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Question Bank of BIOCHEMISTRY



R.A. Joshi

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PREFACE

Biochemistry currently had occupied an eminent position not only as Medical subject but as the main part in other disciplines also. Many foreign editions along with Indian editions are available in the market, but very less books are there which are helpful for the students to prepare for the exam. Having being connected with teaching profession for many years it was felt that students find it difficult to answer in the exam. The answer in the book are given in very lucid, simple, and authentic manner. Hope that it will be beneficial for students.

I am thankful to all my students and colleagues for their valuable suggestions during preparation of the text. I am deeply indebted to my husband Sri A.K. Joshi and children Shreya and Tanmay for their valuable help and cooperation. Last but not the least I am thankful to my publisher for publishing the book.

RASHMI A. JOSHI

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CONTENTS

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1

BIOPHYSICS AND INSTRUMENTATION

Q. Define adsorption and adsorbent.

Ans. Adsorption is the surface phenomenon. The surface of a solid or liquid has a tendency to attract and retain molecules of other species with which it is brought into contact. As these molecules remain only at the surface and do not go deeper into the bulk, their concentration is more at the surface than in the bulk of solid or the liquid, as the case may be. The phenomenon of higher concentration of any molecular species at the surface than in the bulk of a solid (or a liquid) is known as **adsorption.** The forces involved are the molecular forces.

The solid that takes up a gas or vapour or solute from a solution is called the **adsorbent** while the gas or the solute, which is held to the surface of the solid, is called **adsorbate**.

Q. Define imbibition.

Ans. This is a very significant property of hydrophilic substances i.e. adsorption of water molecules. The tenacity with which water molecules are held on the imbibing surface is a function of their water potential and the nature of surfaces (especially charge and the distance between water molecules and the surface). The closer the molecules are to the surface more firmly they will be held. The tenacity with which they are held may be expressed in terms of chemical potential or water potential. The phenomenon of imbibition has three important characteristics

1. Volume change—In imbibition the volume of the system increases i.e. swelling occurs. The total volume of water imbibed plus the imbibing material is less after than before.
2. Production of heat—As the water molecules are arranged on the surface of the imbibiant they loose some of their kinetic energy which then appears as heat in the system.
3. If an imbibing system is confined, the swelling may develop great pressure.

Q. Define Surface tension.

Ans. Surface tension may be defined as the force in dynes acting at right angles to the surface of a liquid along one-centimeter length of the surface.

Q. Define Diffusion.

Ans. Simple diffusion is defined as the random mixing of ions and molecules in a solution due to their kinetic energy. Substances may also diffuse through a membrane, if the membrane is permeable to them. Lipid soluble molecules such as O_2, CO_2, N_2, steroids, small alcohol's and ammonia diffuse through the phospholipid bilayer of the plasma membrane into and out of the cell.Water also diffuses easily through the phospholipid bilayer. Diffusion is important in the movement of oxygen and carbon dioxide between blood and body cells and between blood and air within the lungs during breathing.

Lipid insoluble molecules also are diffused into or out of the cells through small water filled pores of channels formed by integral proteins For example Na^+, K^+, Ca^{++}, Cl^-, HCO_3^- and urea diffuse through the membrane via pores of channels.

Q. Define Facilitated diffusion.

Ans. Many ions urea, glucose, fructose, galactose and certain vitamins, which are lipid insoluble diffuse through the plasma membrane by facilitated diffusion. In this process the substances moves down its concentration gradient from a region of higher concentration to a region of lower concentration with the help of specific integral proteins in the membrane that can serve as water filled channels or transporters (carriers) for each type of substance. The rate of facilitated diffusion is determined by the size of the concentration difference on the two sides of the membrane and the number of channels or transporters available.

The most common example of facilitated diffusion is transport of glucose across plasma membrane. Glucose first attaches to a transporter on the outside of the membrane. Different cells have different glucose transporters.The transporter changes its shape. Glucose passes through the membrane and is released inside the cell. After glucose has entered a cell by facilitated diffusion an enzyme known as kinase attaches a phosphate group to give glucose 6 phosphate. This reaction keeps the intracellular concentration of glucose itself very low so that the concentration gradient also favor facilitated diffusion of glucose into, not out of the cell.

Q. What is Ionic product of water.

Ans. Water is a weak electrolyte, which dissociates to form H^+ and OH^- ions.

$$H_2O \rightleftharpoons H^+ + OH^-$$

The ionization of water is a reversible reaction admitting the law of mass action. The concentration of H_2O in pure water may be calculated to be 1000/18 or 55.5 moles/lit. Since the concentration of H_2O in dilute aqueous solution is essentially unchanged from that in pure H_2O, this figure may be taken as a constant. It is usually in fact incorporated into the expression for the dissociation constant of water to give—

$$[H^+]\,[OH^-] = 1.8 \times 10^{-16} \times 55.5$$
$$Kw = 1.01 \times 10^{-14}$$
$$Kw = 1.01 \times 10^{-14} \text{ at } 25°C$$

This Kw is called as **ionic product of water** and it expresses the relation between the concentration of H^+ and OH^- ions in aqueous solutions. Since in pure water, the concentration of hydrogen and hydroxyl ions must be equal to one another.

$$[H^+] = [OH^-] = 1 \times 10^{-14}$$
$$= 1 \times 10^{-7} \text{ mol } l^{-1}$$

The relation $Kw = [H^+]\,[OH^-]$ is important since if either of $[H^+]$ or $[OH^-]$ is known, the other can be calculated easily.

Q. Define pH.

Ans. Sorensen introduced the term pH as a convenient manner of expressing the concentration of

H^+ ion by means of logarithmic functions.

This pH may be defined as

$$pH = \log \frac{1}{aH^+} = -\log aH^+$$

Where aH^+ is defined as the activity of H^+. There is no distinction in text between activities and concentrations so,

$$pH = \log \frac{1}{H^+} = -\log \left[H^+ \right]$$

If we now apply the term of pH to the ion product expression for pure water we obtain

$$[H^+] \times [OH^-] = 1.0 \times 10^{-14}$$

Taking logarithm of this equation

$$\log [H^+] + \log [OH^-] = \log (1.0 \times 10^{-14}) = -14$$

Multiplying by –1

$$-\log [H^+] - \log [OH^-] = 14$$

If $-\log[OH^-]$ is defined as pOH then

$$pH + pOH = 14$$

The solution is acidic when $[H^+]$ is greater than 10^{-7}, and if $[H^+]$ concentration is less than 10^{-7} the solution is alkaline. The reaction of all biological fluids occur between $[H^+]10^{-1}$ and 10^{-10}.

Q. Differentiate between an acid and a base.

Ans. An acid is a substance, which has a tendency to donate a proton to any other substance and a base as a substance, which has tendency to accept a proton from any other substance. In other words, an acid is a proton donor and base is a proton acceptor.

When an acid loses a proton, the residual part of it has a tendency to regain the proton. Therefore, it behaves as a base. An acid and a base may, therefore be defined, by the general equation.

$$\text{Acid} \rightleftharpoons \underset{(\text{Proton})}{H^+} + \text{Base}$$

The acid and base which differ by a proton as represented by the above relationship are known to form a conjugate pair.

Q. What are conjugate acids and bases?

Ans. If we consider, an example of the ionization of acetic acid in water which may be represented as

$$\underset{\text{Acid}}{CH_3COOH} + \underset{\text{Base}}{H_2O} \rightleftharpoons \underset{\text{Acid}}{H_3O^+} + \underset{\text{Base}}{CH_3COO^-}$$

In this acetic acid donates a proton to water and thus acts as **acid**, water accepts a proton and, therefore, acts as a **base**. In the reverse reaction hydronium ion (H_3O^+) donates a proton to a acetate ion, and therefore, acts as an acid. The acetate ion can accept a proton and, therefore behaves as a base.

Such pairs of substances, which can be formed from one another by gain or loss of protons are known as **conjugate acid-base** pairs. Thus acetic acid is the conjugate acid of acetate ion and acetate ion is the conjugate base of acetic acid. Similarly water is the conjugate base of hydronium ion and hydronium ion is the conjugate acid of water.

Q. What is relative strength of acids and bases?

Ans. The strength of an acid depends upon its tendency to lose protons and the strength of base depends upon its tendency to gain protons. If an acid such as hydrochloric acid, is a strong acid, it will have a strong tendency to donate protons.

$$HCl + H_2O \rightleftharpoons H_3O^+ + Cl^-$$

Thus, the equilibrium lies very much to the right and the reverse reaction, representing the gain of protons by the chloride ions leading to the reformation of HCl, but will take place to a very small extent. Thus, chloride ion is a weak base.

Acetic acid, on the other hand has considerably less tendency to lose protons and is therefore, a much weaker acid. It will ionize very little.

$$CH_3COOH + H_2O \rightleftharpoons H_3O^+ + CH_3COO^-$$

The equilibrium lies mostly towards the left. It follows therefore, that acetate ion must have a much greater tendency than chloride ion to gain protons and hence it is a much stronger base than the chloride ion.

Thus, as a general rule, the stronger acid, the weaker must be its conjugate base and vice versa. If an acid (e.g. HCl) is strong, its conjugate base (Cl^-) is weak). If a base (e.g. CH_3COO^-) is strong, its conjugate acid (CH_3COOH) is weak.

Q. What is ionization constant?

Ans. A weak acid is only partially ionized in aqueous solution. Consider the ionization of the generalized weak acid HA:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

The proton donated by HA is accepted by H_2O to form H_3O^+ hydronium ion. The equilibrium constant is known as **ionization constant** Ka. The $[H_3O^+]$ is same as the hydrogen ion concentration so—

$$HA \rightleftharpoons H^+ + A^-$$

$$Ka = \frac{\left[H^+\right]\left[A^-\right]}{\left[HA\right]}$$

This constant (Ka) is characteristic of the acid concerned and varies only with temperature like other dissociation constants.

Similarly, the ionization of weak base can be repressed as

$$BOH \rightleftharpoons B^+ + OH^-$$

$$Kb = \frac{\left[B^+\right]\left[OH^-\right]}{\left[BOH\right]}$$

Q. Describe the Henderson-Hasselbalch Equation.

Ans. Henderson Hasselbalch have rearranged the mass law as it applies to the ionization of weak acids into useful expression called as **Henderson Hasselbalch equation.** If we consider the ionization of a weak acid

$$HA \rightleftharpoons H^+ + A^-$$

$$Ka = \frac{[H^+][A^-]}{[HA]}$$

Rearranging the equation

$$[H^+] = \frac{Ka\,[HA]}{[A^-]}$$

Taking logarithms of both sides

$$\log [H^+] = \log Ka + \log \frac{[HA]}{[A^-]}$$

and multiplying by –1

$$-\log [H^+] = -\log Ka - \log \frac{[HA]}{[A^-]}$$

$-\log [H^+]$ is defined as pH

$-\log Ka$ is defined as pKa

$$pH = pKa + \log \frac{[Salt]}{[Acid]}$$

This equation is known as Henderson Hasselbalch equation. It enables the calculation of pH values of buffer solutions made by mixing known concentrations of weak acids and its salts. Alternatively it enables calculation of the ratio in which acid and salt must be mixed in order to get a buffer solution of a definite pH.

Q. What are Buffers?

Ans. Buffers are the solution that resists a change in pH on the addition of acid or alkali. Most commonly the buffer solution contains a mixture of weak acid and its conjugate base. For e.g. mixture of acetic acid and sodium acetate or of ammonium hydroxide and ammonium chloride are buffer solutions.

Q. Explain the mechanism how the buffers maintain the constant pH in a solution.

Ans. The buffer solution contains a mixture of weak acid and its conjugate base. When alkali i.e. NaOH is added to the mixture of the acetic acid (CH_3COOH) and sodium acetate (CH_3COONa) the following reactions occur

$$CH_3COOH + CH_3COONa + NaOH \rightarrow 2CH_3COONa + H_2O$$

There will be no free OH^- ions in solution and pH will remain same.

Similarly when HCl is added to buffer solution—

$$CH_3COOH + CH_3COONa + HCl \rightarrow NaCl + 2CH_3COOH$$

The salt (NaCl) is formed and no free H^+ are in solution.

Addition of acid or base does not alter the pH of buffer. The buffer acts almost as if it were absorbing the added free H^+ or OH^- ions.

Q. What are Physiological buffers? Or Describe the physiological importance of buffers.

Ans. Buffers are of great physiological importance. The importance of buffers depend on several factors as molar concentration of buffer components, ratio of concentration of conjugate base to that of weak acid.

In animals complex and vital buffer system is bicarbonate and carbonic acid buffer found in circulating blood. This buffer neutralizes stronger dietary and metabolic acids (HA) converting them into weak bases (A^-) with the increase in H_2CO_3. Stronger bases (B) are also converted into weak acids (BH^+) with the rise in HCO_3^-.

$$HA + HCO_3^- \rightleftharpoons A^- + H_2CO_3$$
$$B + H_2CO_3 \rightleftharpoons BH^+ + HCO_3$$

The buffer ratio in blood remain unchanged by the respiratory elimination of H_2CO_3 as CO_2 or the urinary elimination of HCO_3^-.

The other buffers present in circulating blood are as Phosphate buffer, Haemoglobin and Oxyhaemoglobin buffer.

Q. What are Indicators?

Ans. Indicators are chemical compounds, which change their colour with change in pH of the solution in which they are present. These indicators are dyes, which are weak acids or weak bases. They have one colour, but after ionization change their original colour. Universal indicator is a mixture of indicators, which gives a wide variety of colours over a wide range of the pH. Indicators are used in

(a) Determination of the end point in acid base titrations.

(b) Determination of pH of solutions.

Indicators	**PH range**	**Colour**	
		In acid solution	**In alkaline solution**
Thymol blue	1.2 – 2.8	Red	Yellow
Methyl yellow	2.9 – 4.0	Red	Yellow
Methyl orange	3.1 – 4.4	Red	Orange yellow
Methyl red	4.3 – 6.1	Red	Yellow
Phenol red	6.7 – 8.3	Yellow	Red
Phenolphthalein	8.2 – 10.0	Colourless	Violet red

Q. Define dialysis.

Ans. Dialysis is a process by which the more diffusible materials can be separated from non diffusible materials. If we take water solution of egg albumin and sugar in the upper small container

whose open bottom is covered with a semipermeable membrane. The semipermeable membrane has got selective permeability to water and sugar molecules but not to macromolecules - the egg albumin. This container is suspended partially in water of a large container filled with water. Due to selective permeability, the sugar molecules will ultimately go into the water leaving behind only the albumin and water. As the albumin is impermeable to this membrane, this will rebound from the membrane during the process of dialysis.

Q. What is Osmosis? Define osmotic pressure.

Ans. Osmosis is the net diffusion of a solvent, which is water in living system through a selectively permeable membrane .Osmotic pressure is an important force in the movement of water between various compartments of the body. Normally the osmotic pressure of intracellular fluid is the same as the osmotic pressure of the interstitial fluid outside cell. Because the osmotic pressure on both sides of the membrane are equal. Cell volume remains relatively constant. But the osmotic pressure of blood plasma is greater than the osmotic pressure of the interstitial fluid surrounding capillary walls. This difference tends to draw tissue water into blood capillaries. In conditions that decrease the blood osmotic pressure excess water remain in interstitial fluids. Such condition is called as edema.

Demonstration experiment – By thistle funnel

Take a thistle funnel and cover its mount with parchment paper (semipermeable membrane). Pour sugar solution into the funnel till it reaches the middle of the vertical tube. Dip the inverted funnel in a beaker containing water. Fix the thistle funnel in a vertical position. Mark the level of sugar solution in the vertical tube and observe.

After sometime level of solution in the vertical tube starts to rise. This shows that the water molecules enter into thistle funnel through semipermeable membrane. A considerable rise in the level of the liquid will be noticed. This phenomenon of the passage of pure solvent into a solution through a semipermeable membrane is known as **osmosis**. The flow of the solvent through the semipermeable membrane will continue till equilibrium is reached when the hydrostatic pressure of the liquid column exactly balances the tendency of water to pass inward through the semipermeable membrane. The hydrostatic pressure set up as a result of osmosis is the measure of the osmotic pressure of the solution. For instance, if the liquid rises to a height h, then

$$\pi = h \times \rho$$

where ρ is the density of the solution.

Thus, the osmotic pressure of a solution is equivalent to the pressure needed to prevent the passage of pure water (or any solvent molecule) into the solution of higher concentration (from a solution of lower concentration) through a semipermeable membrane thereby preventing an increase in the volume of that solution. In other words the maximum amount of pressure that can be developed in a solution separated from pure water by a semipermeable membrane is termed as **osmotic pressure.** For exaple a molar solution of sucrose separated from pure water by a semipermeable membrane has osmotic pressure of approximately 22.4 atoms (22.3 hrs) at 0°C.

Osmotic pressure can be calculated by **Vant Hoff's law of osmotic pressure,** which states that

1. The osmotic pressure of a solution is directly proportional to the concentration of the solute in the solution.
2. The osmotic pressure of a solution is directly proportional to the absolute temperature.

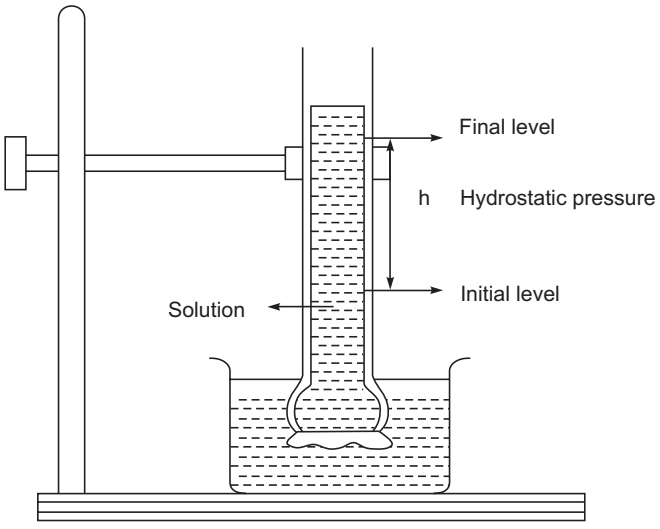


Fig. 1.1. Experiment to demonstrate osmosis by thistle funnel.

Thus they indirectly follows Boyle's and Charles Law

$$PV = nRT$$

Or $$P = \frac{nRT}{V} \quad \text{... (1.1)}$$

But $$n = \frac{\text{grams}}{\text{Mol wt}} = \frac{g}{M}$$

Putting this in eqn (2.1)

So $$P = \frac{gRT}{MV}$$

$$g = \frac{C}{MV}$$

So $$P = cRT$$

Where P is pressure
C is molal concentration
R is gas constant
T is absolute temperature

According to the law of osmotic pressure, 1 molar solution exerts an osmotic pressure of 22.4 liters at 0°C.

The osmotic pressure of substances which ionizes is given by

$$P = iCRT$$

Where i is the **isotonic coefficient** and is given by

$$i = 1 + \alpha\ (n–1)$$

Where α is degree of ionization
n is number of ions produced
The value of i is dependent on the degree of dissociation of the electrolyte.

Unit of osmotic pressure

The unit of osmotic pressure is **osmol or milliosmol.** It is defined as exerting the osmotic pressure of a one molar solution of a non dissociated solute in one liter of solution. The number of osmoles of a nonionized substance in one liter of solution is the weight in grams divided by its molecular weight.

The milliosmolar concentration of glucose in a sample of plasma containing 90mg per 100 ml will be

$$\frac{90 \text{ mg per } 100 \text{ ml} \times 10}{180 \ (\text{Mol. Wt. Of glucose})} = 5 \text{ milliosmol per lit.}$$

Q. Describe the different methods of measurement of Osmotic Pressure.

Ans. The osmotic pressure can be measured in various ways.

1. Mechanical Methods

(*a*) **By putting weights**—The simplest way is to apply adequate pressure (i.e. weight) upon the stronger solution to prevent any rise of volume. That pressure which is just needed to stop the increase of volume of a particular solution is the measure of its osmotic pressure.

(*b*) **By manometer**—The same thing can be done by connecting the apparatus with a suitable manometer in which the pressure will gradually rise till it equalizes with the osmotic pressure of the solution, at which point further rise will stop.

2. Biological Methods

(*a*) **Hamburger's Red Corpuscle Method**—Red cells are kept in the unknown solution for some time after which the cell volume is noted. If the cell volume be reduced, the solution is hypertonic than plasma (hence, water has been drawn out), if the cells swell up, the solution is hypotonic (so that water has entered), if no change, the solution is isotonic. If sufficiently hypertonic, the red cells will gradually swell up and ultimately burst **(Haemolysis)**.

