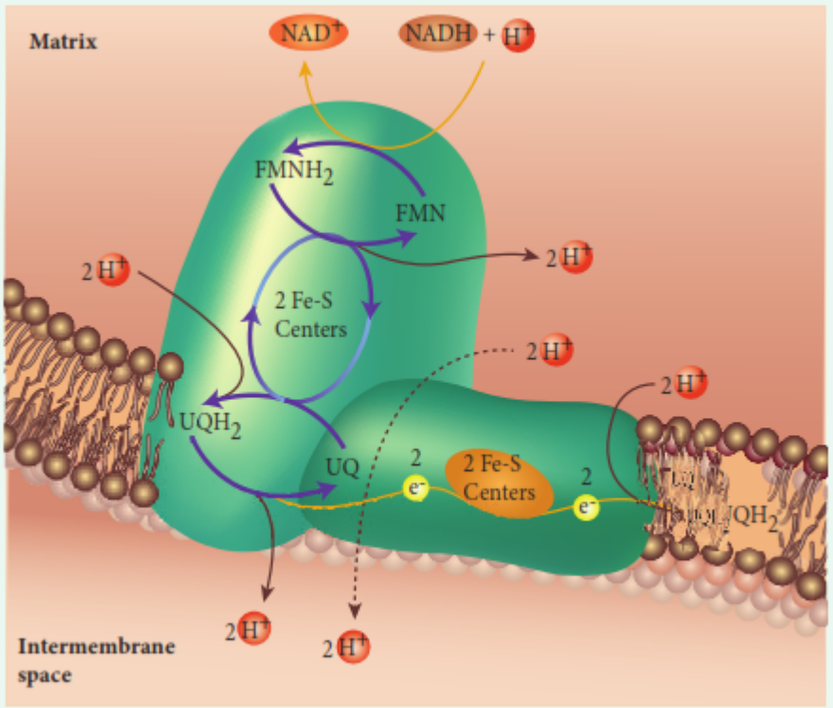


Figure 8.4 NADH dehydrogenase complex

Succinate reductase complex : This complex II contains the succinate dehydrogenase enzyme used in TCA cycle to transform succinate to fumarate and in the process form $FADH_2$. The $FADH_2$ stays in the complex, and gives two electrons, to a series of Fe-S cluster, that is transferred to ubiquionone (Fig.8.5). This complex is not a proton pump, it does not move the hydrogen ions across the membrane from matrix side to the intermembrane side of mitochondria.

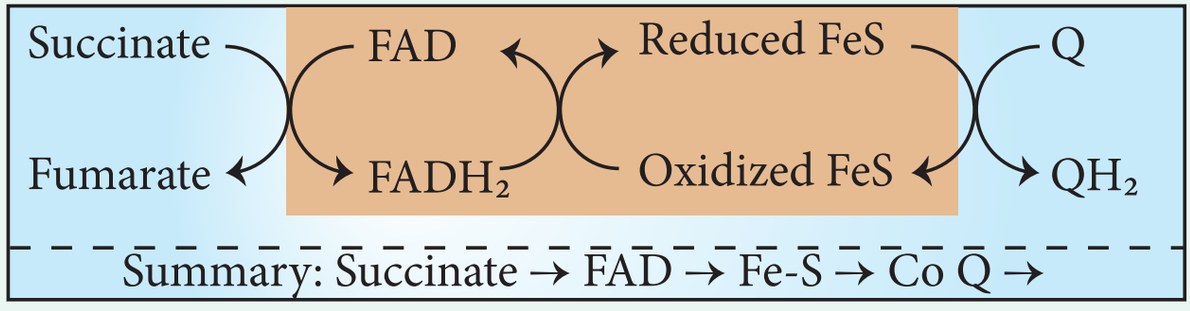


Fig.8.5 Reactions involved in complex II

Coenzyme Q : The succinate dehydrogenase complex of Complex II consists of the TCA cycle enzyme succinate dehydrogenase and two iron sulphur centers. The transfer of electrons from succinate to Coenzyme Q (UQ) is mediated in complex II via the iron sulphur centers. Co Q can accept hydrogen atoms from $FMNH_2$ of NADH dehydrogenase (Complex I) and $FADH_2$ from succinate dehydrogenase (Complex II). Further, electrons from cytoplasmic NADH are also transferred to ubiquinone (UQ) through glycerol 3 phosphate dehydrogenase and acyl coA dehydrogenase (Fig.8.6).

Cytochrome reductase : Electrons from reduced ubiquinone (UQH_2) are passed to cytochrome c via Complex III. Cytochromes are proteins with heme prosthetic group. All cytochromes have the tendency to act as electron carriers. Once the electron is accepted, the iron atom of heme group changes from Fe^{3+} to Fe^{2+} state. The pathways of electrons transfer from UQH_2 to cytochrome c is quite complex. As represented in Fig.8.7, UQH_2 is oxidized to UQ in a multistep process. One electron is transferred to cytochrome c via Rieske Fe-S protein / cyt c1 and the second electron is transferred to Cyt b.

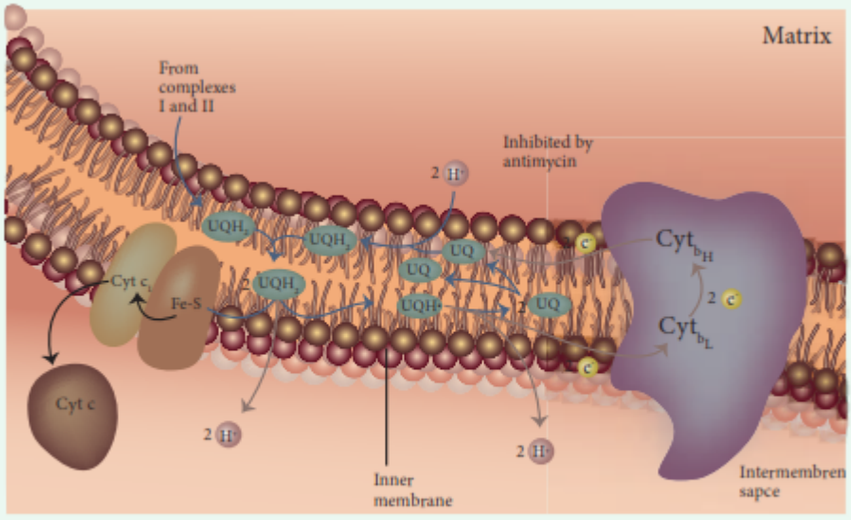


Figure 8.7 Overview of reactions in complex III

One of the two molecule of UQ produced diffuses to the matrix side of mitochondria where it is reduced to form UQH_2. The so formed UQH_2 diffuses to its oxidation site and joins the pool of UQH_2 from complexes I and II. During this process, 2H is released into the intermembrane space.

Cytochrome c oxidase: Cytochrome c oxidase of Complex IV catalyzes the one electron oxidation of four consecutive reduced cytochrome c molecules and the concomitant four electron reduction of one oxygen molecule. Cytochrome c oxidase contains two cytochromes (a and b). Cyt a is paired with copper atom, CuA and cyt a3 is paired with a different copper atom, CuB. Each of the reduced Cytc molecules donates two electrons, one at a time to CuA. The electrons are further transferred to cyt a, cyt a_3, CuB. The transfer of four electrons from cyt c converts oxygen and four protons to two molecule of water (Fig.8.8).

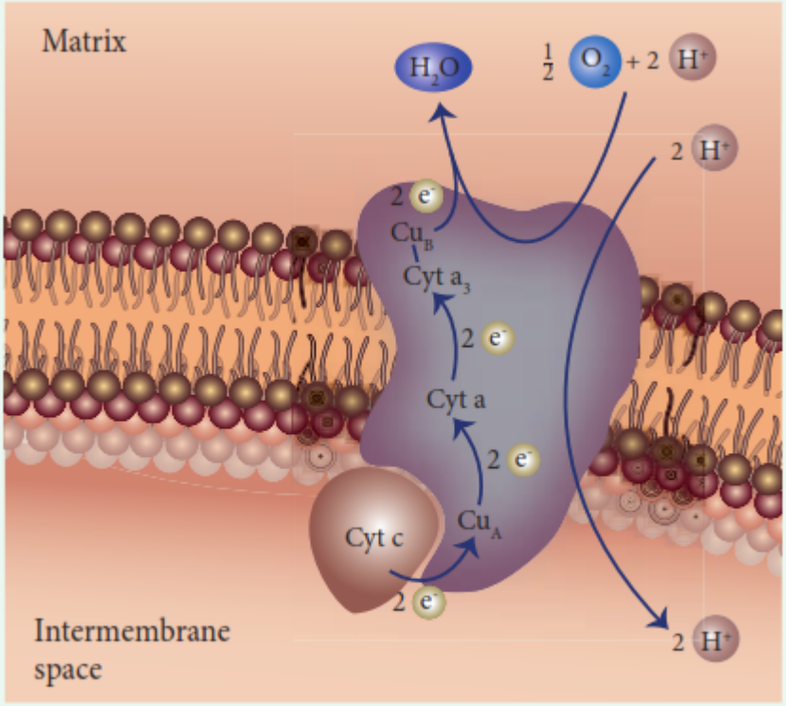


Fig.8.8 Overview of reactions in Complex IV

Complex No.	Complex Name	Polypeptides	Prosthetic groups
Complex I	NADH dehyrogenase	Around 46	FMN, Fe-S Centres
Complex II	Succinate dehydrogense	2 Fe-S Proteins	FAD, Fe-S Centres
Complex III	Cytochrome b c_1 complex	2 Cytocrhomes 1 Fe-S protein 6-8 other proteins	b and c type hemes (cyt b_1, b_H, c_1) Fe-S centres
Complex IV	Cytochrome oxidase	Around 13	a type hemes (cyt a,a_3) 2 Cu

8.3.4 Inhibitors of the Electron transport chain

ETC may be blocked by many inhibitors. ETC inhibitors act by binding a suitable area in the ETC. They prevent electrons from being passed from one carrier to the next one. Each inhibitor binds a particular carrier For example, rotenone and amytal inhibits complex I at NADH dehydrogenase and prevent NADH oxidation. Antimycin A and dimercaprol inhibit ETC at complex III. Poisons such as hydrogen sulphide, Cyanide, azide and carbon monoxide inhibit Complex IV. Oligomycin is a ATP synthase inhibitor. Many of the details of respiratory chain were obtained using inhibitors of ETC. The correct order of the ETC components was determined using inhibitors. Using oxygen electrode, the extent of electron transport was measured. During this process, when electron transport is inhibited, oxygen consumption is diminished. (Fig.8.9).

8.4 OXIDATIVE PHOSPHORYLATION

During aerobic cellular respiration, most of the ATP is generated by oxidative phosphorylation where electrons derived from NADH and $FADH_2$ are oxidised by electron transport through the respiratory chain. In oxidative phosphorylation, carbon fuels are oxidized in TCA cycle to give electrons with high transfer potential. Eventually, this electro-motive force is converted into a proton-motive force and, finally, into phosphoryl transfer potential.

The conversion of electro-motive force into proton-motive force is carried out by three electron-driven proton pumps: NADH-Q oxidoreductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase. These large transmembrane complexes contain multiple oxidation-reduction centers, including quinones, flavins, iron-sulfur clusters, hemes, and copper ions. The final phase of oxidative phosphorylation is carried out by ATP synthase, an ATP-synthesizing assembly that is driven by the flow of protons back into the mitochondrial matrix. Components of this remarkable enzyme rotate as part of its catalytic mechanism.

Oxidative phosphorylation clearly shows that proton gradients are an interconvertible currency of free energy in biological systems. Oxidative phosphorylation is different from substrtate level phosphorylation, as it does not involve phosphorylated chemical intermediates and uses a different mechanism known as chemiosmotic hypothesis.

8.4.1 Chemiosmotic theory

In 1961, Peter Mitchell proposed the Chemiosmotic theory which demonstrates that the energy derived from oxidation of components in the ETC is coupled to the translocation of protons from the inside to the outside of the inner surface of the mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP. As explained earlier, the complexes of the respiratory chain act as proton pumps.

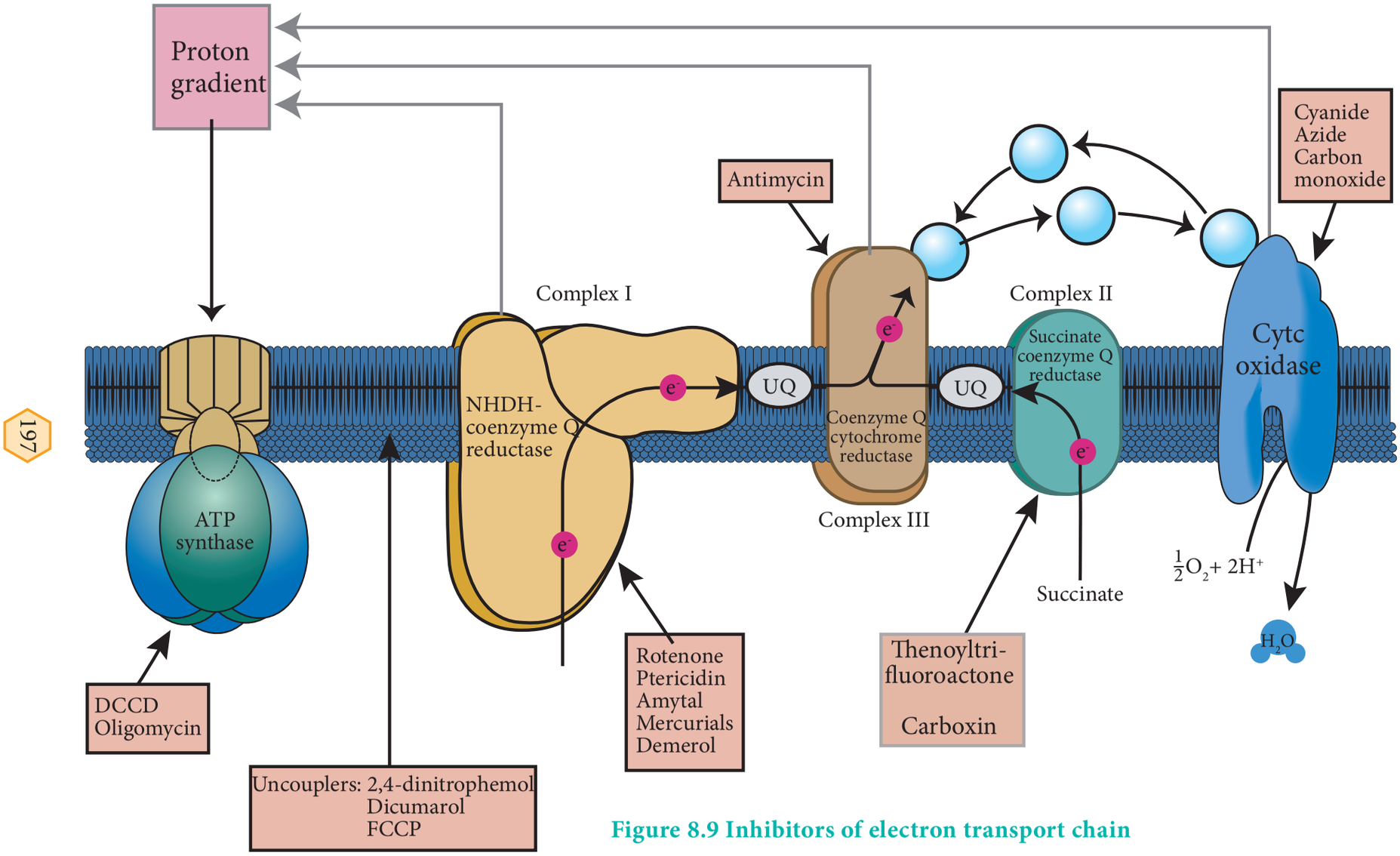


Figure 8.9 Inhibitors of electron transport chain

The inner membrane of mitochondria is impermeable to ions, particularly to protons, which accumulate outside the membrane, thereby creating an electrochemical potential difference across the membrane (ΔμH+). This is called as chemical potential and electric potential.

The chemiosmotic coupling theory has the following features:

1. As electrons pass through the ETC, protons are transported from the matrix and released into the intermembrane space. An electrical potential (ψ) and proton gradient (ΔpH) arise across the inner membrane. The electrochemical proton gradient is called as proton motive force.
2. Protons, now in greater excess in the intermembrane space, pass directly through the innermembrane and back to the matrix, down the concentration gradient, via specific channels, thereby producing ATP.

8.4.2 Role of ATP synthase

The enzyme complex ATP synthase is known as Complex V. It synthesizes ATP using the energy of the proton gradient generated by the electron transport chain. The role of this ATPase is to bring back the protons to the mitochondria. The interior of a mitochondrion is alkaline and the reaction is favourable to drive the synthesis of ATP.

The proton-flow through the F_1F_0 ATPase is required to release ATP from the active site where it was synthesized from ADP and P_i. The ATP made in the interior of the mitochondria must be exchanged for ADP outside the mitochondria to keep the cytosol supplied with ATP. The exchange of mitochondrial ATP for cytoplasmic ADP is catalyzed by the ATP/ADP translocase. The complete transfer of 2 electrons from NADH through the entire ETC to oxygen generates 3 ATPs. One $FADH_2$ feeds electrons into coenzyme Q after the first ATP-generating step. Flavin-linked substrates generate only 2 ATPs per 2 electrons transferred down the chain. Flavin-linked substrates generate only 2ATPs, not only because they feed electrons after the first ATP has already been made, also because $FADH_2$ is not as strong a reducing agent as NADH. There is not enough energy in the oxidation of $FADH_2$ to generate 3 ATPs.

8.5 HIGH ENERGY COMPOUNDS

High energy compounds are usually referred to as high-energy phosphates. Inorganic phosphate groups form high energy bonds which have the ability to break, provide energy, and run the metabolic processes of life. The hydrolysis of high energy phosphate bonds releases energy. For a reaction to be feasible, ΔG (Change in Gibbs free energy) value must be negative. Compounds that contain phosphate are known as high-energy entities with a large negative value of ΔG of the order of −25 or −30 kJ/mol.

8.5.1 Storage form of high energy compounds

Living organisms use two main types of energy storage. First one is energy-rich molecules such as glycogen and triglycerides. They store energy in the form of covalent chemical bonds. Cells synthesize such molecules and store them for later release of the energy. The second form of biological energy storage is electrochemical reactions and takes the form of gradients of charged ions across cell membranes.

8.5.2 ATP as a high energy compound

Adenosine triphosphate (ATP) is the chief carrier of energy in cells. Hydrolysis releases energy from the chemical bonds in ATP to fuel cellular processes. When energy is needed by the cell, it is converted from storage molecules into ATP. The high energy of ATP is because of its two high-energy phosphate bonds. The bonds between phosphate molecules are called phosphoanhydride bonds. Structure of ATP is represented in Figure 8.10.

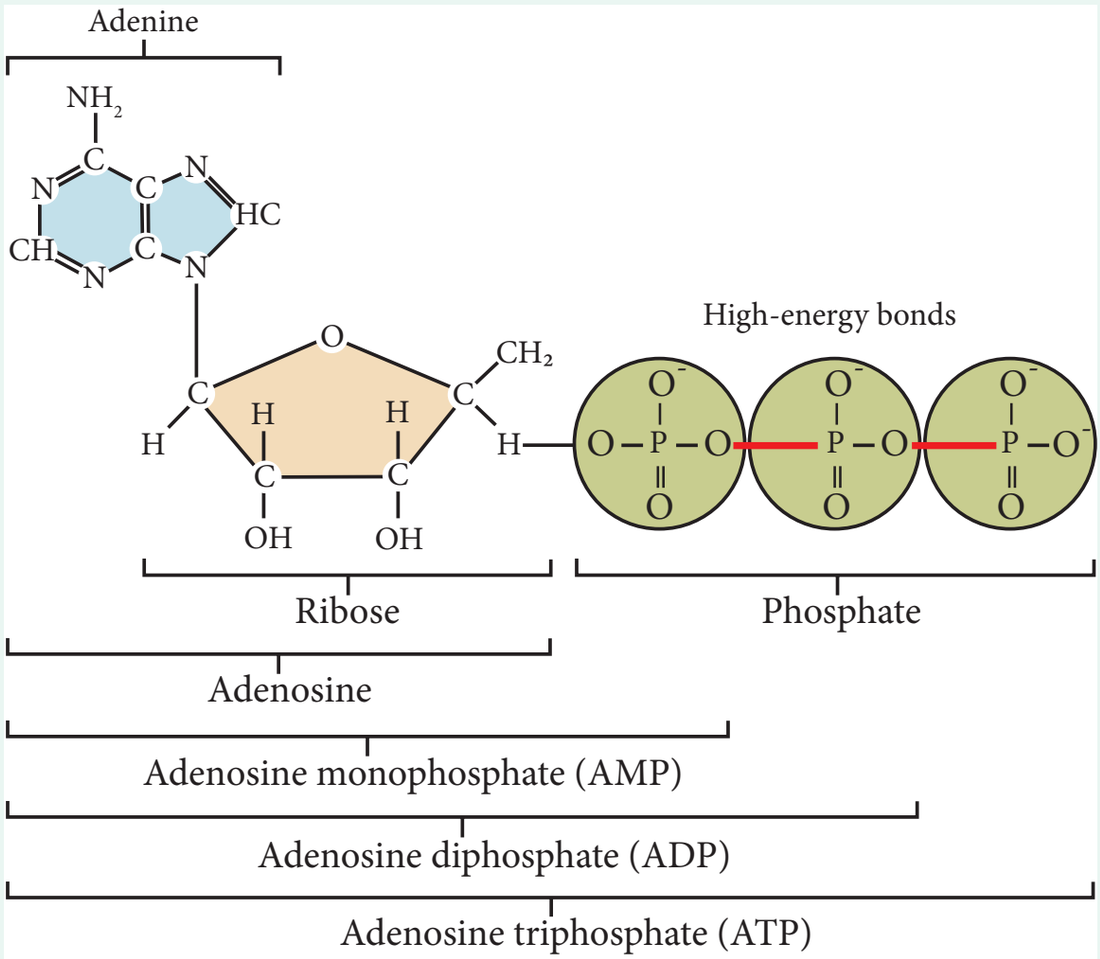


Fig.8.10 Structure of Adenosine phosphates

8.5.2.1 Structure of ATPase (F_1F_0 ATPase)

Mitochondrial ATP synthase is an F-type ATPase. It is quite unique in its structure compared to other ATP synthases derived from chloroplast and eubacteria. Looking into the structure, it is a large enzyme complex found in the inner mitochondrial membrane, which catalyzes the formation of ATP from ADP and Pi. As demonstrated in Fig.8.11, ATP synthase has two discrete components: F_1 (catalytic unit), a peripheral membrane protein, lies in the mitochondrial matrix and is made up of five type of polypeptide chains (α_3, β_3, γ, ε and δ chains), and F_o (o denoting oligomycin-sensitive), is an integral membrane protein which is composed of three subunits, a, b, and c, in the proportion ab_2c_{10-12}.

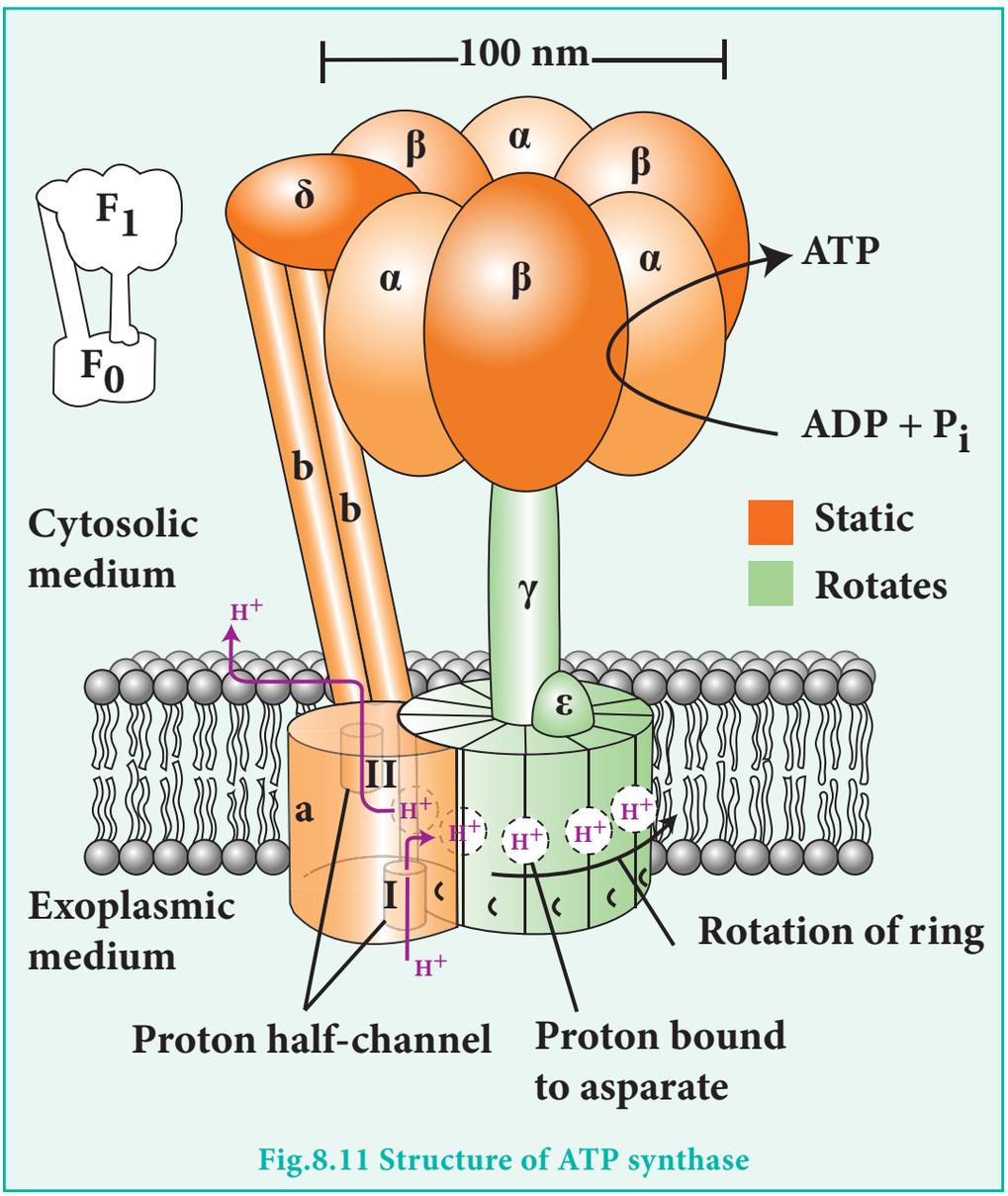


Fig.8.11 Structure of ATP synthase

The critical subunits namely, β possesses ADP / P_i binding sites, α subunit can bind ATP; γ subunit rotates to change to alpha and beta conformations. c subunit of F_0 contains proton half channels; 'a' is the site of proton entry into 'c' subunits.

F1 catalytic unit: Three α and three β chains combine to form hexameric $\alpha_3\beta_3$ that is responsible for catalyzing the synthesis of ATP. The γ and ε polypeptide chains organize to form the central stalk and run to the inner cavity of the hexameric ring. The γ subunit helps in holding the $\alpha_3\beta_3$ and manifests rotating of the machine. This complex harness energy released by the electron transport to drive the synthesis of ATP, whereas, while alone, the F_1 component hydrolyses ATP.

F0 region of proton pump: It consists of 10-14 c subunits organized into a ring-like structure and acts as a proton channel. A single 'c' unit connects F_0 to F_1 unit. F_0 and F_1 are connected at two points through γε central stalk and through the arm formed by 'a' subunit, two b subunits and one δ subunit.

8.5.2.2 Free energy of hydrolysis of ATP

Among phosphate-containing molecules, ATP is an important molecule. It exchanges energy between enzymes. ATP is an unstable molecule which hydrolyzes to ADP and inorganic phosphate. The high energy of this molecule comes from the two high-energy phosphoanhydride bonds.

The hydrolysis of ATP has a $\Delta G^{\circ\prime}$ of –30.5 kJ/mol. In addition, since the cells contain higher concentration of ATP than ADP, the ΔG for the reaction is more negative than the $\Delta G^{\circ\prime}$ value. ATP hydrolysis can therefore donate energy to other systems to allow those systems to perform reactions that would otherwise be thermodynamically unfavorable.