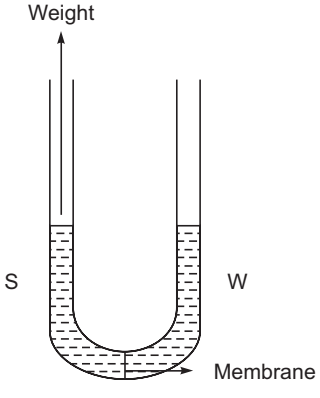


Fig. 1.2. Measuring osmotic pressure

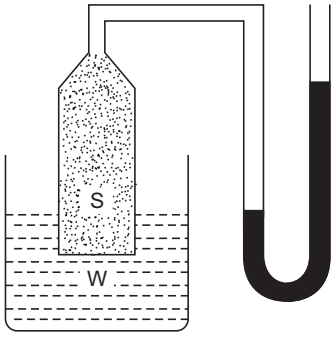


Fig. 1.3. Measuring osmotic pressure by weight with manometer

De veris Plant Cell Method - The same principle is followed as above, only plant cells are used instead of red cells and the comparison is made with the cell sap inside. In hypertonic solutions, the cells will shrink, in isotonic- there will be no change, while in hypotonic solutions the cell will swell up and may burst (Plasmolysis).

3. Physical Methods

(*a*) **Depression in freezing point**—By calculating the depression in freezing point, the osmotic pressure is measured. Higher the concentration, lower will be the freezing point and therefore higher will be the osmotic pressure.

(*b*) **Calculating Vapour Tension**—The higher the concentration, lower will be the rate of evaporation from the solution and higher will be the osmotic pressure.

4. Barger's Capillary Glass Tube Method

Alternate drops of known and unknown solutions separated by air bubbles and are drawn in standard capillary glass tube and after some time the edges of the solution is noted. The edges will shift according to the rate of evaporation, from these data osmotic pressure can be calculated.

Q. What are isotonic, hypertonic and hypotonic solutions?

Ans. Solutions, which have the same osmotic pressure at the same temperature, are said to be isotonic. For example if a solution in which a cell is placed has osmotic pressure equal to that of cell sap. The outer solution is called isotonic solution. If the osmotic pressure of outer solution is more than that of the cell sap the outer solution is known as hypertonic solution and in case the osmotic pressure of the outer solution is less than that of the cell sap the outer solution is called the hypotonic solution.

Q. What is Viscosity?

Ans. Viscosity implies resistance to flow. It is well known that all liquids do not flow equally readily. Those liquids which flow slowly (For example, glycerine, castor oil, honey and coal tar) are said to have high viscosity while those which flow readily (for example, water, alcohol and ether) are said to have low viscosity.

The significance of viscosity may be further elucidated, by considering the flow of a liquid through a narrow pipe. All parts of the liquid do not move with the same velocity. A thin layer immediately in contact with the walls of the tube is almost stationary. Each succeeding thin layer of the liquid moves with gradually increasing velocity, which becomes maximum as the center of the tube, is approached. The resistance that one part of a liquid flowing with one velocity offers to another part of the liquid flowing with a different velocity is known as **viscosity.** Alternatively, viscosity may be looked upon as the force of friction between two layer of a liquid, moving fast with one another with different velocities.

Suppose a cylindrical liquid of area A moves over another similar layer at a distance S with a velocity difference μ. Then the tangential force of friction (f) required to maintain a constant difference of velocity, is given by

$$f = \frac{\eta A \mu}{S}$$

Where η is a constant at a given temperature, depending upon the nature of the liquid and is known as **coefficient of viscosity.**

If A is 1 cm^2, μ is 1 cm per sec, and s is 1 cm then f = η dynes. The **coefficient of viscosity** of a liquid may therefore be defined as the force in dynes per square centimeter required to maintain a difference of velocity of 1 cm per sec between two parallel layer of the liquid held at a distance of 1 cm from one another.

In terms of SI units, coefficient of viscosity would be defined as the force in Newtons per square meter required to maintain a difference of velocity of one meter per second, between two parallel layer of the liquid held at a distance of one meter from each other.

The reciprocal of viscosity is called **fluidity** and is denoted by Φ. Thus,

$$\Phi = \frac{1}{\eta}$$

Unit of viscosity

If F is measured in dynes, S in cm, A in cm^2 and μ in cm sec^{-1}, then the units of η are dynes × cm × cm^2 × cm sec^{-1} = dynes cm^{-2} sec.

For simplicity, the unit of viscosity viz. Dynes cm^{-2} sec, is called **poises.** Still more convenient units of viscosity are centipoises and millipoises (1 millipoise = 0.001 poise and 1 centipoise = 0.01 poise). For example, viscosity (in centipoise) of water is 1.008, chloroform 0.563, ethyl alcohol 1.216 etc.

Q. Define hydrotropy.

Ans. Certain substances have the property of making water insoluble substances soluble in water. This is called **hydrotropy** or **hydrotropic action.** How is this action brought about is not definitely known. It is said that the hydrotropic substances form loose compounds with the insoluble substances and thereby make them soluble and diffusible through membrane. This view is supported by the fact that a quantitative relation is often found between them. For instance, when glycocholic acid forms hydrotropic compound with oleic acid they have molecular ratio of 3:1. Examples of hydrotropic substances of physiological importance are: bile salts and such other compounds of cholic acid lecithin, soaps of higher fatty acids, phenyl acetic acid, benzoic acid, hippuric acid etc. Hydrotropic substances of unknown nature are also found in the intestinal juice, in the intestinal mucosa, in the blood plasma and possibly in other tissues and body fluids. The insoluble substances which are made soluble by addition of hydrotropic substances are fats, certain phospholipids, steroids-specially cholesterol, insoluble soaps, uric acid and inorganic salts of Ca, Hg and possibly of Fe, Cu, Mn etc.

Q. What are Colloids? Describe different colloidal systems.

Ans. Colloids are finely divided particles of any substance with diameters lying within 10-2000 A° range dispersed in any medium.

A colloidal system is a two phase systems consisting of a **continuous phase** or dispersion medium in which extremely minute particles lying within the colloidal range of a second substance termed as discontinuous phase or **dispersed phase** are suspended. The dispersed phase may not necessarily be solid always. It may be liquid or even a gas as well. Similarly dispersion medium may be a gas or a liquid or even a solid. Thus different type of colloidal systems are shown in table.

Table : Different Colloidal Systems

Dispersion Medium	*Dispersed Phase*	*Examples*
Gas	Liquid	Clouds, Moist
Gas	Solid	Smoke, Volcanic dust
Liquid	Gas	Foams, Whipped cream
Liquid	Liquid	Emulsions in milk
Solid	Gas	Starch, Proteins
Solid	Liquid	Jellies, Gel, Cheese
Solid	Solid	Rock Salt

When the dispersion medium is a gas, the colloidal system is called **aerosol**. The systems with solids as dispersed phase and liquid as dispersion medium are known as sols. When the liquid medium is water, the system is called **hydrosol** or **aquasol**.

Q. Write briefly the classification of colloids.

Ans. Substances such as proteins, starch and rubber whose molecules are large enough to be close to the lower limit of colloidal range pass readily into colloidal state whenever mixed with a suitable solvent, such colloids are known as **lyophilic colloids.** These colloids have strong interactions with the dispersion medium. On the other hand, substances like ferric hydroxide, gold and other metals, which are sparingly soluble and whose molecules are much smaller than the lower colloidal limit change into colloidal state readily and are called **lyophobic colloids.** These colloids are much less stable and their residue left on evaporation cannot readily be converted into solution by ordinary means. These are also called as **irreversible colloids.** The main differences between the two types of sols are summed up in table.

Table Differences between Lyophobic and Lyophilic sols

Property	*Lyophobic sols*	*Lyophilic sols*
1. Surface Tension	Surface tension is usually the same as that of the medium (i.e. the liquid in which the particles are dispersed).	Surface tension is generally lower than that of the medium (i.e. the liquid in which the particles are dispersed).
2. Viscosity	Viscosity is about the same as that of the medium.	Viscosity is much higher than that of the medium.
3. Visibility	The particles, though invisible, can be readily detected under ultra microscope.	The particles cannot be readily detected even under ultramicroscope.
4. Migration in an electric field	The particles migrate either towards anode or towards cathode in an electric field.	The particles may migrate in either direction or not at all in an electric field.
5. Action of electrolytes	The addition of small quantities of electrolytes can cause precipitation (coagulation).	The addition of small quantities of electrolytes has little effect. Much larger quantities are needed to cause precipitation.
6. Reversibility	These are irreversible.	These are reversible.
7. Hydration	The particles are not hydrated to any larger extent.	These particles are extensively hydrated. This is due to the presence of a number of polar groups in the molecules of lyophilic colloids as for example, in proteins, polysaccharides etc.

Q. Mention the different methods for purification of colloidal solution.

Ans. The presence of impurities, particularly the electrolytes renders sols unstable. These must be eliminated by suitable means. Two simple methods are generally employed.

Dialysis

It is the process of separating substances in a colloidal state from those present in true solution with the help of fine membranes. The membrane used for this purpose are called as **dialysers.** If a mixture containing colloidal particles as well as particles in a true solution is placed in a parchment bag which is then held in a side vessel containing pure water the substance in true solution pass out while the colloids remain in bag). The distilled water in wider vessel is renewed frequently.

In **electrodialysis** the true solution is an electrolyte. The mixture is placed between two dialysis membranes while pure water is contained in a compartment on each side.

There is one electrode in each compartment by means of which the required EMF is applied. The ions of the electrolyte migrate out to the oppositely charged electrodes while the colloidal particles are held back.

Ultra filtration

The separation of solutes from colloidal system can also be carried out by process called **ultra filtration.** In this technique the pores of filter paper are made smaller by soaking filter paper in a solution of gelatin and subsequently hardening then by soaking in formaldehyde. The pores thus become very small and the colloidal particles may be retained on the filter paper. The treated filters are known as **ultra filters.** This process of separation colloids from solutes is known as **ultra filtration.**

Electrophoresis

If an electric current be passed through a colloidal solution, the positively charged colloid ions will accumulate around the negative pole, while the negatively charged particles will accumulate around the positive pole. In this way they can be separated. Even their rate of movement can be measured by noting the concentration of a particular colloid at different points in the electric field.

Salting out

It is a kind of precipitation of a protein from its solution by saturation or partial saturation with such neutral salts as sodium, chloride, magnesium sulphate or ammonium sulphate. By adding suitable amounts of various salts the colloidal particles can be separated.

Q. Mention the important properties of colloids.

Ans. Heterogeneous character—Colloidal systems are heterogeneous. They consist of two phases; dispersed phase and dispersion medium.

Diffusibility—They are not readily diffusible through parchment or other fine membranes.

Filterability—The colloidal systems readily pass through ordinary filter papers along with any dissolved material. This is because extremely fine filter paper has bigger pores than the colloidal dimensions.

Visibility—It is not possible to see colloidal particles even with the help of most powerful microscope.

Colligative properties—Colloidal particles show colligative properties like osmotic pressure, lowering of vapour pressure, elevation in boiling point and all these properties are dependent on the number of solute particles present in a given weight of solvent.

Optical properties—When a beam of light is passed in colloidal solution it becomes visible as a bright streak. This phenomenon is known as **Tyndal effect.** This phenomenon is due to scattering of light from the surface of colloidal particles. The intensity of the scattered light depends on the difference between the refractive indices of the dispersed phase and the dispersion medium.

The Brownian movement—Robert Brown in 1827, observed that pollen grains in aqueous suspension were in constant motion. Later on when ultra microscope was invented, it was found that the particles of lyophobic solutions are also in a state of ceaseless erratic and random motion.

The kinetic activity of particles suspended in a liquid is called Brownian movement The Brownian movement is due to the bombardment of colloidal particles by molecules of dispersion medium, which are in constant motion like molecule in a gas. Brownian movement offers a visible proof of the random kinetic motion of molecules in a liquid.

Electrical properties—The most important property of colloidal dispersion is that particles carry on electric charge. All the particles in a given colloidal system carry the same charge and the dispersion medium has an opposite and equal charge, the system as a whole being electrically neutral.

Q. What are emulsions?

Ans. Emulsions are colloidal systems in which dispersed phase as well as dispersion mediums are normally liquids. The examples are milk (liquid fat in water) and cod liver oil (water in oil).

Types of Emulsion

(a) **Oil in water type**—In this, oil is the dispersed phase and water is the dispersion medium.
(b) **Water in oil type**—In this water is the dispersed phase and oil is the dispersion medium.

Q. Differentiate between Sols and Gel.

Ans. Several lyophilic sols and a few lyophobic sols as well, when coagulated under certain conditions, change into a semi rigid mass, enclosing the entire amount of the liquid within itself. Such a product is called as **gel.** The process of transformation of a solution into a gel is known as **gelation.** Gel represents a liquid - solid system i.e. a liquid dispersed in a solid. Amongst lyophilic sols, the examples are gelatin, agar - agar etc. Amongst lyophobic sols the examples are silicic acid, ferric hydroxide etc.

Physiological significance

The colloids are of immense physiological value. Some examples are as follows

(1) **Cell protoplasm**—In every cell, there exists cell protoplasm, which is mostly emulsoid. 90% of organic matter in body remains as colloids.
(2) **Milk, Plasma and lymph**—All are emulsoids. Blood is a suspension of red cells in plasma.
(3) **Imbibition of water**—Since emulsoids readily imbibe, water, a good amount of water remains stored in the body in this way.

Q. Define Donnan Membrane Equilibrium.

Ans. This equilibrium occurs when a large non-diffusible charged ion (e.g. a protein ion) is separated by a semipermeable membrane from a diffusible salt

Suppose a solution of salt Na^+P^- of concentration c_1 (where P^- is a protein particle carrying a negative charge) is separated by a semipermeable membrane from a solution of sodium chloride of concentration c_2. During osmosis the chloride ions tend to diffuse from a region of high concentration, c_2 on the right to a region of low concentration c_1 on the left. However the protein ions P^- cannot pass through membrane.

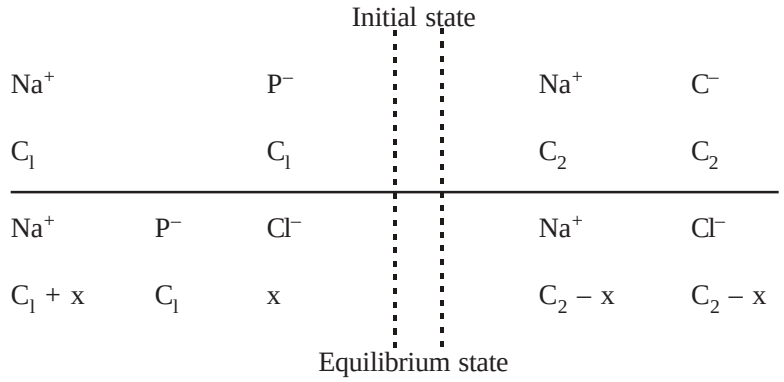


Fig. 1.4. Donnan Membrane equilibrium.

Let x be the concentration change due to diffusion of NaCl across the membrane. In both the compartments the total concentration of positive ions must be equal to the negative ions in each solution. at equilibrium the chemical potential of NaCl must be same on both sides of membranes.

Accordingly,

$$RT \log ({}^{a}NaCl)_l = + RT \log ({}^{a}NaCl)_r$$

Where NaCl represents activity of sodium chloride and subscript l and r refer to the solution left and right of semi permeable membrane. Assuming that the mean ionic activity coefficients in the left and right, we can express Donnan equilibrium as

$$[Na^+]l\ [Cl^-] = [Na^+]r\ [Cl^-]r$$

or $$(c_1 + x)x = (c_2 - x)\ (c_2 - x) \text{ or } (c_2 - x)^2$$

or $$c_1x + x^2 = (c_2)^2 + x^2 - 2\ c_2x$$

or $$x\ (c_1 + 2c_2) = c_2^{\ 2}$$

$$x = \frac{c_2^2}{(c_1 + 2c_2)}$$

It is evident from above equation that the extent of diffusion of sodium chloride i.e. is distinctly affected by the presence of the non-diffusible ion P^-.

(*i*) If the solution behaves ideally the osmotic can be calculated with help of Vant Hoff equation.

$$P = RTC$$

Or $$P = RT\ [(c_1 + x) + c_1 + x] - [(c_2 - x) + c_2 - x]$$

$$P = 2RT\ (c_1 - c_2 + 2x)$$

(*ii*) If the concentration of salt C_2 is small as compared to concentration of non-diffusible ion C_1. The osmotic pressure is given by

$$P = 2RT\ (c_1 + 2x)$$

(*iii*) If the concentration of salt C_2 is large as compared to the concentration of C_1. Then pressure is given by

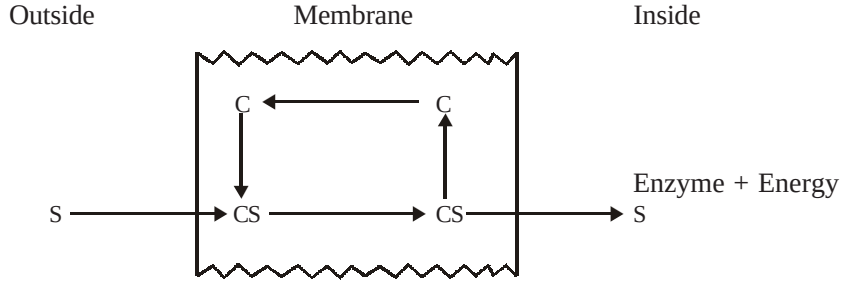
$$P = 2RTC_1$$

This equation does not involve the concentration of chloride ion. In other words the effect of Donnan equilibrium on osmotic pressure is practically eliminated by using a high concentration of salt in the solution. This is precisely the condition under which molar masses of macromolecules should be determined from osmotic pressure measurement.

Q. Write note on Active transport mechanism.

Ans. Active transport mechanism—The active transport is the process of the movement of molecules or ions against the concentration gradient or electrical gradient and requiring energy. The different substances that are actively transported through the cell membranes are H^+ ions, Na^+ ions, K^+ ions, Ca^{++} ions, iron ions, Cl^- ions, I^- ions, ureat ions, several different sugars and amino acids.

Mechanism



The substance S enters the outside surface of the membrane. A specific carrier present in the membrane has natural affinity for the substance S so that at the outer surface of the membrane the carrier and the substance readily combine. Then the combination of the two diffuses through the membrane to the inner surface.

At the inner surface an enzyme catalysed reaction occurs, utilising energy for ATP which is spilt into ADP and inorganic phosphate. This reaction splits the substance away from the carrier. Thus the enzyme catalysed reaction makes the affinity of the carrier for the transported substance very low and thereby diplaces it from the combination. But the released substance is insoluble in the membrane and hence cannot diffuse backward through the lipid matrix of the membrane. Therefore it is released to the inside of the membrane while the carrier alone diffuses back to the outside surface to transport still another molecule of substance in the inward direction.

A special characteristic of active transport is that the mechanism saturates when the concentration of the substance to be transported is very high. This results from limitation either of quantity of carrier available to transport the substance or of enzymes to promote the chemical reaction that release the substance from the carrier.

The carrier substance is believed to the either protein or lipoproteins. The protein moiety providing a specific site for attachment of the substance to be transported and the lipid moiety providing solubility in the lipid phase of the cell membrane. This carrier has got the specificity of the substance to be transported.

Q. What is Chromatography?

Ans. The Russian botanist Michael Tswett, first employed the technique of chromatography. In the process of analysis separations of plant leaf pigments in solution through the use of solid adsorbents was done. He named this process as **chromatography** (Greek: Chroma, colour + graphein,

to write), presumably resulting from the coloured bands that formed in the adsorbents as the components of the pigment mixtures separated from one another.

Chromatography depends essentially on the distribution of solutes between two solvents i.e. a mobile and stationary. The ratio of the amount of solute retained in one solvent (usually the stationary) to the amount of same solute in the other solvent (mobile) measured under the constant conditions of temperature is called as **distribution ratio** (D). This ratio depends upon the nature of the solute to be distributed between two solvents. Three types of distributions are obtained as shown in Fig.

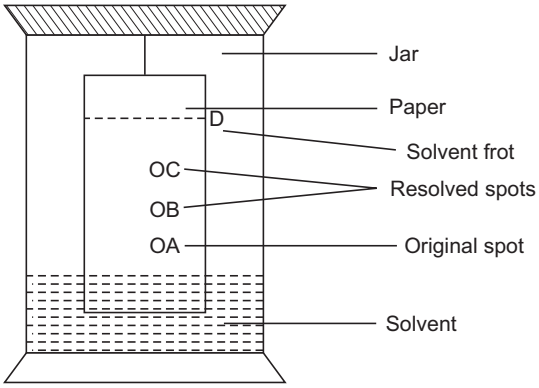


Fig. 1.5. Separation of pigments by paper chromatography.

A substance may thus be characterized by the speed at which it migrates. Migration of a particular solute is measured in terms of its **Rf values.** Rf is the measure of the velocity of migration and is defined as "the quotient of the distance of substance (solute) from the starting point divided by the distance of the solvent from the starting point".

$$Rf = \frac{\text{Distance of the solutes from origin}}{\text{Distance of the solvent traveled}}$$

For example, in paper chromatography of plant pigments, if the spot is applied at position A on paper the two pigments (yellow and green) move upto the position B and C respectively and solvent upto position D after a definite time run then,

$$Rfb = \frac{\text{Distance between A and B}}{\text{Distance between A and D}}$$

And

$$Rfc = \frac{\text{Distance between A and C}}{\text{Distance between A and B}}$$

It is apparent from the above expression that the value of Rf is always one or less than one. Rf value of a substance is a physical constant and can be reproduced.

Q. Describe briefly how the chromatography is classified.

Ans. The various chromatographic methods are classified according to their mobile and stationary phases. For example in gas liquid chromatography, the mobile and stationary phases are gaseous and

liquid respectively, whereas in liquid-liquid chromatography there are immiscible liquids, one of which is bound to an inert solid support. Chromatographic methods may be further classified according to the nature of the dominant interactions between the stationary and mobile phase and the substances being separated. For example, if the retarding force is ionic in character, the separation technique is referred to as **ion exchange chromatography,** whereas if it is a results of the adsorption of the solutes onto a solid stationary phase, it is known as **adsorption chromatography**.

Q. What is Adsorption chromatography. Desribe in brief.

Ans. Adsorption chromatography was the earliest method used in the history of chromatography. It is also known as **column chromatography** as solid columns of inert materials are used for the adsorption of different substances. Mixture to be separated is taken in the form of solution. The solution is then poured down to the column. Molecules are physically adsorbed on the surface of insoluble substances such as alumina (Al_2O_3), charcoal, diatomaceous earth (also called kieselguhr, the silicaceous fossils of unicellular organisms known as diatoms), finely powdered sucrose, or silicagel (silicic acid), through Vander Waals and hydrogen bonding associations. The molecules are then eluted from the column by a pure solvent such as chloroform, hexane or ethyl ether or by a mixture of such solvents. The separation process is based on the partition of the various substances between the polar column material and the non-polar solvent. This procedure is most often used to separate non-polar molecules rather than polar molecules.

Q. What is Hydroxy apatite chromatography?

Ans. Proteins are adsorbed by gels of crystalline hydroxy appetite, an insoluble form of calcium phosphate with empirical formula $Ca_5(PO_4)_3OH$.

The separation of the proteins occurs upon gradient elution of the column with phosphate buffer (the presence of other anions is unimportant). The physicochemical basis of this fractionation procedure is not fully understood but apparently involves the adsorption of anions to the Ca^{2+} sites and cations to the PO_4^{3-} sites of the hydroxy appetite crystalline lattice.

Q. What is Ion Exchange Chromatography?

Ans. In the process of ion exchange, ions that are electrostatically bound to an insoluble and chemically inert matrix are reversibly replaced by ions in solution.

$$R^+A^- + B^- \rightleftharpoons R^+B^- + A^-$$

Here R^+A^- is an anion exchanger, in the A^- form and B^- represents anion in solution. Cation exchangers similarly bear negative charged groups that reversible bind cations. Polyanions and polycations therefore bind to anion and cation exchangers respectively. However proteins both positive and negative charges can bind both cation and anion exchangers depending on their net charges. In purifying a given protein, the pH and the salt concentration of the buffer solution in which the protein is dissolved are chosen so as to immobilize the desired protein on the selected ion exchanger. The impure protein solution is applied to a column in which the ion exchanger has been packed and the column is washed with this buffer solution. Various proteins bind to the ion exchanger with different affinities. As the column is washed, a process known as **elution,** those proteins with relatively low affinities for the ion exchanger move through the column faster than the proteins that bind to the ion exchanger with higher affinities. This occurs because the progress of a given protein through the column is retarded relative to that of the solvent due to interactions between the protein molecules and

the ion exchanger. The greater the binding affinity of a protein for the ion exchanger, the more it will be retarded. Thus proteins that bind highly to the ion exchanger can be eluted by changing the elution buffer to one with a higher salt concentration (and/or a different pH) a process called **stepwise elution.** With the use of fraction collector, purification of a protein can be affected by selecting only those fractions of the column effluent that contains the desired protein.

The purification process can further be improved by washing the protein-loaded column using the method of gradient elution. Here the salt concentration and/or pH is continuously varied as the column is eluted so as to release sequentially the various proteins that are bound to the ion exchanger. This procedure generally leads to a better separation of proteins that does elution of the column by a single solution or stepwise elution.

Many different types of elution gradients have been successfully employed in purifying biological molecules. The most widely used of these is the linear gradient in which the concentration of elutant varies linearly with the volume of solution passed. The solute concentration C in the solution being withdrawn from the mixing chamber is expressed as

$$C = C_2 - (C_2 - C_1)f$$

Where C_1 is the solutions initial concentration in the mixing chamber C_2 is its concentration in the reservoir chamber and f is the remaining fraction of the combined volumes of the solutions initially present in both reservoirs. Linear gradient of increasing salt concentrations is probably more commonly used than all other means of column elution. However, gradients of different shapes or more chambers of different cross sectional areas or programmed mixing devices.

The ion exchangers used in ion exchange chromatography are charged groups covalently attached to support matrix. The chemical nature of the charged groups determines the types of ions that bind to ion exchanger and strength with which they bind. The chemical and mechanical properties of the support matrix govern the flow characteristics ion accessibility and stability of ion exchanges. .

Cellulosic ion exchangers are among the materials most commonly employed to separate biological molecules. The cellulose, which is derived from wood or cotton is lightly derivatized with ionic groups to form the ion exchanger. The most often used cellulosic anion exchanger is **diethyl amino ethyl cellulose** (DEAE) whereas carboxymethyl cellulose (CM) is the most popular cellulosic cation exchanger.

Gel type ion exchangers can have the same sorts of charged groups as do cellulosic ion exchangers. The advantage of gel type ion exchangers is that they combine the separation properties of gel filtration with those of ion exchange. Because of their high degree of substitution of charged groups, these gels have a higher loading capacity than do cellulosic ion exchangers.

One disadvantage of cellulosic and gel type matrices is that they are easily compressed (usually by the high pressures resulting from attempts to increase the elutant flow rate) thereby greatly reducing elutant flow. This problem has been alleviated in recent years by the development of non-compressible matrices such as derivatized silica or coated glass beads. Such materials allow very high flow rates and pressures, even when they are very finely powdered, and permit more effective chromatographic separations.

Q. Describe Paper Chromatography.

Ans. Paper chromatography was developed in 1941 by Archer Martin and Richard Synge, and has played an indispensable role in biochemical analysis due to its ability to efficiently separate small molecules such as amino acids and oligopeptides and its requirement for the only the simplest of equipment.

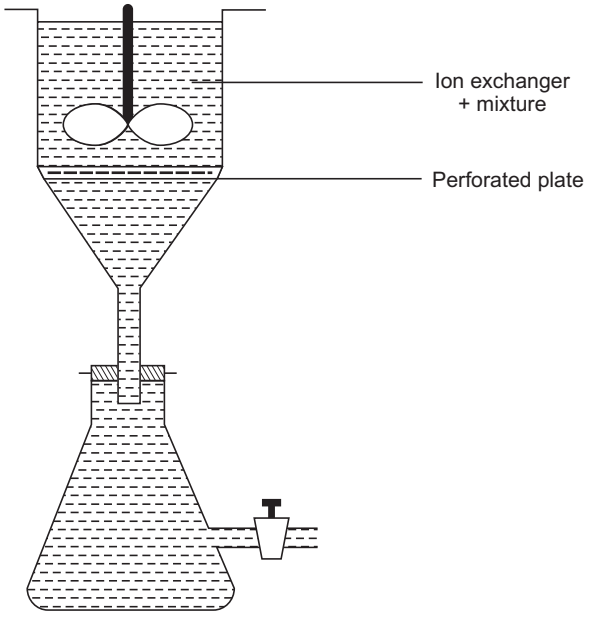


Fig. 1.6 Arrangement for ion exchange chromatography.

In paper chromatography a few drops of solution containing a mixture of the components to be separated are applied (spotted) 1–2 cm above one end of the spirit of filter paper. After drying, that end of paper is dipped into a solvent mixture consisting of aqueous and organic components for example, water/butanol/acetic acid in 4:5:1 ratio. The paper should also be in contact with the equilibrium vapours of the solvent. The solvent soaks into the paper by capillary action because of the fibrous nature of the paper. The aqueous component of the solvent binds to the cellulose of the paper and thereby forms a stationary gel like phase with it. The organic component of the solvent continues migrating, thus forming the mobile phase.

The rate of migration of the various substrates being separated are governed by their relative solubilities in the polar stationary phase and non-polar mobile phase. In a single step of separation process, a given solute is distributed between the mobile and stationary phases according to its partition coefficient an equilibrium constant is defined as

$$Kp = \frac{\text{Concentration in stationary phase}}{\text{Concentration in mobile phase}}$$

The molecules are therefore separated according to their polarities, with non polar molecules moving faster than polar ones. After the solvent front has migrated to an appropriate distance, the chromatogram is removed from the solvent and dried. The separated materials if not coloured, may be detected by several means:

1. Materials may be visualized by spraying the chromatogram with a reagent solution that forms a coloured product upon reaction with the substance under investigation. For example a amino acids and other primary amines react with ninhydrin to form an intensely purple compound secondary amines such as proline also reacts with ninhydrin but forms a yellow compound.
2. Materials that are fluorescent or quench the normal fluorescenes of the paper can be seen under ultraviolet (UV) light.

3. Radioactive labeled materials may be located by a variety of radiation detection methods.

The migration rate of a substance may be expressed according to the ratio.

$$Kf = \frac{\text{Distance traveled by substance}}{\text{Distance traveled by solvent front}}$$

For a given solvent system and paper type, each substance has a characteristic Rf value.

Paper chromatography can be used as preparative technique for purifying small amounts of materials. A solution containing the substance of interest is applied in a line across the bottom of a sheet of filter paper and the entire sheet is chromatographed as described. The substance of interest is located on the chromatogram by applying an appropriate detection technique to a small strip that has been cut from the chromatogram along the direction of solvent migration. Finally, the band of purified substance is cut out and the purified substance is recovered by eluting it from the paper with a suitable solvent.

A complex mixture that is incompletely separated in a single paper chromatogram can often be fully resolved by two-dimensional paper chromatography. In this technique, a chromatogram is made as previously described except that the sample is spotted onto one corner of a sheet of filter paper and the chromatogram is run parallel to an edge of the paper. After the chromatography has been completed and the paper dried, the chromatogram is rotated 90° and is chromatographed parallel to the second edge using another solvent system. Since each compound migrates at characteristic rate in a given solvent system, the second chromatographic step greatly enhance the separation of the mixture into its components.

Q. What is Gel filtration Chromatography?

Ans. In gel filtration chromatography, which is also called as size exclusion or molecular sieve chromatography, molecules are separated according to their size and shape. The stationary phase in this technique consists of beads of a hydrated sponge like material containing pores that span a relatively narrow size range of molecular dimensions. If an aqueous solution containing molecules of various sizes is passed through a column containing such molecular sieves, the molecules that are too large to pass through the pores are excluded from the solvent volume inside the gel beads. These larger molecules therefore traverse the column more rapidly, that is, in a smaller eluent volume, than the molecules that pass through the pores.

The molecular mass of the smallest molecule unable to penetrate the pores of a given gel is said to be the gels exclusion limit. This quantity is to some extent a function of molecular shape because elongated molecules, as a consequence of their higher radius of hydration, are less likely to penetrate a given gel pores than spherical molecule of the same molecular volume.

The behavior of a molecule on a particular gel column can be quantitatively characterized. If Vx is the volume occupied by gel beads and Vo **(void volume)** is the volume of the solvent space surrounding the beads, then Vt, the **total bed volume** of the column, is simply their sum.

$$V_t = Ve + Vo$$

Vo is typically ~35% of Vt.

The elution volume of a given solute, Ve is the volume of solvent required to elute the solute from the column after it has first contacted the gel. The void volume of a column is easily measured as the elution volume of a solute whose molecular mass is larger than the exclusion limit of the gel. The

behavior of a particular solute on a given gel is therefore characterized by the ratio Ve/Vo, the relative elution volume, a quantity that is independent of the size of the particular column used.

Molecules with molecular masses ranging below the exclusion limit of the gel will elute from the gel in the order of their molecular masses, with the largest eluting first. This is because the pore sizes in the gel vary over a limited range so that larger molecules have less of the gels interior volume available to them than smaller molecules do. This effect is the basis of gel filtration chromatography.

There is a linear relationship between the relative elution volume of a substance and the logarithm of its molecular mass range . The molecular mass of unknown substance can be estimated from its position on the plot drawn between molecular masses of known substances . The precision of this technique is limited by the accuracy of the underlying assumption that the known and unknown macromolecules have identical shapes.

The most commonly used materials for making chromatographic gels are **dextran** (a high molecular mass polymer of glucose produced by the bacterium Leuconostoc mesenteroides), **agarose** (a linear polymer of alternating D-galactose and 3, 6 anhydro - L galactose from red algae) and **polyacrylamide.**

Q. Describe Affinity Chromatography.

Ans. In this technique a molecule, known as ligand, which specifically binds to a protein of intrest is covalently attached to an inert and porous matrix. When an impure protein solution is passed through this chromatographic material, the desired protein binds to the immobilized ligand, whereas other substances are washed through the column with the buffer. The desired protein can be recovered in highly purified form by changing the elution conditions such that the protein is released from the chromatographic matrix. The great advantage of affinity chromatography is its ability to exploit the desired proteins unique biochemical properties rather than the small differences in physicochemical properties.

Q. What is Thin Layer Chromatography?

Ans. In thin layer chromatography (TLC), a thin (~ 0.25 mm) coating of solid material is spread on glass or plastic plate. The solid material can be however be one from a variety of substances such as ion exchangers, gel filtration agents and physical absorbents. According to the choice of solvent for the mobile phase, the separation may be based on adsorption, partition, gel filtration or ion exchange processes, or some combination of these. The advantages of thin layer chromatography is convenience, rapidity and high resolution has led to its routine use in the analysis of organic molecules.

Q. What is Reverse Phase Chromatography (RPC)?

Ans. Reverse phase chromatography is a form of liquid-liquid partition chromatography in which the polar character of the phases is reversed relatively to that of paper chromatography. The stationary phase consists of a non-polar liquid immobilized on a relatively inert solid and the mobile phase is a more polar liquid. Reverse phase chromatography was first developed to separate mixtures of non-polar substance such as lipids but has also been found to be effective in separating polar substances such as oligonucleotides and proteins, provided that they have exposed non-polar area. Although non-polar side chains tend to inhabit the water free interiors of native proteins, denaturation results in the exposure of these side chains to the solvent. Even when still in the native state, a significant fraction of these hydrophobic groups are at least partially exposed to the solvent at the protein surface. Consequently, under suitable conditions, proteins hydrophobically interact with the non-polar groups

on an immobilized matrix. The hydrophobic interactions in RPC are strong, so the eluting mobile phase must be highly non-polar to dislodge adsorbed substances from the stationary phase.

Q. What is Hydrophobic Interaction Chromatography?

Ans. The hydrophobic interactions forms the basis not only of RPC but of hydrophobic interaction chromatography (HIC). In HI, the stationary phase is a hydrophilic substance such as an agarose gel, that is only lightly substituted with hydrophobic groups, usually octyl or phenyl residues. The resulting hydrophobic interactions in HIC are therefore relatively weak, so proteins maintain their native structures. The elutants in HIC whose gradients must progressively reduce these weak hydrophobic interactions are aqueous buffers with for example, decreasing salt concentrations, increasing concentrations of detergents or increasing pH. Thus HIC separates native proteins according to their degree of surface hydrophobicity, a criterion that differs from those on which other types of chromatography are based.

Q. Describe High Performance Liquid Chromatography or High Pressure Liquid Chromatography.

Ans. In high performance liquid chromatography (HPLC), a separation may be based on adsorption, ion exchange, size exclusion, HIC or RPC as described above. The separations are greatly improved, however, through the use of high resolution columns and the column retention times are much reduced. The narrow and relatively long columns are packed with a non compressible matrix of fine glass or plastic beads coated with a thin layer of stationary phase. Alternatively, the matrix may consists of silica whose available hydroxyl groups can be derivatized with many of the commonly used functionally active groups of ion exchange chromatography, RPC, HIC or affinity chromatography. The mobile phase is one of the solvent systems previously discussed including gradient elutions with binary or even ternary mixtures. In the case of HPLC, however the mobile phase is forced through the tightly packed column at pressures of upto 5000 psi leading to greatly reduced analysis time. The elutants are detected as they leave the column by such methods as UV absorption, refractive index or fluorescence measurements.

The advantages of HPLC are

1. Its high resolution, which permits the routine purification of mixtures that have defined separation by other techniques.
2. Its speed, which permits most separations to be accomplished in significantly <1h.
3. Its high sensitivity, which in favourable cases, permits the quantitative estimation of less than piomole quantities of materials.
4. Its capacity for automation.

Q. What is Gas Liquid Chromatography?

Ans. The gas liquid chromatography was developed largely since 1951. It is the most preferred method for rapidly and accurately analyzing many volatile substances. In essence the volatile material is injected into a column containing a liquid absorbent supported on an inert solid. The basis for separation of the components of the volatile material is the difference in the partition coefficient of the components as they are carried through the column by an inert gas such as helium. The actual apparatus is quite simple and is shown in figure. The column is first flushed out with carrier gas to remove previously injected material and to form a stable baseline. The sample is introduced at A. The carrier gas transport the injected volatile material into the column, where the components partition into

the liquid absorbent and separate, eventually a fraction passes through a suitable detecting device, which sends signals to a recorder, which in turn converts the signal into a useful sequence of peaks. There are two main types of detecting devices. The thermal conductivity cell is a detecting device based on the principle that heat is conducted away from a hot wire by a gas passing over it. Two fine coils of wire with a high temperature coefficient of resistance are placed in two parts of the metal block (C' and C). Suitable electrical resistors are inserted in the circuit of C' and C to form a Wheatstone bridge circuit. When current is passed through the bridge, the wires C' and C are heated. Final equilibrium temperature of the wires depends on the thermal conductivity of the gas passing over the wire coil. If the gas is same, the wires will have the same temperature and the same resistance, and therefore the bridge is balanced, if an effluent gas now passes through C' while only the carrier gas passes through C, the wire temperature will differ. The resistance in turn will be changed and the bridge becomes unbalance. The extent of unbalanced is measured with a recording potentiometer as indicated.

The second type of detector device is a hydrogen flame ionization detector. It has extreme sensitivity, a wide linear response and is insensitive to water. In theory, when organic material is burned in hydrogen flame, electrons and ions are produced. The negative ions and electrons move in a high voltage field to an anode and produce a very small current, which is changed to a measurable current by appropriate circuitry. The electrical current is directly proportional to the amount of material burned.

The use of gas chromatography has revolutionized the analysis of fats, fatty acids, flavor components, gaseous mixtures and any compound which can be converted into a volatile material.