The phosphate bond holds usable energy. The hydrolysis of a phosphate which is responsible for release of free adenosine from AMP has a $\Delta G^{\circ\prime}$ of –14 kJ/mol; this is not considered to be a high-energy phosphate bond; and is emphasized by drawing the bond as a straight line.

The net result is that ATP hydrolysis can act as a thermodynamic driving force to allow thermodynamically unfavorable reactions that do not involve phosphate transfer to proceed. Though ATP is the most widely used high-energy compound, there are other high-energy compounds of physiological significance. An example is the high-energy thioester bond of acetyl-CoA.

8.6 UNCOUPLERS

Chemicals such as 2,4 dinitrophenol (DNP) act as uncoupling agents, which means they stop the synthesis of ATP, but ETC still continues and so oxygen is still consumed. DNP and other uncouplers are lipid soluble small moieties and can bind H^+ ions and transport them across membranes.

Summary

In eukaryotes, reactions of aerobic energy occur in mitochondria. An inner membrane separates the mitochondria into two spaces: matrix and intermembrane space. ETC process in the innermembrane oxidises NADH and succinate, utilizing oxygen and generates ATP. The scheme of respiratory chain and its coupling to ATP production can be summarized as follows:

1. Chemical reactions that transfer electrons between reactants are called oxidation-reduction reactions, or redox reactions.

2. During oxidation, a substance loses electrons, or is oxidized. In reduction, a substance gains electrons, or is reduced.

3. Electron transport chain is a step wise process comprising of four complexes. A series of flavoproteins, cytochromes, iron-sulfur clusters and quinone constitute the electron transport chain.

4. Complex I transfers electron from NADH to the electron carrier ubiquinone and complex II transfers electrons from succinate to ubiquinone

5. Complex III is comprised of three important cytochromes known as cytochromes bL, bH and c plus an iron sulphur center. These transfer electrons from reduced ubiquinone (UQH2) to chytochrome c

6. Complex IV contain two cytochromes (a and a3), two copper atoms, and transfers electrons to oxygen

7. Several poisons such as MPTP, rotenone, azide and cyanide inhibit the electron transport chain

8. During oxidative phosphorylation electrons derived from NADH and FADH2 combine with oxygen and the energy released is used to drive the synthesis of ATP from ADP.

9. ATP synthase is a reversible coupling device that can convert the energy of the electrochemical proton gradient into chemical bond energy or vice versa.

10. The transfer of energy from NADH/FADH2 to O2 yields high energy of ΔG equal to -52.5kcal/mol from each pair of electrons transferred.

EVALUATION

I Multiple Choice Questions

1. During aerobic cellular respiration, most of the ATP made, is generated by

   a. Substrate level phosphorylation
   b. Pyruvate kinase
   c. Glycolysis
   d. Oxidative phosphorylation

2. The prosthetic group of NADH dehydrogenase is

   a. FMN
   b. NADH
   c. FAD
   d. NADPH

3. The chemiosmotic theory involves all except

   a. A membrane impermeable to protons
   b. Electron transport by the respiratory chain pumps protons out of mitochondria
   c. Proton flow into the mitochondria depends on ADP + Pi
   d. Only proton transport is strictly regulated. other positively charged ions can easily diffuse freely across the mitochondrial membrane

4. Which of the following accepts only one electron?

   a. Cytochrome b
   b. Coenzyme Q
   c. FMN
   d. FAD

5. Loss of electrons can be termed as

   a. Metabolism
   b. Anabolism
   c. Oxidation
   d. Reduction

6. Which complex does not pump protons

   a. NADH dehydrogenase complex I
   b. Succinate dehydrogenase complex II
   c. cytochrome reductase complex III
   d. cytochrome oxidase complex IV

7. An uncoupler of oxidative phosphorylation is

   a. DNP
   b. DTT
   c. azide
   d. Rotenone

II Give short answer for the following

1. Explain Redox reactions
2. Outline the structure of mitochondria
3. Describe the inhibitors of electron transport chain
4. What do you understand by oxidative phosphorylation

5. Describe high energy phosphate bonds.

III Answer the following

1. Give a diagrammatic representation of electron transport chain showing the flow of electrons
2. Describe the structural features of ATP synthase.

Students Activity

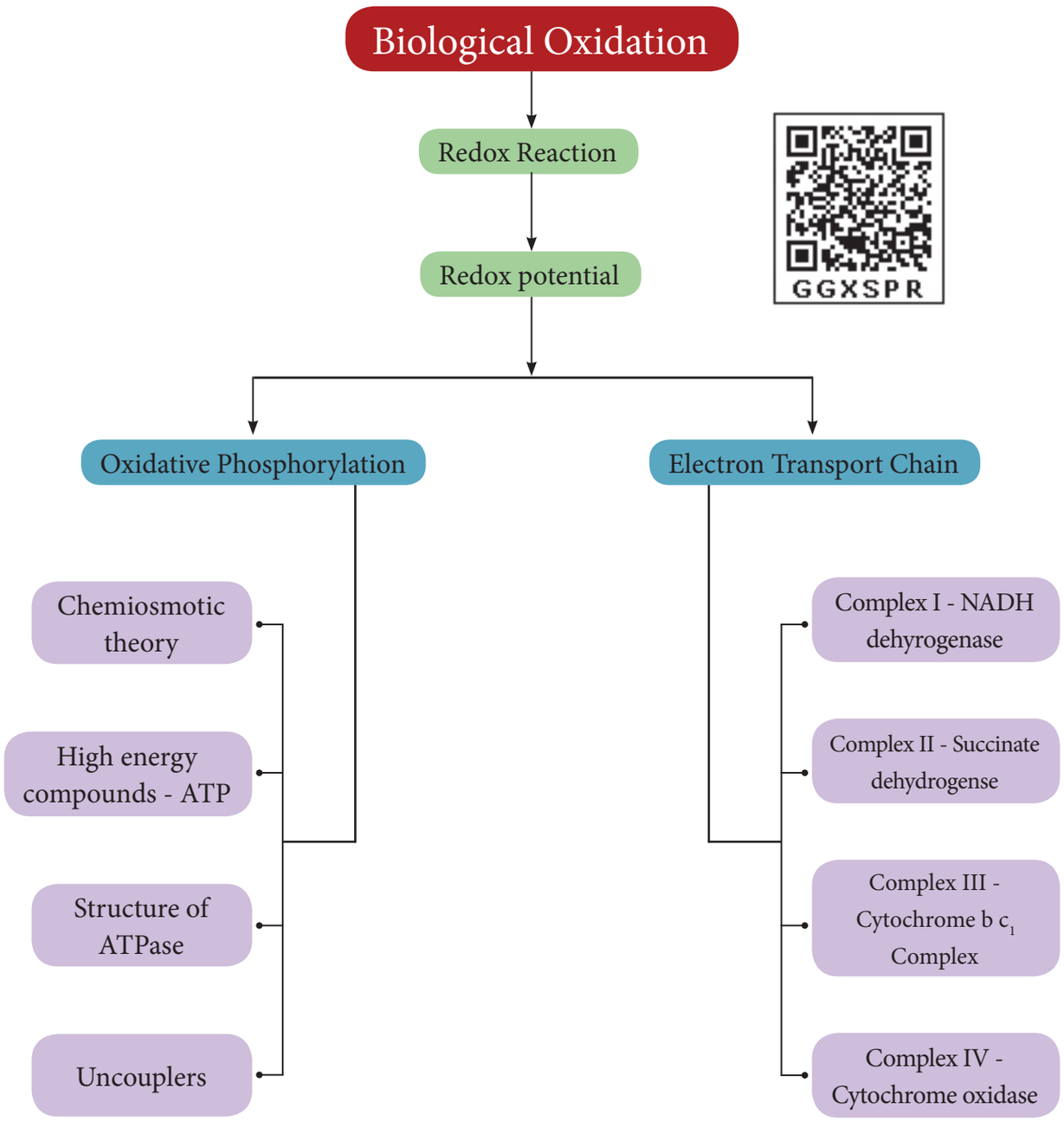
Using a cardboard, prepare a mitochondrion and label its parts.

Using a chart, schematically present the components of electron transport chain.

References

1. Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's illustrated Biochemistry, 30th Edn. Mc.Graw Hill Publishers.
2. Lehninger, Nelson DL and Cox MM. Principles of Biochemistry. II Edn.
3. Mckee T, McKee JR. Biochemistry. The Molecular basis of life. Third Edn. Mc.Graw Hill Publishers.
4. Voet D, Voet J, Pratt CW. Fudamentals of Biochemistry. Wiley Publishers.
5. Talwar GP. Srivastava LM. Text book of Biochemistry and human biology, Prentice Hall India Learning Private Limited; 3 edition (2002)
6. S.Nagini, Textbook of Biochemistry.

CONCEPT MAP



UNIT 9

ENZYME KINETICS

Jon Jakob Berzelius James B. Sumner

In 1835 by the Swedish chemist Jon Jakob Berzelius who termed their chemical action catalytic. It was not until 1926, however, that the first enzyme was obtained in pure form, a feat accomplished by James B. Sumner of Cornell University. Sumner was able to isolate and crystallize the enzyme urease from the jack bean. His work was to earn him the 1947 Nobel Prize.

TDJMEM

Learning Objectives

After studying this unit the students will be able to

- Derive the Michaelis – Menten equation.
- Understand the types of enzyme inhibitors.
- Explain the differences between competitive, non-competitive and un-competitive inhibition

INTRODUCTION

In Class XI you have already learnt about enzymes as biocatalysts. Enzymes are generally considered as essential components for biological reactions.

One of the most captivating areas of study in chemical kinetics is enzyme catalysis. The phenomenon of enzyme catalysis usually results in a very large increase in reaction rate and high specificity. By specificity, we mean that an enzyme is capable of selectively catalysing certain reactants, called substrates.

The specificity of an enzyme for one of the two enantiomers depends on which one fits better than the other into the active site of the enzyme (Figure 9.1).

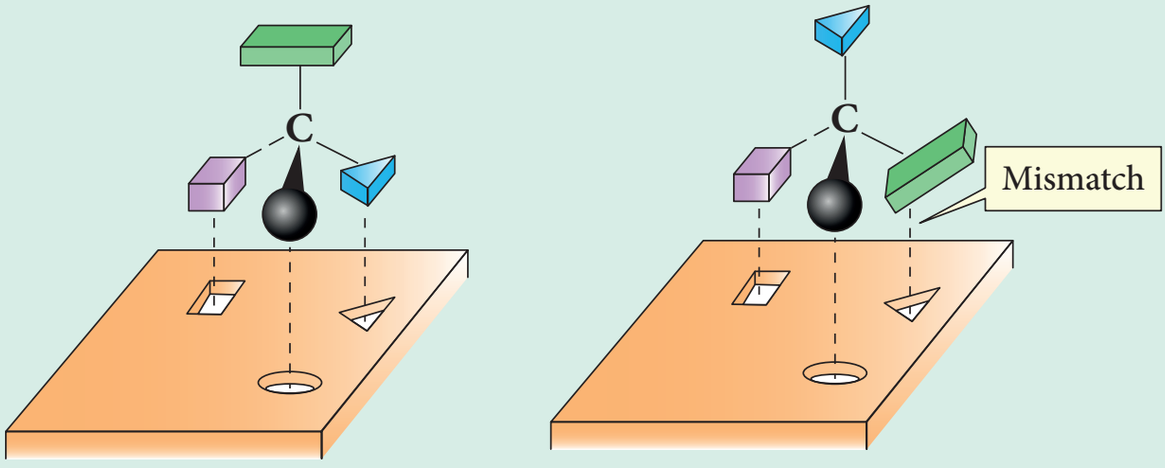


Figure 9.1 Specificity of an enzyme for its substrate

Activation energy is a term coined in 1889 by the Swedish scientist Arrhenius to describe the minimum energy which must be available to the reactants to result in a chemical reaction. An uncatalyzed reaction requires a higher activation energy than does an enzyme-catalyzed reaction (Figure 9.2)

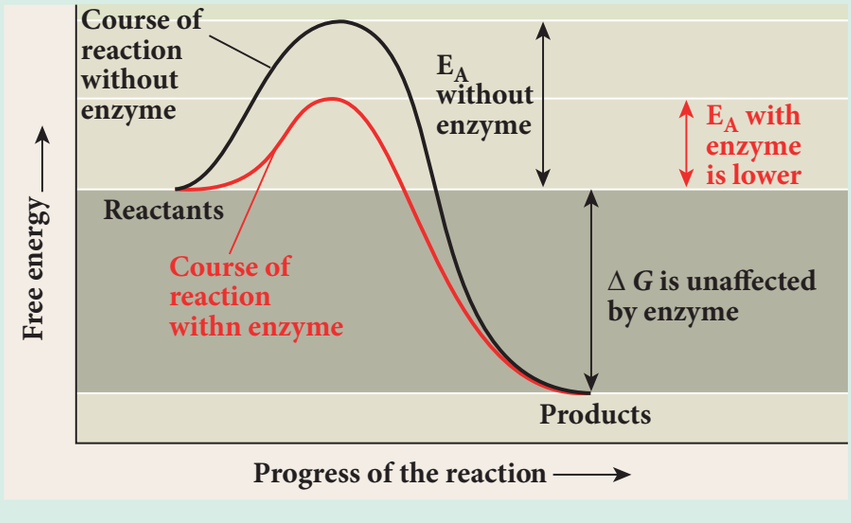


Figure 9.2 Enzymes lower the activation energy barriers.

One important factor which affects the enzyme activity is temperature. All enzymes have the highest activity at a particular temperature which is known as optimum

temperature. Above this temperature enzymes get destroyed and below this they remain inactive (Figure 9.3a). The second important factor is pH. Enzymes work at a specific pH known as optimum pH. For example, digestive enzymes work well at acidic pH and intracellular enzymes work better at neutral pH (Figure 9.3b). The other factors are enzyme and substrate concentration, if the initial concentration is high it gradually increases the reaction rate (Figure 9.3c).

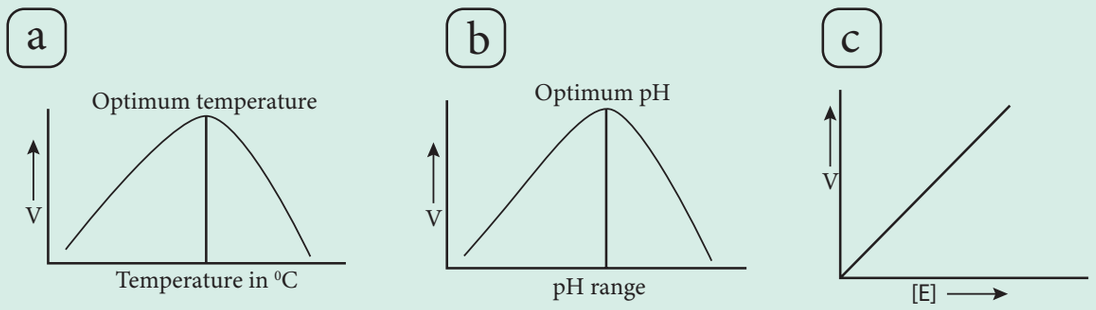


Figure 9.3 Factors affecting enzyme activity

9.1 DERIVATION OF MICHAELIS - MENTEN EQUATION

To understand the mechanism of any catalytic reaction, one should study the kinetic behaviour of the reaction systems, where the rate of the reaction can be obtained at various concentrations of the enzyme and the substrate. Formation of enzyme substrate complex is a critical event in an enzyme-catalyzed reaction. Michaelis and Menten proposed that the binding of the substrate and the enzyme is reversible and derived a kinetic model for a simple single substrate enzymatic reaction. This laid the basis for understanding the various factors that influence the rate of the reaction, such as concentration of substrate, temperature and pH of the system.

The kinetics of enzyme catalyzed reactions is explained by Michaelis- Menten equation. It can be derived with the following assumptions:

1. The concentration of enzyme is much lower than the concentration of the substrate.
2. The initial rate (or initial velocity), designated v_0, when [S] is much greater than the concentration of enzyme [E], is measured
3. None of the products formed revert to the initial substrate.
4. A steady-state condition is reached between the formation of ES complex and its decomposition.

The enzyme (E) combines with the substrate (S) to form ES complex with the rate constant k_1. The ES complex can dissociate either into 'E' and 'S' with the rate constant k_2 or it can form the product (P) with the rate constant k_3.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

If, only the initial period of the reaction is considered, the product concentration [P] is negligible and the formation of ES from product can be ignored.

The velocity of the overall enzymic process

$$v = k_3[ES] \quad ...(1)$$

Rate of formation of ES = $k_1[E]\,[S]$

Rate of breakdown of ES = $[k_2+k_3]\,[ES]$

Under steady state conditions,

Rate of formation of ES = Rate of breakdown of ES

(i.e) $k_1[E][S] = [k_2 + k_3]\,[ES]$

$$[ES] = \frac{(k_1[E][S])}{(k_2 + k_3)}$$

$$[ES] = \frac{([E][S])}{\frac{(k_2 + k_3)}{k_1}} = \quad ...(2)$$

Where, $K_m = \frac{(k_2 + k_3)}{k_1}$, is a constant known as Michaelis constant.

The concentration of uncombined enzyme [E], is equal to the total enzyme concentration [ET] minus concentration of ES complex, (i.e) [E] = [ET] – [ES]

Substitute this value of [E] in equation (2), we get

$$[ES] = \frac{(\{[E_r]-[ES]\}\,[S])}{K_m} \quad ...(3)$$

$$[ES]\,K_m = [E_T]\,[S] - [ES]\,[S]$$

$$[ES]K_m + [ES]\,[S] = [E_T]\,[S]$$

$$[ES]\,(K_m + [S]) = [E_T]\,[S]$$

$$\therefore [ES] = \frac{([E_T][S])}{(K_m + [S])}$$

Substitute this value of [ES] in equation (1)

$$v = \frac{(k_3[E_T][S])}{(K_m + [S])} \quad ...(4)$$

The velocity becomes maximum velocity (V_{max}) when the catalytic sites on the enzyme are saturated with substrate.

(i.e) when [S] >>> K_m;

K_m + [S] = [S]

Therefore, $V_{max} = \frac{(k_3[E_r][S])}{([S])} = k_3[ET]$

Substitute this value of $k_3[ET]$ in equation (4)

$$V = \frac{(V_{max}[S])}{(K_m + [S])} \quad ...(5)$$

This equation is known as Michaelis - Menten equation.

Enzymes may be denatured by excessive heat, extreme pH or various chemicals. Such extremities or chemicals cause enzymes to denature by the disruption and possible destruction of both the secondary and tertiary structures. Such changes are irreversible.

9.1.1. Significance of Michaelis - Menten Equation:

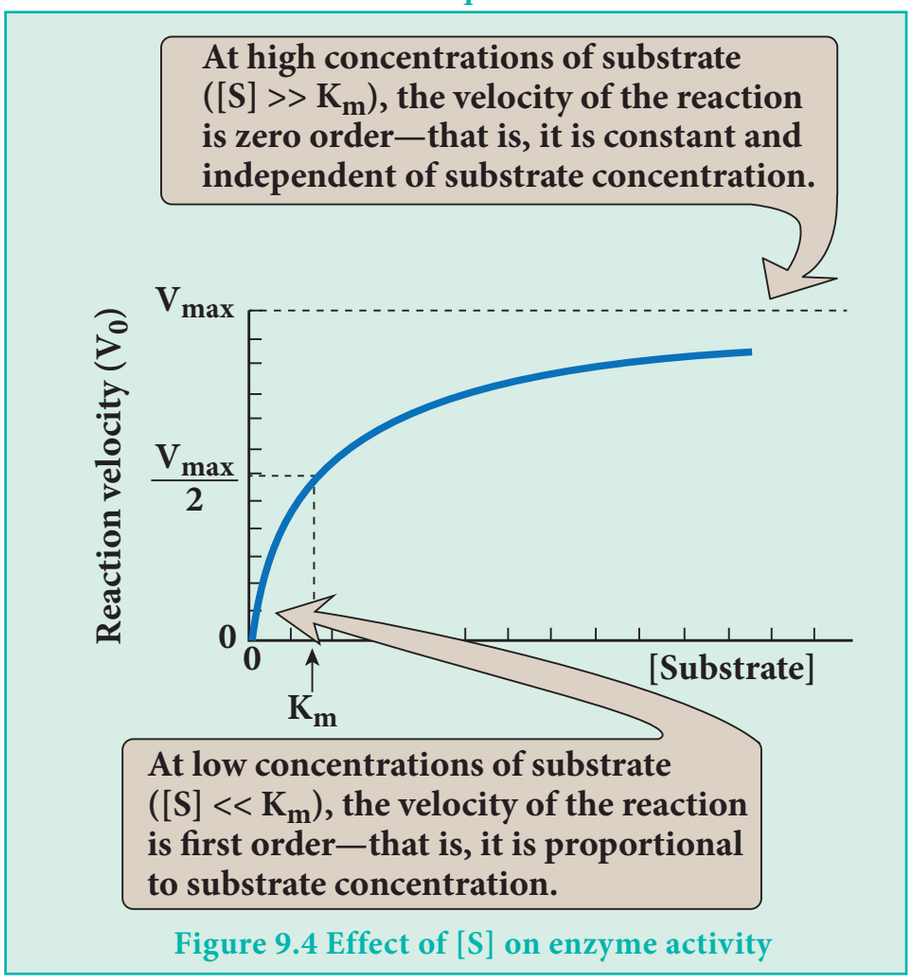


Figure 9.4 Effect of [S] on enzyme activity

Michaelis - Menten equation accounts for the three different phases of the substrate - concentration graph.

i) When [S] in very much less than K_m:

The Michaelis-Menten equation is

$$v = \frac{(V_{max}[S])}{(K_m + [S])}$$

When [S] << K_m; the equation becomes

$$v = \frac{(V_{max}[S])}{(K_m)}$$

When both the constants 'V_{max}' and 'K_m' are replaced by another constant 'K' the above equation becomes v = K[S]

i.e. the velocity is directly proportional to the substrate concentration (first order) (Fig 9.4)

ii) When [S] is very much greater than K_m:

$$v = \frac{(V_{max}[S])}{(K_m + [S])}$$

When [S] >> K_m the equation becomes,

$$v = \frac{(V_{max}[S])}{([S])} = V_{max}$$

The velocity is independent of substrate concentration (zero order) (Fig 9.4)

iii) When [S] = K_m : (Derivation of K_m):

$$v = \frac{(V_{max}[S])}{(K_m + [S])}$$

When K_m = [S]

$$v = \frac{(V_{max}[S])}{([S]+[S])} = \frac{(V_{max}[S])}{(2[S])} = ½\ V_{max}$$

Thus K_m = [S] at ½ V_{max}

9.1.2. Significance of K_m : (Michaelis-Menten Constant)

K_m is defined as the substrate concentration at half maximal velocity.

At ½ V_{max}, K_m = [S]

K_m is also defined as

$$K_m = \frac{(k_2 + k_3)}{k_1}$$

k_1 is the rate constant for the formation of ES complex,

k_2 is the rate constant for the dissociation of ES complex into E & S and

k_3 is the rate constant for the dissociation of ES complex into P.

K_m is a measure of the strength of ES complex. A high value of K_m indicates weak binding and a low value of K_m indicates strong binding between enzyme and substrate. The K_m values of a few enzymes are given below.

ENZYME	SUBSTRATE	K_m(mM)
Aspartate aminotransferase	Aspartate α-Ketoglutarate Oxaloacetate Glutamate	0.9 0.1 0.04 4
Threonine deaminase	Threonine	5
Arginyl-tRNA synthetase	Arginine $tRNA^{Arg}$ ATP	0.003 0.0004 0.3
Pyruvate carboxylase	HCO_3^- Pyruvate ATP	1.0 0.4 0.06
Penicillinase	Benzylpenicillin	0.05
Lysozyme	Hexa-*N*-acetylgulucosamine	0.006

Note: For a given enzyme K_m is a constant for a specific substrate.

9.1.3. Lineweaver - Burk Equation:

The Michaelis - Menten equation is

$$v = \frac{(V_{max}[S])}{(K_m + [S])} \quad \ldots(1)$$

Taking reciprocals on both sides of equation (1)

$$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{([S])} + \frac{1}{V_{max}} \quad \ldots(2)$$

This is the equation for a straight line., $y = mx + c$

Equation (2) is known as Lineweaver - Burk equation.

A plot of $\frac{1}{v}$ versus $\frac{1}{([S])}$ is called a double - reciprocal plot (or) Lineweaver - Burk plot (LB plot) which yields a straight line.

This line, on extrapolation, cuts the 'X' axis at $\frac{-1}{K_m}$ and 'Y' axis at $\frac{1}{V_{max}}$.

The slope = $\frac{K_m}{V_{max}}$.

From these data both K_m and V_{max} can be determined (Fig 9.5)

For most of the enzymes, K_m lies between 10^{-1} and 10^{-7} M.

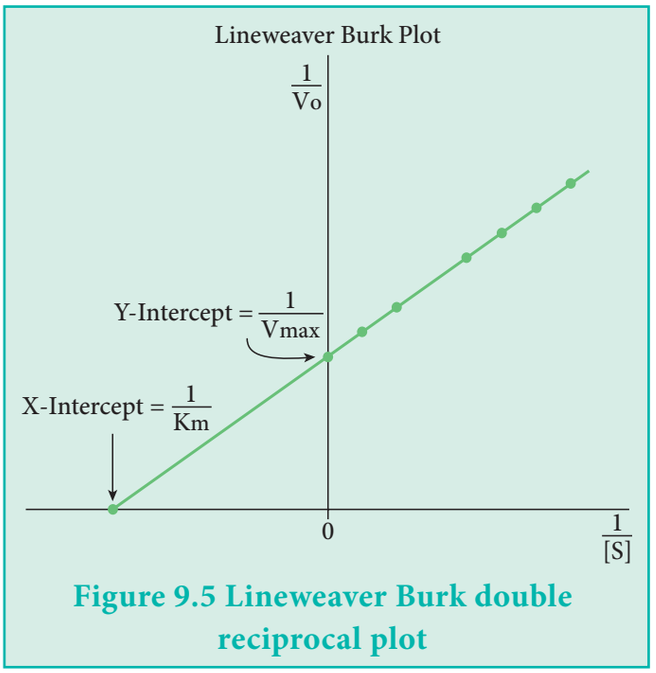


Figure 9.5 Lineweaver Burk double reciprocal plot

Significances of V_{max} :

1) Vmax is the maximal velocity of the enzyme catalysed reaction.
2) This condition is attained when the catalytic sites on the enzyme are saturated with substrate. (i.e) when [S] >>> K_m.
3) $V_{max} = k_3$ [ET], where k_3 = rate constant for the dissociation of ES complex into product; [ET] = Total enzyme concentration.
4) k_3 = kcat = $\frac{V_{max}}{[ET]}$

The turnover number of an enzyme (kcat) is defined as the number of substrate molecules converted into product by an enzyme molecule in unit time when the enzyme is fully saturated with the substrate. The turnover numbers of most enzymes fall in the range of 1 to 10^4 sec^{-1}. The turnover number of different enzymes are given as follows :

S. NO.	ENZYME	TURNOVER NUMBER (SEC^{-1})
1	Carbonic anhydrase	6,00,000
2	Penicillinase	2,000
3	DNA polymerase I	15
4	Lysozyme	0.5

Haem is an iron-containing prosthetic group. It may function as an electron carrier and oxygen carrier in haemoglobin. It is also found in catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide to water and oxygen.

9.2. ENZYME ACTION:

Active Site:

It is a three dimensional area or region on the surface of the enzyme molecule where the substrate binds. It is also known as 'catalytic site' or 'substrate site'

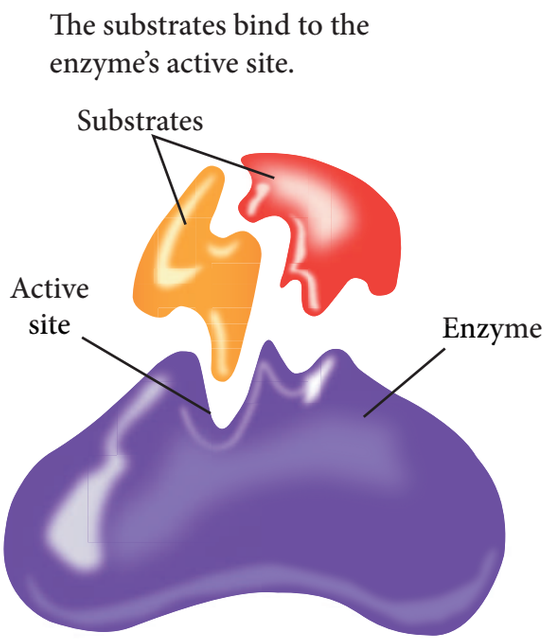


Figure 9.6 Active site of an enzyme

Although the enzymes differ widely in their properties, the active site present in their molecule possesses some common features. These are listed below :

The active site occupies a relatively small portion of the enzyme molecule.