Q. Describe briefly Electrophoresis.

Ans. Electrophoresis, the migration of ions in an electric field, is widely used for the analytical separation of biological molecules. The laws of electrostatics state that the electrical force $F_{electric}$ on an ion with charge q in an electric field of strength E is expressed by

$$F_{electric} = qE \quad \text{... (1)}$$

The resulting electrophoretic migration of the ion through the solution is opposed by a frictional force.

$$F_{frictional} = vf \quad \text{... (2)}$$

Where v is the rate of migration (velocity) of the ion and f is the frictional coefficient. The frictional coefficient is a measure of the drag that the solution exerts on the moving ion and is dependent on the size, shape and state of solvation of the ion as well as the viscosity of the solution in a constant electric field, the forces on the ion balances each other:

$$qE = vf \quad \text{... (3)}$$

So that each ion moves with a constant characteristic velocity. An ions electrophoretic mobility μ is defined as

$$\mu = \frac{v}{E} = \frac{q}{f} \quad \text{... (4)}$$

The use of electrophoresis to separate various proteins was first reported in 1937 by Swedish biochemist Arne Tiselius. In the technique he introduced, moving boundary electrophoresis, a protein solution is placed in a U shaped tube and a protein free buffer solution is carefully layered over both ends of protein solution. Electrodes are immersed in the buffer on either side of the protein solution, thereby generating an electric field that causes the charged protein molecules to migrate towards the

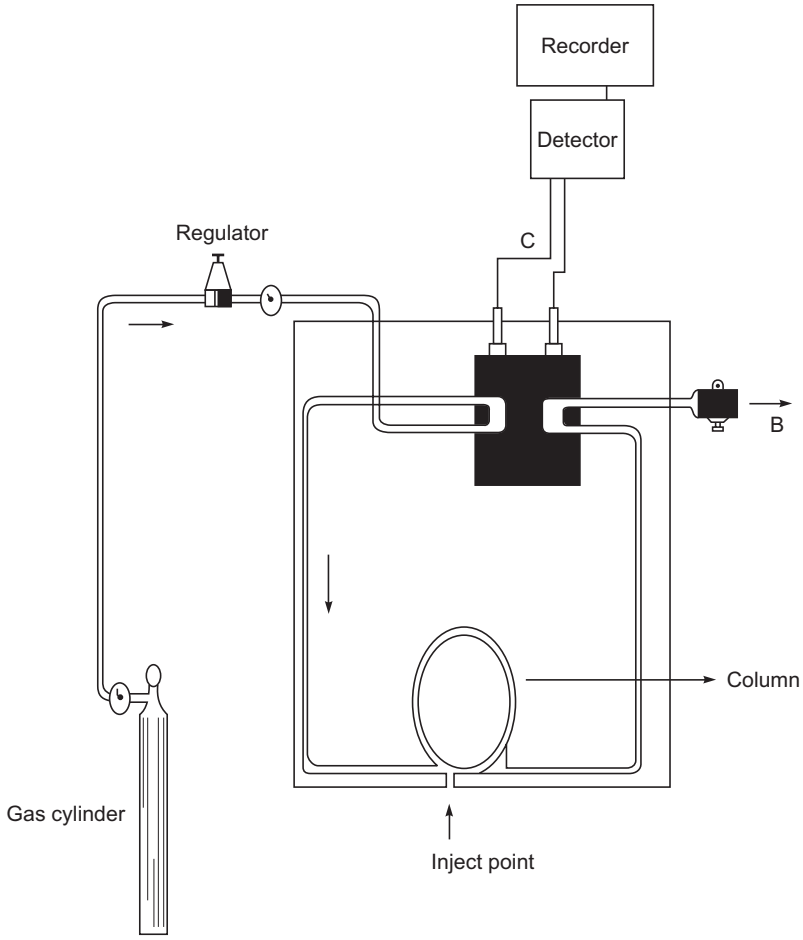


Fig. 1.7 G.L.C apparatus.

electrodes of opposite polarity . Different proteins move at different rates as a result of their diverse charges and frictional coefficients so that the leading and trailing edges of the migrating protein columns of each species form separate moving boundaries (front) in the buffer solution. Moving boundary electrophoresis was one of the few powerful analytical techniques available in the early years of protein chemistry. However preventing the convective mixing of the migrating proteins necessitates a cumbersome apparatus that requires very large samples. Moving boundary electrophoresis, has therefore been supplanted by zone electrophoresis, a technique in which the sample is constrained to move in a solid support such as filter paper, cellulose or gel. This largely eliminates the convective mixing of the sample that limits the resolution achievable by moving boundary electrophoresis. Moreover, the small quantity of material that is used in zone electrophoresis permits the various sample components to migrate as discrete bands (zones).

Q. Describe Paper Electrophoresis.

Ans. In paper electrophoresis, the sample is applied to a point on a strip of filter paper or cellulose acetate moistened with a buffer solution. The ends of the strip are immersed in separate reservoirs of buffer in which the electrodes are placed.

Upon application of a direct current the ions of the sample migrate towards the electrodes of opposite polarity at characteristic rates to eventually form discrete bands. An ion's migration rate is influenced, to some extent, by its interaction with the support matrix, but is largely a function of its charge. Upon completion of the electrophoretogram the strip is dried and the sample components are located using the same detection methods employed in paper chromatography.

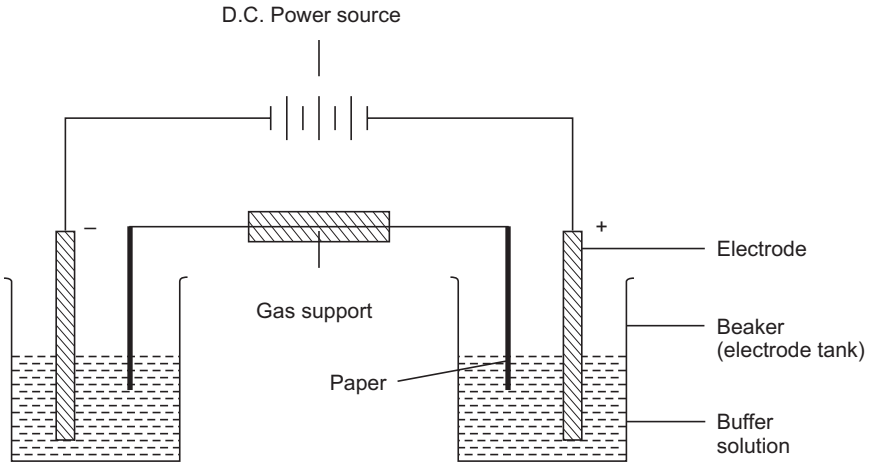


Fig. 1.8. Apparatus and arrangement for paper electrophoresis.

Q. Describe Gel Electrophoresis.

Ans. Gel electrophoresis is among the most powerful and yet conveniently used methods for macromolecular separations. The gels in common use, polyacrylamide and agarose have pores of molecular dimensions whose sizes can be specified. The molecular separations are therefore based on gel filtration as well as the electrophoretic mobilities of the molecules being separated.

In polyacrylamide gel electrophoresis (PAGE), gels are made by the free radical-induced polymerization of acrylamide and N, N' methylene bisacrylamide in the buffer of choice . In the simplest form of PAGE, a tube 3 to 10 cm long, in which the gel has been polymerized, is suspended vertically between an upper and a lower buffer reservoir. The gel may also be cast as a thin rectangular slab in which several samples can be simultaneously analyzed in parallel lanes. The samples are dissolved in a minimal amount of a relatively dense glycerol or sucrose solution to prevent it from mixing with the buffer in the upper reservoir and is applied in preformed slots on the top of gel. The buffer, is same in both reservoirs and the gel, has a pH such that the macromolecule have net negative charges and hence migrate to the anode in the lower reservoir. A direct current of ~ 300V is passed through the gel for a time, sufficient to separate the macromolecular components into a series of discrete bands, the gel is removed from its holder, and the bands are visualized by first staining (or radioactive counting or immuno blotting) and then under UV light.

Q. Define Sedimentation.

Ans. The rate at which a particle sediments in the ultracentrifuge is related to its mass. The force, $F_{sedimentation,}$ acting to sediment a particle of mass m that is located at a distance r from a point

about which it is revolving with angular velocity ω, is the centrifugal force ($m\omega^2r$) on the particles less the buoyant force ($Vp\rho\omega^2r$) excreted by the solution.

$$F_{sed} = m\omega^2r - Vp\rho\omega^2r$$

Here Vp is the particle volume and ρ is the density of solution. However, the motion of the particle through the solution, is opposed by the frictional force.

$$F_{friction} = vf$$

Where $v = \frac{dr}{dt}$ is the rate of migration of the sedimenting particle and f is the friction coefficient can be determined from measurements of its rate of diffusion.

Under the influence of gravitional (centrifugal) force, the particle acclerates until the force on it exactly balances.

$$M\omega^2r - Vp\ \rho\ \omega^2r = vf$$

The mass of 1 mol of particles, m is

$$M = mN$$

Where N as Avogadro number (6.022×10^{23}). This, a particles volume Vp, may be expressed in terms of its molar mass.

$$Vp = Vm = \frac{M}{N}$$

Where Vp is the particles **partial specific volume,** is the volume change when 1g (dry weight) of particles is dissolved in an infinite number of the solute.

Q. What is Photometry?

Ans. Photometry is the technique used to study the concentration of substances by adopting the property of absorption of light of a definite wavelength by molecules or particles. Small quantities of substances are used for simple and rapid estimation to maintain high order of accuracy. Photometry cannot help in separating substances but help in estimating substances.

When monochromatic light is passed through the solution of a substance, it comes out with diminished intensity since a part of it is absorbed. The absorption of light by the substances obeys Beer Lambert's law.

Beer's law state that the intensity of a ray of monochromatic light decreases exponentially as the concentration of absorbing material increases.

$$\text{Log}\ \frac{I_o}{I} = K_1C$$

Where Io is the intensity of light entering the solution and I is the intensity of light emerging from the solution of concentration C (g/lit or mg/100 ml) and K_1 is constant.

Lamberts Law state that the proportion of light absorbed by an absorbing substance is independent of the incident light.

$$\text{Log}\ \frac{I_o}{I} = K_2I$$

Where I is the length of the solution traversed by combing the two laws.

$$\text{Log}_{10} \frac{I_o}{I} = KCI$$

The logarithmic ratio of Io and I is called the **optical density** (OD) or **extinction E** or **absorbance.** Substituting E in the above equation E = KCI. Therefore, when light passes into a solution of an absorbing substance in a non-absorbing solved, the extinction E is directly proportional to the depth of the solution (I) and the concentration (C) of the absorbing substance.

Transmission is the ratio of the transmitted light to that of the incident light.

$$T = \frac{I}{I_o}$$

Percentage transmission is the transmission when Io is equal to 100.

Thus,

$$E = KCI = \text{Log}_{10} \frac{I_o}{I}$$

$$\therefore \quad E = \text{Log}_{10} \frac{100}{I} \quad (\text{where } I_o = 100)$$

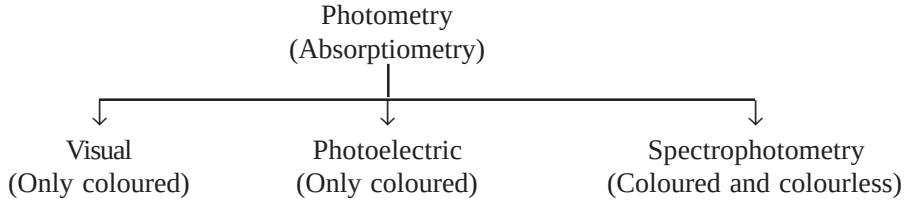
$$\therefore \quad E = \text{Log}_{10} 100 - \text{Log}_{10} I$$

$$\therefore \quad E = 2 - \text{Log}_{10} I$$

$$\therefore \quad E = 2 - \text{Log}_{10} T$$

(I is the percentage transmission T)

The above principle is employed in estimating the substances in all instruments used in photometry. If the product of the reaction is coloured, the wavelength of light used is in the region of the spectrum (400 – 800 mμ). Even colourless products can be estimated by photometry for which a special instrument called as spectrophotometer is required.



Q. What is Colorimetry and what are colorimeters?

Ans. The coloured solutions are estimated by colorimetry. The principal colorimeters are of two types

(1) Visual colorimeters

(2) Photoelectric colorimeters

Visual Colorimeters

In the visual colorimeters, the length of the column through which light passes is changed with the help of plungers so that the intensity of transmitted light of both the standard, and the test as seen through the eye piece is equal. The intensity of incident light (I_o) is also same for both.

If C_1 the concentration of the standard, I_1 the length of the solution column corresponding to standard and I_2 the length corresponding to the test solution are known, the only other unknown is C_2, the concentration of the test sample is calculated.

$$E_{std} = KC_1I_1$$

$$E_{std} = KC_2I_2$$

We known $$E = \text{Log}_{10} \frac{I_o}{I}$$

Since I_o and I are same, Estd and Etest are also the same so

$$KC_1I_1 = KC_2I_2$$

$$\therefore \quad \frac{C_1}{C_2} = \frac{I_2}{I_1}$$

The unknown concentration C_2 can be easily calculated now.

Photoelectric Colorimeters

In this type of colorimeters no colour matching is done. A photocell and a galvanometer are used for measuring the actual intensity of the transmitted light. The electrical energy formed from the light energy by the photocell is measured in a galavanometer. The percentage transmission or optical density is read from the galvanometer. The light of desired wavelength is set up by means of suitable filters.

$$E_{std} = KC_1I_1$$

$$E_{std} = KC_2I_2$$

$I_1 = I_2$, since the lengths of solutions are constant

$$\therefore \quad \frac{\text{Estd}}{\text{Etest}} = \frac{C_1}{C_2}$$

If the extinctions due to the known concentration (C_1) of the substances and that of the test are known, the concentration of the unknown (C_2) can be easily calculated.

A graph is drawn which have the actual concentration and the extinction of the standard by plotting the different concentrations and extinctions of the standard substances.

The extinction, if any, produced by reagent blank should be substracted from that due to the test and the standard.

Spectrophotometry

The spectrophotometers are helpful to analyze coloured as well as colourless samples. This technique uses prisms as monochromator and also the wavelength of incident light can be adjusted precisely. Ultraviolet spectrophotometers work at wavelengths below 400 mμ whereas infra - red spectrophotometers work at the wavelengths above 800 mμ. The former is more useful in biochemical laboratory in the estimation of many dehydrogenase enzymes, which needs the extinction to be taken at about 340 mμ and at extinction at about 260 mμ the purines and pyrimidines of nucleic acid are estimated. Beckmann model DK works from 185 to 3500 mμ and model DU from 210 to 1000 mμ. The spectrophotometers provides the use of smaller volumes of liquids and even samples of solids and gases which can never be used in photoelectric colorimeter.

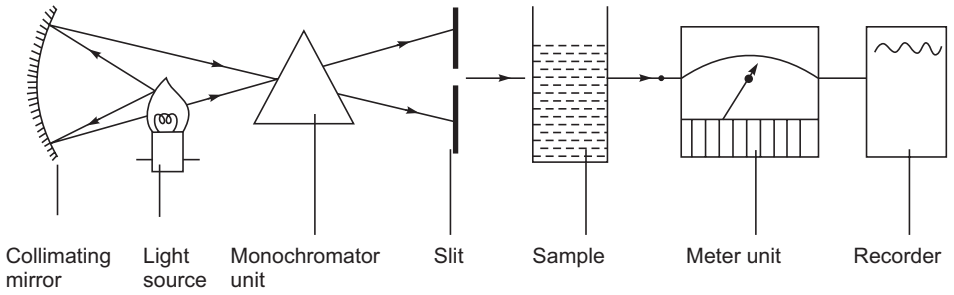


Fig. 1.9. Essential components of a spectrophotometer.

Q. What is Fluorimetry?

Ans. The fluorimetric technique allows measurement of concentrations as low as 1ng in suitable cases. The emission of electromagnetic radiations is shared by the processes of fluorescence, phosphorescence and luminescence. If the excited state persists for less than 10 nanoseconds the process is called **fluorescence** but for excited states with longer survival times, the term **phosphorescence** is used.

The fluorescent light produced as a result of the irradiation on substances like thiochrome, riboflavin etc, is used in fluorimeter and it is emitted in all directions. The photocell is placed at right angles to the path of incident beam. The suitable wavelength for the exciting or primary radiation is selected from the light source and then falls on to a solution of substance under study in a cuvette. The fluorescent light is emitted in all directions but it is usual to select that part emerging at right angles to the incident beam, the unabsorbed part of which passes directly through the cuvette and enters a light trap. The appropriate wavelength is selected from the emitted or secondary light and the beam then falls on the detector. The output from this is amplified and displayed. Fluorimeters are usually divided into two types

(1) Filter fluorimeters

(2) Spectrofluorimeters

In filter fluorimeter one or more individual filters make the selection of the primary wavelength. In spectrofluorimeters, the wavelength selection is by monochromators, which usually employ diffraction gratings but some prism instruments are available.

Flame Photometry

In flame photometry a solution containing the substance to be determined is passed under carefully controlled conditions as a very fine spray into the air supply of a burner. In the flame the solution evaporates and the substance is first converted to the atomic state in which the electrons in the outermost shell are in their lowest energy state closest to the nucleus, the ground state. As the temperature rises, the thermal energy of the flame excites these electrons. If the energy supplied increases it can become great enough for the electrons to escape from the atom to form an ion. The electrons in the higher energy orbits are in a metastable state and are prone to lower energy orbits including the ground state. In doing so the energy previously absorbed is released as quanta of light the wavelength of which are characteristic of substance, thus giving rise to the emission spectrum. The more energy supplied the more complex is the spectrum.

In the emission flame photometry the relatively low energy source only excites a few elements mainly the alkali metals. A portion of the light, which is emitted in all directions is collected by a

reflector and falls on a detector. The detector output is directly proportional to the concentration of the substance in the flame. Under the usual conditions only a small proportion of the atoms present in the flame are excited into emission, 1 to 5 percent in the case of alkali metals and less for the other elements. Most atoms, therefore, remain in the ground state. This technique has been mostly used to determine sodium and potassium and recently also lithium.

Q. What are Auto analyzers, mention its parts and their functioning?

Ans. Autoanalysers are involved in the automation to routine biochemical analysis of hospital. In this instrument about 60 to 300 samples of blood or serum per hour for as many as 18 constituents can be analyzed.

The principal parts of auto analyzers are the following

(1) Sampler
(2) Proportionating pump
(3) Dialyser
(4) Constant Temperature unit
(5) Colorimeter
(6) Recorder

(1) **Sample:** This module holds the batch of samples awaiting analysis in separate cups on a circular tray, which is rotated at intervals. A probe connected by plastic tubing to the proportionating pump enters each sample serially. The volume of sample aspirated is determined by the pumping rate and the adjustable dwell time of the probe in the sample.

(2) **Proportionating pump:** This module determines the relative flow rates of sample and all reagents and replaces the use of different sizes of pipettes in manual methods. The pumping technique involves the peristaltic action produced by a series of rollers passing along an array of parallel plastic 'Pump tubes'. Each roller compresses all tubes so that the rate flow in each tube is proportional to the square of the pump tube diameters.

(3) **The Dialyser:** This module achieves the separation of small and large molecules by allowing the former to pass through a semipermeable membrane from the donor (sample) stream of liquid and air bubbles to a recipient stream of liquid again segmented by air bubbles. The dialysis rate depends on the temperature but complete passage of small molecules into the recipient stream is rarely achieved and may be only a few percent of the total. The analytical process then requires that a constant fraction should dialyse and this is not always the case when simple aqueous and protein containing solutions are compared. Care must be taken to ensure that the output from the recipient stream is the one, which enters the remainder of the analytical system. If the sample stream is greatly diluted, the difference may not be seen easily.

(4) **Maintenance of constant temperature:** The constant temperature is maintained by using a incubator baths. The incubator baths consist of glass delay coil mounted in a thermostatically controlled oil bath. This is sealed and stirred constantly. The incubator bath maintain the reaction mixture at a constant temperature for a defined time and thus bring about the required chemical change under controlled conditions.

(5) **Flow through colorimeter:** The colorimeter is to measure the intensity of colour produced in the reaction and provide a graphical display of change in colour with time.

(6) **The recorder:** The servo-potentiometer recorder is used to record the ratio of the responses from the two detectors and these responses are proportional to the intensity of light reaching the detectors.

Functioning of the instrument

The flow of the analytical stream is directed through plastic tubing from one module to another. The samples are then loaded into the cup of the sampler and the channels of the proportionating pump aspirate them as well as dilute them. The diluted serum samples are led through one of the dialyser units. The pump then introduces suitable reagents through the other side of the dialyser unit. Thus the two streams running side by side are being separated only by dialysing membrane. A portion of dialysable constituents of serum passes across the membrane to the reagent stream. Further treatment like incubation at suitable temperature is given in the temperature bath. The intensity of the colour developed is measured in a colorimeter and recorded in the recorder. Suitable standards are also treated in the same manner.

Recently, multiple analyzers have been introduced with multiple channels to estimate as many as 18 selected constituents including the non dialysable proteins, enzymes and electrolytes. About 60 to 300 samples can be investigated per hour simultaneously for 18 parameters. Auto analyzers have gained much popularity for routine biochemical analysis in clinical laboratories for their speed, flexibility of the methodology, increased quantum of clinical, biochemical analysis, easy operations and accuracy of results.

Q. What are Radioisotopes?

Ans. The most useful radioisotopes are ^{14}C, ^{35}S, ^{32}P and ^{3}H. These are β-ray emitters, that is when the nuclei of these atoms disintegrate, one of the products is an electron which moves with energies characteristic of the disintegrating nucleus. the so called β-rays interact with the molecules through which they traverse, causing dissociation, excitation, or ionization of the molecules. It is the resultant ionization property which is used to measure quantitatively the amount of radioisotope present.

Units—A curie is the amount of emitter, which exhibits 3.7×10^{10} disintegrations/sec (dps). More common units are a millicurie, mc (10^{-3} curie) and a microcurie; μc (10^{-6} curie).

Specific activity—This is defined as disintegrations/minute per unit of substance (mg, mole etc.).

Dilution factor—The factor is defined as

$$\frac{\text{Specific activity of precursors fed}}{\text{Specific activity of compound isolated}}$$

This factor is used frequently to express the precursor relation of a compound in the biosynthesis of a second compound. Thus, in the sequence A → B → C → D, the dilution factor for C → D would be small, whereas for A it would be large. Therefore a small dilution factor would indicate that compound C fed to a tissue has a better precursor relationship to the final product than compound A with a large dilution factor.

Percentage of incorporation

This is used to compare the proximity of a precursor compound. If labeled compound A is administered to an experimental system and some of the radioactivity is incorporated into compound D, the percentage of incorporation is expressed as curies (or microcuries) in D divided by curies (or microcuries) in A × 100 [(i of D/Ci of A) × 100].

Measurements

Liquid scintillation counting is probably the most popular technique for measuring radioisotopes. The technique is based on the use of a scintillation solution containing fluors and a multiplier phototube. The scintillation solution converts the energy of the radioactive particle into light, the

multiplier phototube responds to the light by producing a charge which can be amplified and counted by a scaling circuit.

In liquid scintillation counting, the radioactive substance is usually dissolved in a suitable organic solvent containing the fluor. Alternately, the radioactive sample, which can consist of filter paper containing the sample, is suspended or immersed in the scintillation fluid. Under these conditions the energy of the radioactive particle is first transferred to the solvent molecule, which may then ionize or become excited. It is the electronic excitation energy of the solvent which is transferred to the fluor (solute) when the excited molecules of the solute return to the ground state they emit quanta of light that are detected by the phototube.

Scintillation counting is particularly useful for determining the weak β particles of triticom (3H) and carbon 14(^{14}C). The efficiency of counting these particles can be as high as 50 and 5% respectively.

2

ENZYMES

Q. Define enzyme.

Ans. An enzyme is a protein that is synthesized in a living cell and catalyze or speeds up a thermodynamically possible reaction so that the rate of the reaction is compatible with the biochemical process essential for the maintenance of a cell. The enzyme does not modify the equilibrium constant or the ΔG of a reaction. Being a protein an enzyme loses its catalytic properties if subjected to agents like heat, strong acids or bases, organic solvents or other conditions which denature the protein.

OR

Enzymes are soluble, colloidal organic catalysts formed by living cells, specific in action, protein in nature, inactive at 0°C and destroyed by moist heat at 100°C.

Q. Differentiate between intracellular and extracellular enzymes.

Ans. The enzymes, which are used in the cells, which make them, are said to be **intracellular enzymes**. These enzymes correspond to the old "organized ferments". Those enzymes, which are produced by other cells and are secreted to other parts of the body i.e. digestive juice are called as **extracellular enzymes**. These enzymes correspond to the old "unorganized ferments".

Q. What is a coenzyme?

Ans. Some enzymes are purely protein in nature and depend for activity on their structure while certain enzymes require for their function, one or more non-protein part. These are termed as **coenzymes, cofactors** or **prosthetic groups**. If such a compound is firmly attached to enzyme proteins than it is called as coenzyme. Certain coenzymes exist in free state in solution and contact enzyme protein, only at the time of reaction.

Q. What are apoenzymes?

Ans. The term **apoenzyme** refers to the protein part of the enzyme. The apoenzyme in combination with its prosthetic group constitutes a complete coenzyme or **holoenzyme**.

Holoenzyme =	Apoenzyme	+	Coenzyme
	(Proteinous nature)		(Non proteinous nature)

Q. Discuss the classification of enzymes.

Ans. The enzymes are broadly classified as

1. Oxidoreductase

Enzymes which are concerned with biological oxidation and reduction between two substrates

and therefore with respiration and fermentation processes.

$$A\ (reduced) + B\ (oxidized) = A\ (oxidized) + B\ (reduced)$$

The class includes not only the dehydrogenases and oxidases, but also the peroxidases, which use H_2O_2 as the oxidant, the hydrolases, which introduce hydroxyl groups and the oxygenases, which introduce molecular O_2 in place of a double bond in the substrate.

2. Transferases

Enzymes, which catalyze the transfer of one C group or one radical R, from one molecule to another molecule.

$$A.R + B = A + R.B$$

The group includes

(*a*) **Transphosphorylase:** Transfer phosphoryl group from one molecule to another.

$$ATP + D\text{-hexose} \xrightarrow{\text{Hexokinase}} D\text{-hexose-6 phosphate} + ADP$$

(*b*) **Transacylase:** Transfer acyl group from one molecule to another.

$$\text{Acetyl CoA} + \text{Choline} \xrightarrow{\text{Choline acetyl transferase}} \text{CoA} + \text{Acetyl choline}$$

Besides these, it also includes transaminases, transglycosidases and transmethylases.

3. Hydrolases

Enzymes which catalyze hydrolysis of ester, ether, peptide glycosyl, acid anhydride by addition of water. This group includes the extracellular digestive enzymes and many intracellular enzymes. For example β galactosidase acts on, β – D galactoside, pepsin, renin and chymotrypsin acts on peptide bonds, lipases and phosphatases acts on esterases.

$$\beta - D \text{ galactoside} + H_2O \rightarrow \text{An alcohol} + D \text{ galactose}$$

4. Isomerases

Enzymes which catalyze interconversion of optical, geometric or positional isomers. This class includes racemases, epimerases, cis-trans isomerases and enzymes catalyzing interconversion of aldehyde and ketones.

$$\text{L alanine} \xrightleftharpoons{\text{Racemases}} \text{D alanine}$$

$$\text{Trans retinene} \xrightleftharpoons{\text{Cistrans isomerases}} \text{Cis retinene}$$

5. Lyases

Enzymes that catalyze removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

$$\begin{array}{l} C - C \rightleftharpoons C = C + X - Y \\ \quad | \quad\ | \\ \quad X \quad Y \end{array}$$

This class includes aldehyde lyases, carbon oxygen lyases (fumerases).

$$\text{L malate} \xrightleftharpoons{\text{Fumerase}} \text{L fumarate} + H_2O$$

6. Ligases

Enzymes that catalyze the linking together of two compounds couple to the breaking of a pyrophosphate bond in ATP or a similar compound. These enzymes catalyze formation of C – S bonds (succinate thiokinase), C – N bonds (glutamine synthetase), C – C bonds (acetyl CoA carboxylase, succinate thiokinase)

$$\text{GTP + Succinate + CoA} \xrightleftharpoons{\text{Glutamine synthetase}} \text{GDP + Pi + Succinyl CoA}$$

$$\text{ATP + L-glutamate + } NH^{4+} \xrightleftharpoons{\text{Acetyl CoA carboxylase}} \text{ADP + Orthophosphate + L glutamine}$$

Q. Describe in brief the different factors affecting enzyme actions.

Ans. The enzymes are colloidal, proteinous organic molecules in nature. A number of factors affect the enzme ctivity. Some of the important factors are given as follows—

Effect of Temperature

In a chemical transformation

A – B →	A ----- B →	A + B
Initial	Transition	Final
state	state	state

There is an activation of a population of A – B molecules to an energy rich state which is called as **transition state.** When reacted, the bond holding A and B will be so weakened that it will break leading to the formation of products A and B. The rate of reaction will thus be proportional to the concentration of the transition state species. The concentration of the transition state species, in turn, depends on the critical thermal kinetic energy required to produce transition state species of the reacting molecules. The important feature of an enzyme catalyzed reaction is that an enzyme lowers the activation energy.

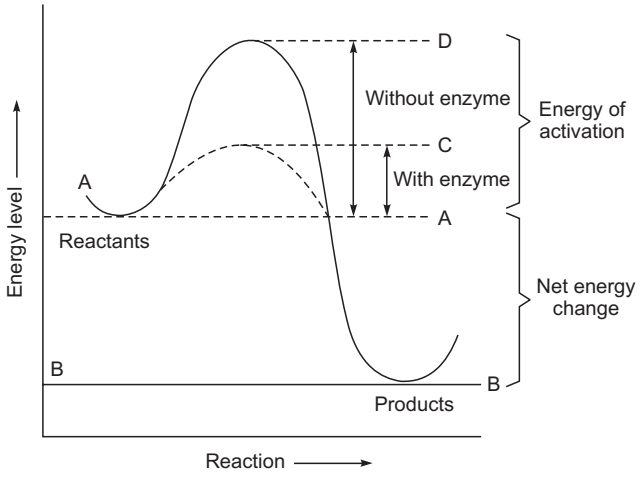


Fig. 2.1. The energy of activation of a biochemical reaction in presence and absence of a specific enzyme.

Because of the protein nature of an enzyme, thermal denaturation of the enzyme protein with increasing temperature will decrease the effective concentration of an enzyme and consequently

decrease the reaction rate. Up to perhaps 45°C the predominant effect will be an increase in reaction rate as predicted by chemical kinetic theory. Above 45°C an opposing factor, namely thermal denaturation will become important, until at 55°C rapid denaturation will destroy the catalytic function of the enzyme protein.

Q^{10} or temperature coefficient

The rate of an enzyme catalyzed reaction increases depending over a strictly limited range of temperature owing to increased kinetic energy of the reacting molecules. The kinetic energy of the enzyme exceeds the energy barriers for breaking the weak hydrogen and hydrophobic bonds that maintain its secondary and tertiary structure. The factor responsible for the increase of the rate of a biologic process for a 10°C temperature rise is the **Q^{10} or temperature coefficient.**

Effect of pH

Since enzymes are proteins, pH changes will profoundly affect the ionic character of the amino and carboxylic groups on the protein and will therefore markedly affect the catalytic site and conformation of an enzyme. The low or high pH values can cause considerable denaturation and hence inactivation of the enzyme protein. Moreover, since many substances are ionic in character (e.g. ATP, NAD^+, Amino acids and CoASH) the active site of an enzyme may require particular ionic species for optimum activity. These effects are probably the main determinants of a typical enzyme activity - pH relation. Thus a bell shaped curve is obtained with relatively small plateau and with sharply decreasing rates on either side as indicated in figure. The plateau is usually called the **optimal pH point**.

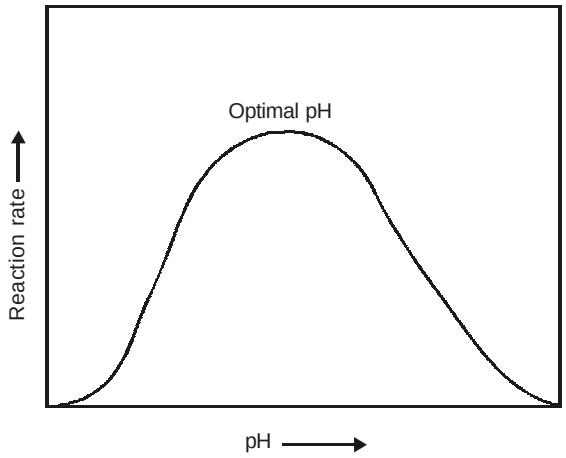


Fig. 2.2. Effect of pH on enzyme catalyzed reaction.

At the optimum pH the enzyme (E) will react with substrate (S^+) as

$$E^- + H^+ \rightleftharpoons ES$$

At low pH values E^- will be protonated and loose its negative charge and cannot combine with substrate

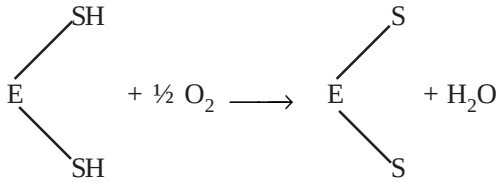
$$E^- + H^+ \longrightarrow E$$

Similarly at high pH values, S^+ will loose its positive charge and cannot react with enzyme.

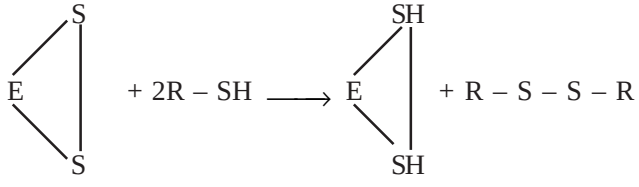
$$S^+ \longrightarrow S + H^+$$

Oxidation

The sulfhydryl (SH) groups of many enzymes are essential for enzyme activity. Oxidation of these groups by many oxidizing agents including the oxygen of air forms the disulfide (S – S) linkages and results in loss of enzyme activity.



The enzyme activity may be regained by reduced sulfhydryl compounds such as cysteine or glutathione (R – SH)



Radiation

Enzymes are highly sensitive to short wave length (high energy) radiation such as X-, β-, or γ-rays. The high energy radiation forms peroxides which causes oxidation of the enzymes resulting in loss in the enzyme activity.

Coenzymes and Activators

Almost all the enzymes work efficiently in presence of some other substances, which may be either organic or inorganic; commonly called as **coenzymes** and **activators** respectively. In absence of coenzymes and activators the enzymes may be inactive or sluggish. For example activators Cl^-, Mg^{++}, Ca^{++}, Mn^{++} etc. may take part in the formation of enzyme substrate complex. Some enzymes which are called activators i.e. enterokinase converts trypsinogen into trypsin by removing hexapeptide from trypsinogen.

Q. Describe the Michaleis Menton equation OR State the effect of enzyme concentration and substrate concentration.

Ans. The rate of an enzyme catalyzed reaction depends directly on the concentration of the enzyme. With a fixed concentration of enzyme and with increasing substrate concentration, an important relationship is observed. With fixed enzyme concentration, an increase of substrate will result at first in a very rapid rise in velocity or Reaction State. As the substrate concentration continues to rise, the increase in the rate of reaction begins to slow down until, with a large substrate concentration, no further change in velocity is observed.

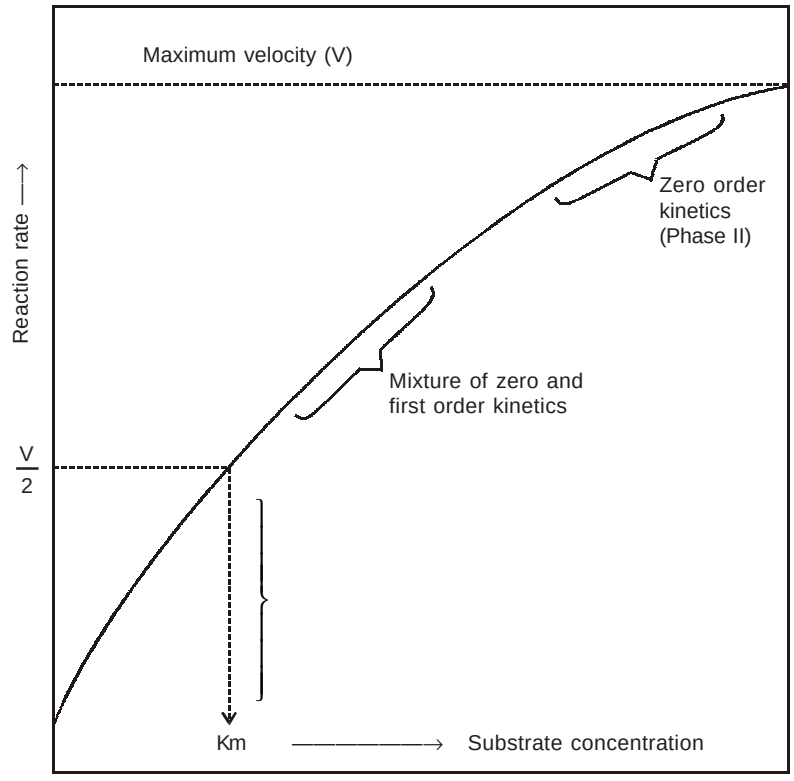


Fig. 2.3 Effect of substrate concentration on reaction rate assuming that enzyme concentration is constant.

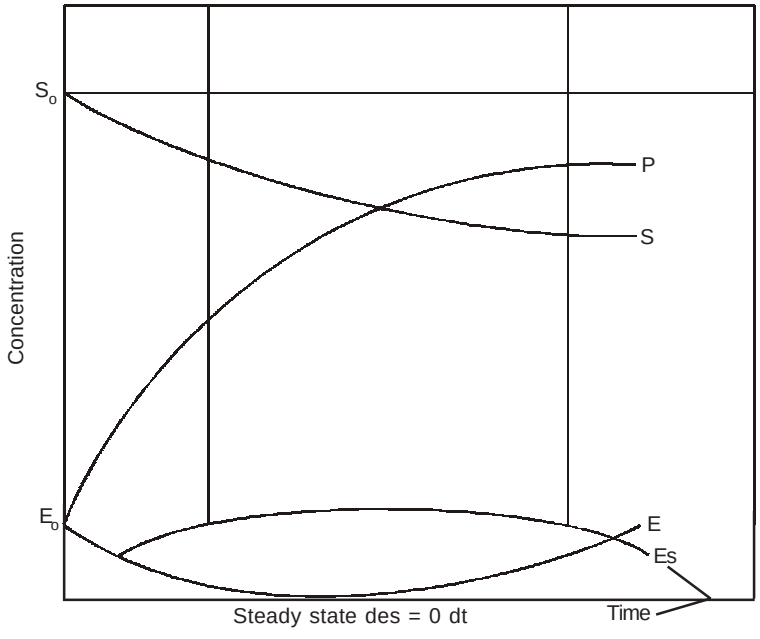


Fig. 2.4 Hyperbolic Curve.

As the number of substrate molecule increases, the sites are covered to a greater degree until at saturation no more sites are available, the enzyme is working at full capacity and now the rate is independent of substrate concentration.

The mathematical equation that defines the quantitative relationship between the rate of an enzyme reaction and the substrate concentration and thus fulfils the requirement of the rectangular hyperbolic curve is the **Michaelis – Menton equation.**

$$V = \frac{V\,max\,[S]}{Km + [S]} \quad \ldots (1)$$

In this equation V is the **observed velocity** at given substrate concentration [S]; Km is the **Michaelis constant** expressed in units of concentration (mole/litre); and *Vmax* is the **maximum velocity** at saturating concentration of substrate. Equation (1) is readily derived by employing the Briggs – Haldane assumption of steady state kinetics by considerating of the following steps:

1. A typical enzyme catalyzed reaction involves the reversible formation of an enzyme substrate complex (ES), which eventually breaks down to form the enzyme E again and the product P. This is represented in equation (2).

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_4}{\overset{K_3}{\rightleftharpoons}} E + P \quad \ldots (2)$$

   Where K_1, K_2, K_3 and K_4 are the rate constants for each given reaction.
2. After a few milliseconds after the enzyme and substrates have been mixed a concentration of *ES* builds up and does not change as long as *S* is in large excess and $K_1 \geq K_3$. This condition is called the **steady state** of the reaction, since the rate of decomposition of *ES* just balance the rate of formation. Recognizing that the rate of formation of *ES* is equal to the rate of decomposition of *ES*, we can write

Rate of formation of [*ES*] = Rate of decomposition of [*ES*]

$$K_1[E][S] + K_4\,[E][P] = K_2[ES] + K_3\,[ES] \quad \ldots (3)$$

and therefore,

$$[E]\,(K_1[S] + K_4[P]) = [ES]\,(K_2 + K_3) \quad \ldots (4)$$

$$\frac{[ES]}{[E]} = \frac{K_1[S] + K_4[P]}{K_2 + K_3}$$

$$\frac{[ES]}{[E]} = \frac{K_1[S]}{K_2 + K_3} + \frac{K_4[P]}{K_2 + K_3} \quad \ldots (5)$$

We can simplify this equation by considering that since we are examining equation (1) at an early stage of the enzyme catalyzed reaction, *P* will be very small and hence the rate of formation of *ES* by the reaction

$$E + P \rightarrow ES$$

will be extremely low. Thus, the term $K_4[P]/\,(K_2 + K_3)$ can be ignored and equation (5) simplify to

$$\frac{[ES]}{[E]} = \frac{K_1[S]}{K_2 + K_3} \quad \ldots (6)$$

The three constant K_1, K_2 and K_3 can be combined into a single constant, Km by the relationship

$$\frac{K_2 + K_3}{K_1} = Km \qquad \text{... (7)}$$

and thus equation (6) can be further simplified to

$$\frac{[E]}{[ES]} = \frac{Km}{[S]} \qquad \text{... (8)}$$

3. We are now faced with the problem of converting (*E*) and (*ES*) into easily measurable values. We can resolve this problem if we consider that the total enzyme concentration $[E]_t$ in the reaction consist of the enzyme, [*E*] which is free plus that which is combined with the substrate, [*ES*]. The free enzyme concentration [*E*] therefore is $[E]_t - [ES]$ and

$$\frac{[E]}{[ES]} = \frac{[E]_t - [ES]}{[ES]} = \frac{[E]_t}{[ES]} - 1$$

$$\frac{[E]_t}{[ES]} - 1 = \frac{Km}{[S]} \qquad \text{... (9)}$$

Since these terms still cannot be readily determined by the usual techniques available, we must resort to the following relationships. The maximum initial velocity (*Vmax*) is attained when the total enzyme $[E]_t$ is completely complexed with saturating amounts of *S*, or

V = *K*[*ES*] and thus,

$$\frac{Vmax}{V} = \frac{[E]_t}{[ES]} \qquad \text{... (10)}$$

Finally the ratio *Vmax/V* can now be substituted for $[E]_t/[ES]$ to yield.

$$\frac{Vmax}{V} = \frac{Km}{S} + 1 \qquad \text{... (11)}$$

Inverting and rearranging, we obtain

$$V = \frac{Vmax\,[S]}{Km + [S]} \qquad \text{... (l2)}$$

This equation is **Michaelis Menton** equation. The constant *Km* is important, since it provides a valuable clue to the mode of action of an enzyme catalyzed reaction.