The active site is neither a point nor a line or even a plane but is a 3- dimensional entity. It is made up of groups that come from different parts of the linear amino acid sequence.

Usually, the arrangement of atoms in the active site is well defined, resulting in a marked specificity of the enzymes. Although cases are known where the active site changes its configuration in order to bind a substance which is only slightly different in structure from its own substrate.

The active site binds the substrate molecule by relatively weak forces.

The active sites in the enzyme molecules are grooves or crevices from which water is largely excluded. It contains amino acids such as aspartic acid, glutamic acid, lysine, serine etc. The side chain groups like -COOH, $-NH_2$, $-CH_2OH$ etc., serve as catalytic groups in the active site. Besides, the crevice creates a micro-environment in which is essential for catalysis.

(i) Mode of enzyme action

In order to explain the mode of enzyme action, the following models have been proposed.

i) Fischer's lock and key model: According to this model the substrate binds with the enzyme at the active site just like a key fits into a lock. This leads to the formation of enzyme-substrate complex (ES complex). This complex decomposes to give the product and free enzyme.

In fact, the enzyme-substrate union depends on a reciprocal fit between the structure of the enzyme and the substrate. Since two molecules (the substrate and the enzyme) are involved, this hypothesis is also known as the concept of intermolecular fit. The enzyme-substrate complex is highly unstable and almost immediately this complex decomposes to form the product. (Fig 9.7).

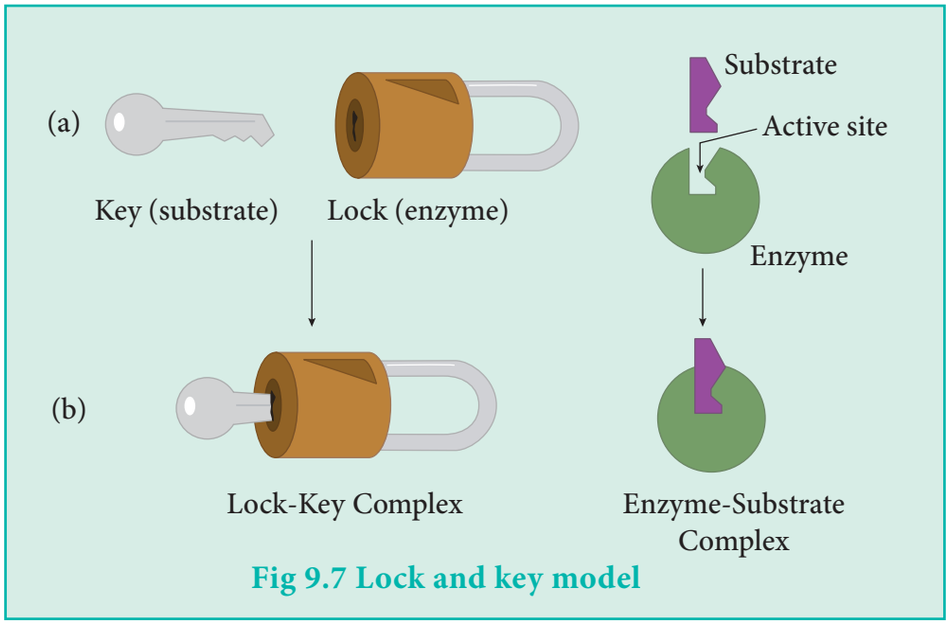


Fig 9.7 Lock and key model

(ii) Koshland's induced fit model: According to this model, the initial interaction between enzyme and substrate is relatively weak. These weak interactions quickly induce conformational changes in the enzyme that strengthen binding. This is the more accepted model for enzyme-substrate complex than the lock-and-key model. The failure of Fischer's model is the rigidity of the active site. Unlike the lock-and-key model, the induced fit model shows that enzymes are flexible structures in which the active site continually changes by interacting with the substrate (Fig 9.8).

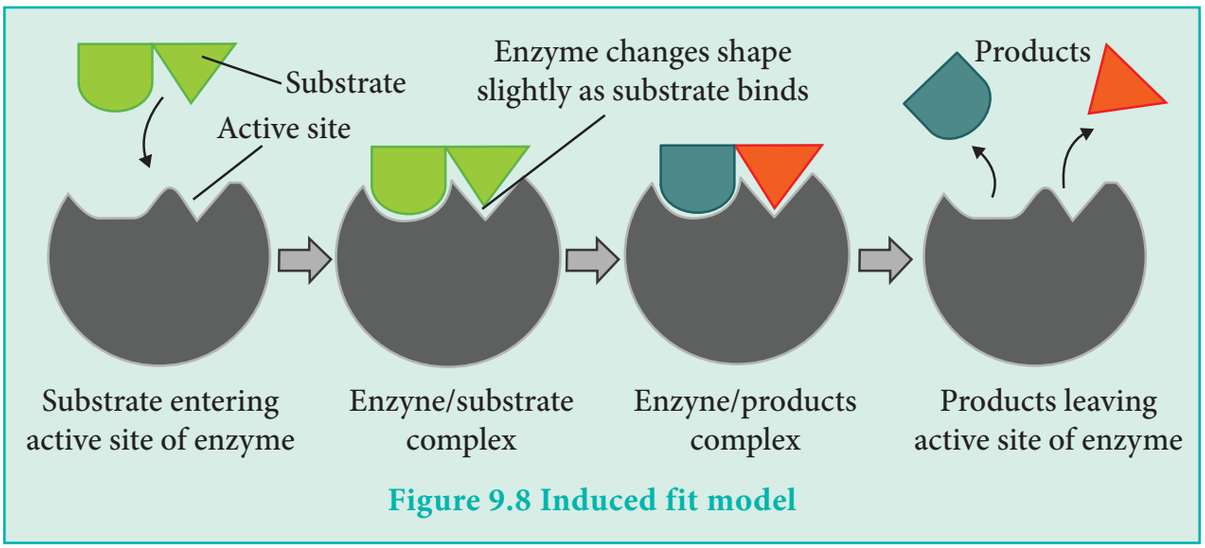


Figure 9.8 Induced fit model

(ii) Mechanism of enzyme action :

The enzyme-substrate union results in the release of energy. It is this energy which, in fact, raises the energy level of the substrate molecule, thus inducing the activated state (Fig 9.2). In this activated state, certain bonds of the substrate molecule become more susceptible to cleavage.

Evidences Proving the Existence of an ES Complex: The existence of an ES complex during enzymatically catalyzed reaction has been shown in many ways :

1. The ES complexes have been directly observed by electron microscopy and x-ray crystallography.
2. The physical properties of enzymes (solubility, heat sensitivity) change frequently upon formation of an ES complex.
3. The spectroscopic characteristics of many enzymes and substrates change upon formation of an ES complex. It is a case parallel to the one in which the absorption spectrum of deoxyhemoglobin changes markedly, when it binds oxygen or when it is oxidized to ferric state.
4. Stereospecificity of highest order is exhibited in the formation of ES complexes. For example, D-serine is not a substrate of tryptophan synthetase. As a matter of fact, the D-isomer does not even bind to the enzyme.
5. The ES complexes can be isolated in pure form. This may happen if in the reaction, $A + B \rightarrow C$, the enzyme has a high affinity for the substrate A and also if the other reactant B is absent from the mixture.
6. A most general evidence for the existence of ES complexes is the fact that at a constant concentration of enzyme, the reaction rate increases with increase in the substrate concentration until a maximal velocity is reached.

9.3. Enzyme Inhibition

A substance which binds with an enzyme and brings about a decrease in catalytic activity of that enzyme is called an enzyme inhibitor. This process is known as enzyme inhibition. The enzyme inhibitor may be organic or inorganic in nature. Enzyme inhibition is classified into three types as follows :

1) Reversible inhibition
2) Irrerversible inhibition.
3) Allosteric inhibition

9.3.1. Reversible inhibition :

When the inhibitor binds noncovalently with the enzyme and the inhibitor dissociates rapidly from the enzyme inhibitor complexes, it is known as reversible inhibition. It is further subdivided into

1. Competitive inhibition
2. Noncompetitive inhibition
3. Uncompetitive inhibition.

9.3.1.1. Competitive inhibition (or) substrate analogue inhibition :

It is a type of reversible inhibition in which the inhibitor resembles the substrate and binds to the active site of the enzyme.

Since the competitive inhibitor resembles the substrate, it is otherwise known as substrate analogue.

In competitive inhibition, the enzyme can bind substrate (ES complex) or inhibitor (EI)

The inhibitor thus competes with the substrate to combine with the enzyme.

A competitive inhibitor decreases the rate of catalysis by reducing the proportion of enzyme molecules bound to a substrate.

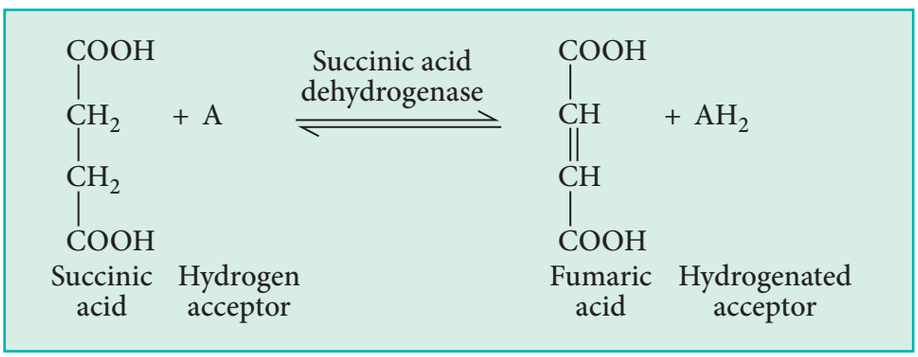
The degree of inhibition depends upon the relative concentrations of both substrate and inhibitor.

Competitive inhibition can be overcome by a sufficiently high concentration of substrate.

In competitive inhibition K_m value increases, whereas V_{max} remains unchanged.

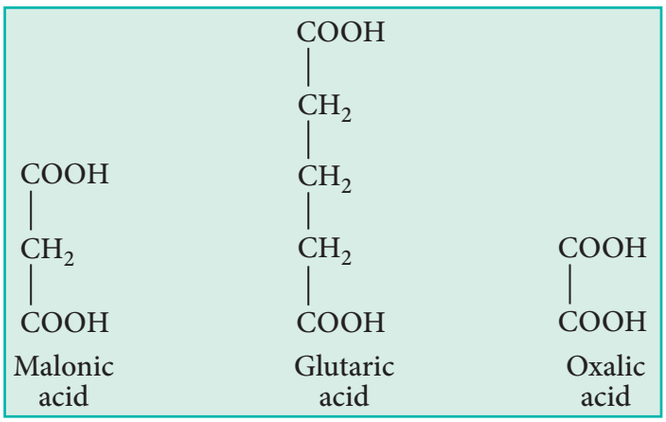
Example :

The action of malonate on succinate dehydrogenase (SDH)

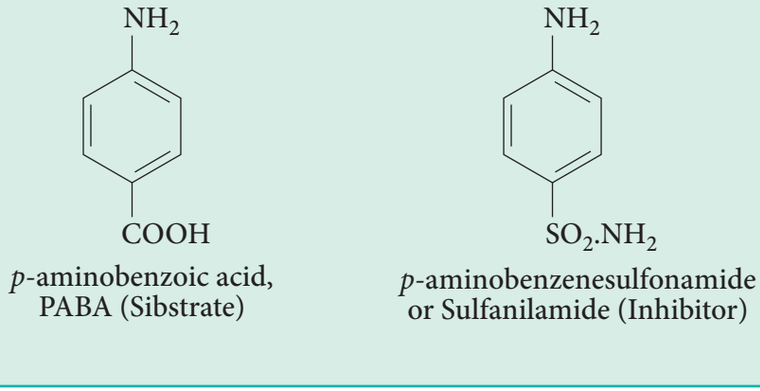


Oxalic, malonic and glutaric acids are competitive inhibitors of SDH. Among them, malonic acid is the most potent competitive inhibitor.

The pharmacological action of many drugs may be explained by the principle of competitive inhibition. For example, sulphonamides are very commonly used antibacterial drugs.



Bacteria synthesise the vitamin folic acid from P-aminobenzoicacid (PABA). Bacterial cell wall is impermeable to folic acid. Sulphanilamides and other sulpha drugs are structural analogues of PABA and hence inhibit folic acid synthesis in bacteria and they die. This drug is nontoxic to human cells, because human beings lack the enzymes necessary for folic acid synthesis and it is needed as a vitamin in the diet.



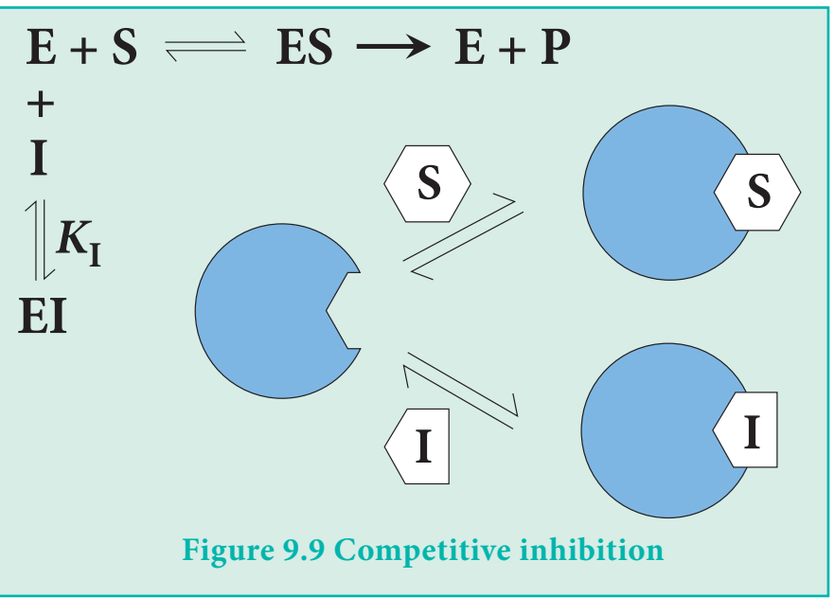


Figure 9.9 Competitive inhibition

The action of competitive inhibitor may be given as follows (Figure 9.9)

The inhibitor constant (K_i) for competitive inhibition is

$$K_i = \frac{([E]\ [I])}{([EI])}$$

9.3.1.2. Noncompetitive inhibiton

It is also a reversible inhibition in which the inhibitor has no structural resemblance with the substrate and binds at a site other than the active site on the enzyme surface.

In noncompetitive inhibition both the substrate and inhibitor can bind simultaneously to an enzyme molecule.

The inhibitor binds with the enzyme as well as ES complex which leads to the formation of EI and ESI complexes, respectively.

Noncompetitive inhibition cannot be overcome by increasing the substrate concentration.

In noncompetitive inhibition K_m value is unchanged while V_{max} is decreased.

Example :

i) Various heavy metals (Ag^{+}, Pb^{2+}, Hg^{2+}) can non-competitively inhibit various enzymes. For example, urease is highly sensitive to all these heavy metal ions.

ii) H_2S and cyanide strongly inhibit the action of iron containing enzymes like cytochrome oxidase.

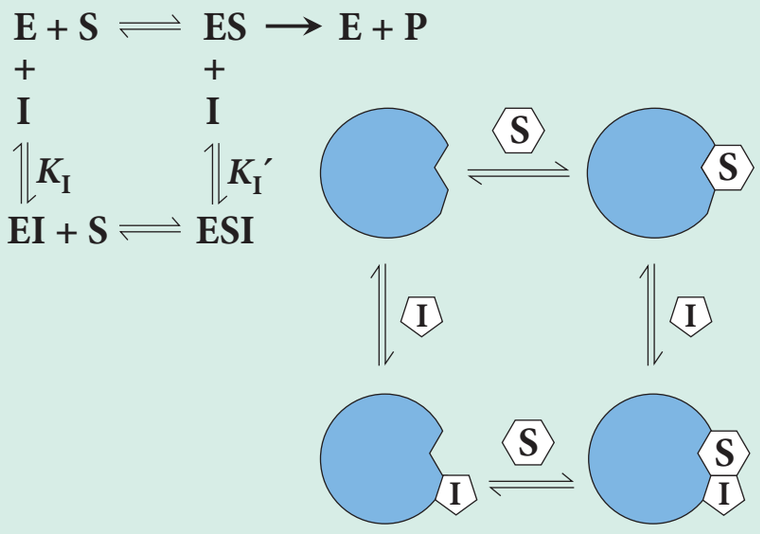


Figure 9.10 Non-competitive inhibition

The action of a non-competitive inhibitor is given as follows (Figure 9.10)

The noncompetitive inhibitor gives two K_i values as follows.

$$K_i = \frac{([E]\ [I])}{([EI])}$$

$$K_i = \frac{([ES]\ [I])}{([ESI])}$$

9.3.1.3. Uncompetitive inhibition :

It is also a type of reversible inhibition in which the inhibitor does not have any affinity for free enzyme, but binds only with the enzyme-substrate complex.

Uncompetitive inhibitor decreases both K_m and V_{max} values of the enzyme.

Example : Inhibition of placental alkaline phosphatase by phenyl alanine is an example of uncompetitive inhibition.

The action of uncompetitive inhibitor may be given as follows (Figure 9.11)

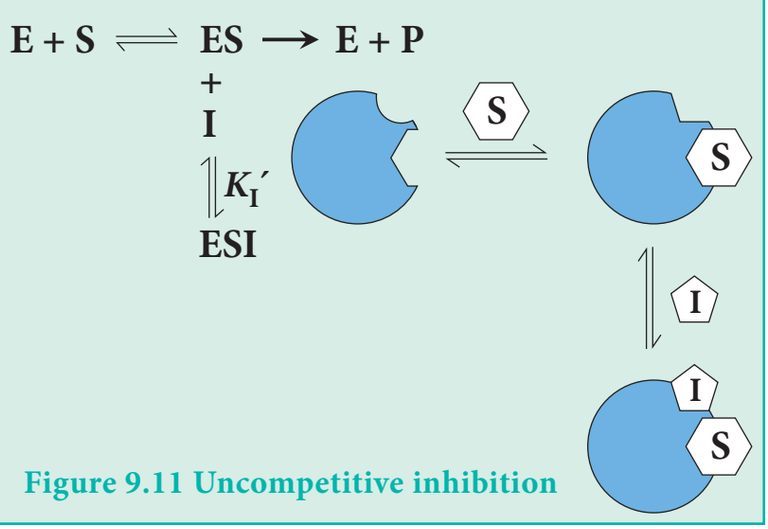


Figure 9.11 Uncompetitive inhibition

Uncompetitive inhibition is rare in one-substrate reactions but common in two-substrate reactions.

The degree of uncompetitive inhibition may increase with increase in substrate concentration.

The inhibitor constant (K_i) of uncompetitive inhibition is

$$K_i = \frac{([ES]\ [I])}{([ESI])}$$

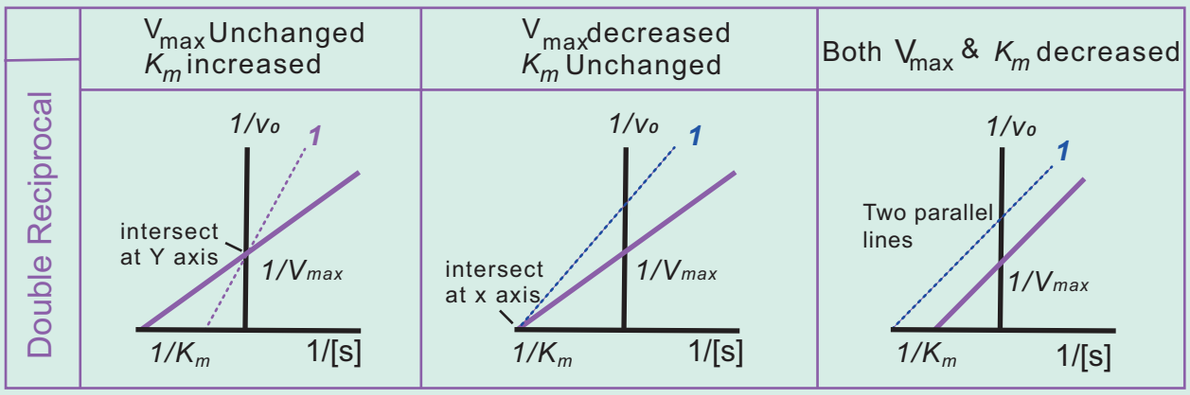


Figure 9.12 Lineweaver Burk plots for competitive, non-competitive and uncompetitive inhibition.

Differences Between Competitive And Noncompetitive Inhibition

COMPETITIVE INHIBITOR	NON-COMPETITIVE INHIBITOR
Inhibitor resembles the substrate (substrate analogue)	Inhibitor has no structural resemblance with the substrate.
Inhibitor binds at the active site.	Inhibitor binds at a site other than active site
Enzyme binds either with substrate or inhibitor	Enzyme binds with both substrate and inhibitor
Reversible	Irreversible
Can be overcome by increasing substrate concentration.	Cannot be overcome by increasing substrate concentration
K_m value will be increased	K_m remains unchanged
V_{max} remains unchanged.	V_{max} value will be decreased

Example: Action of malonate on succinate dehydrogenase.	Example: Action of cyanide on cytochrome oxidase.
Applied in drug action	Applied in toxicological studies.

9.3.2 Allosteric inhibition: (Greek allo = 'other'; stereos = 'space' or 'site')

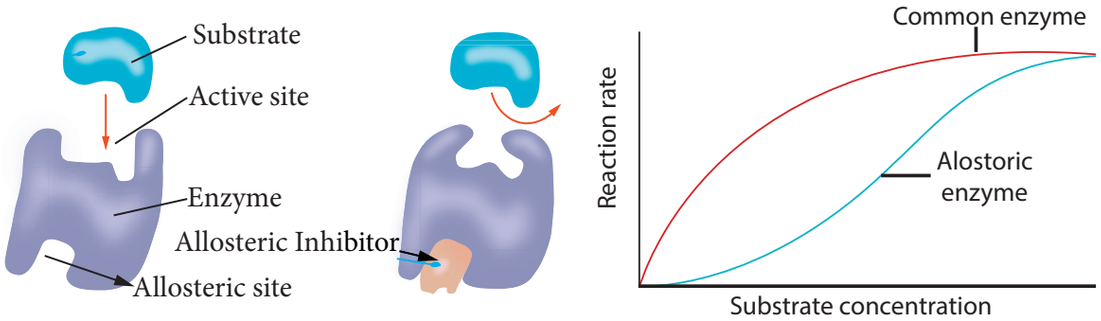


Figure 9.13 Allosteric inhibition

The allosteric inhibitor binds to an enzyme at a site other than the active site (Allosteric site). This leads to a conformational change which reduces the affinity of the enzyme for substrate (Fig 9.13). Allosteric enzymes do not exhibit Michaelis Menten kinetics. A graph of v_0 versus [S] gives a sigmoidal curve.

9.3.3. Irrversible enzyme inhibition :

When the inhibitor binds tightly to the enzyme either covalently or noncovalently and dissociates very slowly from the enzyme - inhibitor complex, it is known as irreversible inhibition (Fig 9.14)

Example :

1) Action of nerve gases on acetylcholine esterase.
2) Action of iodoacetamide on enzymes containing –SH group by modifying cysteine.

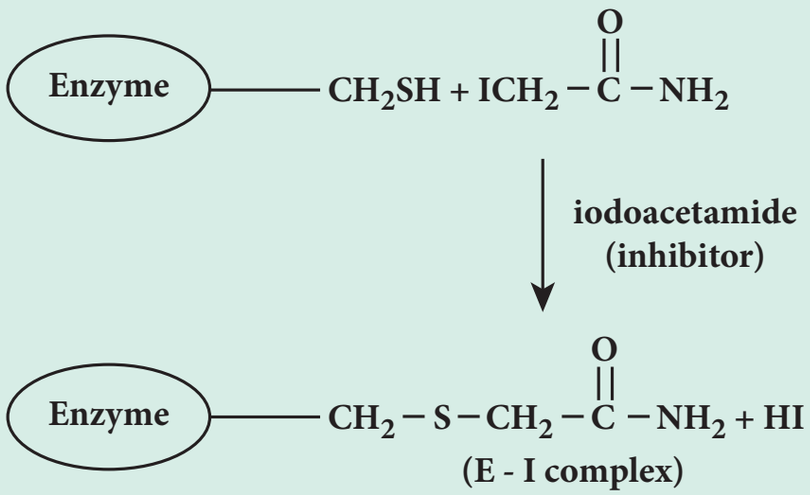


Fig 9.14 Irreversible Inhibition

Summary

1. Enzymes increase the rate of a reaction by lowering the energy of activation.
2. The active site of an enzyme is a pocket or cleft containing aminoacid side chains that participate in the binding of substrate and the catalytic reaction.
3. Binding of substrate causes a conformational change in the enzyme (induced fit) that favours catalysis.
4. When a substrate binds to the active site, an enzyme–substrate (ES) complex is formed which subsequently dissociates to enzyme and product.
5. Most enzymes show Michaelis-Menten kinetics, and a plot of the initial reaction velocity vo against substrate concentration([S] has a hyperbolic graph.
6. Michaelis Menten derived the equation for a single substrate enzyme catalyzed reaction assuming steady state conditions where there is no change in [ES].
7. Lineweaver Burk used the double reciprocal linear plot to determine V_{max} and K_m more accurately.
8. Inhibitors are molecules that can reduce the rate of an enzyme catalyzed reaction. There are two types of inhibitors; reversible and irreversible inhibitors.
9. Competitive (V_m unchanged, K_m increased), non-competitive (V_m decreased, K_m unchanged) and uncompetitive inhibitors (Both V_m and K_m decreased) are reversible inhibitors.
10. Allosteric enzymes do not show Michaelis Menten kinetics, instead they show sigmoidal kinetics. Allosteric inhibitors bind to a site other than the active site.
11. Irreversible inhibitors bind tightly to the enzyme, either covalently or noncovalently, and dissociates very slowly from the enzyme - inhibitor complex.

Hirudin: An enzyme from leech saliva which keep blood flowing without being clotted from the host body. This helps leech suck as much blood as it needs. Hence leech therapy uses them to minimize pain of varicose veins by sucking accumulated blood.

EVALUATION

I. Choose the correct answer from the four given alternatives

1. ES complex formation is
   a) a reversible reaction
   b) an irreversible reaction
   c) an energy consuming reaction
   d) a complete reaction
2. According to Michaelis Menten theory
   a) only a single substrate is involved
   b) the concentration of substrate is much greater than that of enzyme
   c) an intermediate enzyme substrate complex is formed
   d) all the above
3. The reciprocal form of M-M equation was considered by
   a) Lineweaver – Burk
   b) Fischer
   c) Koshland
   d) Dixon
4. Lock and Key theory was proposed by
   a) Dixon
   b) Fischer
   c) Koshland
   d) Michaelis Menten
5. An exact structural similarity with the substrate is needed for a
   a) competitive inhibitor
   b) uncompetitive inhibitor
   c) noncompetitive inhibitor
   d) irreversible inhibitor
6. In uncompetitive inhibition inhibitor binds only to
   a) enzyme
   b) substrate
   c) ES-complex
   d) Active site
7. The number of molecules of substrate converted to product per enzyme molecule per second is called the …
   a) Turnover number
   b) Optimum number
   c) Maximum reaction rate
   d) K_m

8. The active site of an enzyme is generally a

a) cleft
b) Indentation
c) Hole
d) Tube

9. Which one of the following conditions is least likely to denature an enzyme?

a) a high temperature
b) an extreme pH
c) heavy metal ions
d) a low temperature

10. According to lock and key & induced fit models which of the following is not true?

a) Induced fit model is more specific than lock and key model
b) In lock and key model the active site is more RIGID than induced fit model
c) In induced fit model the active site can undergo conformational change
d) In lock and key model, the enzyme is considered as a lock and substrate is considered as a key

11. Which of the following is the best description of an enzyme?

a) they allow chemical reactions to proceed very quickly
b) they increase the rate at which a chemical reaction approaches equilibrium
c) they make a reaction thermodynamically favorable
d) all of the above e. none of the above

12. Given an enzyme with a K_m = 10m M and V_{max} = 100 m mol/min. If [S] = 100 m M, which of the following will be true?

a) A 10 fold increase in Vmax would increase velocity 10 fold y
b) A 10 fold decrease in Km would increase velocity
c) Both (a) and (b)
d) A 10 fold increase in V_{max} would decrease velocity 20 fold.