Lineweaver Burk Double Reciprocal Plot

It is difficult to estimate Vmax from the position of an asymplote, as in the rectangular hyperbola, linear transformations of the Michaelis Menton equation are often used.

$$\frac{1}{V} = \frac{1}{Vmax} + \frac{Km}{Vmax} \cdot \frac{1}{[S]}$$

($y = b + mx$) gives straight line where *m* is the slope and *b* is *y* intercept of the regression of *y* on *x*.

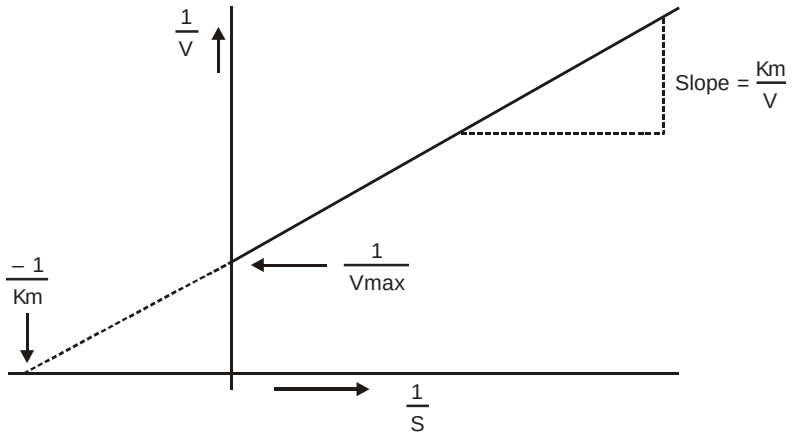
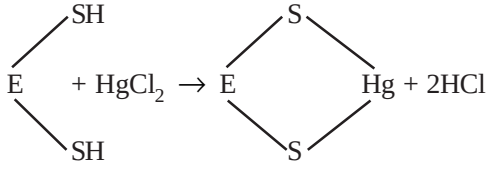


Fig. 2.5. Lineweaver Burk plot.

Q. Describe the actions of Inhibiting agents.

Ans. The activity of enzymes are inhibited by certain chemical agents which are usually salts of mercury, silver, gold and salts of heavy metals or fluorides. For example many enzymes (known as –SH enzymes) depend for their activity on the presence of free –SH group. They can be inactivated by mercuric chloride, which reacts with free –SH groups thus:



This type of inhibition is non-specific. But there are other types of inhibition and their kinetic assumptions have also been described by kineticists. The enzyme inhibition is broadly classified as irreversible inhibition and reversible inhibition.

Q. What are Irreversible inhibitions?

Ans. In irreversible inhibition, the inhibitor forms a covalent bond with a specific function, which is usually an amino acid residue, which may, in some manner be associated with the catalytic activity of the enzyme. The inhibitor cannot be released by dilution or dialysis.

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

$$+$$

$$I$$

$$\downarrow$$

$$EI$$

Example of irreversible inhibitors include diisopropyl fluorophosphate which reacts irreversibly with serine proteases such as chymotrypsin and iodoacetate which react essentially with sulfhydryl group of an enzyme such as triose phosphate dehydrogenase.

$$E - SH + I\,CH_2COOH \rightleftharpoons E - S - CH_2COOH + HI$$

Q. What are Reversible Inhibitions?

Ans. This type of inhibition involves equilibrium between the enzyme and the inhibitor, the equilibrium constant (*Ki*) being a measure of the affinity of the inhibitor for the enzyme. Three distinct type of reversible inhibition are known and they are :

Competitive inhibition
Non competitive inhibition
Uncompetitive inhibition

Q. Define Competitive inhibition.

Ans. Compounds that may or may not be structurally related to the natural substrate combine reversibly with the enzyme at or near the active site. The inhibitor and the substrate therefore compete for the same site according to the reaction.

$$\begin{array}{l} E + S \rightleftharpoons ES \rightarrow E + P \\ + \\ I \\ \Updownarrow Ki \\ EI \end{array}$$

ES and EI complexes are formed but EIS complex is never produced.

Q. Define Non-competitive inhibition.

Ans. Compounds that reversibly bind with either the enzyme or the enzyme substrate complex are designated as non-competitive inhibitors and the following reactions describe these events.

$$\begin{array}{lll} E + S \underset{Km}{\rightleftharpoons} ES \rightarrow E + P \\ + & + \\ I & I \\ \Updownarrow Ki & \Updownarrow \\ EI \underset{Km}{\overset{IS}{\rightleftharpoons}} & EIS \end{array}$$

Non competitive inhibition therefore differs from competitive inhibition in that the inhibitor can combine with ES and S can combine with EI to form in both instances EIS. This type of inhibition is not completely reversed by high substrate concentration since the closed sequence will occur regardless of the substrate concentration. Since the inhibitor binding site is not identical to nor does it modify the active site directly the Km is not altered.

Q. Differentiate between Competitive and Non Competitive Inhibition

Competitive Inhibition	*Non competitive Inhibition*
1. It is reversible reaction.	It can be reversible or irreversible.
2. Inhibitor and substrate resemble each other in structure.	There is no structural similarity between substrate and inhibitor.
3. Inhibitor binds at active site.	Inhibitor do not bind at active site.
4. Vmax is same.	Vmax is lowered.
5. Km is increased.	Km is unaltered.
6. Inhibitor cannot bind with ES complex.	Inhibitor can bind with ES complex.
7. It lower the substrate affinity to enzyme.	It does not change substrate affinity for the enzyme.
8. It forms the EI complex.	It forms EI and ESI complex.
9. Michaelis Menton equation changes to $V = \frac{Vmax\,[S]}{Km\left[\frac{1+(I)}{Ki}\right] + S}$	Michaelis Menton equation changes to $V = \frac{Vmax\,[S]}{Km\left[\frac{1+(I)}{Ki}\right] + [Km + S]}$

Q. What is Uncompetitive inhibition?

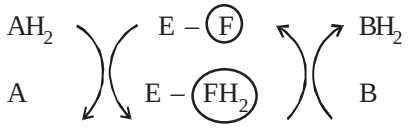
Ans. Compounds that combine only with the ES complex but not with the free enzyme are called **uncompetitive inhibitors.** The inhibition is not overcomed by high substrate concentrations; the K'm value is consistently smaller that the Km value of unhibited reaction which implies that S is more effectively bound to the enzyme in presence of the inhibitor. The sequence of typical reaction is

$$E + S \underset{Km}{\rightleftharpoons} ES \rightarrow E + P$$
$$+$$
$$I$$
$$\Updownarrow Ki$$
$$EIS$$

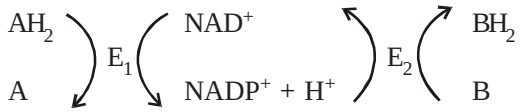
Q. What are Cofactors?

Ans. A large number of enzymes require an additional component before the enzyme protein can carry out its catalytic functions. The general term cofactor encompasses this component. Cofactor may be divided rather loosely into three groups, which include (a) prosthetic groups (b) coenzymes and (c) metal activators.

A **prosthetic group** is usually considered to be a cofactor firmly bound to the enzyme protein. Thus, for example, the porphyrin moiety of the hemoprotein peroxidases and the firmly associated flavin-adenine dinucleotide in succinic dehydrogenase are prosthetic groups.



A **coenzyme** is a small, heat stable organic molecule, which readily dissociates off an enzyme protein and in fact can be dialyzed away from the protein. Thus NAD^+, $NADP^+$, tetrahydrofolic acid and thiamine pyrophosphate are examples of coenzymes. The function of a coenzyme namely to interact with different enzymes can be depicted as



With NAD^+ / $NADP^+$ oscillating between E_1 and E_2.

The requirements of a large number of enzymes for metallic, mono or divalent cations represent the metal activator group as K^+, Mn^{2+}, Mg^{2+}, Ca^{2+} or Zn^{2+}. These may be either loosely or firmly bound to an enzyme protein, presumably by chelation with phenolic, amino, phosphoryl, or carboxyl groups.

Q. What are Anti enzymes?

Ans. If certain enzymes are repeatedly injected into an animal, a substance will be produced in the animal's serum, which can prevent the normal action of the enzymes injected. This substance is called an **anti enzyme** for example antipepsin, antitrypsin etc.

Q. What are Allosteric enzymes?

Ans. The regulatory or allosteric enzymes are oligomeric type of enzymes with distinct regulatory and catalytic site. They show sigmoid kinetics and usually can be predicted to catalyze the reaction at a branch point in a metabolic pathway. The characteristic kinetic factor for most allosteric enzymes is a typical relationship of activity and substrate concentration. Mostly enzyme possess independent substrate binding sites i.e. the binding of one molecule of substrate has no effect on the intrinsic dissociation constants of the vacant sites. Such enzymes yield normal hyperbolic curves. However, if the binding of one substrate or effector molecule induces structural or electronic changes that results in altered affinities for the vacant sites, the velocity curve will no longer will follow Michaelis Menton kinetics and the enzyme will be classified to be an allosteric enzyme. Generally, allosteric enzymes yield sigmoidal velocity curves. The binding of one substrate (or effector) molecule facilitates the binding of the next substrate (or effector) molecule by increasing the affinities of the vacant binding sites. This phenomenon is called as **cooperative binding** or **positive cooperativity** with respect to substrate binding or positive homotropic response. It signifies that an effector other than the substrate is being bound at a specific regulatory site, which increases the affinities of the vacant binding sites.

Q. Describe the Template or Lock and Key model of enzyme action.

Ans. This model was originally proposed by Fischer, which states that the active site already exists in proper conformation even in absence of substrate. Thus the active site by itself provides a rigid, pre shaped template, fitting with the size and shape of the substrate molecule. Substrate fits into active site of an enzyme as the key fits into lock and hence it is called as Lock and Key model. This model proposes that substrate binds with rigid preexisting template of the active site, provides additional groups for binding other ligands. But this cannot explain change in enzymatic activity in presence of allosteric modulators.

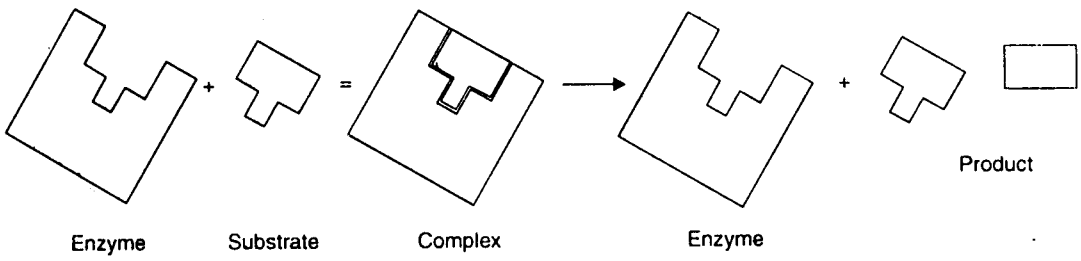


Fig. 2.6. Template or Lock and Key model.

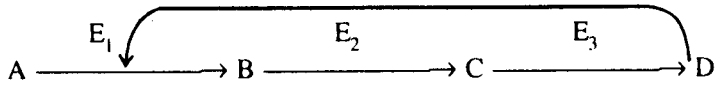
Q. Discuss the Induced fit or Koshland model of enzyme action.

Ans. This model was proposed by Koshland in 1963. The important feature of this model is the flexibility of the region of active site. According to this theory, active site does not possess a rigid, preformed structure on the enzyme to fit the substrate. On the contrary the substrate during its binding induces conformational changes in the active site to attain the final catalytic shape and form. This explains several matters related to enzyme action such as (1) why enzymes become inactive on denaturation (2) saturation kinetics (3) competitive inhibition and allosteric modulation.

Q. Write a note on the Regulation of Enzyme Activity.

Ans. There are several means by which the activity of a particular enzyme is specifically regulated.

1. **Irreversible covalent activation:** Some enzymes are secreted in an inactive form called as proenzymes or zymogens. At the site of action specific peptide bonds are hydrolyzed either enzymatically or by pH change to convert it into active form e.g. pepsinogen to pepsin; trypsinogen to trypsin. After hydrolysis when it is activated, it cannot be reconverted into proenzyme form.
2. **Reversible covalent modifiction:** By the addition of or removal of a phosphate or adenylate certain enzymes are reversibly activated and inactivated as per the requirement. Protein kinase of muscle phosphorylates phosphorylase kinase, glycogen synthetase by making use of ATP.
3. **Allosteric modulation:** In this type of regulation, the inhibitor binds to the enzymes at a site other than the active site but on a different region in the enzyme molecule.
4. **Regulation of enzyme synthesis:** The concentration of enzyme is an important factor responsible for enzymatic activity. Since enzymes are protein in nature, their synthesis is under control of genes responsible for their synthesis. The regulation of synthesis takes place by feed back inhibition. In this the end products inhibit the activity of the enzyme as shown—



A high concentration of D typically inhibits conversion of A $\rightarrow$ B. This involves not simple backing up of intermediates but the activity of D to bind to and inhibit E_1. D thus acts as negative allosteric effector or feed back inhibitor of E_1. The kinetics of feed back inhibition may be competitive non competitive, mixed etc. It is the commonest way of regulation of a

biosynthetic pathway. Feed back regulation generally occurs at the earliest functionally reversible step unique in the biosynthetic pathway. It is of following types:

(*a*) **Cumulative feed back inhibition:** In this the inhibitory effect of two or more end products on a single regulatory enzyme is strictly additive.

(*b*) **Concerted or Multivalent feed back inhibition:** In this type, complete inhibition occurs only when two or more end products are present in excess.

(*c*) **Cooperative feed back inhibition:** In this type, a single end product present in excess inhibits the regulatory enzyme, but the inhibition due to two or more end products far exceed the additive effects of cumulative feed back inhibition.

Q. What are Isoenzymes?

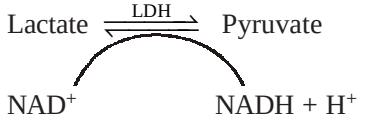
Ans. The enzymes that occur in a number of different forms and differ each other chemically, immunologically and electrophoretically are called **isozymes.** They are present in serum and tissues of mammals, amphibians, birds, insects, plants and unicellular organisms. The examples of isozymes include dehydrogenases, several oxidases, transaminases, aldolases transphosphorylase etc.

Characteristics of Isozymes

Isozymes catalyze the same reaction but they can be distinguished by physical methods such as electrophoresis or by immunological methods. The difference between some isozymes are due to difference in the quaternary structure of the enzyme for e.g. lactate dehydrogenase exist in 5 isozymic forms. The isozymic forms of lactate dehydrogenase are tetramers each is made from two types of units H and M. The molecular weight of active lactate dehydrogenase is 1,30,000. Only the tetrameric molecule possess catalytic activity. The subunits are expressed in the following five ways.

HHHH
HHHM
HHMM
HMMM
MMMM

The synthesis of these subunits are controlled by distinct genetic loci. The lactate dehydrogenase catalyze the transfer of two electrons and one hydrogen ion from lactate to NAD



Basically, two different types of LDH occur. One type, which predominates in the heart, is called as heart LDH. The other type is characteristics of many skeletal muscles are called muscle LDH.

Isozymes are wide spread in nature, with over a hundred enzyme now known to ccur in two or more molecular forms.

Q. What are Coenzymes?

Ans. Many reactions of substrates are catalyzed by enzymes only in the presence of a specific non protein organic molecule called as **coenzyme.** It combines with the apoenzyme to form **holoenzyme.** The **apoenzyme** is the proteinious part. The coenzymes are also regarded as

cosubstrates. Thus the coenzymes are heat stable, dialyzable non-protein organic molecule and are the prosthetic group of enzymes.

Coenzymes are broadly classified as

I. Based on chemical characteristics:
- A. Containing an aromatic hetero ring: ATP, NAD, NADP, FMN, $B_6 - PO_4$.
- B. Containing a non aromatic hetero ring: Biotin, lipoic acid.
- C. Containing no hetero ring: Sugar phosphate, coenzyme Q.

II. Based on functional characteristics:
- A. Group transferring coenzymes: ATP, Sugar phosphates, Thiamine pyrophosphate, Coenzyme A, $B_6 - PO_4$, Biotin.
- B. Hydrogen transferring coenzymes: NAD, NADH, FAD, FMN, Coenzyme Q.

III. Based on nutritional characteristics:
- A. Containing B vitamins: Coenzyme A, Biotin, Folic acid, $B_6 - PO_4$, B_{12}.

Q. Mention the important functions of coenzymes.

Ans. The function of coenzymes is usually to accept atoms or groups from a substrate and to transfer them to other molecules. They are less specific than enzymes and so the same coenzyme can act as such in a numbers of different reactions. NAD and NADP function as hydrogen acceptors in dehydrogenation reactions. The coenzymes are responsible to carry out different reactions along with the enzymes.

Q. Write briefly a note on Coenzyme A.

Ans. Coenzyme A is composed of adenosine triphoshphate, pantothenic acid and β – mercapto ethylamine. So it is also called as coenzyme form of pantothenic acid (a vitamin). It is a group transferring enzyme and the reacting group is sulfhydryl (–SH) group. The action involves the acceptance of acyl group by the sulfhydryl group to form acetyl coenzyme A (CH_3CoS. CoA).

Functions of Coenzyme A

Coenzyme A is the carrier of acyl groups i.e. acetyl, succinyl benzoyl etc. Some of the pantothenic acid is bound to protein in the form of acyl carrier protein. This can be regarded as coenzyme A in which the adenine dinucleotide is replaced by protein. Acyl carrier protein chiefly functions on the synthetic processes e.g. of fatty acid and cholesterol. It is also required in the oxidative decarboxylation of pyruvic acid and α ketoglutaric acid, in the breakdown and synthesis of cholesterol which is involved in bile acids, bile salts, steroid hormones and vitamin D formation.

Q. Discuss in brief how the enzymes are isolated and purified.

Ans. Since enzymes are very unstable molecules, their isolation from the tissues, is to be done under controlled conditions of pH, ionic strength and temperature. The standard procedures for extracting and purifying an enzyme are the same as for any other protein. Enzyme assay is performed at each step of isolation, to ensure that the biological activity of the molecule is not lost during procedure manipulation. The step involved in enzyme isolations are described as follows.

1. **Enzyme extraction:** The plant or animal tissue from which the enzyme is to be extracted is homogenised with mortar and pestle, a tissue homogeniser, a blender or by ultrasonic vibrations. Generally the extraction is done under cold (around 4°C) as most enzymes are inactive at room temperatures. Extraction is done with a buffer of specific pH and ionic strength. EDTA (ethylene diamine tetraethyl amine) is normally added in the extraction medium

to solubilize the membrane and to chelate heavy metals which could otherwise inhibit enzyme activity. Small amounts of thiols such as beta mercaptoethanol is added, to prevent oxidation of disulphide bonds in the enzymes.