13. The relationship between Km and Vmax is known as

a) Haldane equation
b) MichaelisMenten equation
c) Numerical solution approach
d) Gibbs-Helmholtz equation

14. A competitive inhibitor of an enzyme is usually

a) a highly reactive compound
b) a metal ion such as Hg2+ or Pb2+
c) structurally similar to the substrate.
d) water insoluble

15. The types of inhibition pattern based on Michaelis-Menten equation are
    a) competitive
    b) non-competitive
    c) uncompetitive
    d) all of the above
16. In a Lineweaver-Burk Plot, competitive inhibitor shows which of the following effect?
    a) It moves the entire curve to right
    b) It moves the entire curve to left
    c) It changes the x-intercept
    d) It has no effect on the slope
17. An allosteric inhibitor of an enzyme usually
    a) participates in feedback regulation
    b) denatures the enzyme
    c) is a hydrophobic compound
    d) causes the enzyme to work faster
18. The enzyme inhibition can occur by
    a) reversible inhibitors
    b) irreversible inhibitors
    c) Both (a) and (b)
    d) None of these
19. Non-competitive inhibitor of an enzyme catalyzed reaction
    a) decreases Vmax
    b) binds to Michaelis complex (ES)
    c) both (a) and (b)
    d) can actually increase reaction velocity in rare cases
20. The active site of an enzyme remains
    a) at the center of globular proteins
    b) rigid and does not change shape
    c) complementary to the rest of the molecule
    d) none of the above

II. Say True or False.

1. Enzyme substrate complex is a permanent stable complex.
2. Malonate is the competitive inhibitor of succinate dehydrogenase.
3. An enzyme substrate complex is formed in all the enzymatic reactions.
4. The degree of competitive inhibition cannot be decreased byincreasing the concentration of the substrate.
5. An uncompetitive inhibitor has affinity towards E-Scomplex.

III. Match the following

1. Enzymes - $\frac{1}{V}$ vs $\frac{1}{S}$
2. ES complex – affinity of enzyme for substrate
3. k_m - Unstable and highly energetic
4. Line weaver Burk plot - Biocatalysts

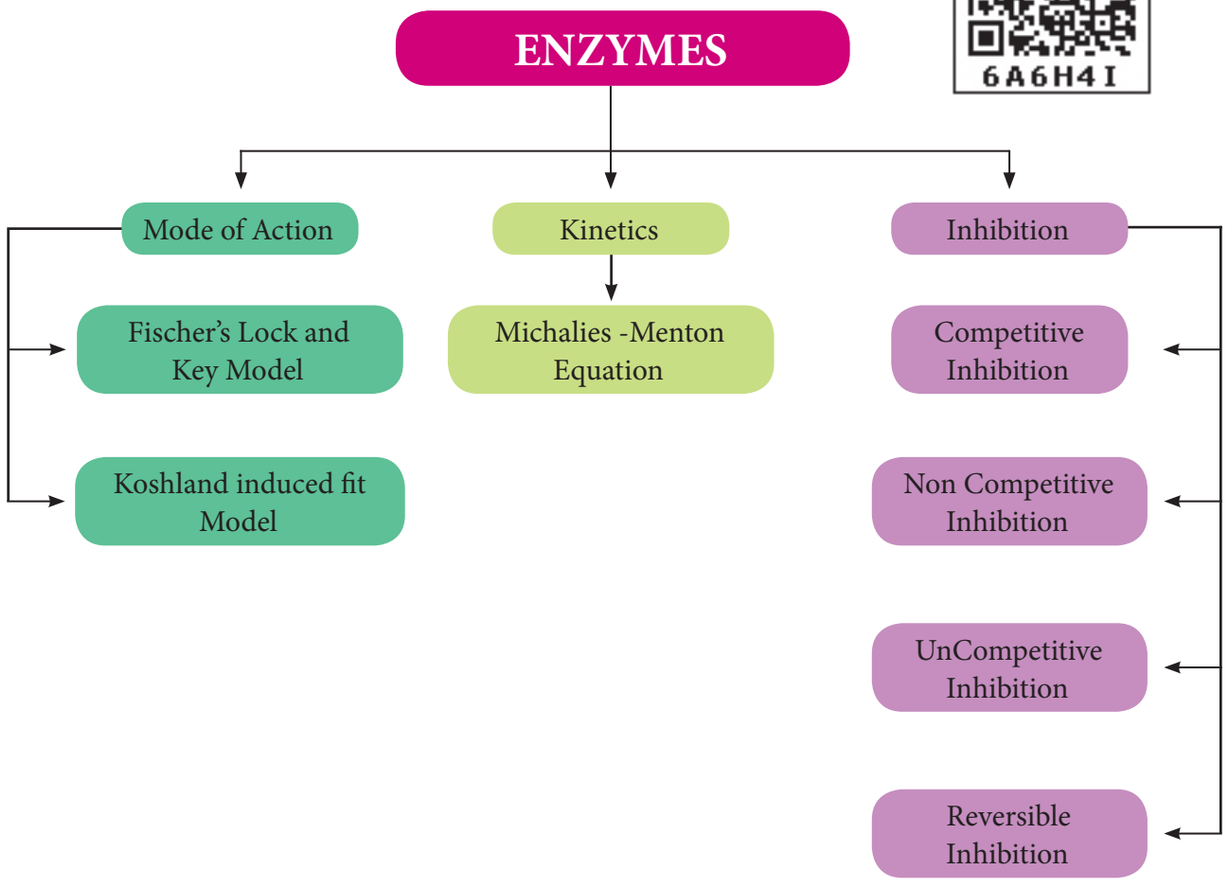
IV. Give short answers for the following

1. Define K_m
2. What is the nature of active site according to lock and key theory ?
3. What is competitive inhibition ?
4. What is induced fit theory ?
5. What is irreversible enzyme inhibition ?

V. Answer the following

1. Derive MM equation.
2. How is the LineWeaver Burk plot arrived at?
3. Explain the concept of competitive inhibition
4. What is the action of malonate on succinate dehydrogenase ?
5. Compare competitive and non-competitive inhibition.

CONCEPT MAP



UNIT
10

IMMUNOLOGY

Edward Jenner,

Edward Jenner, an English Physician and Scientist, is known as the Father of Immunology because of his contribution to the invention of a vaccine against small pox which saved many lives.

BSY2F5

Learning Objectives

After studying this chapter, one should be able to

- Understand the basic concepts of infection.
- Identify a few infectious diseases.
- Understand the differences between innate and adaptive immunity.
- List out the functions of antibodies.
- Carry out blood grouping tests
- Carry out blood grouping tests

10.1. INTRODUCTION TO IMMUNOLOGY

"A healthy life is a wealthy life" and "Prevention is better than cure" are the proverbs frequently used to define the status of human health which is closely related to immunity and the immune system. Immunity is defined as the state of resistance to disease caused by specific microorganism or their toxic products. The immune system is the system of specialized cells and organs that protect an organism from diseases and infectious organisms. It is also called as the host defense system.Immunology is the study of all aspects of the immune system in an organism.

The concept of immunity can be traced back to 430BC, when Thucydides observed that individuals who had recovered from the plague would not get it a second time and could nurse other affected patients. The earliest recognised and written evidence of inducing immunity was practiced by the Chinese and Turks in the 15th Century. They either inhaled the dried crusts derived from small pox pustules or inserted them into wounds in the skin. Lady M.W. Montagu, the wife of the British ambassador to Constantinople, applied this technique on her own children and found the results to be positive. In 1798, a vaccine (from the Latin word 'vacca' , meaning 'cow') for small pox was developed by Edward Jenner. He inoculated an 8-year old boy James Phipps with material obtained from a cowpox lesion. The results were conclusive of prevention of small pox infection. Friedrich Henle was the first to discover that germs caused disease and the isolation of infectious bacteria was done by his pupil Robert Koch. Louis Pasteur developed vaccine for chicken cholera, anthrax and rabies. Modern immunology begins with the research of Metchnikoff, who discovered the phenomenon of phagocytosis in starfish and extrapolated it to macrophages in humans as cells that engulf infectious agents.

Variolation was the method used to immunize individuals against smallpox by infecting them with substance from the pustules of patients.

10.2. INFECTION

Infection is defined as the invasion and multiplication of pathogenic organisms in the host. An infection without symptoms is represented as subclinical; and with symptoms it is represented as clinically apparent. Illness caused through pathogenic organisms is termed as infectious disease or communicable disease or transmissible disease.

Epidemiology

Epidemiology is the branch of medical science that deals with the geographical distribution and timing of infectious disease occurrences. The study also includes the modes of transmission and maintenance in nature, with the goal of recognizing and controlling outbreaks.

The spectrum of occurrence or prevalence of disease in a defined population includes sporadic, endemic, epidemic and pandemic.

Sporadic refers to a disease that occurs infrequently and irregularly without a geographic focus. Examples of sporadic diseases include tetanus, rabies, and plague.

Endemic disease is an infectious disease which is restricted to a population in a given geographical region only and the constant rate of presence for years.

Epidemic refers to an increase in the number of cases of a disease in a particular geographical region within a short span of time when compared to the previous year infection rate. Influenza (common cold) is a good example of a common epidemic disease.

Pandemic refers to an epidemic that has spread over several countries or continents, usually affecting a large number of people i.e. a wide geographical region. AIDS is an example for pandemic since it is present in many countries.

Transmission

Transmission of an agent causing an infectious disease can be direct or indirect. The transfer of an infectious agent directly into the body is known as direct transmission. There are four types of **direct contact transmission**.

1. **Physical contact** between hosts (Influenza, Skin infections).
2. **Direct contact** with body fluids or tissues of an infected individual (HIV, HPV).
3. **Droplet contact** in which large infectious particles sprayed into the air from the respiratory tract of an infected individual (pneumonia, mumps, measles).
4. **Droplet nuclei contact** in which small infective dried droplet particles that are suspended in the air are taken in by a host, and are capable of traveling to the lung (TB, chickenpox).

Indirect transmission is the transfer of a pathogen by a vector or vehicle. Malaria is an example of a vector borne disease. Examples of diseases spread through vehicle-borne transmission are food-borne diseases and waterborne diseases eg: Cholera. Zoonosis occurs when diseases are transferred from animals to people. Zoonotic diseases include anthrax from sheep and plague from rodents.

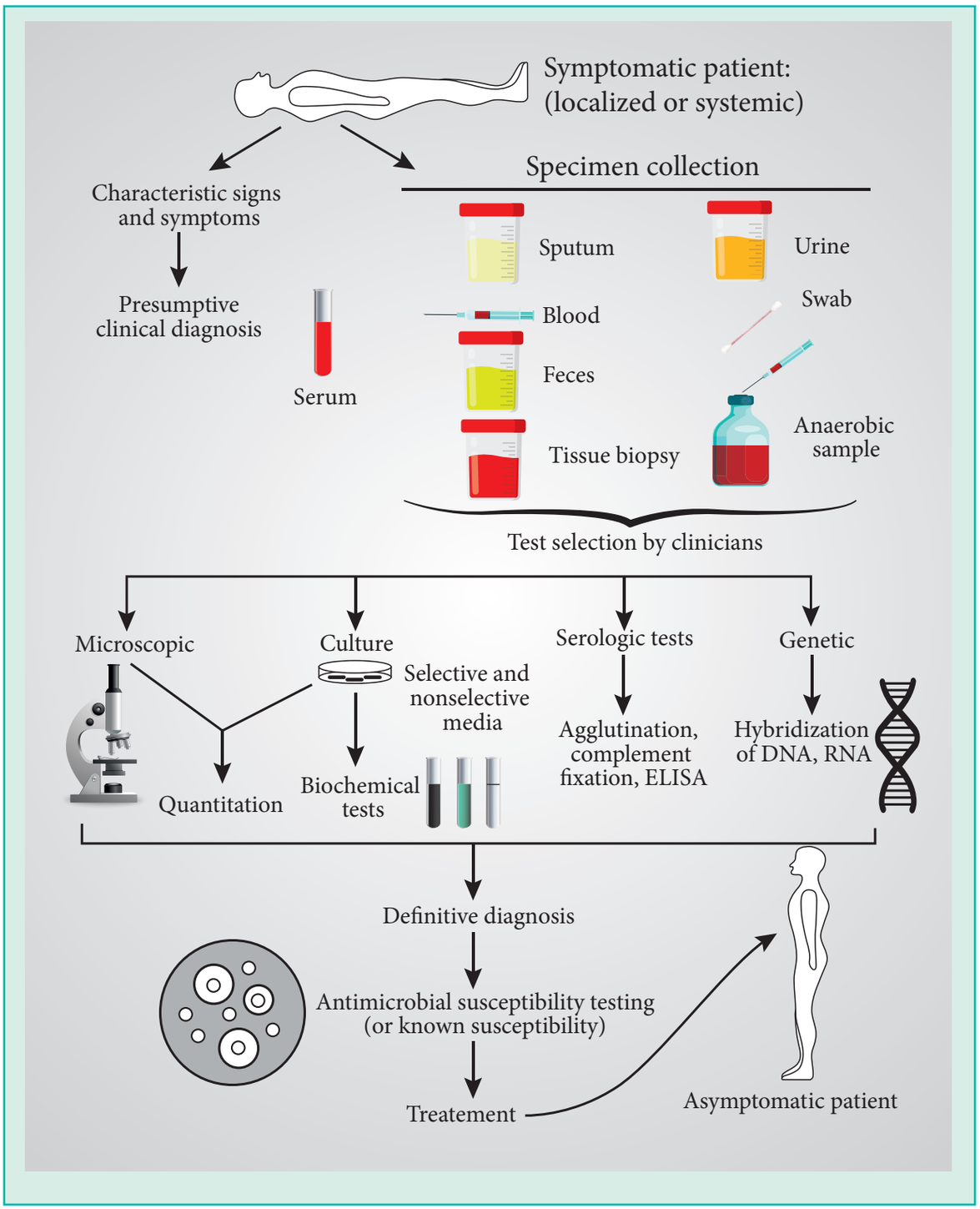
Etiology

Etiology is the study of cause or origin of disease. The etiologic agent or causative agent is responsible for the cause of a disease. A pathogen or infectious agent is a biological agent that causes disease or illness to its host organism. Pathogenic organisms are of five major types; bacteria, virus, fungi, worms, and protozoa.

Diagnosis

Laboratory tests may identify organisms directly (e.g., visually, using a microscope, growing the organism in culture) or indirectly (e.g., identifying antibodies to the organism). They use a sample of blood, urine sputum,stool, throat swab or other fluid or tissue from

the infected individual. This sample may be stained and examined under a microscope, cultured, tested for antibodies, tested for a microorganism's antigens or tested for genetic material (such as DNA or RNA) from the microorganism.



Treatment and Prevention

Antibiotics, anti-virals, anti-fungals, and anti-parasitic agents along with quorum quenching methods are being used to treat infectious diseases depending upon the nature of infection. Many infectious diseases can be prevented by personal hygiene and vaccines.

Pus is a thick protein rich fluid called as liquor puris. It consists of dead white blood cells and infected agents. It is a natural product formed during immunological reactions against infecting agents. It might be yellow or green or brown in colour and with foul odour. Appearance of pus at the site of surgery indicates an infection.

10.2.1. Bacterial Infections

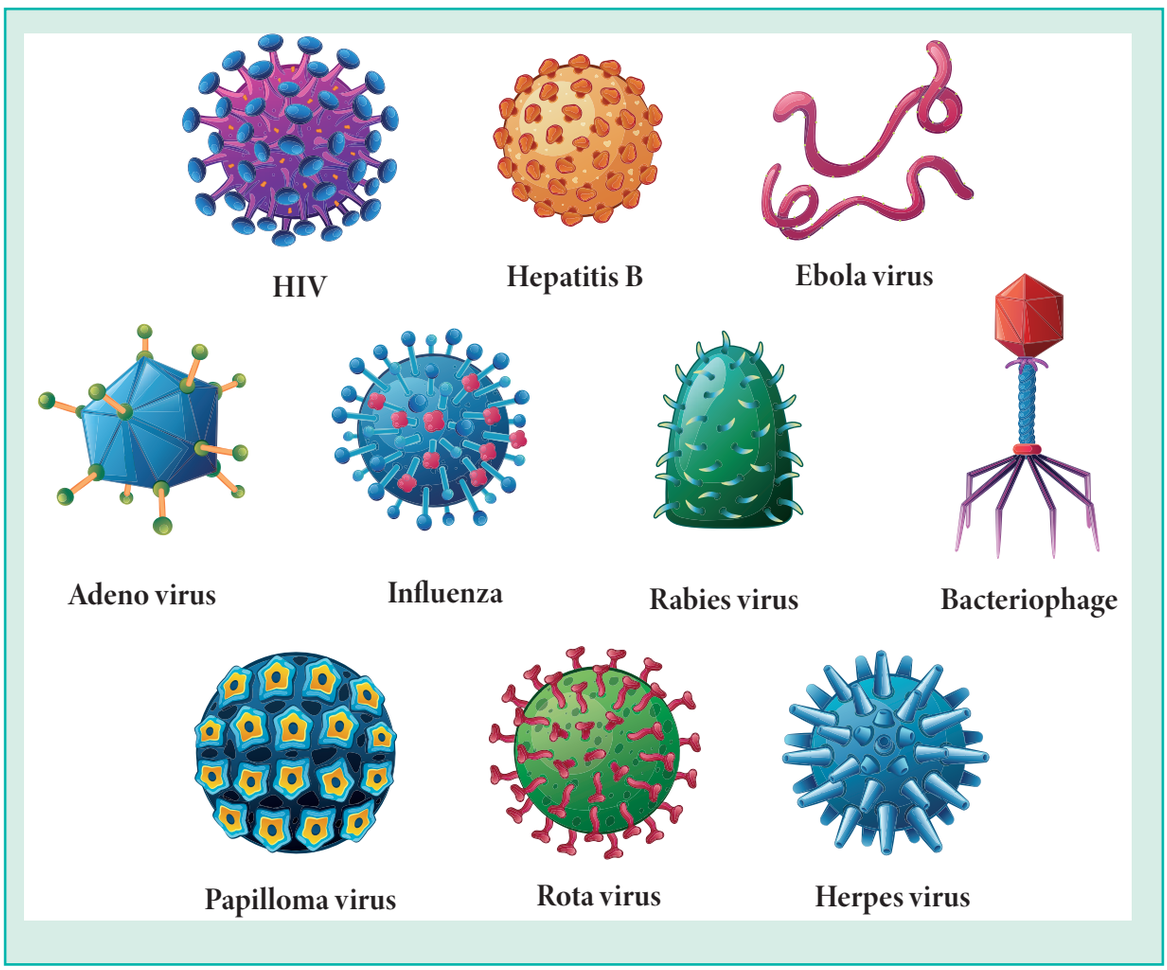
Bacterial infections include any type of illness caused by bacteria. Based on the structure and shape,there are three major groups of bacteria namely, Bacillus (cylindrical forms), Coccus (spherical forms) and Spiral. Humans and animals have abundant normal flora (microbes) that usually do not produce disease under normal healthy condition. These bacteria are referred to as good bacteria or healthy bacteria or normal flora. Harmful bacteria that cause bacterial infections and disease are called pathogenic bacteria. Bacterial diseases occur when pathogenic bacteria enter into the body and begin to reproduce and to grow in tissues that are normally sterile. Harmful bacteria may also emit toxins that can damage the body.

Name of Disease	Pathogen	Epidemiology	Incubation Period	Symptoms	Therapy
Pulmonary Tuberculosis	*Mycobacterium tuberculosis*	Airborne and Droplet infection	2-10 weeks	Coughing; chest pain and bloody	Streptomycin, para-amino salicylic acid, rifampicin
Diphtheria	*Corynebacterium diphtheriae*	Airborne and Droplet infection	2-6 days	Inflammation of mucosa of nasal chamber, throat etc. respiratory tract blocked	Diphtheria antitoxins, Penicillin, Erythromycin
Cholera	*Vibrio cholerae*	Direct and oral with contaminated food and water	6 hours to 2 – 3 days	Acute diarrhoea and dehydration	Oral rehydration therapy and tetracycline
Leprosy	*Mycobacterium leprae*	Slowest infectious and contagious	2-5 years	Skin hypopigmentation, nodulated skin, deformity of fingers and toes.	Dapsone, rifampicin, Clofazimine

Tetanus (Lock Jaw)	*Clostridium tetani*	Through injury	3-21 days	Degeneration of motor neurons, rigid jaw muscles, spasm , paralysis	Tetanus-antitoxins
Plague	*Yersinia pestis*	Indirect and inoculative (vector is rat flea)	2-6 days	Bubonic plague affects lymph nodes; Pneumonic plague affects lungs and Septicemic plague causes anaemia	Tetracycline, streptomycin, Chloromycetin

10.2.2. Viral Infections

Viruses are acellular obligate intracellular parasites. They contain only one type of nucleic acid, it may be either single or double stranded DNA or RNA. Viral diseases range from minor ailments such as the common cold to severe diseases such as Rabies and Acquired Immune Deficiency Syndrome (AIDS). They may be sporadic like Mumps, endemic like Infectious hepatitis, epidemic like Dengue fever or pandemic like Influenza.



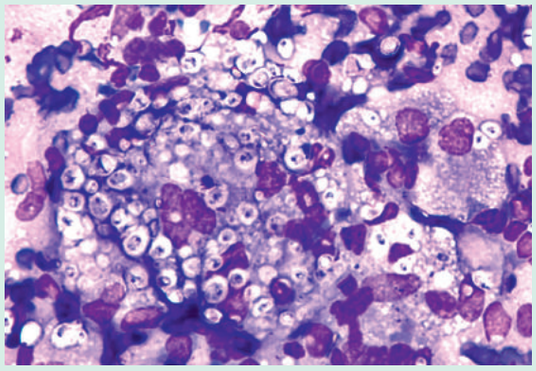
Name of	Pathogen Epidemiology Incubation	Disease	period	Symptoms	Therapy
Poliomyelitis	Polio-virus	Direct and oral	7-14 days	Damages motor neurons causing stiffness of neck, convulsion, paralysis of generally legs	Physiotherapy
Measles	Rubella-virus	Contagious and Droplet infection	10 days	Rubeolla (skin eruptions), coughing, sneezing	Antibiotics and sulpha drugs
Mumps	Mumps-virus	Contagious and Droplet infection	12-26 days	Painful enlargement of parotid salivary glands	Antibiotics
Rabies (Hydrophobia)	Rabies-virus	Indirect and inoculative	10 days to 1- 3 months	Spasm of throat and chest muscles, fears from water, paralysis and death	Pasteur-treatment
Influenza (Flu)	Myxovirus influenzae	Air borne and pandemic	24-48 Hours Lasts for 4-5 days	Bronchitis, sneezing bronchopneumonia, leucopenia, coughing	Antibiotic therapy
Hepatitis (Epidemic Jaundice)	Hepatitis-B virus	Direct and oral	20-35 days	Damage to liver cells releasing bilirubin, jaundice	Hepatitis-B vaccine
Chikungunya	chikungunya virus	infected *Aedes aegypti* mosquitoes (vector)	3 weeks	rash, muscle pain, fever and sever joint pain	Antipyretics, analgesics, fluids, and rest
Dengu fever	*Dengu virus*	infected *Aedes aegypti and Aedes albopictus* mosquitos (vector)	3 and 14 days	Fever, Headache, Rash appearing between the second and fifth day of fever, Platelets reduction	Intravenous (IV) fluids, acetaminophen

AIDS	Human Immunodeficiency Virus (HIV)	Direct contact with infected blood, semen or vaginal fluids	12 years	flu-like symptoms such as fever, sore throat and fatigue after few weeks of infection	antiretroviral treatment (ARVT)

10.2.3. Fungal Infections

Fungi are eukaryotic protista, recognized as causative agents of human disease earlier than bacteria. Fungal infections (mycosis) are most common among those patients who use antibiotics for prolonged period of time. These antibiotics not only kill pathogenic bacteria but also target the normal flora of human body (useful bacteria) and give rise to fungal growth. Human fungal infections are usually of two types: superficial and deep infection. Fungi causing superficial mycoses are specialized saprophytes, with the capacity to digest keratin. Superficial mycoses are of two types - surface infections (only on dead layers of skin) and cutaneous infections (cornified layer).

Mucormycosis molds



Histoplasma fungi

Disease Name	Pathogen	Epidemiology	Symptoms	Therapy
Candidiasis or yeast infections	*Candida* yeasts *Candida albicans*	Direct contact	itching and swelling, redness and soreness	Nystatin , Clotrimazole and fluconazole
Jock itch or Tinea cruris (Dermatophytoses)	*Trichophyton rubrum* and *T. mentagrophytes*	direct contact with an infected person	redness in the groin, buttocks, or thighs, itching	miconazole, clotrimazole, ketoconazole

Athlete's foot or Tinea pedis (Dermatophytoses)	*T. mentagrophytes, Trichophyton rubrum*	Direct contact	redness or blisters, peeling or cracking skin	topical antifungal ointments, itraconazole, terbinafine
Tinea capitis or scalp ringworm (Dermatophytoses)	*Trichophyton tonsurans, T. schoenleinii, T. violaceum*	Direct contact	round patches of dry scale, alopecia	Griseofulvin, Terbinafine
Mucormycosis	*Mucor and Rhizopus*	Air borne	Opportunistic pathogen	Amphotericin B

10.3 IMMUNITY

Immunity refers to the ability of the immune system to defend against diseases caused by microbes or foreign substances which are products like toxins from microbes. Immunity depends upon various factors like host resistance, dosage of organism injected and virulence of the organisms.

10.3.1. Classification

Immunity is mainly classified into innate and acquired immunity. Innate or nonspecific or natural immunity refers to the basic resistance to disease that an individual is born with. Acquired or specific or adaptive immunity requires the activity of a functional immune system, involving cells called lymphocytes and their products. Innate defense mechanisms provide the first line of defense against invading pathogens until an acquired immune response develops. In general, most of the microorganisms encountered by healthy individual are readily cleared within a few days by non-specific defense mechanisms without enlisting a specific immune response. When an invading microorganism eludes the nonspecific host defense mechanism, a specific immune response occurs.

10.3.1.1. Innate (Natural) Immunity

Innate immunity may be considered at the level of the species, race or individual. Species immunity refers to the total or relative refractoriness to a pathogen, shown by all members of a species. For instance, all human beings are totally insusceptible to plant pathogens and to many pathogens of animals such as rinderpest.. This immunity is something a person obtains by birth, for the reason that he belongs to the human species. The mechanisms of species immunity may be due to physiological and biochemical differences between the tissues of the different host species, which determine whether or not a pathogen can multiply in them.

Within a species, different races may show differences in susceptibility to infections. This is known as racial immunity. It has been reported that the African-descendants in the USA are more susceptible to tuberculosis than the Americans. The differences in innate immunity exhibited by different individuals in a race are known as individual immunity. It is well documented that homozygous twins exhibit similar degrees of resistance or susceptibility to lepromatous leprosy and tuberculosis.

10.3.1.2. Components involved in Innate Immunity

Components involved in innate immunity include skin, mucus, cells like neutrophils, macrophages, natural killer cells and soluble factors like complements, cytokines, and acute phase proteins.

Skin and mucus provide anatomical barrier, cells like macrophages and neutrophils provide phagocytic barrier and soluble factors like complement, acute phase proteins provides physiological and inflammatory barriers.

10.3.1.3 Mechanism involved in Innate immunity

Innate immunity is provided by four types of defensive barriers namely, anatomical, physiological, phagocytic and inflammatory barriers.

Anatomical Barriers

Anatomic barriers that tend to prevent the entry of pathogens are an organism's first line of defense against infection. The skin and the surface of mucous membranes are included in this category because they are effective barriers to the entry of most microorganisms. The outer epidermis of skin contains several layers of tightly packed epithelial cells which prevents entry of pathogens. The outer epidermal layer consists of dead cells and is filled with a waterproofing protein called keratin. The inner dermis layer of skin, which is composed of connective tissue, contains blood vessels, hair follicles, sebaceous glands, and sweat glands. The sebaceous glands are associated with the hair follicles and produce an oily secretion called sebum. Sebum consists of lactic acid and fatty acids, which maintain the pH of the skin between 3 and 5; this pH inhibits the growth of most microorganisms.

The conjunctivae , the alimentary, respiratory, and urogenital tracts are lined by mucous membranes. These membranes consist of an outer epithelial layer and an underlying layer of connective tissue. The viscous fluid called mucus, which is secreted by epithelial cells of mucous membranes, entraps foreign microorganisms. In the lower respiratory tract, the mucous membrane is covered by cilia, the hair like protrusionsof the epithelial cell membranes. The synchronous movement of cilia propels mucus-entrapped microorganisms from these tracts.

Physiological Barriers

The physiological barriers that contribute to innate immunity include temperature, pH, and various soluble and cell associated molecules. Many species are not susceptible

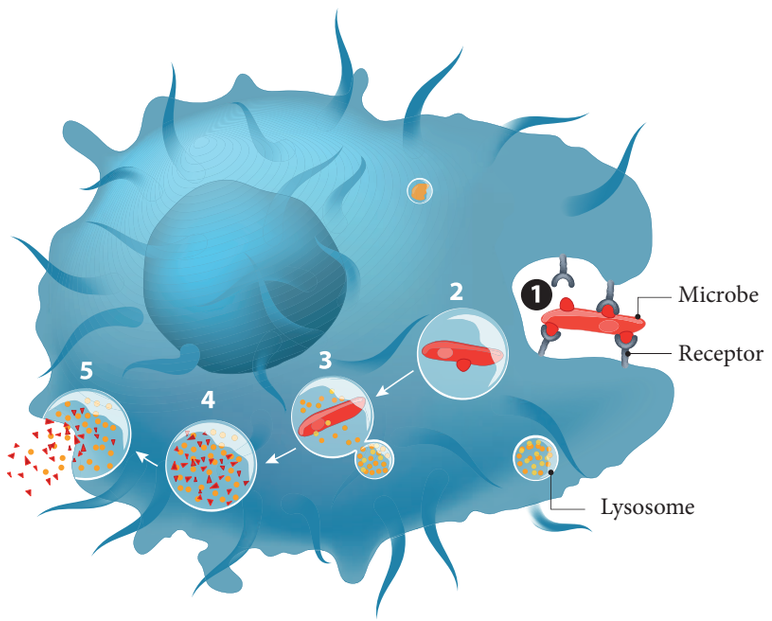
to certain diseases simply because their normal body temperature inhibits growth of the pathogens. Gastric acidity is an innate physiologic barrier to infection because very few ingested microorganisms can survive the low pH of the stomach contents.

A variety of soluble factors contribute to innate immunity, among them are the soluble proteins lysozyme, interferon, and complement. Lysozyme, a hydrolytic enzyme found in mucous secretions and in tears, is able to cleave the peptidoglycan layer of the bacterial cell wall. Interferon comprises a group of proteins produced by virus-infected cells which prevents viral infection of neighbouring cells. Complement lyse bacteria by forming membrane attack complex.

The newborn babies (neonates) are susceptible to some infections that do not affect adults. The reason is that their stomach contents are less acidic i.e higher pH than those of adults. The pH variation provides optimum medium for the growth of pathogenic microorganisms.

Phagocytic Barriers

Another important innate defense mechanism is the ingestion of extracellular particulate material by phagocytosis. The process of phagocytosis was discovered by Metchnikoff. The term phagocytic denotes the engulfment and digestion of whole cells. The two major cell types in the body which are associated with the engulfment and digestion of microorganism are the polymorphonuclear leucocytes and the macrophages. Minor cell types are the eosinophils. The process of phagocytosis involves the following steps:



1.Binding and absorption | 2.Phagosome formation | 3. Phagosome and lysosome to from a phagolysosome | 4. Digestion | 5. Release of microbial products

1. Attachment

   Attachment is the adherence of a bacterium to the cell membrane of the phagocytic cell. Some bacteria are easily attached to the phagocytic cell. Example , *Mycobacterium tuberculosis*.

2. Phagosome formation

   After attachment the phagocyte extend small pseudopodia around the infecting bacterium. The pseudopodia fuse to form an endosome which contains a bacterium surrounded by the cell membrane. This structure is called as phagosome.

3. Phagolysosome formation

   After engulfment, the lysosome containing the hydrolytic enzymes fuses with phagosome to form phagolysosome. In this step, lysosomal enzymesare discharged to phagosome which is vital for the lysis of bacteria.

4. Lysis

   A number of antimicrobial and cytotoxic substances produced by phagocytes can destroy phagocytosed microorganisms.

Oxygen dependent killing mechanisms

During phagocytosis, a metabolic process known as the 'respiratory burst' occurs in activated phagocytes. This process results in the activation of a membrane bound oxidase that catalyzes the reduction of oxygen to superoxide anion, a reactive oxygen intermediate that is extremely toxic to ingested microorganisms. Activated phagocytes begin to express high levels of nitric oxide synthase (NOS), an enzyme that oxidizes L-arginine to yield L-citrulline and nitric oxide (NO). Much of the antimicrobial activity of phagocytes against bacteria, fungi, parasitic worms, and protozoa is due to nitric oxide and substances derived from it.

Oxygen independent killing mechanisms

Activated phagocytes also synthesize lysozyme and various hydrolytic enzymes whose degradative activities do not require oxygen. In addition, activated phagocytes produce a group of antimicrobial and cytotoxic peptides, commonly known as defensins. Cathepsin G is an example for defensins. These molecules are cysteine-rich cationic peptides that form circularized defensin which inturn form ion-permeable channels in bacterial cell membranes to lyse bacteria. Lactoferrin chelates iron from the medium and prevents the growth and proliferation of iron dependent bacteria. Lysozyme splits mucopeptide in bacterial cell wall and lysed bacteria.

5. Exocytosis

   Finally the killed organisms are digested by hydrolytic enzymes and the degraded products are released to the exterior by the process of exocytosis.

Inflammatory Barriers

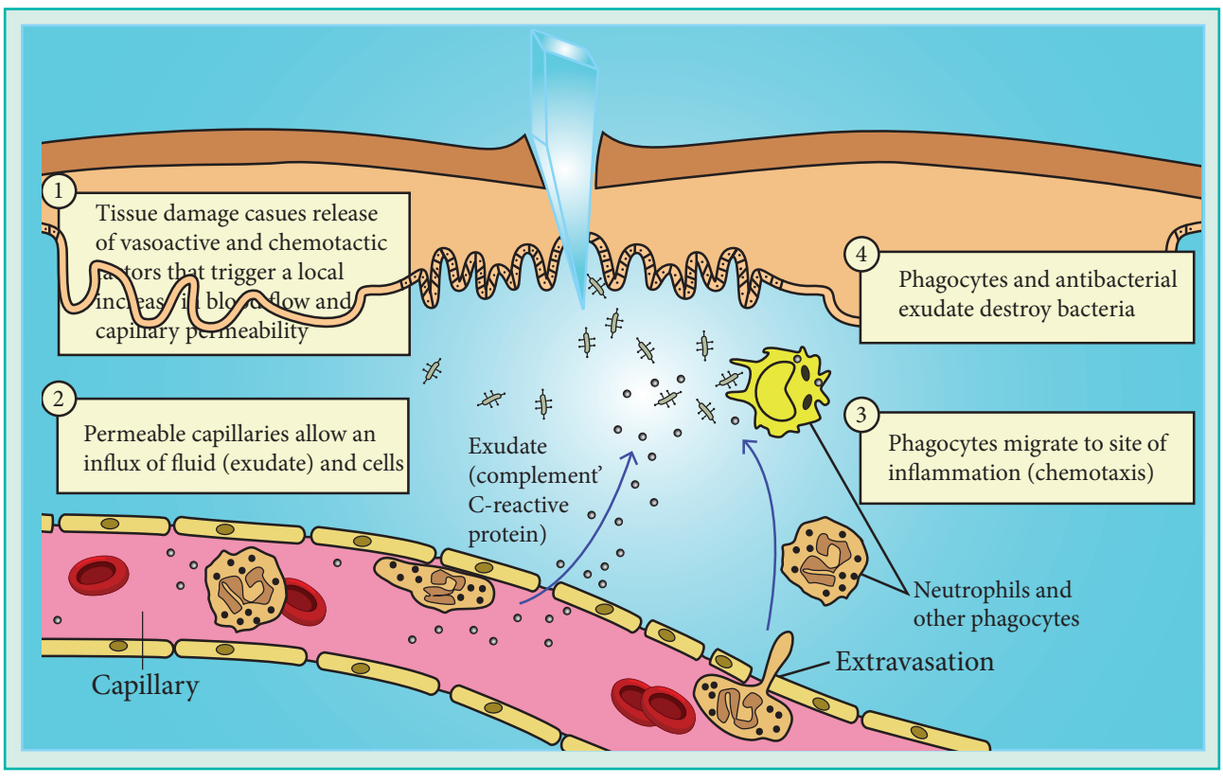
This barrier is created by the inflammatory response. Tissue damage caused by a wound or by an invading pathogenic microorganism induces a complex sequence of events collectively known as the inflammatory response. Inflammatory response is described by the "four cardinal signs of inflammation" as rubor (redness), tumor (swelling), calor (heat) and dolor (pain). Presently a fifth sign, functiolaesa (loss of function),is included.

The following are the sequential steps that occur during inflammation:

Vasodilation occurs in nearby capillaries resulting in enlargement of the capillary network. The enlarged capillaries are responsible for tissue redness (erythema) and an increase in tissue temperature.

An increase in capillary permeability facilitates an influx of fluid and cells from the enlarged capillaries into the tissue. The fluid that accumulates (exudate) has much higher protein content than fluid normally released from the vasculature. Accumulation of exudate contributes to tissue swelling (edema).

Influx of phagocytes from the capillaries into the tissues is facilitated by the increased permeability of the capillaries. The emigration of phagocytes is a multistep process that includes adherence of the cells to the endothelial wall of the blood vessels (margination), followed by their emigration between the capillary endothelial cells into the tissue (diapedesis or extravasation), and, finally, their migration through the tissue to the site of the invasion (chemotaxis). As phagocytic cells accumulate at the site and begin to phagocytose bacteria, they release lytic enzymes, which can damage nearby healthy cells. The accumulation of dead cells, digested material, and fluid forms a substance called pus.



Inflammation that develops at the site of infection induces the acute phase response. This generalized response is characterized by fever, changes in vascular permeability and changes in biosynthesis, metabolism and catabolism in many organs. These changes result in a rise in the concentration of certain proteins in the blood and a drop in the concentration of other proteins. These proteins, termed as acute phase proteins or acute phase reactants. These include C- reactive protein (CRP), fibrinogen and serum amyloid A protein. The concentration of CRP in the blood increases from a normal level of 1 mg / ml to as much as 1000 mg / ml during the acute phase response. It function in clearance of nuclear material released from killed microbes and killed host cells during inflammation by binding to DNA, chromatin and histones.

10.3.2. Acquired (Adaptive) Immunity

The form of immunity that is mediated by lymphocytes and stimulated by exposure to infectious agents is adaptive immunity. It reflects the presence of a functional immune system that is capable of specifically recognizing and selectively eliminating foreign microorganisms and molecules. It is characterized by four characteristics namely antigenic specificity, diversity, immunologic memory, and self and non-self recognition.

Antigenic specificity

Immune responses are directed toward and able to distinguish between distinct antigens or small parts of macromolecular antigens. This fine specificity is attributed to lymphocyte antigen receptors that may bind to one molecule but not to another with only minor structural differences from the first. Antibodies can differentiate between two molecules that differ by only a single aminoacid.

Diversity

Diversity is the result of variability in the structure of the antigen binding sites of lymphocyte receptors for antigens. Diversity allows the adaptive immune system to specifically recognize billions of uniquely different structures on foreign antigens.

Immunologic memory

Immunologic memory is mediated by memory cells. Memory cells are clonally expanded progeny of T and B cells formed during the primary response following initial exposure to antigen. Memory cells are more easily activated than naive lymphocytes and mediate secondary response on subsequent exposure to antigen. They survive in a functionally quiescent state for many years, even after the elimination of the antigen. Due to this attribute, the immune system can confer life long immunity to many infectious agents.

Self and non-self recognition

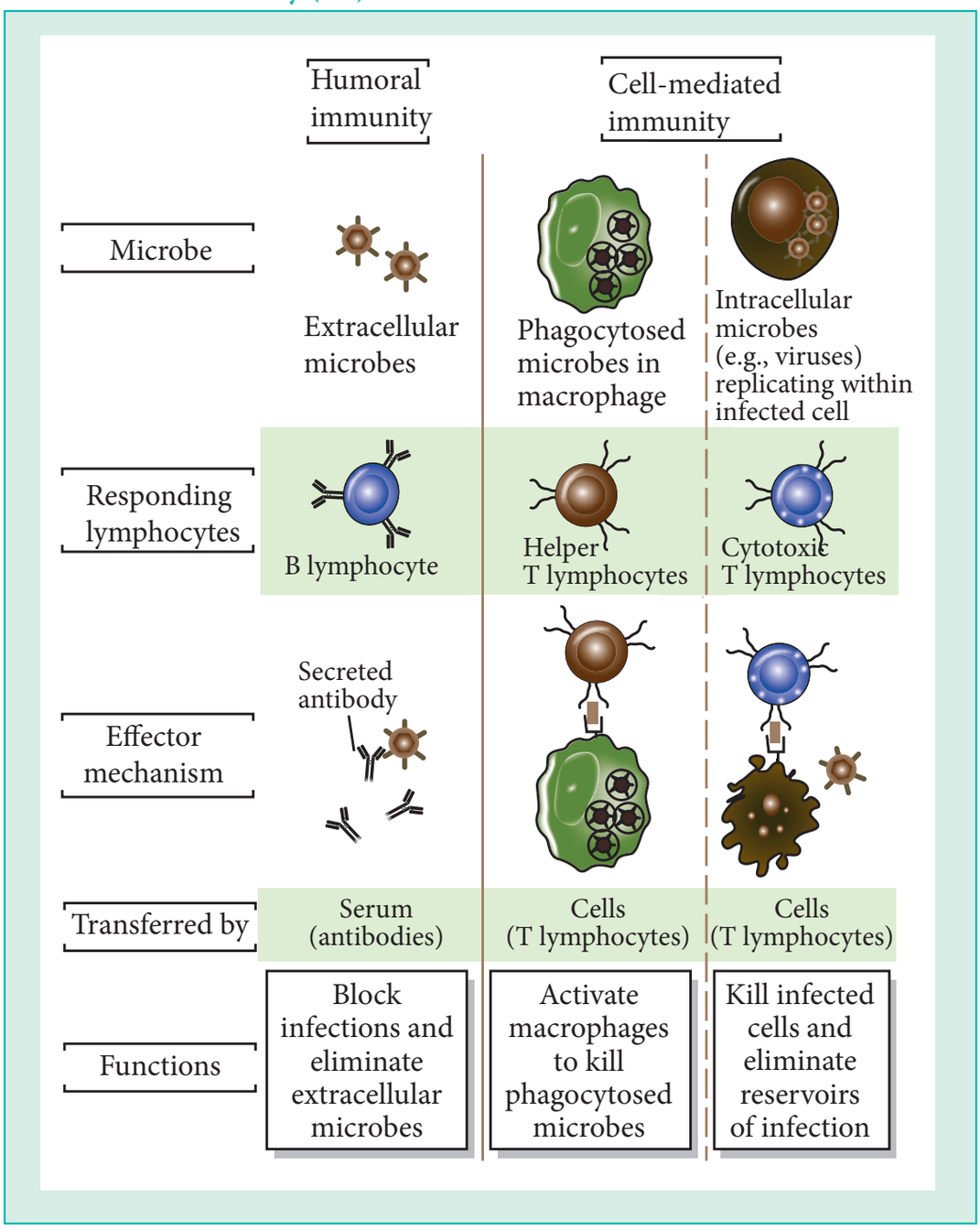
The immune system is able to distinguish self (host) from nonself (foriegn) antigens and respond only to non-self molecules. The production of immune cells , only against non- self molecules, is achieved by selection procedure like, positive selection and negative

selection during the maturation process of lymphocytes in bone marrow and thymus.

Acquired immunity does not occur independent of innate immunity and vice versa. For example, the phagocytic cells crucial to nonspecific immune responses are intimately involved in activation of the specific immune response. Similarly, the soluble factors produced during a specific immune response, have been shown to augment the activity of these phagocytic cells.

Acquired immunity, on the basis of components involved in immunity, is classified into two types namely, Humoral immunity and Cell mediated immunity.

10.3.2.1 Humoral Immunity (HI)



An overview of Acquired Immunity - Humoral and Cell mediated Immunity

The term humoral is derived from the Latin humor meaning "body fluid".Thus humoral immunity refers to immunity that can be conferred on a non-immune individual by administration of serum antibodies from an immune individual. Humoral immunity is mediated by molecules in blood and mucosal secretions called antibodies that are produced by cells called B lymphocytes.

Antibodies recognize microbial antigens, neutralize infectivity of the microbes and target microbes for elimination by various effector mechanisms. Antibodies themselves are specialized and different types of antibodies may activate by different mechanisms, for example, IgG and IgM antibodies promote phagocytosis and IgE antibodies trigger the release of inflammatory mediators from leukocytes such as mast cells. Binding of antibody to antigen on a microorganism also can activate the complement system, resulting in lysis of the microbes.

Humoral immunity is the principal defense mechanism against extracellular microbes and their toxins because secreted antibodies can bind to these microbes and toxins and assist in their elimination.

10.3.2.2 Cell Mediated Immunity (CMI)

Cell Mediated immunity (cellular immunity) is mediated by T lymphocytes. There are two types of T-cells mainly T_H and $T_{C,}$ which differ by their surface marker CD4 and CD8, respectively. T-cells are activated by antigen presenting cells (APC) after processing antigens. Both activated T_H cells and T_C cells serve as effector cells in CMI. Cytokines secreted by T_H cells can activate various phagocytic cells, enabling them to phagocytose and kill microorganisms more effectively. Cytotoxic T lymphocytes (CTL) participate in CMI by killing altered self cells. They also play an important role in the killing of virus infected cells and tumor cells.

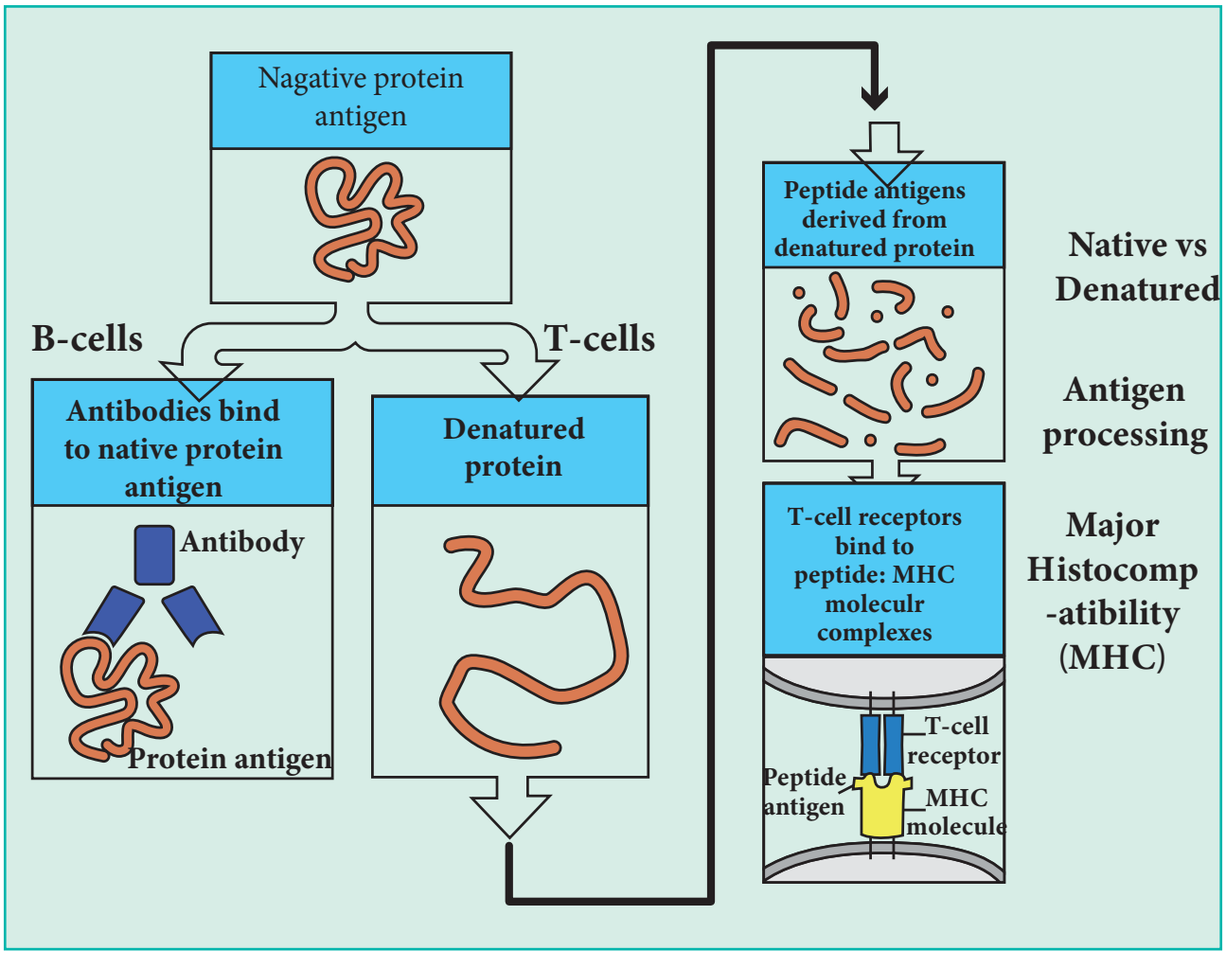
Intracellular microbes, such as virus and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell mediated immunity.

Antigen presenting cells are a functionally defined group of cells which are able to take up antigens and present them to T lymphocytes. Eg: Dendritic cells, activated macrophages, and activated B-cells.

A Collective and coordinated response to the introduction of foreign substances in an individual, mediated by the cells and molecules of the immune system is referred to as immune response. An adaptive immune response that occurs upon the first exposure of native lymphocytes with a foreign antigen is known as primary immune response. An adaptive immune response that occurs upon the second or subsequent encounter of primed lymphocytes with a given antigen is known as secondary immune response.

10.4. ANTIGENS

Antigens are foreign substances that have an ability to induce antibody generation. Antigenic determinant or epitope is the region of the antigen recognized by antibodies or T-Cell Receptor (TCR) of T cells. There are two types of epitopes namely B cell epitope and T cell epitope. B cell epitope is the region of antigen recognized by antibodies. T cell epitope is the region of antigen recognized by TCR of T cells.



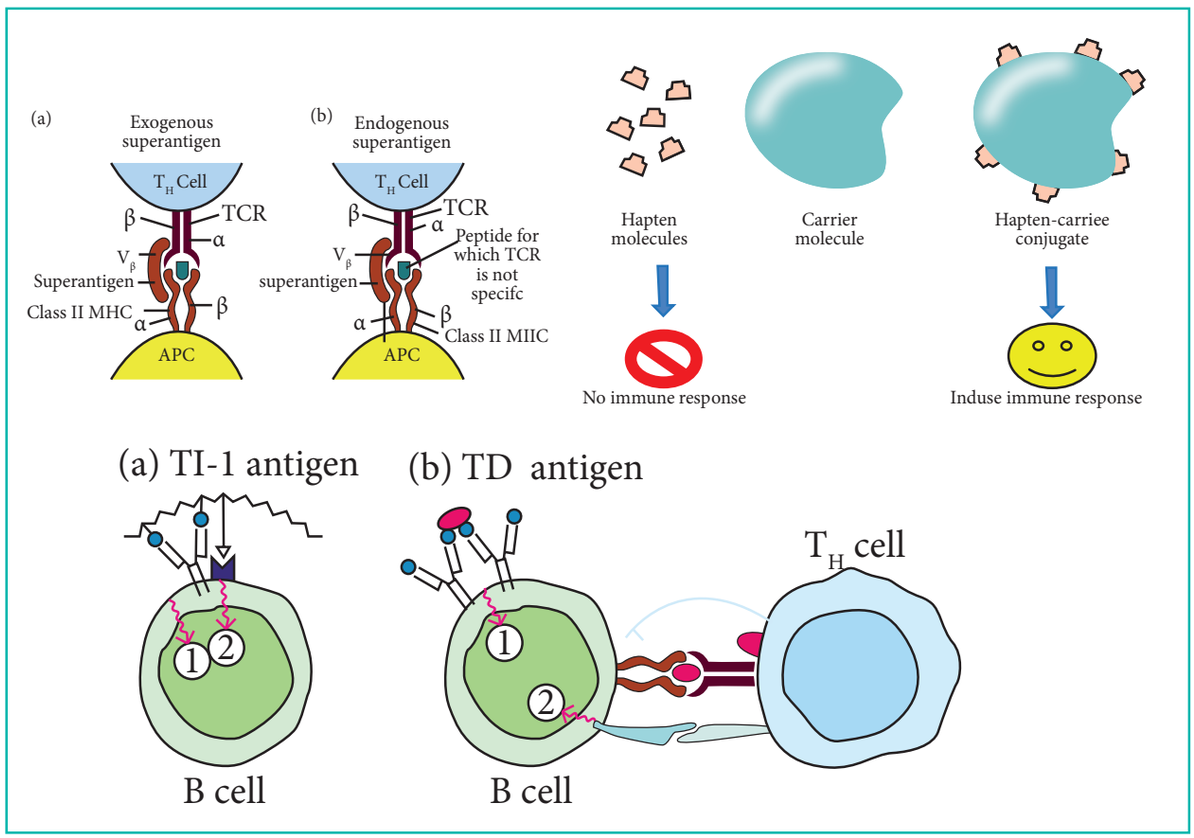
10.4.1. Types of antigens

Sl. No.	Types of Antigens	Nature	Example
1.	Sequestered antigens	These antigens are secluded or sequestered in capsule i.e. it is not exposed to immune system during development or when produced.	Lens proteins and sperm proteins.
2.	Neoantigens	They are newly produced antigens i.e. normal agents become antigens. They are formed due to the change in the chemical, physical and biological status of the agents.	Penicillin can be converted to neoantigen when it is bound with protein.

3.	Heterophile or Heterogenic or Cross reactive antigen	These antigens interact with antibodies produced against another antigen.	Forssman antigen. These antigens are present on mucosal cells of GI tract of human and RBCs of horse.
4.	Mitogenic antigens	Since some mitogens (they induce cell division) activate immune cells, they can be considered as mitogenic antigens.	Lipopolysaccharides induce B cell proliferation, Concanavalin – A (CON A) and Phytohemagglutinin (PHA) induce T cell proliferation and Pokeweed mitogen (PWM) induces both T and B cell proliferation.
5.	Superantigens	These antigens activate T cells non-specifically.	TSST-1 (Toxic shock syndrome toxin – 1) produced from *Staphylococcus sp.* . - Exogenous superantigens Mouse mammary tumor viral (MMTV) antigens - Endogenous superantigens
6.	Exogenous antigens	These antigens are usually secretory products of microbes or soluble antigens. They are usually present outside the host cell	Diphtheria toxin from *Cornebacterium diptheriae*
7.	Endogenous antigens	They are usually microbes which are present inside the cell or particulate antigen.	: Virus and endotoxins

Sl. No.	Types of Antigens	Nature	Example
8.	T cell dependent antigens	These antigens require T cells especially T_H cells to induce immune response.	Soluble antigens
9.	T cell independent antigens	These require T cells partially or not to induce immune response. T cell independent antigen – I (TI-1) does not require T	Lipopolysaccharides – Example for TI-I Cell wall polysaccharides

		cell to induce immune response. T cell independent antigen – 2 (TI-II) does not require direct contact of T cells but cytokines produced by them to induce immune response.	and glycoproteins – Example for TI-2.
10.	Allergens	Antigens responsible for allergic response are called allergens.	Pollens
11.	Autogens	Those antigens which are capable of inducing autoimmune disorders are known as autogens.	Immunoglobulins for Rheumatoid Arthritis.
12.	Self antigens	They are not originally antigens. They are normal cell surface components and proteins of normal host.	Host proteins
13.	Haptens	Haptens are otherwise known as incomplete antigens or partial antigens because they are unable to elicit immune response by itself, but they can gain this ability when they bind with a carrier molecule.	Dinitrophenol (DNP)



10.4.2. Factors influencing the antigenicity of antigens

Ability of an antigen to induce an immune response is known as antigenicity. The factors that influence antigenicity are mainly of two types i.e. factors contributed by antigens and factors contributed by host cells.