2. **Filtration and centrifugation:** The tissue extract is filtered using cheese cloth or filter paper to remove cell debris and fibers etc. The clear supernatant is then centrifuged in cold using a refrigerated centrifuge. The centrifugation can be used for purification as well as characterization of protein. The technique operate on the principle that molecules of different masses and shapes move through a solution at different rates in the presence of an applied gravitational field, obtain by spinning the rotor of the centrifuge. High speed centrifugation (around 20000 × g) remove cell debris and also the bigger cell organelles such as chloroplasts and mitochondria. A very high centrifugation in ultra centrifuge at about 100,000 × g removes all the cell organelles and only cytosolic proteins remain in the supernatant. This supernatant can be used for further purification of cytosolic enzymes.
3. **Precipitation:** Like any other protein, enzymes are also highly charged molecules and they can be precipitated out with proper charge breaking chemicals. Once their charges are removed, they form aggregates and settle down as precipitates. Acids, bases, salts and organic solvents are often used to precipitate out proteins. For enzymes, the most commonly used precipitant is ammonium sulphate. The salt, either as solid powder or saturated solution is added slowly to the enzyme preparation to achieve desired concentration of the salts. Most proteins are precipitated out between 30 to 70 % of ammonium sulphate. The enzymes settle down as precipitate and can be removed by centrifugation. The precipitate is dissolved in a small amount of buffer of desired pH and ionic strength and is dialyzed to remove excess of ammonium sulphate and other low molecular weight contaminants. About four folds enzyme purification is typically obtained with ammonium sulphate precipitation.
4. **Purification of enzymes:** The dialyzed enzyme preparation is purified further by a variety of techniques. However the most common used techniques are chromatography and electrophoresis.

Adsorption or column chromatography is used to separate other proteins from the specific enzyme. Several spleen enzymes of human beings have been separated by this method. The column is packed with hydroxypatite and enzymes are eluted with phosphate buffer (pH 6.8) of 0.05 to 0.5 M conc. Filtering the enzyme preparation through a column packed with sephadex or any other gel is also used to purify enzymes. This process is called molecular exclusion chromatography or molecular sieve chromatography. However, many enzymes are purified using affinity chromatography. In this process, the enzymes are separated according to their biological specificity. The procedure is similar to that of column chromatography. The column is packed with inert material plus a small molecular weight compound to which the enzyme binds covalently. Thus the enzyme is freed from this binding by using a suitable elutant.

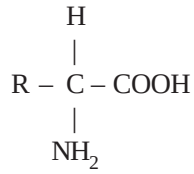
In electrophoretic purification of enzymes, they are separated according to their net charge in the presence of an externally applied electric field.

3

PROTEINS

Q. What are Amino acids?

Ans. Amino acids are the monomers of proteins. All amino acids found in living system, plants and animals are L– (α) amino acids. They have a primary amino and a carboxyl group attached to the same carbon atom. The only exception to this is Glycine, which has two hydrogen atom attached to same carbon.



α amino acid

There are over 200 different amino acids found in nature but proteins of plants, animals and microbes contain only 20 amino acids.

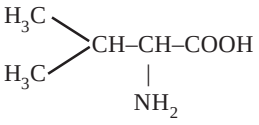
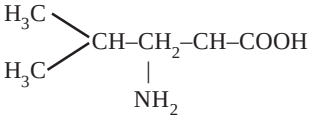
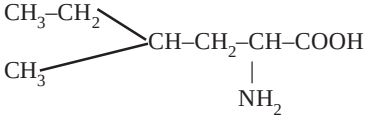
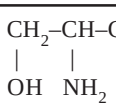
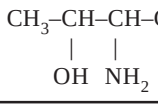
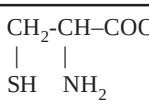
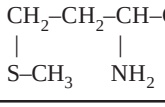
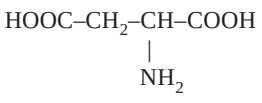
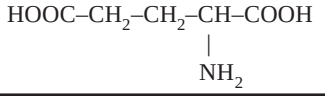
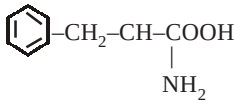
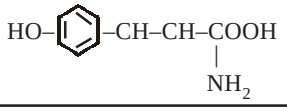
Q. Discuss briefly the Classification of Amino acids.

Ans. A variety of classification of amino acids can be done. Either they can be classified according to presence of acidic, basic or neutral groups or upon their chemical structure i.e. presence of polar, nonpolar groups and so on. Generally amino acids are classified into 7 classes. Table 3.1 includes trivial name, symbol and structural formulas of 20 amino acids.

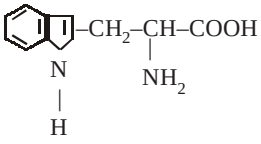
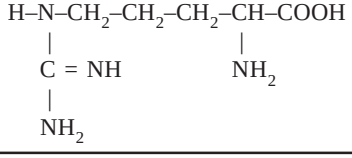
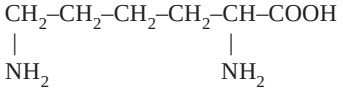
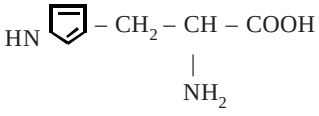
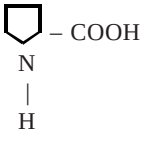
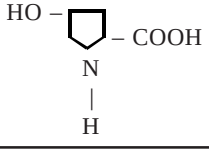
Table 3.1 Classification of α amino acids found in proteins

Group	*Trivial Name*	*Three letter code*	*One letter code*	*Structural formula*
I	**With Aliphatic Side Chains**			
	Glycine	Gly	G	$H–CH(NH_2)–COOH$
	Alanine	Ala	A	$CH_3–CH(NH_2)–COOH$

contd.

Group	*Trivial Name*	*Three letter code*	*One letter code*	*Structural formula*
	Valine	Val	V	
	Leucine	Leu	L	
	Isoleucine	Ile	I	
II	**With Side Chains Containing Hydroxyl (OH) groups**			
	Serine	Ser	S	
	Threonine	Thr	T	
III	**With Side Chains Containing Sulfur Atom**			
	Cysteine	Cys	C	
	Methionine	Met	M	
IV	**With Side Chains Containing Acidic Groups**			
	Aspartic acid	Asp	D	
	Glutamic acid	Glu	E	
V	**Containing Aromatic Rings**			
	PhenylAlanine	Phe	F	
	Tyrosine	Tyr	Y	

contd.

Group	*Trivial Name*	*Three letter code*	*One letter code*	*Structural formula*
	TryptoPhan	Trp	W	
VI	**With Side Chains Containing Basic Group**			
	Arginine	Arg	R	
	Lysine	Lys	K	
	Histidine	His	H	
VII	**Imino acids**			
	Proline	Pro	P	
	Hydroxy Proline	Hyp	–	

Q. How the amino acids are classified nutritionally?

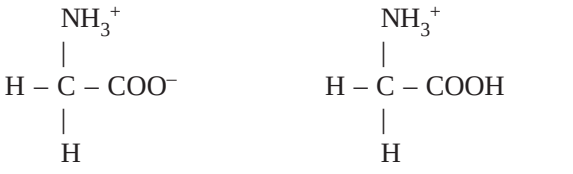
Ans. Nutritionally amino acids are of three types:

1. **Essential Amino acids:** These are the amino acids, which are not synthesized in the body tissue but must be taken in diet. This class of amino acids are leucine, valine, isoleucine, phenyl alanine, threonine, tryptophan, methionine and lysine.
2. **Non-Essential Amino acids:** These can be synthesized by the body and may not be the requisite component of diet for example glycine, alanine, serine, aspartic acid.
3. **Semi-Essential Amino acids:** These are growth promoting factors, they are not synthesized in sufficient quantity during growth. They include arginine and histidine. They become essential in growing children, pregnancy and lactating women.

Q. Mention some important general properties of amino acids.

Ans. Zwitter ion and Isoelectric pH

The amino acids are also called as **ampholytes.** They contain both acidic and basic groups and can react with both alkalies and acids to form salts. For example in glycine the COOH group is the acidic group or proton donor and the NH_2 group is the basic group or protein acceptor. Such amino acids in the crystalline state exist as inner salts or Zwitter ions as a result of H^+ ions passing from the COOH to the NH_2 group.



Zwitter ion Amino acid (Glycine)

The Zwitter ion is an ampholyte as it is both proton donor and proton acceptor. Thus pI is defined as that pH at which amino acids does not migrate or have least migration in an electric field. At this pH the amino acid molecule exists in the Zwitter ion form. pI can be calculated as

$$pI = \text{isoelectric pH} = \tfrac{1}{2}(pk_1 + pk_2)$$

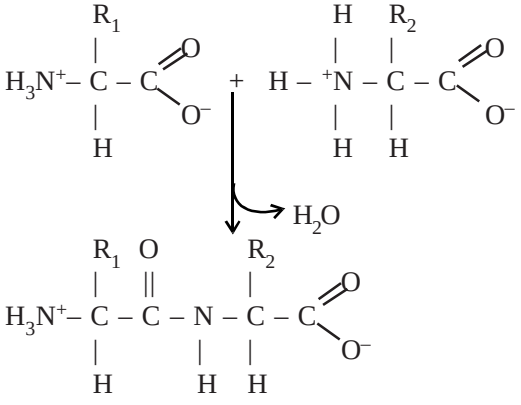
Where pk_1 is the pH at which the carboxyl group is half titrated and pk_2 is the pH at which the NH_3^+ group is half titrated.

Proteins containing acidic and basic groups exists in solution as Zwitter ions and have their own isoelectric pH. Above the isoelectric pH they act as acids and forms negative ions, whereas below the isoelectric pH they acts as bases and form positive ions

$$\text{Protein}^+ \rightleftharpoons H^+ + \text{Protein}^+ \rightleftharpoons H^+ + \text{Protein anion}$$

Peptide bonds

The α amino acids polymerize, at least conceptually through the elimination of a water molecule.



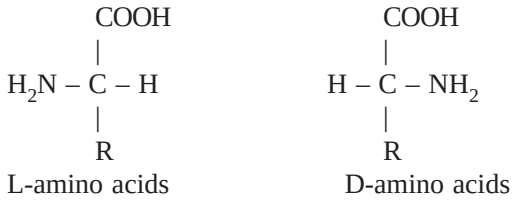
The resulting CO–NH linkage is known as a peptide bond. Polymers are composed of two, three, a few and many amino acids are known as dipeptides, tripeptides, oligopeptides and polypeptides. These substances however are often referred to simply as peptides. These polypeptides range in length

from 40 to over 4000 amino acid residues and since the average mass of an amino acid residue is ~1100, have molecular masses that range from ~4 to over 440 KD.

Isomerism

Two types of isomerism are shown by amino acids basically due to the presence of asymmetric carbon atom. Glycine has no asymmetric carbon atom in its structure hence it is optically inactive.

(*a*) **Stereoisomerism**—All amino acids except glycine exist in D and L form. In D amino acids-NH_2 group is on the right hand while in L amino acids it is oriented to the left.



Neutral proteins of animals and plants generally contain L-amino acids. D-amino acids occur in bacteria.

(*b*) **Optical isomerism**—All amino except glycine have asymmetric carbon atom. Few amino acids like isoleucine and threonine have an additional asymmetric carbon in their structures. All amino acids except glycine rotate the plane of polarized light. Optically active molecules have an asymmetry such that they are not superimposable on their mirror image in the same way that a left hand is not superimposable on its mirror image ,a right hand. This situation is characteristic of substance that contain tetrahedral carbon atoms that have 4 different substituents. The central atoms in such atomic constitutions are known as **asymmetric centres** or **chiral centres** and are said to have property of **chirality:**

Acid base properties

Amino acids and proteins have conspicuous acid-base properties. The a amino acids have two or for those with ionizable groups, three acid-base groups.

The pH at which a molecule carries no net electronic charge is known as its isoelectric point pI. For a amino acids, the application of the Henderson Hasselbalch equation indicates that to a high degree of precision.

$$PI = \tfrac{1}{2}\ (pK_i + pK_j)$$

Where K_i and K_j are the dissociation constants of the two ionizations involving the neutral species.

Q. List important physical properties of amino acids.

Ans.

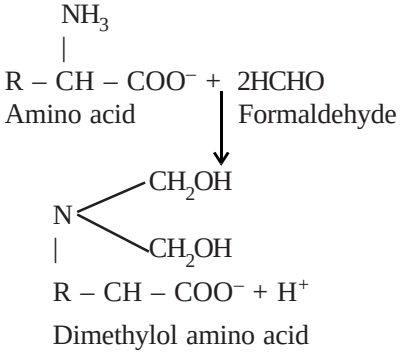
1. Amino acids are colourless, crystalline substances more soluble in water, but tyrosine is only soluble in hot water.
2. They are also soluble in polar solvents (water and alcohol) but are insoluble in non-polar solvents (benzene or ether).
3. Their melting point is above 200°C.
4. The aromatic amino acids can absorb UV light.
5. They have a high dielectric constant. They posses a large dipole moment.

Q. Mention chemical properties of amino acids.

Ans. The chemical reactions of amino acids are due to different functional groups in them. Some of the important chemical reactions are as follows:

Ninhydrin Reaction—Ninhydrin is a powerful oxidizing agent, which causes decarboxylation of amino acids yielding CO_2, NH_3 and an aldehyde. The reduced ninhydrin reacts with liberated ammonia to form blue coloured compounds called Rheummann's purple whereas imino acids Proline and hydroxy proline gives a yellow colour with ninhydrin. This reaction is used for the estimation of free amino groups in amino acids, peptides and proteins.

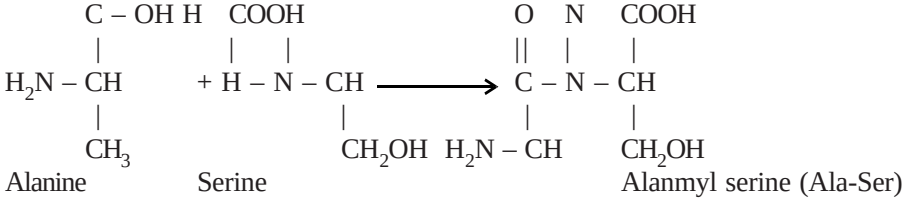
Formal Titration—Sorensen's formal titration method is used for the estimation of free carboxyl group in amino acid and mixtures of amino acid. Amino acids are neutral in solution. If formaldehyde is added to a solution of amino acid, an adduct is formed at the amino group, leaving the carboxyl group free and the molecule becomes acidic in reaction. Free carboxyl group thus can be titrated.



Dimethylol amino acid

By this method one can determine the rate of digestion of proteins by determining the increase in carboxylic groups which accompanies during enzymatic hydrolysis.

Formation of peptide bonds—Peptide bond formation involves removal of one mole of water between the α amino acid and the carboxyl group of second amino acid.



Alanine Serine Alanmyl serine (Ala-Ser)
(A dipeptide)

A peptide formed consists of two or more amino acids linked by a peptide bond. Polypeptides are formed by long peptide chains containing large numbers of peptide bonds. Polymers of upto 100 amino acids are termed polypeptides and those with more than 100 polypeptides generally termed proteins.

Q. Write a note on the role of amino acids as biologically important molecules.

Ans. Besides their role in proteins, amino acids and their derivatives have many biologically important functions. A few examples of these substances are γ amino butyric acid (GABA),

Histamine, Dopamine, Thyroxine, Citrulline, Ornithine, β Cyanoalanine, Homo cysteine, Azaserine and S Adenosyl methionine. This alternative uses of amino acids is an example of the biological opportunism. Nature tends to adapt materials and processes that are already present to new functions.

Amino acids and their derivatives often function as chemical messengers in the communications between cells. For example, glycine, γ aminobutyric acid (GABA, a glutamate decarboxylation product) and dopamine (a tyrosine product) are neurotransmitters. Histamine (the decarboxylation product of histidine) is a potent local mediator of allergic reactions and thyroxine (a tyrosine product) is an iodine containing thyroid hormone that generally stimulates vertebrate metabolism.

Certain amino acids are important intermediates in various metabolic processes. Among them are citrulline and ornithine intermediates in urea triosynthesis. Homocysteine an intermediate in amino acid metabolism and S adenosyl methionine, a biological methylating reagent.

Nature diversity is remarkable. About 250 different amino acids have been found in various plants and fungi. For the most part, their biological role are obscure although the fact that many are toxic suggests that they have a protective function. Indeed some of them, such as azaserine are medically useful antibiotics.

Q. What are Proteins? Write some of the important functions of them.

Ans. Proteins are defined as the high molecular weight mixed polymers of α amino acids joined by peptide linkages. Proteins are the essence of life processes. They are the fundamental constituents of all protoplasm and are involved in the structural integrity and functions of living cells.

Functions of proteins

Proteins are at the centre of the action in the biological processes.

1. They function as enzymes, which catalyze the complex set of chemical reactions that are collectively referred to as life.
2. Protein serve as regulators of these reactions both directly as components of enzymes and indirectly in the form of chemical messengers known as hormones as well as the receptors for these hormones.
3. Proteins act to transport and store biologically important substances such as metal ions, O_2, glucose, lipids and many other molecules.
4. Proteins in the form of muscle fibre and other contractile assemblies, generate the coordinated mechanical motion of numerous biological processes, including the separation of chromosomes during cell division and the movement of eyes.
5. Proteins, such as rhodopsin in the retina of eye, acquire sensory information that is processed through the action of nerve cell proteins.
6. The proteins of immune system such as immunoglobulins, form an essential biological defense system in higher animals.

Q. Classify proteins on the basis of shape and size.

Ans. The proteins are differentiated on the basis of their different shapes and sizes as follows-

Fibrous proteins—This is simple protein. When the axial ratio of length, width of a protein molecule is more than 10, it is called as fibrous protein e.g. Collagen, Scleroprotein.

Globular proteins—When the axial ratio of length, width of a protein molecule is less than 10, it is called as globular protein e.g. Myoglobin, Haemoglobin, Ribonuclease etc.

Q. Classify proteins on the basis of functional properties.

Ans. The proteins are differentiated with regard to the functions they perform.

Defense proteins—Involved in defense mechanisms for e.g. Immuno-globulins.

Respiratory proteins—Involved in the function of respiratory for e.g. Haemoglobin, Myoglobulin, Cytochromes.

Contractile proteins—Involved in muscle contractions, and relaxation. e.g. protein of skeletal muscles.

Hormones—Proteins acting as hormones.

Enzymes—Proteins acting as enzymes.

Structural proteins—Involved in structural integrity of cells e.g.proteins of skin, cartilage, nail.

Q. Classify proteins on the basis of solubility and its physical properties.

Ans. This is the most acceptable scheme of classification of proteins. According to this scheme proteins are classified on the basis of their solubility and physical properties and are divided in three different classes.

1. **Simple proteins**—These are the proteins, which on complete hydrolysis yield only amino acids.
2. **Conjugated proteins**—These are which in addition to amino acid contain a non-protein group called prosthetic group in their structure.
3. **Derived proteins**—These are the proteins formed from native protein by the action of heat, physical forces or chemical factors.

Q. What are simple proteins? Mention some of the important simple proteins.

Ans. Simple proteins are the proteins, which on complete hydrolysis yield only amino acids.These are further sub classified on the basis of the solubilities and heat coagulable properties. Their properties depend on the shape and size of the molecule. Major subclasses are

Fibrous proteins

These are animal proteins, which are highly resistant to digestion by proteolytic enzymes. These are water-soluble. In this group are found keratin's, collagen's and elastins.

Keratins—Keratin is a mechanically durable and chemically unreactive proteins occurring in all higher vertebrates. It is a principal component of their horny outer epidermal layer, comprising upto 85% of the cellular protein, and its related appendages such as hair horn, nails and feathers. Keratins have been classified as either α keratin, which occur in mammals or beta keratin which occur in birds and reptiles. All hard keratin on hydrolysis yield histidine lysine and arginine. Human hair has a higher content of cysteine than that of other species and is called as a keratin.

Elastin—Elastin is a protein with rubber like elastic properties whose fibres can stretch to several times their normal length. It is the principle protein component of elastic yellow connective tissue that occurs in the lungs, walls of large blood vessels such as the aorta, and elastic ligaments such as those in the neck. They are rich in non polar amino acids as alanine, leucine, valine and proline. Elastins are hydrolyzed by pancreatic elastase enzyme.

Collagen—Collagen occurs in all multicellular animals and is the most abundant protein of vertebrates. It is an extracellular protein that is organized into insoluble fibers of great tensile strength. This suits collagen to its role as the major stress bearing component of connective tissues and bone as long, thin, partially crystalline substance. It is insoluble in all neutral solvents. It contains high proportion of hydroxy proline and hydroxy lysine and on boiling it forms gelatin.

Globular proteins

Globular proteins comprise a highly diverse groups of substances that, in their native state, exists as compact spheroidal molecules. Enzymes along with transport and receptor proteins are globular proteins.

Albumins—These are the proteins, which are soluble in water and dilute salt solutions. They are coagulable by heat and changes to such products that are insoluble in water and solutions of salt. Albumins have low isoelectric pH of pI of 4.7 and therefore they are acidic proteins at pH 7.4. The albumin's may be precipitated out of solution by saturating the solution with ammonium sulphate.