Factors contributed by Antigens:

The factors are: size, foreignness, chemical nature, complexity, heterogeneity and susceptibility for antigen processing.

Size

Antigens with greater than 10,000 Daltons molecular weight are found to be effective antigens For example: Hemoglobin is more antigenic than penicillin.

Foreignness (Alien)

It refers to the distance in the phylogenetic tree i.e. evolutionary distance. Highly distant organisms are found to have more antigenicity than less distant ones. For example: Protein from gorilla is found to have less antigenicity than the protein from fish to humans because fish is more evolutionarily distant from gorilla.

Chemical Nature

Depeneding on the chemical nature, antigenicity varies.

antigenicity than carbohydrates, lipids and nucleicacids. Solubility also plays an important role.

Less soluble antigens have more antigenicity.

; For example, proteins have greater

Complexity

As the complexity of the antigens increase antigenicity also increases. For example, Primary structure of proteins has lesser antigenicity than tertiary structure of the same protein.

Heterogenicity

In the case of multimeric proteins, heteromultimeric proteins have more antigenicity than homomultimeric proteins. This was mainly because of presence of different types of epitopes in heteromultimeric proteins.

Susceptibility for Antigen Processing

Those antigens which are found to be easily processed by APCs, have greater antigenicity than those antigens which are not easily processed. For example: Horse RBCs have greater antigenicity than asbestos.

Factors contributed by Host

These factors include route of entry, genotype and dose.

Route of entry

Route of entry is also important to provide antigenicity and disease. If a microorganism enters through an adverse route it is degraded by the immune mechanism and is less antigenic. When it enters through its natural route, it will cause disease and it also escapes from the immune system partially. For example: *Vibrio Cholerae* entering through the circulatory system will not cause diarrhea; but if it enters through GI tract, it causes diarrhea.

Genotype

The genetic constitution (genotype) of an immunized animal influences the type of immune response the animal manifests, as well as the degree of the response. For instance, human beings are protected against some diseases, yet affected by other diseases. It purely depends upon the genotype.

Dose

To induce antigenicity, an optimum amount of infecting agent or antigenis required and this optimum amount is known as optimum dose. When microbes enter above and below this optimum dose level, fluctuation occurs in antigenicity.

10.5. ANTIBODIES (IMMUNOGLOBULINS)

Antibodies are proteins secretedby B-cells. Antibodies combine chemically with substances which the body recognizes as alien, such as bacteria, virus, and foreign substances in the blood.

Antibodies protect the host against potential parasites by:

1) directly inhibiting binding sites of virus or various enzymes and toxins produced by bacteria, 2) agglutination,
3) opsonization which facilitates removal by phagocytes,
4) lysis of susceptible organisms via complement fixation, and
5) inducing inflammation.

10.5.1. Antibody structure

The general structure of antibodies are explained with the example of IgG. The IgG molecule is composed of two identical heavy (H) chains and two identical light (L) chains. The H and L chains are linked by disulfide bridges.

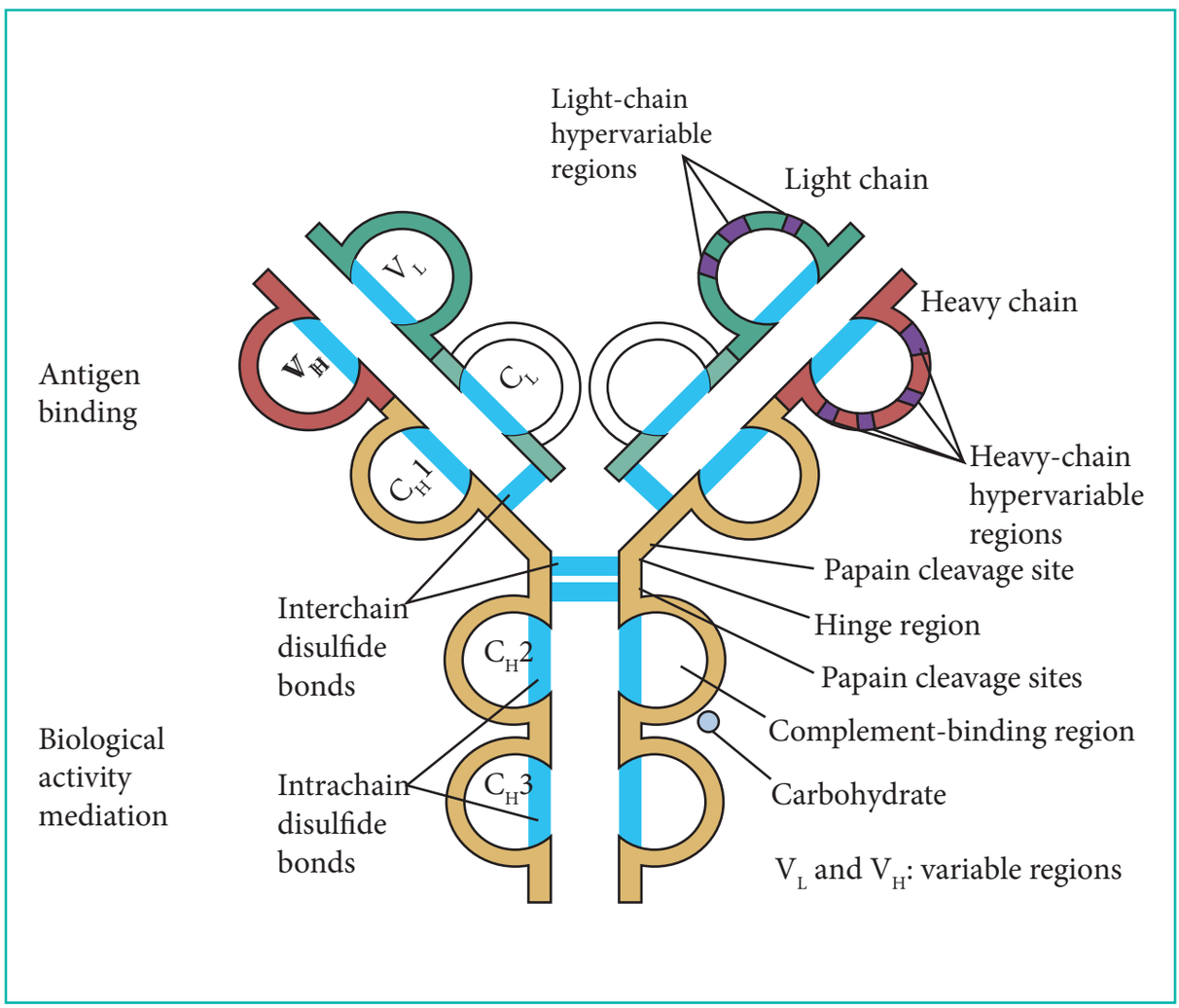
The H chain contains four or five domains. Each domain of immunoglobulin molecule consists of 110 amino acid residues. The amino terminal of heavy chain , shows great sequence variation to other antibodies for different antigens and was therefore called the variable (V) region. The remaining part of the heavy chain is called as constant (C) region. Each of the different heavy chain constant region sequences is called an isotype. The length of the constant region is approximately 330 amino acids for gamma, delta and

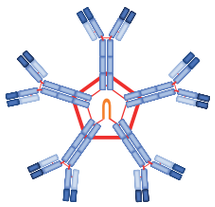
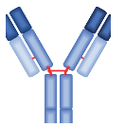
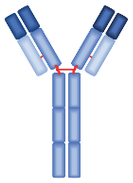
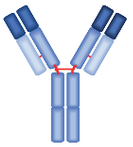
alpha and 440 amino acids for mu and epsilon. In humans there are two subclasses of alpha heavy chains and four subclasses of gamma chains .

Light chain domain consisting of 110 amino acids was found to have varying amino acid sequences in different antibodies for different antigens at amino terminal end. This region was called as the variable (V) region. The carboxy terminal half of the molecule, is called the constant (C) region. There are two types of light chains , kappa and Lambda . In humans 60% of the light chains are kappa and 40% are lambda. A single antibody molecule contains either kappa chains or lambda chains, but never both.

The gamma, delta, and alpha heavy chains contain an extended peptide sequence between the C_{H1} and C_{H2} domains. This region, called the hinge region, is rich in proline residues and is flexible.

A paratope (antigen-binding site) is a part of an antibody which recognizes and binds to an antigen. It is a small region of the antibody's variable region, and contains parts of the antibody's V_H domain of heavy and V_L domain of light chains. This region is also called as complementarity determining region (CDR). 2. Types of Antibodies - Classification



The Five Immunoglobulin(Ig) Classes					
	IgM Pentamer	IgG Monomer	Secretory IgA Dimer	IgE Monomer	IgD Monomer
			 Secretory Component		
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900000	150000	385000	200000	180000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	No	Yes	No	No	No
Fixes complement	Yes	Yes	No	No	No
Fc binds to		Phagocytes		Mast cells land basophils	
Function	Main antibody of primary responses, best at fixing complement, the monomer form of IgM serves as the B cell receptor.	Main blood antibody of secondary responses, neutralizes toxins, opsonisation	Secreted into mucus, tears, Saliva, Colostrum	Antibody of allergy and antiparasitic activity	B Cell receptor

10.6 ANTIGEN-ANTIBODY REACTIONS

Antigen-Antibody reactions refer to the interaction between antigens and antibodies. The reactions between antigens and antibodies occur in three stages.

The primary stage is the initial interaction between antigen and antibody without any visible effects.

The secondary stage leading to demonstrable events such as precipitation, agglutination, lysis of cells, killing of live antigens, neutralization of toxins and other biologically active antigens, fixation of complement, immobilization of motile organisms and enhancement of phagocytosis.

The tertiary stage reactions lead to neutralization of destruction of antigens or to tissue damage. They include humoral immunity against infectious disease as well as clinical allergy and other immunological disease.

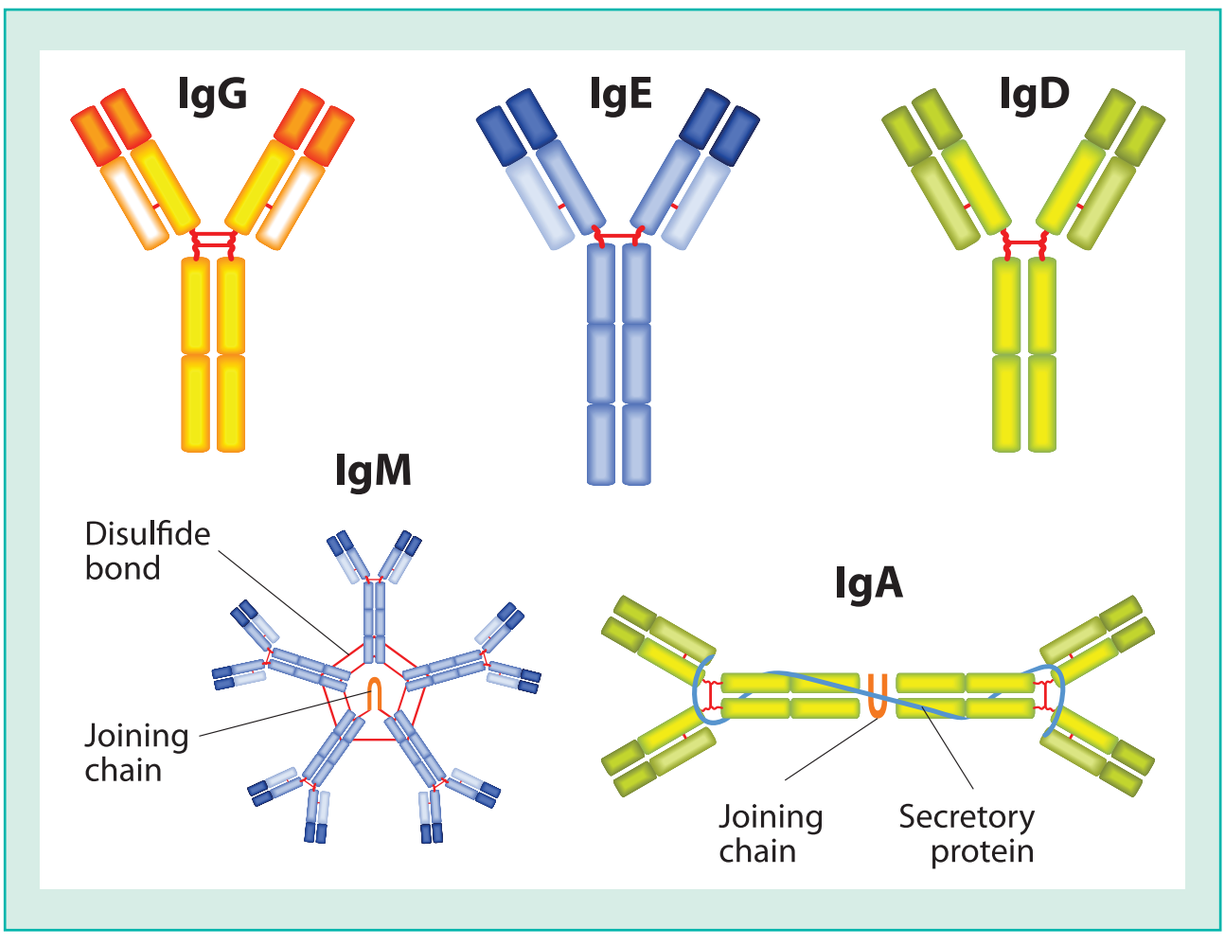
Antigen-antibody reactions have the following general characteristics:

1. The reaction is specific. However cross reaction may occur.
2.Entire molecules react and not fragment.
2. There is no denaturation of the antigen or the antibody during the reaction.
3. The combination occurs at the surface.
4. The combination is firm but reversible. The firmness of the union is influenced by the affinity and avidity of the reaction.

Affinity refers to the intensity of attraction between single epitope of the antigen and paratope of antibody molecules.

Avidity is the total strength of the bond after the formation of the antigen antibody complexes. Generally IgG possess greater affinity and IgM possess higher avidity

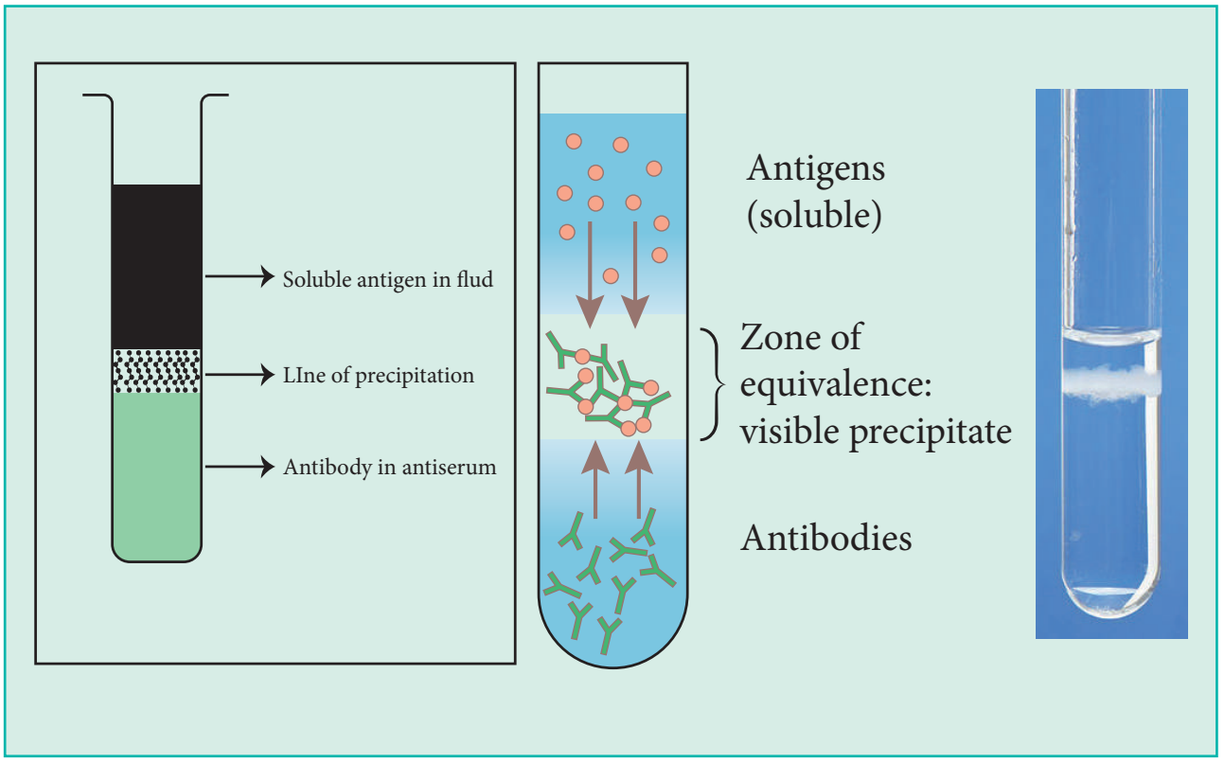
5. Both antigen and antibodies participate in the formation of agglutinates or precipitates.
6. Antigens and antibodies can combine in varying proportions.



10.6.1. Precipitation

The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops a visible precipitation. Antibody that forms precipitation is known as precipitin. This process is called as precipitation reaction. Formation of an Ag-Ab lattice depends on the valency of both antigen and antibody. Zone of equivalence is a point at which the maximum precipitation occurs. This reaction is widely used in several immunological techniques.

Ring test: In this test, over a column of antiserum (antibody), the antigen solution is layered. A precipitate forms at the junction of the two liquids.



10.6.2. Agglutinations

The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins. Agglutination reactions are similar in principle to precipitation reactions; they depend on the cross linking of polyvalent antigens. Just as an excess of antibody inhibits precipitation reactions, such excess can also inhibit agglutination reactions; this inhibition is called the prozone effect.

Haemagglutination (Slide Agglutination)

In typing for the ABO antigens, RBCs are mixed on a slide with antisera to the A or B blood-group antigens. If the antigen is present on the cells, they agglutinate, forming a visible clump on the slide. Determination of which antigens are present on donor and recipient RBCs is the basis for matching blood types for transfusions. At neutral pH, red blood cells are surrounded by a negative ion cloud that makes the cells repel one

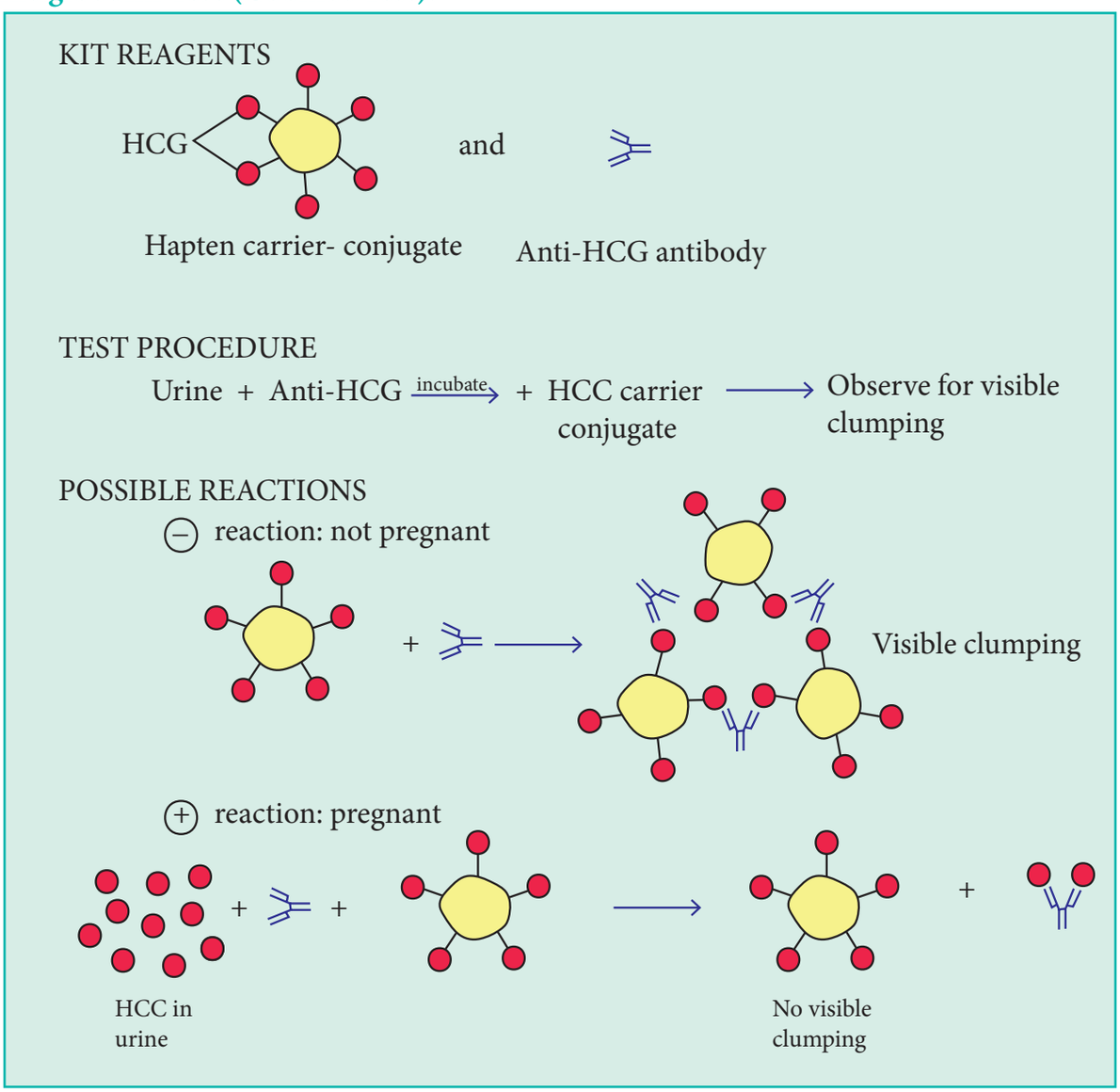
another.This repulsive force is called zeta potential. Because of its size and pentameric in nature, IgM can overcome the zeta potential and cross link red blood cells, leading to agglutination. The smaller size and bivalency of IgG makes it less able to overcome the zeta potential. For this reason, IgM is more effective than IgG in agglutinating red blood cells.

Bacterial Agglutination (Tube Agglutination)

A bacterial infection often elicits the production of serum antibodies specific for surface antigens on the bacterial cells. The presence of such antibodies can be detected by bacterial agglutination reactions.

Widal test is used for the diagnosis of typhoid fever. In typhoid patients, the serum contains antibodies to *Salmonella typhi*. In Widal test, two antigens are used. They are antigen H, the flagellar antigen and O antigen, the somatic antigen. When antiserum of the patient is added to the antigens, the antigens are clumped and identified.

Antiglobulin Test (Coombs Test):



The antiglobulin test was devised by Coombs, Mourant and Race for the detection of anti-Rh antibodies that do not agglutinate Rh positive erythrocytes in saline. When sera containing incomplete anti-Rh antibodies are mixed with Rh positive red cells, the antibody globulin coats the surface of the erythrocytes, though they are not agglutinated. When such erythrocytes coated with the antibody globulin are treated with a rabbit antiserum against human gammaglobulin (antiglobulin or Coombs serum), the cells are agglutinated. This is the principle of the antiglobulin test.

10.7. BLOOD GROUPS

A blood type is a classification of blood, based on the presence and absence of antibodies in blood and inherited antigenic substances on the surface of red blood cells. These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system.

10.7.1. ABO System

Karl Landsteiner discovered ABO blood group system. He was awarded Noble Prize in 1930 for his discovery. The membranes of human red cells contain a variety of blood group antigens**,**which are alsocalled **agglutinogens.** The most important and best known of these are theA and B antigens. Antibodies against red cell agglutinogens are called **agglutinins.** Antibody-A and Antibody-B are examples of agglutinins. Landsteiner law states that if an agglutinogen is present on the RBC of an individual, thecorresponding agglutinin must be absent in the plasma of that individual and vice-versa.

There are four main blood groups defined by the ABO system:

Blood group A people have A antigens on the red blood cells with anti-B antibodies in the plasma .Blood group B people have B antigens with anti-A antibodies in the plasma

Blood group O people have no antigens, but both anti-A and anti-B antibodies in the plasma .Blood group AB people have both A and B antigens, but no antibodies

Rh System

Another group of antigens found on the red blood cells of people is the Rh factor (Rhesus monkey, in which these antigens were first discovered). The Rh blood group system was discovered in 1940 by Karl Landsteiner and A.S. Weiner. It is the second most important blood group system, after the ABO blood group system. There are five different antigens (C, D, E, c and e) in this group, but D antigen predominantly presents and is medically important. Thus Rh antigen is always referred to as the Antigen-D. If this Rh antigen is present on a person's red blood cells, the person is Rh positive**;** if it is absent, the person is Rh negative**.**

Thus the common major blood group systems are of eight blood groups:

A RhD positive (A+)

A RhD negative (A-)

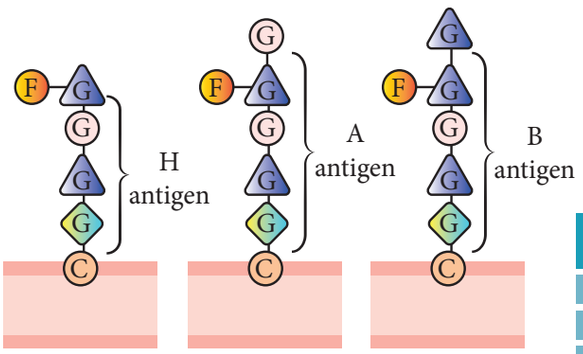
B RhD positive (B+)

B RhD negative (B-)

O RhD positive (O+)

O RhD negative (O-) AB RhD positive (AB+) AB RhD negative (AB-)

10.7.2. Antigens and Natural antibodies of ABO blood groups



Genotype	Antigen on RBCs	Antibody in Plasma
I^AI^A, I^Ai	A	Anti-B
I^BI^B, I^Bi	B	Anti-A
ii	O	Anti-A and anti-B
I^AI^B	AB	Neither anti-A and anti-B

F = fucose
G = N-acetylgalactosamine
G = glucose
G = galactose
C = ceramide

Each person inherits two genes (one from each parent) that control the production of the ABO antigens. The genes for A or B antigens are dominant to the gene for O. The O gene is recessive, simply because it does not code for either the A or the B red blood cell antigens. The genes for A and B are often shown as I^A and I^B and the recessive gene for O is shown as the lowercase i. A person who is type A, therefore, may have inherited the A gene from each parent (I^A I^A), or the A gene from one parent and the O gene from the other (I^Ai). Likewise, a person who is type B may have the genotype I^BI^B or I^Bi. It follows that a type O person inherited the O gene from each parent , whereas a type AB person inherited the A gene from one parent and the B gene from the other (I^A I^B).

The immune system exhibits tolerance to its own red blood cell antigens. People who are type A, for example, do not produce anti-A antibodies. However, they do make antibodies against the B antigen and, conversely, people with blood type B make antibodies against the A antigen. This is believed to result from the fact that antibodies made in response to some common bacteria present in the gut (normal flora) which are similar to A or B antigens. People who are type A, therefore, acquire antibodies that can react with B antigens by exposure to these bacteria, but they do not develop antibodies that can

react with A antigens because tolerance mechanisms prevent this. People who are type AB develop tolerance to both of these antigens, and thus do not produce either anti-A or anti-B antibodies. Those who are type O, by contrast, do not develop tolerance to either antigen; therefore, they have both anti- A and anti-B antibodies in their plasma.

Isoantibodies: Iso means belonging to the same species. Isoantibody is an antibody produced by oneindividual that reacts with the antigen of another individual of the same species. Antibody – A andAntibody– B are called Isoantibodies. Both of these anti-A and anti-B antibodies are of

Ig M type. Rh antibody (Antibody - D) is of Ig G type.

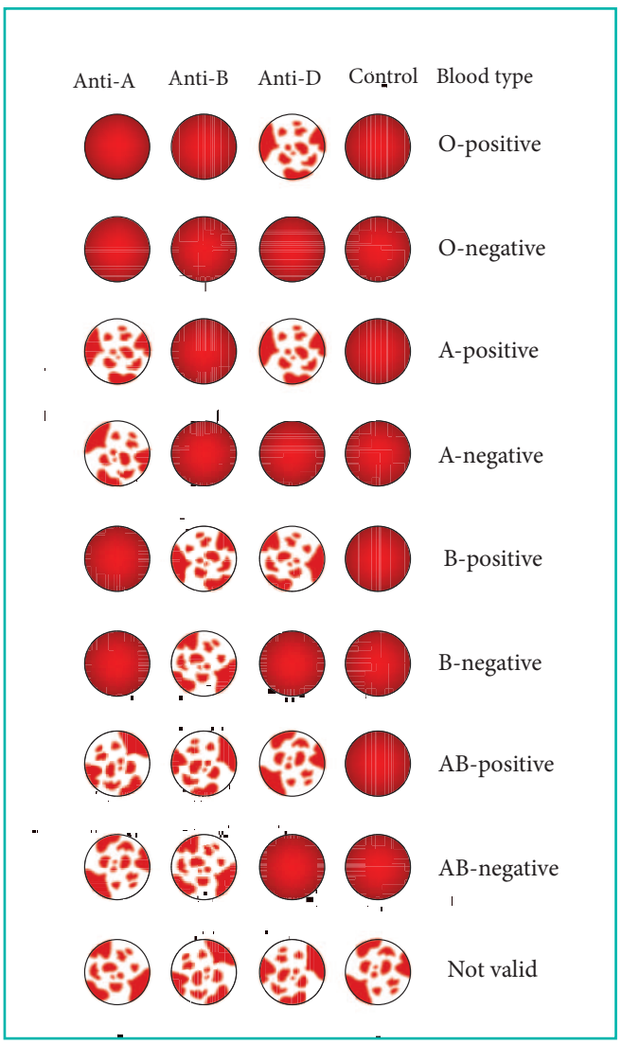
Isoantigens: An antigen of an individual which is capable of eliciting an immune response in individuals ofthe same species who are genetically different and who do not possess that antigen is called isoantigen. It is also otherwise known as alloantigens. A-Antigen and B-Antigen are examples of isoantigens.

Natural antibodies: Humans form antibodies against the blood group antigens they do not express.These antibodies are called naturally occurring antibodies or isoagglutinins. Antibody production starts at 3 months of age, reaches its highest level during adult and decreases with advancing age.

Test Procedure

Basically, a sample of blood is mixed separately with anti-A antibodies, anti-B antibodies and Rh antibodies. If the red cells clump together with anti-A antibodies, then it indicates the presence of A antigens in the red blood cells and the person belongs to A group. Similarly, if agglutination reaction occurs with anti-B antibodies then it indicates the presence of B antigen.

When agglutination is found in both anti-A and anti-B antibodies it indicates that the person belongs to AB group. If no agglutination is found with both antibodies of A and B then it indicates the absence of antigens and the person belongs to O group. Similarly if an anti-Rh antibody shows agglutination with the given blood then it indicates the presence of Rh antigens on the blood cells. Hence the person is Rh positive. If no agglutination is seen then the person is Rh negative.



Activity 10.1

Prepare the flow chart of the history of immunology

Activity 10.2

Cut an apple in half. Cover one half of the apple with food wrapper and leave the other half uncovered. Using a dropper, release several drops of food coloring on each half of the apple. Answer the following questions: What happened to the uncovered half of the apple and to the covered half?. How does the food wrapper provide a model of the human skin?

Activity 10.3 Creating Personnal Health Record

You can check on the status of your health habits by creating a personal health record for your own use.

Taking Care of Your Immune System

S.No.	Activities
1.	Eat a well-balanced and healthful diet.
2.	Get plenty of exercise and rest.
3.	Brush your teeth and bathe or shower regularly
4.	Keep your home clean.
5.	Avoid tobacco, drugs and alcohol.
6.	Get vaccinations that prevent diseases.

Do the following activities: Look at the list of behaviors in the table above. Write each of the behaviorsat the top of a separate piece of paper. Write down your habits related to each behavior during a typical week. Do you think that your weekly habits are healthy?

Activity 10.4

On a separate sheet of graph paper, graph the data of antibody production. You should assume that on 0th day the body was invaded by an unknown antigen. Then you can also assume that the person was exposed for a second time to the same antigen on day 40.

Time	Antibody Units	Time	Antibody Units	Time	Antibody Units	Time	Antibody Units
0	0	16	80	32	0	48	150
4	10	20	20	36	0	52	300
8	70	24	24	40	0	56	260
12	120	28	0	44	40	60	200

answer the following questions from the graph:

How does the first part of the graph (days 0-28) compare to the second part of the graph (days 28-60)?

Which do you think is the response being made by the memory cells?

Summary

The immune system protects the host from infectious agents. Entry, survival and proliferation of pathogenic microbes are referred to as infections. Bacterial, viral and fungal pathogens predominantly cause infectious diseases. Recovery from these infections is naturally achieved with the help of the natural defence system of the host , that is the immune system. The ability of the immune system to protect the host from infections is known as immunity.

There are two major types of immunity, namely innate and acquired immunity. Innate immunity is a type of immunity obtained by birth but it is non-spectific in its action. Innate immunity is provided by four barrier systems namely anatomical, physiological, phagocytic and inflammatory barriers.

Acquired immunity is a specific type of immunity which is provided by immune cells like T-Cells, B-Cells, K-Cells, NK-Cells etc., and immune components like antibodies. Acquired immunity is further divided into humoral immunity and cell mediated immunity. Antibodies playa vital role in humoral immunity. Immune cells like T-Cells play a vital role in cell mediated immunity.

Antigens are foreign substances which have an ability to induce production of immune response products.Depending upon their nature and origin, there are about thirteen different types of antigens. Antigenic determinant or epitope is the region of the antigen recognized by antibodies or T-Cell Receptor (TCR) of T cells. The ability of an antigen to induce an immune response is known as antigenicity. The factors that influence antigenicity are mainly of two types i.e. factors contributed by antigens and factors contributed by host cells.

Antibodies are a proteins produced by B cells in response to a specific antigen. The IgG molecule is composed of two identical heavy (H) chains and two identical light (L) chains. There are about five isotypes of antibodies namely Ig G, Ig M, Ig A, Ig E and Ig D. The H and L chains are linked by disulfide bridges. Antigen-Antibody reactions refer to the interaction between antigens and antibodies.

The interaction between an antibody and a soluble antigen in aqueous solution forms a lattice that eventually develops a visible precipitation. Antibody that forms precipitation is known as precipitin. The interaction between antibody and a particulate antigen results in visible clumping called agglutination. Antibodies that produce such reactions are called agglutinins.

A blood type is a classification of blood, based on the presence and absence of antibodies in blood and inherited antigenic substances on the surface of red blood cells. Karl Landsteiner discovered ABO blood group system. The membranes of human red cells contain a variety of blood group antigens, which are also called agglutinogens. The most important and best known of these are the Aand B antigens. Antibodies against red cell agglutinogens are called agglutinins. Antibody-A and Antibody-B are examples of agglutinins. Another group of antigens found on the red blood cells of people is the Rh factor which leads to the determination of the second major blood group system i.e. Rh system. There are eight common types of blood groups in combination of ABO and Rh blood grouping systems. Blood grouping tests are used for avoiding transfusion reactions and transplantation rejections.

EVALUATION

I. Choose the correct Answer

1. Who developed first vaccine for Rabies?

   a. Edward Jenner b. Rober Koch

   c. Louis Pasteur d. Lady Montagu

2. Which one of the following diseases is a pandemic disease?

   a. AIDS b. Common cold

   c. Rabies d. plague

3. Find the suitable vaccine for tuberculosis from the following vaccines:

   a. DPT b. MMR

   c. BCG d. TDP

4. One of the four cardina sign of inflammatory response “dolor” refers to

   a. Swelling b. Redness

   c. Heat d. Pain

5. Which type of the biomolecule is more antigenic in nature?

   a. Protein b. Carbohydrates

   c. Lipid d. Nucleic acid

6. Name of the protein produced in response to and counteracting a specific antigen from B-cells.

   a. antibody b. Interferon

   c. complement d. acute phase protein

7. What is the name of the region of antibody which recognizes and binds to antigen?

a. paratope b. agretope

c. epitope d. none

8. Which test is used for the diagnosis of typhoid fever.

a. Widal test b. ring test

c. tube test d. none

9. Antibodies against red cell agglutinogens are called as _____.

a. precipitin b. agglutinin

c. hapten d. epitope

10. What will be the blood group of a person with the genotype I^BI^B or I^Bi in ABO system?

a. A Group b. B Group

c. O Group d. AB group

11. Who discovered Rh factor?

a. Landsteiner and Weiner b. Louis Pasteur

c. Landsteiner and Koch d. None

12. How many types of constant region of heavy chain identified?

a. 2 b. 3

c. 4 d.5

13. Which one of the following immunity is non-specific in nature?

a. acquired immunity b. humoral immunity

c. innate immunity d. None

14. Name the process carried out by macrophages to lyse bacteria.

a. pinocytosis b. phagocytosis

c. transcytosis d. oxidation

15. What is the alternative name for antiglobulin test?

a. VDRL test b. Cooms test

c. Rabies test d. Koch test

16. Number of antigen binding sites in IgM antibody are

a. 7 b. 8

c. 9 d. 10

17. Which antibody crosses placenta?

a. Ig G b. Ig A

c. Ig M d. Ig E

18. Which type of light chain of antibody predominates?

a. Kappa b. Lamda

c. Gamma d. Alpha

19. What is the average number of amino acids present in a domain of antibody?

a. 440 b. 330

c. 220 d. 110

20. Which one of the following is a superantigen?

a. Autogen b. TSST-1

c. toxoid d. hapten

21. What is the causative agent causing Athlete's foot?

a. Bacteria b. virus

c. fungus d. helminthus

22. The causative agent for AIDS is

a. HPV b. Hepatitis virus

c. HIV d. SV

23. What is the name of the vaccine administered to prevent polio?

a. Salk vaccine b. Sabin vaccine

c. Both a and b d. None

24. Which vaccine used to prevent Tuberculosis?

a. TT b. DPT

c. BCG d. MMR

25. Who is the "Father of Immunology"?

a. Edward Jenner b. Rober Koch

c. Louis Pasteur d. Lady Montagu

II. Match the followings

1. Ig G - Complement fixation
2. Ig M - Colostrum

3. RA Factor - Allergy

4. Ig E - Opsonisation

5. Ig A - Rheumatoid Arthritis

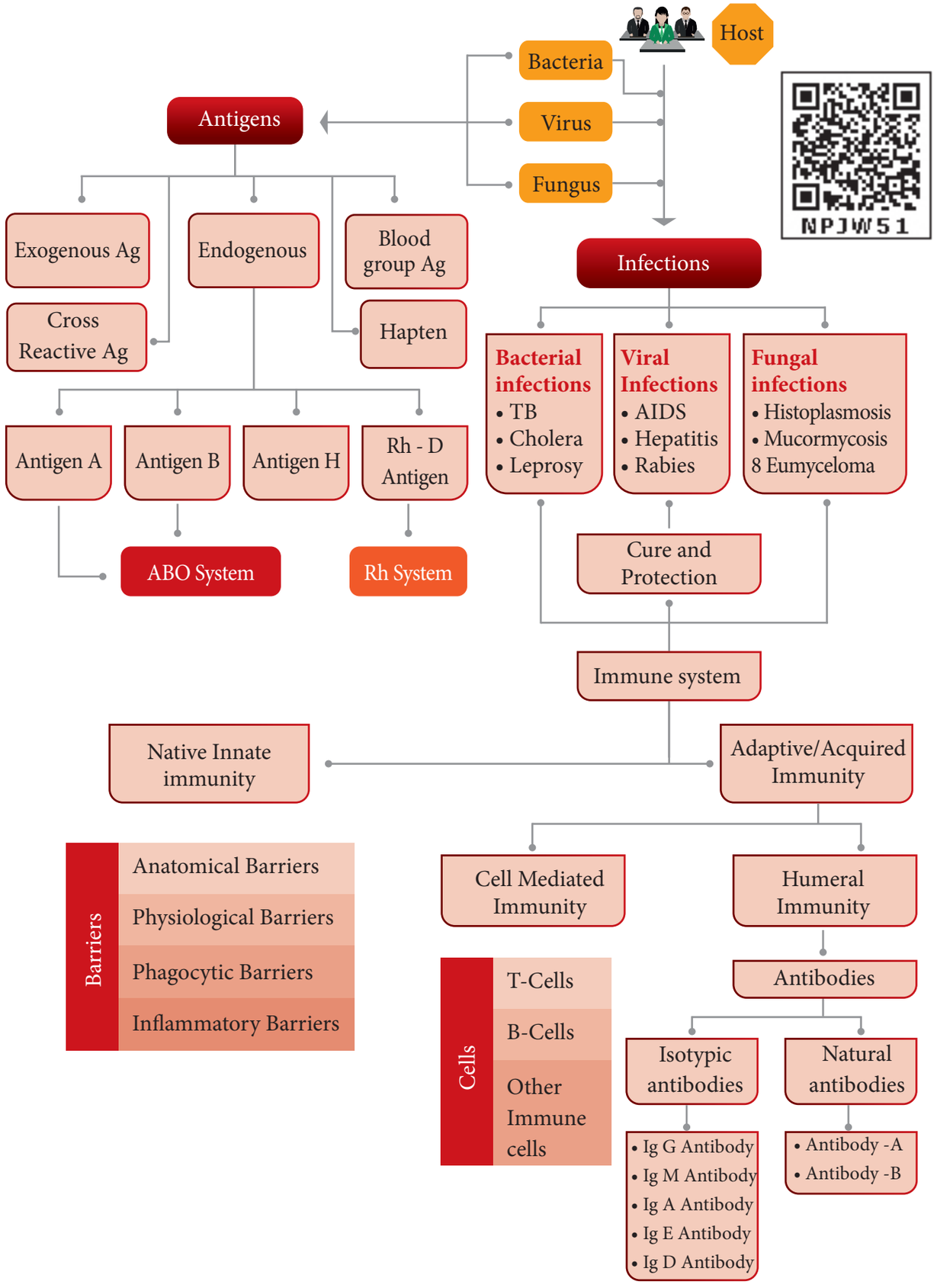
III. Answer the following questions

1. Define epitope
2. What do you meant by phagocytosis?
3. What are haptens?
4. What is the significance of immunologic memory?
5. Give four examples for viral infections.
6. Write short notes on fungal infections.
7. Elaborate the events occuring during inflammatory response.
8. Compare the events of humoral and cell mediated immunity.
9. List out the general characteristics of antigen-antibody reactions.
10. How to identify blood group?
12. Define isoantibodies.
13. What are acute phase proteins?
14. What is the role of tears in immunology?
15. List out minor blood group systems.
16. Explain the tube agglutination test.
17. Define allergen.
18. Define Epidemiology.
19. List out five viral diseases with treatment.
20. Define etiology.
21. Write a note on different diagnostic procedures for infectious disease.
22. What are the different transmission modes of infections?
23. Define variolation.
24. Briefly explain the steps involved in phagocytosis.
25. Differentiate different types of antibodies with a neat table.

Reference Books

1. Punt J, Stranford S, Jones P and Owen J, (2018). Kuby's Immunology, 8th edition, W. H. Freeman and Company, New York. (ISBN: 9781464189784)
2. Delves P, Martin S, Burton D and Roitt I M (2017). Roitt's Essential Immunology, 13th edition, Wiley-Blackwell Scientific Publication, Oxford. (ISBN: 978-1-118-41606-8)
3. Abbas A K, Lichtman A H and Pillai S (2015). Cellular and Molecular Immunology, 8th edition, Saunders Publication, Philadelphia. (ISBN: 978-0-323-22275-4)

CONCEPT MAP



NPJW51

+2 PRACTICALS

DHSKIS

Important instructions

- The students are required to have an observation and a record note book
- The students are advised to use overcoat and safety glass in laboratory.
- They are not permitted to taste or touch any reagent. If any reagent falls on skin, it must be immediately washed with water.
- The students should not inhale any gas or vapour directly.
- To transfer any solutions use droppers and for salts use spatula. During heating of a test tube, the open end should not face any student.
- For any accident in lab, immediately report to the teacher - incharge.
- Follow the systematic procedure carefully during analysis.
- Try to understand the Bio-chemistry in each test clearly.
- At the beginning and after finishing of each lab session wipe your bench tops using disinfectant solutions .
- Never pipette by mouth any chemical reagents. Doing so is strictly prohibited Pipetting is to be carried out with the aid of a mechanical pipetting device.
- Do not contaminate chemical while doing experiments.
- Speak quietly and avoid unnecesary movement around the laboratory t prevent distractions that may cause accidents.

II QUANTITATIVE ANALYSIS

What is Titration?

To determine the concentration of an unknown sample, we use a common laboratory technique called volumetric analysis. In this quantitative technique, the concentration of the particular substance present in an unknown sample solution can be determined by measuring the volume of another solution that reacts with it. The solution that has been taken in the burette is called the titrant and the solution taken in the conical flask is called the analyte.

What is end point of a titration?

When the reaction between fixed volume of titrant and the analyte becomes complete, the titration is said to be reached the end point. This end point can be identified using suitable indicators.

What are indicators?

An indicator is a chemical substance that undergoes a sharp colour change at the endpoint. In acid-base titrations, the endpoint is usually determined using acid-base indicators or PH indicators. Acid Base indicators are either weak organic acids or weak organic bases.

What is a standard solution?

A solution whose concentration is known is called a standard solution. It can be prepared by dissolving a known quantity of the substance in a definite volume of the solvent.

How do we determine the strength of a given acid or base?

Determination of the strength is based on the Law of Equivalents. According to this law, the number of equivalence of the substance to be titrated is equal to the number of equivalence of the titrant used.

Consider an acid-base titration in which V_1 ml of an acid solution of normality N_2 is required to neutralize N_2 ml of a base of normality N_2, then according to normality equation

$$V_1N_1=V_2N_2$$

From the above equation the normality of unknown solution can be calculated by measuring the volume of standard solution consumed.

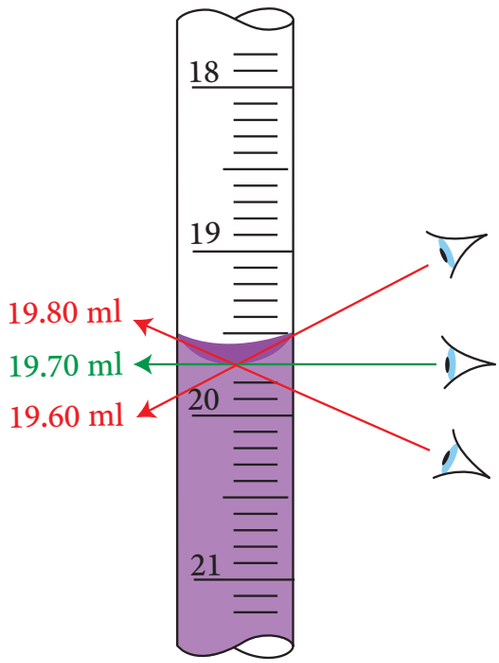
Apparatus Used in Volumetric Analysis

the following are the apparatus required in volumetric analysis.

(i) Graduated-burette.
(ii) 20 ml pipette
(iii) Conical flask.
(iv) Funnel, weighing bottle, wash bottle, etc.,.

Before a burette is filled with the solution, it is thoroughly washed, so that no greasy matter is sticking inside or outside the burette. Then rinse the burette with a small volume of titrant solution to be taken in it. Reject this solution through the stop-cock. After that,the burette is filled with titrant solution with the help of a funnel inserted in the top. The funnel must then be taken out after filling the burette.Care must be taken that no air bubbles remain in the narrow bottom tip of the burette. If air bubbles found in it, should be removed by running out the solution rapidly into the reagent bottle. Burette reading forms the most important aspect of the experiment, therefore, burette reading should be read very carefully.

In case of colourless solutions lower meniscus is read, while in case of coloured solutions, level is read from the upper meniscus. This is due to the reason that in case of coloured solutions lower meniscus is not visible clearly.Take reading of the burette placing your eye exactly in front of meniscus (Fig.) of the solution.



Pipette

The pipette is used for accurate measurements of definite volume of solution. It consists of a long narrow tube with cylindrical bulb in the middle and a jet at its lower end. On the upper part of the stem, there is an etched circular mark. Before a pipette is filled with the solution, it is washed and thoroughly rinsed with the solution to be measured with it. The upper part of pipette is then held by the thumb and middle finger of the right hand, the lower end is dipped into the liquid and the solution is sucked into the pipette until the liquid level is about 2 cm above the mark. The open end of pipette is then closed with index finger. The liquid is allowed to run slowly until the lower edge of meniscus just touches the mark. The solution is then allowed to run freely out of the pipette in the titration flask.

1. DETERMINATION OF BLOOD GROUPING

Aim

To determine the blood group of the given blood sample.

Principle

This test is based on the antigen antibody complex formation between the antigen present on the RBC and the antibody present in the serum.

Reagents and Equipments

1. Anti A, 2. Anti B, 3. Anti D, 4. Sterile lancet, 5. White marble tiles, 6. Sticks for mixing and 7. Alcohol

Procedure

The middle finger of the individual to be tested for blood group is cleaned with alcohol and the excess of alcohol is wiped out with sterile cotton. A clean white tile is taken and it is divided into four columns marked as A, B, D and C.

A small drop of anti A is added in the portion A, anti B in the portion B and anti D in the portion D. The portion C is used for positive or negative control. A small prick is made on the cleaned finger, the first drop of blood coming out is wiped off and second drop of blood is collected directly on the region marked as A, B and D. Immediately, blood is mixed with the corresponding antibody by using the sterile stick and observed for any agglutination in the form of clump formation.

If the clump is observed in the region marked as A, the blood group is A and the formation of clump in B shows presence of B blood group. If the clump is formed in both A and B the blood group is of AB type. If there is no clump formation in both the blood group, it is of O type. The clump formation in D portion is observed carefully. The formation of clump in the D region shows presence of Rh positive blood group and if there is no clump formation it shows the presence of Rh negative blood group.

Clump formation in the regions A and D shows the presence of A+ blood group and clump formation in the regions B and D shows the presence of B+ blood group. If clump is formed in all the three regions it shows the presence of AB+ blood group. No agglutination in regions A & B and agglutination in region D shows O+ blood group. No agglutination in all the three regions shows the presence of O- blood group. The following chart shows the type of blood group and the agglutination with anti-bodies.

If the clump formation is observed immediately in A blood group type it can be denoted as A_1 type, if it is not immediate then the blood group is of A_2.

S.No.	Antibody	Clump formation	Type of blood group
1.	Anti A	Yes	A Positive
	Anti D	Yes	
2.	Anti A	Yes	A Negative
	Anti D	No	
3.	Anti B	Yes	B Positive
	Anti D	Yes	
4.	Anti B	Yes	B Negative
	Anti D	No	
5.	Anti A & B	Yes	AB Positive
	Anti D	Yes	
6.	Anti A & B	Yes	AB Negative
	Anti D	No	
7.	Anti A & B	No	O Positive
	Anti D	Yes	
8.	Anti A & B	No	O Negative
	Anti D	No	

Result:

The blood group of the person is found to be _____

2. COLORIMETRIC ESTIMATION

2.1 ESTIMATION OF PROTEIN (BIURET METHOD)

Aim

To estimate the amount of protein present in the given plasma sample.

Principle

The-CO-NH- group of protein forms a purple coloured complex with copper ion in alkaline medium. The colour intensity is measured at 540nm. Since all proteins contain peptide bond, this method is fairly specific and there is little interference with other compounds.

Reagents required

Stock Biuret reagent

Dissolve 45g of Rochelle's Salt (Sodium potassium tartarate) in about 400ml of 0.2N sodium hydroxide and add 15g of copper sulphate. Stir it continuously until the solution is complete. Add 5g of potassium iodide and make upto 1 litre with 0.2N sodium hydroxide.

Biuret solution for use

Dilute 200ml of stock biuret reagent to 1 litre with 0.2N sodium hydroxide containing 5gm of potassium iodide.

Stock Standard Solution

1g of protein(egg albumin) is weighed and made upto 100ml with distilled water.

Concentration = 10mg/ml

Working standard solution

10ml of the stock is diluted to 100ml using distilled water.

Concentration = 1mg/ml

Procedure

Estimation of protein

0.5-2.5ml of standard protein solution is pipette out into five different test tubes (S1-S5). The concentrations of protein in the tubes are 0.5-2.5mg. 0.1ml of plasma solution is taken in two test tubes labelled as T_1 and T_2. The volume in all the tubes are made upto 5ml using distilled water. A blank is also prepared simultaneously by adding 5ml of distilled water. Then 3ml of biuret reagent is added to all the test tubes including blank. The tubes are mixed well. The tubes are then maintained at room temperature for 10 minutes. The optical density is measured at 540nm.

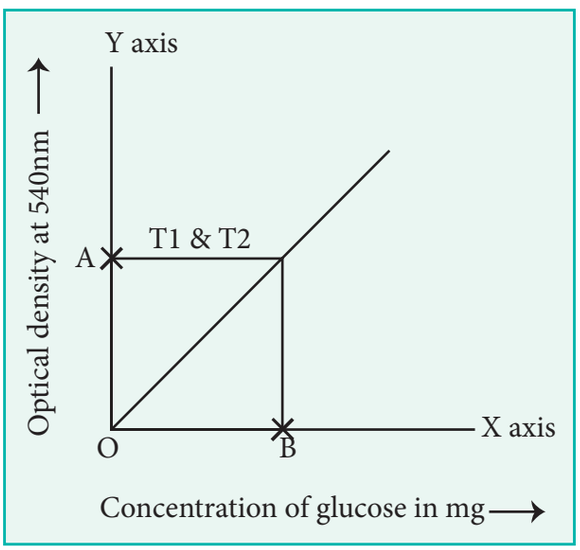
From the values obtained, a standard graph is drawn using concentration of protein in X-axis and optical density in the Y-axis. From the graph the amount of protein present in the given plasma is calculated.

Protocol for protein estimation

S. No.	Reagents required	Blank B	Standard					Plasma	
			S1	S2	S3	S4	S5	T_1	T_2
1.	Standard protein (ml)	-	0.5	1.0	1.5	2.0	2.5	-	-
2.	Concentration of protein (mg)	-	0.5	1.0	1.5	2.0	2.5	-	
3.	Plasma(ml)	-	-	-	-	-	-	0.1	0.1
4.	Distilled water(ml)	5	4.5	4.0	3.5	3.0	2.5	4.90	4.90
5.	Biuret reagent (ml)	3	3	3	3	3	3	3	3
6.	Optical density at 540nm								

Graph

Let the optical density of tubes T_1 & T_2 be A and the corresponding protein concentration is B as shown in the graph.



Calculation

For T_1 & T_2

The optical density A of plasma corresponds to B mg of protein.

0.1ml of plasma contains B mg of protein

Therefore,

100ml of plasma will contain = 100 x B/0.1mg of protein

= Z mg of protein

Result:

The amount of protein present in the given plasma sample= Z mg.

2.2 ESTIMATION OF GLUCOSE (ORTHOTOLUIDINE METHOD)

Aim

To estimate the amount of glucose present in the given blood sample.

Principle

A solution of orthotoluidine in glacial acetic acid when treated with glucose produces a blue coloured product with an absorption maximum at about 640nm. The values obtained represent the true glucose level.

Reagents required

1. Stock Solution

100mg of glucose is weighed and made upto 100ml with distilled water.

Concentration of glucose = 1mg/ml

2. Working standard solution

10ml of stock solution is diluted to 100ml with distilled water.

Concentration of glucose = 100µg/ml.

3. Orthotoluidine reagent

12.5mg of thiourea and 12g of boric acid are dissolved in 50ml of distilled water by heating over a mild flame. 75ml of redistilled orthotoluidine reagent and 375ml of analar acetic acid are mixed separately. The two solutions are mixed and the total volume is made upto 500ml with acetic acid. The reagent is kept overnight at 4˚C.

4. Preparation of Blood sample

0.2ml of blood sample is taken in a centrifuge tube. To this 0.3ml of 10% sodium tungstate, 0.3ml of 2/3 N sulphuric acid and 3.2ml of distilled water are added to precipitate the proteins. It is kept aside for 10 minutes and then centrifuged at 3000 rpm for 10 min. 1ml of the supernatant is taken for the estimation of glucose.

Procedure

Estimation of glucose

0.2-1.0ml of standard glucose solutions are pipetted out into five different test tubes labelled S1-S5 with the concentration of 20-100µg. 1ml of the deproteinised supernatant is pipette out into different test tubes labelled as T_1 and T_2. Final volume is made upto 1ml using distilled water in all the standard tubes. 4ml of orthotoluidine reagent is added to all the test tubes. A blank is also prepared simultaneously comprising 1ml of distilled water and 4ml of orthotoluidine reagent. All the test tubes are heated for 20 minutes in a boiling

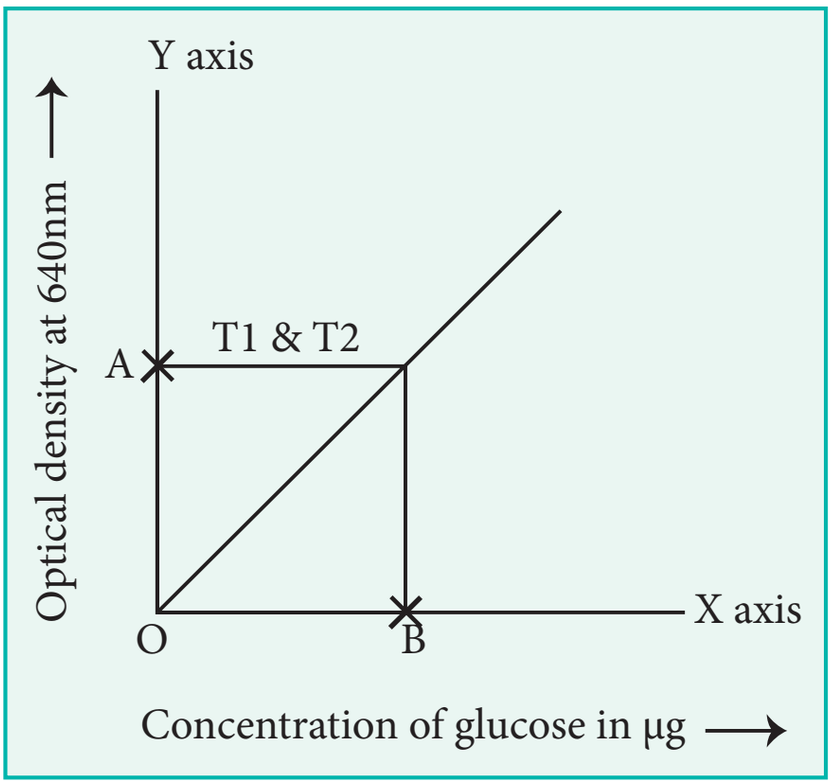
water bath. The blue colour developed is measured at 640nm using a colorimeter.

A standard graph is drawn with optical density in Y- axis vs. Concentration of glucose in axis. The amount of glucose present in the given blood sample is then calculated.

Protocol for glucose estimation

S. No.	Reagents required	Blank B	Standard					Plasma	
			S1	S2	S3	S4	S5	T_1	T_2
1.	Standard glucose (ml)	-	0.2	0.4	0.6	0.8	1.0	-	-
2.	Concentration of glucose (mg)	-	20	40	60	80	100	-	-
3.	supernatant(ml)	-	-	-	-	-	-	1.0	1.0
4.	Distilled water(ml)	1	0.8	0.6	0.4	0.2	-	-	-
5.	Orthotoluidine reagent (ml)	4	4	4	4	4	4	4	4
Tubes are kept in boiling water for 20 minutes and cooled.									
6.	Optical density at 640nm								

Graph



Calculation

For T_1 & T_2

The optical density A of T_1 & T_2corresponds to B µg of glucose.

1.0 ml of supernatant contains B µg of glucose

Therefore,

4ml of supernatant will contain = 4 x B/1.0 µg of glucose

= Z µg of glucose

0.2ml of blood contains Z µg of glucose.

Therefore,

100ml of blood will contain = 100 x Z/0.2 µg of glucose

= C mg of glucose (1000µg= 1mg)

Result:

The amount of glucose present in 100ml of the given blood sample = is _____ mg.

3. ESTIMATION OF CALCIUM (TITRIMETRIC METHOD)

Aim

To estimate the amount of calcium present in the given serum sample.

Principle

Calcium is precipitated as Calcium Oxalate with ammonium oxalate. The precipitate is washed with ammonia to remove the chloride ions. The washed precipitate is then made to react with 1N sulphuric acid. The liberated oxalic acid is now estimated by titrating against standardised potassium permanganate. The amount of oxalic acid liberated is proportional to the amount of calcium.

Reagents required

1. **Ammonium Oxalate solution (4%)**

   4g of ammonium oxalate dissolved in 100ml of distilled water.

2. **Ammonia Solution (2%)**

   2ml of ammonia of specific gravity 0.88 is diluted to 100ml with distilled water.

3. **Potassium Permanganate(0.1N)**

   This is prepared by dissolving 3.16 g of potassium permanganate in 1 litre of distilled water.

4. **Standard oxalic acid solution (0.1N)**

   It is prepared by dissolving 630mg of oxalic acid in 100ml of distilled water.

5. **Sulphuric acid(1N)**

Procedure

Standardisation of Potassium Permanganate

10ml of oxalic acid is pipetted out into a clean conical flask and 10ml of dilute sulphuric acid is added and heated at 60˚C . It is titrated against potassium permanganate in the burette. The end point is the appearance of pale permanent pink colour. Titrations are repeated for concordant values.

Precipitation of Calcium Oxalate

2ml of serum is taken in a centrifuge tube and 2ml of distilled water is added followed by 1ml of 4% ammonium oxalate. The contents are mixed and allowed to stand overnight at 4˚C for complete precipitation of calcium. The precipitate is separated by centrifugation and the supernatant is discarded. To the precipitate 3ml of 2% ammonia is added and centrifuged. This procedure is repeated thrice and the supernatant is tested for the presence of chloride. 10ml of 1N sulphuric acid is added and warmed for solubilisation. This solution is now titrated against potassium permanganate and the volume consumed is noted.

10ml of 1N sulphuric acid is treated as blank and titrated against potassium permanganate. The end point is the appearance of pale permanent pink colour. Titrations are repeated for concordant values and the amount of calcium present is then calculated.

Tabular Column

TITRATION-I

=Standardisation of Potassium permanganate

Standard Oxalic acid Vs Potassium permanganate

S. No.	Volume of Oxalic acid(ml)	Burette		Volume of Potassium Permanganate (ml)	Indicator
		Initial (ml)	Final(ml)		
1.	10	0	x	x	Self
2.	10	0	x		

Volume of oxalic acid V_1 = 10ml

Normality of oxalic acid N_1 = 0.1N

Volume of Potassium permanganate = x ml

Normality of potassium permanganate = ?

Normality of potassium permanganate N_2 = $\frac{V_1N_1}{V_2}$

= $\frac{10 \times 0.1}{X} = N_2$

= *YN*

TITRATION-I I

Estimation of Calcium in Serum

S. No.	Volume of Oxalic acid(ml)	Burette		Volume of Potassium Permanganate (ml)	Indicator
		Initial (ml)	Final(ml)		
1.	**Test Solution** Oxalic acid liberated from calcium oxalate +10ml of sulphuric acid	0	X1	X1-X2	Self
2.	**Blank Solution** 10ml of sulphuric acid	0	X2	(X3)	

Calculation

The amount of calcium present in the given sample can be calculated by using the equation.

1ml of 0.1N potassium permanganate is equivalent to 0.2mg of calcium. Therefore, X3 ml of 'YN' potassium permanganate is equivalent to

0.2 x X3 x Y / 1 x 0.1 = Z mg of Calcium.

2ml of serum contains Z mg of Calcium.

Therefore, 100ml of serum contains 100 x Z/2 mg of calcium.

Result:

The amount of calcium present in the given serum sample is _____ mg/100ml.

ESTIMATION OF PROTEIN BY LOWRY'S METHOD

Aim:

To estimate the amount of total protein in the given sample.

Principle:

It is the most commonly used method for determination of proteins in cell free extracts because of its high sensitivity and quantities as low as 20µg of proteins can be measured. The CO-NH (peptide bonds) in polypeptide chain reacts with copper sulphate in an alkaline medium to give a blue coloured complex. In addition, tyrosine and tryptophan residues of proteins cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method

Reagents:

1. 5% TCA
2. 0.1N sodium hydroxide
3. **Lowry's reagent:** Solution A: 2.0% sodium carbonate in 0.1N sodium hydroxide; Solution B: 0.5% copper sulphate in 1% sodium potassium tartarate solution (freshly prepared); Mix 50ml of solution A and 1ml of solution B prior to use.
4. **Folin'sciocalteu reagent:** A commercial preparation is diluted with water in the ratio of 1:2 before use.
5. **Standard protein solution:** Standard BSA containing 20mg/dl is prepared.

Procedure:

Take 0.1ml of unknown solution and make it to 1ml with 0.1N sodium hydroxide. Add 4.5ml of Lowry's agent shake well and allow standing for 10 minutes. To this 0.5ml of Folin'sciocalteu reagent is added, mix well and kept in room temperature for 20 mins. Record the absorbance at 660 nm.In another set of tubes take suitable aliquots of BSA solution (in a range of 20-100µg) make the total volume to 1ml with 0.1N sodium hydroxide and develop the colour as described above. Draw a standard curve of absorbance at 660 nm versus µg of BSA. From this standard curve determine the amount of protein in the sample tube and calculate the amount of protein per gram of the sample.

S.No.	Particular/ Reagents	B	S_1	S_2	S_3	S_4	S_5	T
1.	Standard solution (concentration of working 2mg/dl)	0	0.2	0.4	0.6	0.8	1.0	-
2.	Concentration (mg)	0	40	80	120	160	200	-
3.	Volume of Test solution (ml)	-	-	-	-	-	-	1
4.	Volume of NaOH (ml)	1	0.8	0.6	0.4	0.2	0	0
Mix the contents in the test tubes								
5.	Volume of Folin's reagent (ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Incubate all the test tubes @ room temperature								
6.	Optical density @ 660nm							

Result:

The amount of protein present in the given sample (1ml) was found to be (mg)

ESTIMATION OF CHOLESTEROL BY ZAK'S METHOD

Aim:

To determine the amount of cholesterol present in the given sample by Zak's method

Principle:

In this method, cholesterol is treated with concentrated sulphuric acid. Cholesterol in the presence of sulphuric acid undergoes dehydration to form 3, 5-cholestadiene. This is inturn oxidised and sulphonated to form red colouredCholestapolyenesulphonic acid in the presence of Fe3+ ions. The intensity of red colour formed is proportional to the amount of cholesterol present in the serum. The colour intensity is measured by using a green filter at 540nm.

Reagents:

1. **Stock ferric chloride reagent:** 840mg of was weighed and dissolved in 100 ml of glacial acetic acid.
2. **Ferric chloride diluting reagent:** 8.5ml of stock ferric chloride was diluted to 100ml with glacial acetic acid.
3. **Standard cholesterol solution:** 100mg pure dry cholesterol was dissolved in 100ml of acetic acid.
4. **Working standard:** Dilute the stock solution by 10 fold.

Procedure:

Different aliquot of working standard ranging from 0.5ml to 2.5ml was taken. The total volume of each tube was made up to 5 ml with ferric chloride diluting reagent. To 0.1ml of unknown solution add 4.9ml of ferric chloridediluting reagent. Mix well and centrifuge 2.5ml of supernatant.Add3 ml of concentrated sulphuric acid. Test tubes are incubated at room temperature to 20-30 mins. Intensity of the red colour is measured at 560nm against blank.

S.No.	Particular/ Reagents	B	S_1	S_2	S_3	S_4	S_5	T
1.	Standard solution (concentration of working solution : 10 mg /dl)	0	0.5	1.0	1.5	2.0	2.5	-
2.	Concentration (mg)	0	50	100	150	200	250	-
3.	Volume of Test solution (ml)	-	-	-	-	-	-	1
4.	Volume of $FeCl_3$ diluting agent (ml)	5	4.5	4.0	3.5	3.0	2.5	4
Mix the contents in the test tubes								
5.	Volume of conc H_2SO_4 (ml)	3	3	3	3	3	3	3
Mixall the test tubes and leave at room temperature for 10 mins								
6.	Optical density @ 540nm							

Result:

The amount of cholesterol present in the given sample is _____(mg)

ESTIMATION OF ASCORBIC ACID (VITAMIN C)

Aim:

To estimate the vitamin C content in given samplecolorimetrically.

Reagents:

1. **Indophenol reagent:** 20mg of 2,6–dichlorophenolindophenols was dissolved in 10ml of warm distilled water.
2. **DNPH-Thiourea mix reagent:** 2gm of 2,4 – dinitrophenyl hydrazine and 1gm of Thiourea was dissolved in 100ml of 9N sulphuric acid.
3. **5% TCA:** 5gm of Tricholorocetic acid was dissolved in 100ml of dissolved water.
4. **85% sulphuric acid:** 85ml of concentrated sulphuric acid is made up to 100 ml with distilled water.
5. **Standard ascorbic acid:** 100mg of ascorbic acid dissolved in 100ml of distilled water.

Procedure:

To 2ml of the unknown sample, 1 drop of the indophenols reagent was added. Then 0.5ml of DNPH-Thiourea mix reagent was added and the mixture was incubated at 60°C for 1 hour. The tubes were then cooled in an ice bath and 2.5ml of 85% sulphuric acid was added to it while shaking. The red colour developed was measured at 540 nm after 30 minutes. Standard graph was prepared by taking standard ascorbic acid at a concentration of 100µg/ml.

Observation

S.No.	Particular / Reagents	B	S_1	S_2	S_3	S_4	S_5	T
1.	Standard Solution (10mg/dl)	0	0.2	0.4	0.6	0.8	1	-
2.	Concentration (mg)	0	20	40	60	80	100	-
3.	Distilled water (ml)	1	0.8	0.6	0.4	0.2	0	-
4.	Volume of Test Solution (ml)	-	-	-	-	-	-	1
5.	Indophenol (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6.	DNPH (ml)	1	1	1	1	1	1	1
Keep it in the boiling water bath and warm it for 30 min								
7.	Volume of H_2SO_4(ml)	3	3	3	3	3	3	3
8.	Optical density @ 540nm							

Result:

Vitamin C content of the given food material was found to be (µg/ml)

ESTIMATION OF DEOXYRIBO NUCLEIC ACID(DNA)

Aim:

To quantify the deoxyribonucleic acid content in the given sample

Principle:

The deoxyribose moiety of the DNA forms a hydroxyl levulaldehyde in TCA solution. This reacts with diphenylamine to give a blue colour which can be read at 565 nm.

Reagents:

1. **Dische's reagent:** Dissolve 1.5g of crystalline diphenylamine in 100 ml of glacial acetic acid and add 1.5 ml of conc. sulphuric acid.The reagent is stored in dark bottles. At the time of use add 0.1 ml of aqueous acetaldehyde (16mg/ml) for each 20 ml of the reagent.
2. 1N perchloric acid:
3. 5mMSodium hydroxide:
4. **Standard DNA solution:** A stock solution is prepared by dissolving DNA (0.4 mg/ml) in 5mMsodium hydroxide. Working standards are prepared by mixing a measured volume of the stock standard with an equal volume of 1N perchloric acid and heating at 700C for 15 minutes.

Procedure:

A standard curve is made by pipetting different aliquots of standard DNA solution,containing 50-250µg of DNA into clean tubes.Separately, 0.1-1ml of the acid extract is taken in clean tubes. To this, 2.0ml of the freshly prepared Dische's reagent is added and the total volume in each tube is made 5 ml with glass distilled water.The initial turbidity formed disappears gradually. The tubes are covered with glass marbles and heated in a boiling water bath for 10-15 min.The solution is then cooled under tab water and the blue colour developed is measured at 565 nm in a spectrophotometer.The control or blank contains only water and the Dische's reagent.

S.No.	Particular/ Reagents	B	S_1	S_2	S_3	S_4	S_5	T
1.	Standard solution concentration (mg)	0	0.4	0.8	1.2	1.6	1.8	-
2.	Concentration (mg)	0	160	320	480	640	800	-
3.	Test solution (ml)	-	-	-	-	-	-	1
4.	citrate buffer (ml)	2	1.6	1.2	0.8	0.4	0.2	1
5.	Diphenyl amine (ml)	4	4	4	4	4	4	4
Keep in bailing water bath fan 10 mins cool and take reading at 595 nm.								
6.	Optical density at 595 nm							

Result:

Amount of DNA present in given sample is _____ µg

ESTIMATION OF UREA BY DIACETYL MONOXIME METHOD

Aim:

To estimate the amount of urea present in the given sample

Principle:

Under acidic conditions when urea is heated with compounds containing two adjacent carbonyl groups such as diacetylmonoxime coloured products are formed. On heating, it decomposes to give hydroxylamine and diacetyl, which then condenses with urea to give rose purple diazinederivative.The colour intensity is measured colorimetrically at 520nm.

Reagents:

1. **Acid reagent:Reagent A:** 5g of ferric chloride is dissolved in 20ml water and 100ml of orthophosphoric acid (85%) is slowly added with stirring and the volume is made up to 250ml with water;Reagent B:200ml of conc. sulphuric acid is added to 800ml water;0.5ml of reagent A is added to 1 litre of Reagent B.
2. **Colour Reagent:** Reagent C: Dissolve 20g diacetylmonoxime in 1 litre of water;Reagent D: Dissolve 5g thiosemicarbazidein l litre of water;Mixed 67ml of C and 67ml of D and made this volume upto1 litre with distilled water.
3. **Urea Stock solution:** Dissolve 100mg Urea in 100 ml of distilled water.

Procedure:

Into a series of test tubes, aliquots of working standard solutions (0.2,0.4,0.6,0.8 and 1.0ml) are taken 1.0ml of unknown sample is taken for the test and the volumes are made up to 2.0ml with distilled water. 2.0ml of water serves as blank. 2.0ml of colour reagent and 2.0ml of acid reagent are added to all the tubes and placed in a boiling water bath for 20 min. The tubes are cooled and pink colour developed is read at 520nm.

S.No.	Particular – Reagents	B	S_1	S_2	S_3	S_4	S_5	T
1.	Standard Solution (10mg/100ml)	-	0.2	0.4	0.6	0.8	1	-
2.	Concentration (mg)	0	20	40	60	30	100	-
3.	Volume of test solution (ml)	-	-	-	-	-	-	1
4.	Distilled water (ml)	2	1.8	1.6	1.4	1.2	1	1
5.	Acid reagent (ml)	2	2	2	2	2	2	2
6.	Colouring reagent (ml)	2	2	2	2	2	2	2
Boil for 15 – 20 minutes								
7.	Optical density at 520 nm							

Result:

The amount of urea present in the given sample is (mg/dl)

ESTIMATION OF AMINO ACID BY SORENSEN'S FORMOL TITRATION METHOD

Aim:

To estimate the amount of amino acid present in the given solution by formol titration

Principle:

The carboxyl group of amino acid cannot be accurately measured by titration with alkali because it reacts with a basic amino acid group to form zwitter ions of amphoteric nature at neutral pH. They may not be decomposed at the end point. But in the presence of formaldehyde with alkali, the amino acid forms an amino acid formol complex. The complex is acidic because of the basic character of amino acid group is suppressed and hence it can be titrated with alkali using phenolphthalein as indicator.

Reagents:

1. **Stock glycine solution:** 7g of glycine is made up to 100ml with distilled water.
2. 630mg of oxalic acid is made up to 100ml with distilled water.
3. Sodium hydroxide solution (0.1N): 4g of made up to 1000ml with distilled water.
4. 40% Formaldehyde
5. Phenolphthalein indicator.

Procedure:

Standardisation of sodium hydroxide: 0.1N of oxalic acid was prepared (0.63g of oxalic acid was dissolved in 100 ml of water). 10ml of oxalic acid was pipetted out into a conical flask and titrated against sodium hydroxide using phenolphthalein as indicator. End point is the appearance of pale pink colour. The experiment was repeated for concordant values.

Estimation of amino acid: 10 ml of formaldehyde and 10ml of distilled water are pipetted out into a conical flask. This is taken as blank. This is titrated against standard sodium hydroxide using phenolphthalein as indicator. A permanent pink colour obtained is the end point. Burette reading is noted and the titration is repeated for concordant values.

10ml of 0.1 N glycine, 10ml of formaldehyde and 10ml of distilled water are pipette out into a conical flask. The solution is allowed to stand for ten minutes to complete the reaction. The above mixture is titrated against sodium hydroxide using phenolphthalein as indicator. A pale pink colour was obtained. End point was noted and the experiment was repeated for concordant values. Similarly the titration is repeated with the unknown glycine and the amount of glycine in the unknown sample is calculated.

Titration: 1 Sodium hydroxide vs oxalic acid

S.No.	volume of solution in conical flask	Burette readings (ml)		Volume of NaOH consumed (ml)	Concordant volume
		Initial	Final		
	10	0			
	10	0			

Titration : 2 Blank titration (sodium hydroxide vs formaldehyde)

S.No.	volume of solution in conical flask	Burette readings (ml)		Volume of NaOH consumed (ml)	Concordant volume
		Initial	Final		
	20	0			
	20	0			

Titration : 3 Sodium hydroxide vs unknow solution

S.No.	volume of solution in conical flask	Burette readings (ml)		Volume of NaOH consumed (ml)	Concordant volume
		Initial	Final		
	30	0			
	30	0			

Calculation

Titration 1

Volume of oxalic acid (V1) = 10ml
Normality of oxalic acid (N1) = 0.1N
Volume of NaOH (V2) =
Normality of NaOH (N2) = N1V1 / V2

Titration 2

Volume of NaOH (V3) =

Titration 3

Volume of NaOH (V4) =
Net Volume of NaOH (V5) = V4 – V3

Normality of NaOH (N2) =

Volume of amino oxide (V6) = 10ml
Normality of amino oxide = N2 V5 / V6
Normality = (Weight / L) / equivalent weight
Weight /L = Normality x equivalent weight

Result:

The amount of glycine present in the whole of the given solution was found to be …….....

Glossary - கலைச்சொற்கள்

Acidosis	அமிலத் தேக்கநோய்
Activator	இயக்குவிப்பான்
Active site	கிளர்வு மையம்
Active transport	செயல்மிகு கடத்தல்
Agglutination	திரட்சி வினை
Albinism	வெளிறுதல்(தோல், கண்)
Amphipathic	நீர்வெறுக்கும்
Antibody	எதிர்காப்பு மூலங்கள்
Antigen	உடற்காப்பு ஊக்கி
Antiporters	எதிர் திசைக் கடத்திகள்
Atherosclerosis	தமனி தடிப்பு
Avidity	இனையும் திறன்
Bacillus	உருளைவடிவ பாக்டீரியா
Bee-sting	தேனீ கொடுக்கு
Bile salt	பித்த உப்புகள்
Biocatalyst	உயிரூக்கிகள்
Biogenic	உயிரிவழித்தோற்ற
Biosynthesis	உயிர்த் தொகுப்பு
Blood vessel	இரத்த குழல்கள்

Cartilage	குருத்தெலும்பு
Catabolism	சிதைமாற்றம்
Cataract	கண்புரை
Cereals	தானியங்கள்
Chemotaxis	வேதித் தூண்டியக்கம்
Choroids	விழியடிகரும்படலம்
Coccus	கோளவடிவ பாக்டீரியா
Competitive inhibition	போட்டித்தன்மையுள்ள தடுத்தல்
Convulsion	வலிப்பு
Cortex	புறணி
Crenation	செல் சுருங்குதல்
Cytosol	உயிரணுக்கணிகம்
Debranching enzyme	கிளை நீக்கும் நொதி
Devoid	காலியான
Diarrhoea	வயிற்றுப் போக்கு
Duodenum	சிறுகுடலின் முன்பகுதி
Duodenum	முன்சிறுகுடல்
Electron transport chain	எலக்ட்ரான் நகர்வுச் சங்கிலி
Electrophiles	எலக்ட்ரான் கவர் காரணிகள்

Elongation factors	நீளமாக்கும் காரணிகள்
Emaciated	உடல் இளைத்தல்
Endoplasmic reticulum	எண்டோபிளாச வலைப் பின்னல்
Erythrocyte	இரத்த சிவப்பு அணுக்கள்
Essential fatty acids	இன்றியமையாத கொழுப்பு அமிலங்கள்
Facilitated diffusion	புரதவழிக் கடத்தல்
Fatty acids	கொழுப்பு அமிலங்கள்
Fibril	நுண்ணிழை
Foreign bodies	நோய்க்கிருமிகள்
Gastro intestinal tract	இரைப்பைக் குடல்
Ghosts	கூடுகள்
Goitre	முன்கழுத்துக் கழலை
Gun cotton	வெடிபஞ்சு
Hemolysis	இரத்தச்சிதைவு
Haemorrhage	இரத்தப்போக்கு
Hepatitis	மஞ்சள் காமாலை
Host	ஓம்புயிரி
Hydrophilic	நீர் விரும்பும்
Hydrophobic	நீர் வெறுக்கும்

Hyperglycaemia	மிகை இரத்த சர்க்கரை
Hypoventilation	மந்த சுவாசம்
Immunity	நோய் எதிர்ப்பாற்றல்
Infections	நோய்த் தொற்று
Inflammation	அழற்சி
Inhibitor	தடுப்பான்
Initiation factors	துவக்கக் காரணிகள்
Jaundice	மஞ்சள் காமாலை
Kidney	சிறுநீரகம்
Leukaemia	இரத்தப் புற்றுநோய்
Lipolysis	கொழுப்புச் சிதைவு
Liver	கல்லீரல்
Lung alveoli,	நுரையீரல் சிற்றறைகள்
Lymphoma	நிணநீர் நாளப்புற்று
Macrophage	பெருவிழுங்கணுக்கள்
Membrane	செல் சவ்வு
Membrane transport	செல்சவ்வுக் கடத்தல்
Meningitis	மூளைக்காய்ச்சல்
Metabolism	வளர்சிதை மாற்றம்

Mutation	திடீர்மாற்றம்
Neoplasm	உயிரணுப் புற்று
Neurotransmission	நரம்புத் தூண்டல்
Non-competitive Inhibition	போட்டித் திறனற்ற தடுத்தல்
Nucleophiles	கருகவர்க் காரணிகள்
Nuts	கொட்டைகள்
Obesity	உடல் பெருத்தல்
Organelle	உள்ளுறுப்புகள்
Passive transport	செயலற்ற கட்த்தல்
Pathogen	நோய்க்கிருமி
Peripheral proteins	புற அமை புரதங்கள்
Phagocytosis	செல் விழுங்குதல்
Pinocytosis	செல்குடித்தல்
Polymerisation	பலபடியாக்கல்
Precursor	முதல்நிலைப் பொருட்கள்
Proteolytic enzymes	புரதச் சிதைவு நொதி
Pulses	பருப்பு வகைகள்
Reducing power	ஒடுக்கும் திறன்
Satiety value	நிறைவுத் தன்மை

Sclera	விழிவெண்படலம்
Sequential process	படிநிலைகள்
Serum	இரத்த திரவம்
Sheath	உறை
Sickle cell anaemia	அரிவாளணுச்சோகை
Signal	சமிக்ஞை
Skeletal muscles	எலும்பு தசை
Spiders	சிலந்திகள்
Starvation	பட்டினி
Stem cell	ஆதாரச் செல்
Strand	இழை
Stress	மன உளைச்சல்
Symporters	ஒரு திசைக் கடத்திகள்
Symptoms	அறிகுறிகள்
Syphilis	பால்வினை நோய்
Ternary complex	முக்கூட்டுப் பொருள்
Thoracic duct	வயிற்றுக் குழல்
Toxic	நச்சுத்தன்மை கொண்ட
Transamination	அமினோ மாற்றம்

Transcription	படியெடுத்தல்
Translation	மரபுக்குறியீட்டு பெயர்ப்பு
Trans membrane proteins	செல்சவ்வு குறுக்கு புரதங்கள்
Uncompetitive inhibition	போட்டித் தன்மையற்ற தடுத்தல்
Uniporters	ஒரு பொருள் கடத்திகள்
Vascular bundles	கடத்துதிசுக் கற்றை
Vesicle	சிறு கொப்புளம்
Villi	குடலுறிஞ்சி
Whole grains	முழுதானியங்கள்

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