Globulins—Globulins are water insoluble but soluble in dilute neutral salt solution. They are also heat coagulable. They are precipitated from solution by ammonium sulphate. Serum globulins, fibrinogens and muscle myosin are examples of globulins.

Protamines—These are small molecules and are soluble in water, dilute acids, alkalies and non coagulable by heat. Their isoelectric pH is around 7.4 and they exist as basic proteins in the body. They combine with nucleic acid to form nucleoproteins Salmine, Sardimine and Cyprimine of fish sperms and testis are examples of protamines.

Histones—These are rich in arginine and histidine. They are soluble in water, dilute acids and salt solutions but insoluble in ammonia. They do not readily coagulate on heating. The protein part of haemoglobin, globin is a typical protein having predominance of histidine and lysine instead of arginine. Nucleoproteins and globin of haemoglobin are histones.

Gliadins (Prolamines)—These are alcohol soluble plant proteins and are insoluble in water or salt solutions and absolute alcohol. They are rich in proline. The examples are gliadins of wheat and hordein of barley.

Glutelins—These are plant proteins insoluble in water or neutral salt solutions but soluble in dilute acids or alkalies. They are rich in glutamic acid. They are large molecules and can be coagulated by heat. The examples are oryzenin of rice and glutelin of wheat.

Q. What are Conjugated proteins? How they are classified further.

Ans. Conjugated proteins are simple proteins combined with a non-protein group called prosthetic group. Protein part is called **apoprotein** and the entire molecule is called **holoprotein.**

Nucleoproteins

The nucleoproteins are compounds made up of simple basic proteins such as protamine or histone with nucleic acid prosthetic group. They are proteins of cell nuclei and chief constituents of chromatin. Deoxyribonucleo proteins contain DNA as prosthetic group and are found in nuclei, mitochondria and chloroplasts. Ribonucleoproteins occur in nuclei and ribosome granules. They have RNA as prosthetic group. The examples are nucleohistone and nucleo prolamine.

Glycoproteins

The glycoproteins have the carbohydrate moiety as prosthetic group. The polypeptide chain is attached to one or more heterosaccharide units by covalent bonds. About 600-700 units are attached to the peptide chain, one per 6.4 amino acid residue. The glycoproteins are secreted by the submaxiallary glands of various animals.

Mucoproteins

Mucoproteins are simple proteins combined with mucopolysaccharide such as hyaluronic acid and the chondroitin sulphate. Water soluble mucoproteins have been obtained from serum, egg white and human urine. These water soluble mucoproteins are not easily denatured by heat or readily precipitated

by picric acid or trichloroacetic acid. These are important constituents of the ground substance of connective tissue. They are present as tendomucoid, osseomucoid and chondro proteins in tendons, bones and cartilage respectively.

Chromoproteins

These are the proteins that contain coloured substance as prosthetic group.

Haemoglobins—All haemoproteins are chromoproteins which carry heme as the prosthetic group which is red coloured pigment found in hemoglobin, cytochromes, catalase, peroxidase.

Other proteins—Flavoprotein is cellular oxidation reduction protein which has riboflavin as its prosthetic group.

Visual purple—It is a protein in retina in which the prosthetic group is a carotenoid pigment.

Phospho protein—These are the proteins with phosphoric acid as inorganic phosphate. But these are not the phosphate containing substances as nucleic acids and phospholipids. Casein and ovovitellin are the two important groups of phosphoproteins found in milk and egg yolk respectively. They contain about 1% of phosphorus. They are sparingly soluble in water and very dilute acid is cold but readily soluble in dilute alkali.

Lipoproteins

These are the proteins, which have lipids as their prosthetic groups. These lipids are lecithin, cephalin, fatty acids etc. Phospholipid protein complex is also called as lecithoprotein. They are found in milk, blood cell nuclei, egg yolk cell membrane etc.

Metalloproteins

They contain an metal ion as their prosthetic group. Ferritin contains Fe, carbonic anhydrase contain Zn as their prosthetic groups.

Q. What are derived proteins?

Ans. This class of proteins includes those products formed from the simple and conjugated proteins. It is not a well-defined class of proteins. These are produced by various physical and chemical factors and derived into two major groups.

Primary derived proteins

These are the denatured or coagulated proteins. Their molecular weight is same as that of native proteins, but they differ in solubility, precipitation and crystallization. The heat, X-ray vigorous shaking, acid, alkali cause denaturation of proteins and they form primary derived proteins. These are synonymous with denatured proteins.

Proteans—These are insoluble products formed by the action of water, very dilute acids and enzymes. These are predominantly formed from certain globulins and differ from globulins in being insoluble in dilute salt solution. For example myosan is derived myosin, elestan from elastin and fibrin from fibrinogen.

Metaproteins—They are formed from further action of acid and alkalies on proteins. They are generally soluble in dilute acids and alkalies but insoluble in neutral solvents.

Coagulated proteins—The coagulated proteins are insoluble products formed by the action of heat or alcohol on native proteins as cooked meat, cooked egg.

Secondary derived proteins

These are the proteins, which are formed from the hydrolysis of protiens at their peptide linkages. They are roughly called as proteoses, peptones and peptides. They differ in their molecular size.

Proteoses—These are the hydrolytic products of proteins, which are soluble in water and are coagulated by heat. They can be precipitated by ammonium sulphate.

Peptones—These are the hydrolytic product of proteoses. They are soluble in water but are not coagulated by heat and not precipitated by ammonium sulphate.

Peptides—These are composed of only a small number of amino acids joined by peptide bonds. These are water-soluble and are not coagulated by heat and are not salted out of solution. but they can be precipitated by phosphotungstic acid. These are named according to the number of amino acids present in them. Dipeptides are made up of two amino acids, tripeptides are made of three amino acids and polypeptides are made up of more than three amino acids. The number of amino acids depends on the molecular weight of native protein molecule.

Q. Discuss the Structural Organization of Proteins.

Ans. The proteins have four structural organizations.

Primary structure

This structural level is in the linear sequence in which the amino acids are held together by peptide bonds in the peptide chain. The peptide bonds forms the backbone and side chains of amino acid residues project outside the backbone chain. The free NH_2 group of the terminal amino acid is called as N terminal and the free COOH end is called as C terminal end. The numbering of amino acids stars from the N terminal end.

Secondary structure

In the secondary structure the peptide chain assumes a three dimensional structure by folding or coiling, zigzag linear or mixed forms. The linkages or bonds involved in the secondary structure formation are hydrogen bonds and disulphide bonds.

The secondary structures are of two types

(*a*) **α helix**—A peptide chain when forms regular helical coils is called as α helix. These coils are stabilized by hydrogen bonds. The α helixes can be either right handed or left handed. But only right handed α helix has been found in protein structure. Each amino acid residue advances by 0.15 nm along the helix and 3.6 amino acid residue are present in complete turn. The distance between two equivalent points on turn is 0.54 nm and is called as pitch.

(*b*) **β - pleated sheet structure**—This structure is formed when hydrogen bonds are between carbonyl oxygens are amide hydrogens of two or more adjacent extended polypeptide chains.

Thus the hydrogen bonding in β - pleated structure is inter chain. The structure is not absolutely planar but is slightly pleated due to angles of bonds. The adjacent chains in β - pleated sheet structure are either parallel or anti-parallel depending on whether the amino to carbonyl peptide linkage of the chains runs in the same or opposite direction. In both parallel and anti-parallel β - pleated sheet structures, the side chains are on opposite sides of the sheet.

Tertiary structure

Tertiary structure of a protein is its three dimensional folding of its arrangement i.e. polypeptide chain with the secondary structure which may be further folded forming many sizes. Such structural conformation is called as tertiary structure. It is only such conformations, which are biologically active and protein in this state is called as native protein. The tertiary structure is constituted by steric relationship between the amino acids located far apart but brought closer by folding.

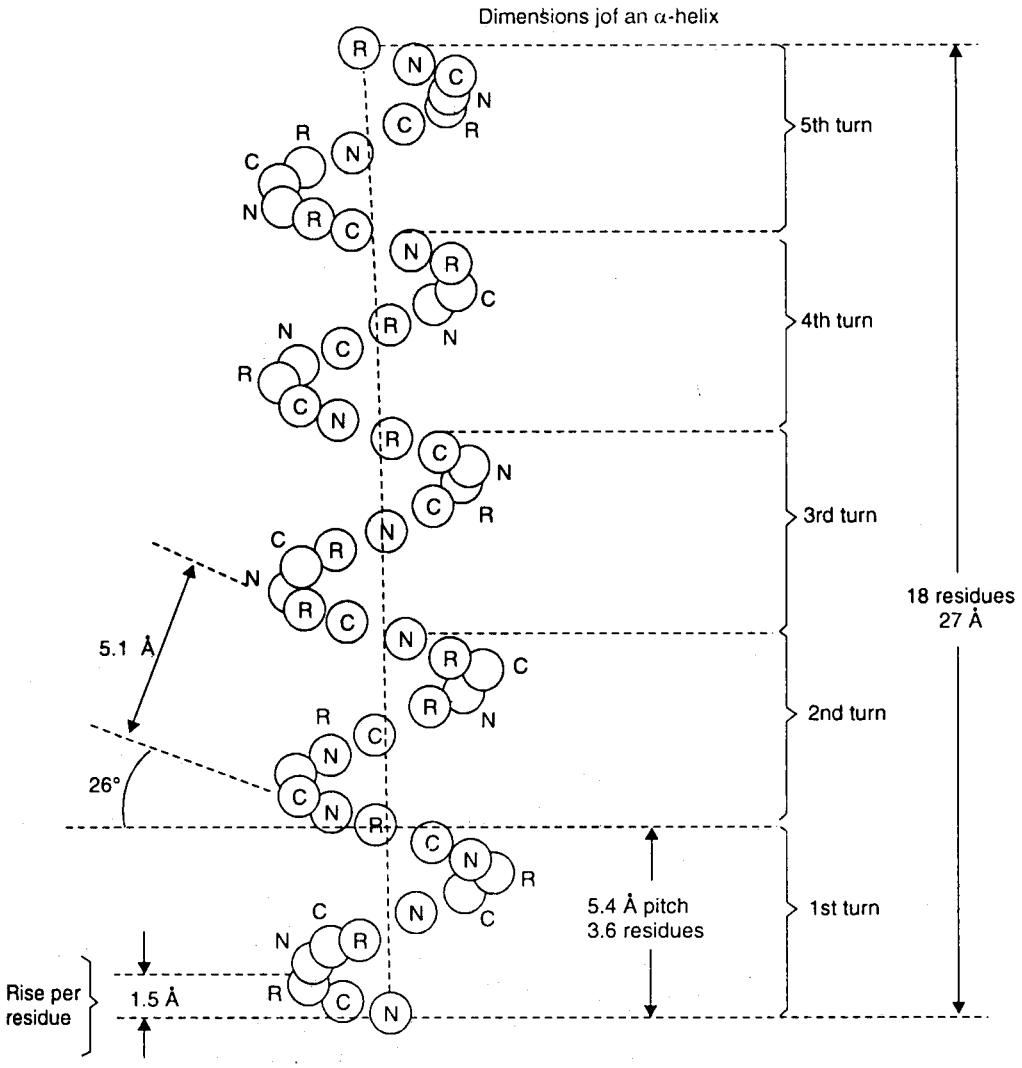


Fig. 3.1. α helix model of protein coiling showing intra chain bonding.

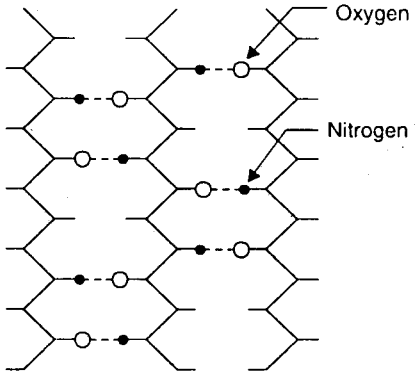


Fig. 3.2. β-Pleated sheet.

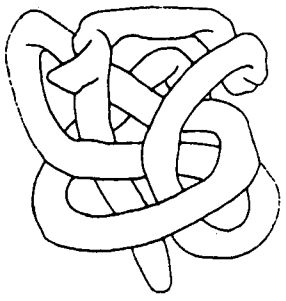


Fig. 3.3. Tertiary Structure of protein.

Quaternary structure

Proteins are composed of more than one polypeptide chain. These polypeptide subunits associate in a geometrically specific manner. The special arrangement of these subunits is known as a proteins quaternary structure.

A multisubunit protein may consist of identical or non-identical polypeptide chains for example haemoglobin has the subunit composition $\alpha_2 \beta_2$. The proteins with identical subunits are referred to as **oligomers** and these identical subunits as **protomers.** A protomer may therefore consist of one, polypeptide chain or several unlike polypeptide chains. In this sense, hemoglobin is a dimer (oligomer of two protomers) of α β-protomer.

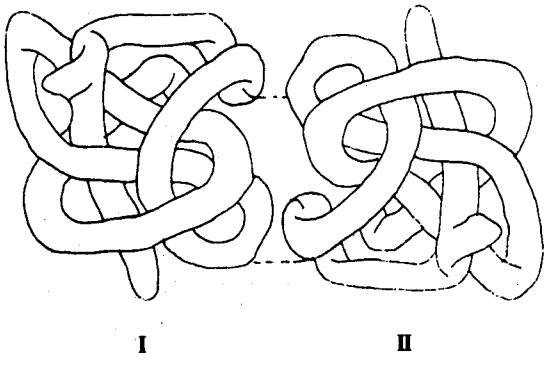


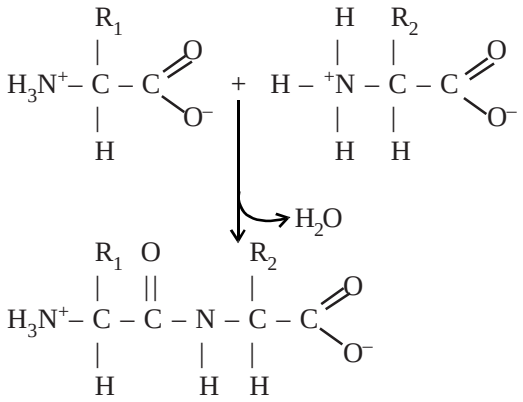
Fig. 3.4 (I and II). A tetramer of protein units illustrating the quarternary structure.

Q. Mention the bonds responsible for the formation of protein molecules.

Ans. The proteins are made up of different types of bonds. The primary structure of proteins is made up of peptide bond, but the other structures are formed by other types of bonds. A brief account of these bonds are given as follows—

Peptide bonds

The α amino acids polymerize, at least conceptually through the elimination of a water molecule.



The resulting CO-NH linkage is known as a peptide bond. Polymers are composed of two, three, a few and many amino acids are known as dipeptides, tripeptides, oligopeptides and polypeptides. These substances however are often referred to simply as peptides. These polypeptides range in length from 40 to over 4000 amino acid residues and since the average mass of an amino acid residue is ~1100, have molecular masses that range from ~4 to over 440 KD.

Hydrogen bonds

These are weak, low energy, non covalent bonds sharing a single hydrogen by two electronegative atoms i.e. O and N. The H bonding in secondary structure occur regularly. The internal hydrogen bonding groups of a protein are arranged such that nearly all-possible hydrogen bonds are formed. This regularity allows the protein to assume a helical configuration or sheeted structure.

Disulphide bonds

These are formed between two cysteine residues. They are strong, high energy, covalent bonds.

Hydrophobic interactions

These interactions occur between non-polar side chains of amino acids as leucine, alanine and isoleucine. They constitute the major stabilizing forces for tertiary structure forming a compact three-dimensional structure.

Ionic or Electrostatic interactions

These are formed between oppositely charged polar side chains of amino acids such as acidic and basic amino acids.

Van der waal forces

These occur between non-polar side chains.

Q. Discuss few general properties of Proteins.

Ans. Taste—Proteins are tasteless. But their hydrolytic products are bitter in taste.

Odour—They are odourless. On heating till they obtain dryness they turn brown and give off odour of burning feather.

Molecular weight—The proteins are generally of large molecular weight. Proteins in the solution generally dissociate into components of low molecular weight which may be re-associated on restoring the original conditions. This suggests that proteins are built up by aggregation of definite units.

Heat coagulation—Proteins coagulate forming an insoluble coagulum. Coagulation is maximum at the isoelectric pH of protein. During coagulation protein undergoes a change called as denaturation. Denatured proteins are soluble in extremes of pH and their maximum precipitation occurs at their isoelectric pH.

Hydration—The polar groups of proteins becomes hydrated in presence of water and swell up when electrolytes, alcohols or sugars are added to protein solutions. There is competition of water and the degree of hydration of protein is decreased. They dehydrate protein and precipitate it from solution.

Viscosity—The viscosity of protein varies widely with the kind of protein and its concentration in the solution. The viscosity is closely related to the molecular shape. The fibrous proteins (long molecules) are more viscous than the globular proteins.

Q. Write few important Colour Reaction of Proteins.

Ans. Proteins produce colour in certain reactions. These reactions are due to characteristic groups of particular amino acids present in them.

Biuret Reaction—When the proteins are heated in presence of very dilute copper sulphate in alkaline medium, purple – voilet colour is obtained. The colour depends upon the presence of 2 or more peptide linkages. Biuret test is due to coordination of cupric ions with the unshared electron pairs of peptide nitrogen and the oxygen of water to form a coloured coordination complex.

Xanthoproteic Reaction—The aromatic amino acids such as tyrosine, tryptophan and phenylalanine present in proteins give yellow precipitate when heated with conc. HNO_3. On addition of alkali, the precipitate turns orange due to nitration of the aromatic ring. Collagen and gelatin do not give a positive reaction.

Millon's Test—This is specific for tyrosine present in protein. Protein gives a white precipitate with Millon's reagent (10% mercurous chloride in H_2SO_4) on heating. On addition of sodium nitrate the precipitate turns pink red.

Sakaguchi Test—This is specific test for arginine of the protein. This reagent is alcholic α naphthol and a drop of sodium hypobromite. Guanidine group of arginine ($HN=C-NH_2$) gives red colour with the reagent.

Nitroprusside Reaction—Proteins with free –SH group of cysteine give reddish colour with sodium nitroprusside in ammonical solution. Many proteins give this test positive after heat coagulation or denaturation indicating liberation of free –SH groups.

Ninhydrin Reaction—Ninhydrin is a powerful oxidizing agent and causes oxidative decarboxylation of α amino acid producing an aldehyde with one carbon less than the parent amino acid. The reduced ninhydrin hydrindatin then reacts with amino acids which has been liberated and the molecule of ninhydrin forms a blue coloured compound. A molecule of CO_2 is evolved indicating the presence of α amino acid.

Hopkins Cole Reaction—Proteins containing tryptophan gives this test positive. The reagent contain glyoxylic acid so it is also called as glyoxylic acid reaction. Gelatin, collagen do not contain tryptophan and hence do not give test as positive.

Lead Acetate Test—This test is specific for sulphur containing amino acid. The protein containing S-containing amino acids is boiled with strong alkali to split out sulphur as sodium sulphide, which reacts with lead acetate to give black precipitate of lead sulphate.

Q. Write a note on biological significance of proteins.

Ans. The importance of proteins as biologically important molecule depends upon their chemical and physical structure and location inside the cell, various proteins perform various functions.

Structural proteins are generally inert to biochemical reactions. They maintain the natural form and position of the organs; the cell wall and primary fibrous constituent of the cell have structural proteins. Collagen is the most abundant known protein in animals, forming a major part of the skin, cartilage, ligament, tendon and bone. The scales of fish and reptiles and hairs, feathers, horns hoof and claws are made up of the protein keratin. Capacity of motion and flexibility in the organisms is the virtue of certain proteins with great tensile strength. There are contractile proteins. Catalytic proteins are the most active proteins of an organism. The properties of living cells depend upon the biological processes, which are regulated by enzymes. The major portion of an enzyme is made up of proteins. These proteins are mostly spherical in shape and are termed globular proteins. Some enzymes are simple proteins containing only amino acids, others are complex proteins. Enzymes catalyze a variety of reactions in the living cells. Certain proteins, especially in the animals are involved in the transport of many essential biological factors to different parts of the organisms. Haemoglobin transporting O_2 from one part of the body to the other is an example of a carrier protein.

4

CARBOHYDRATES

Q. Define Carbohydrates.

Ans. The name carbohydrates indicates that they are hydrates of carbon and contain carbon, hydrogen and oxygen. Carbohydrates or saccharides are essential components of all living organisms and are, infact, the most abundant class of all biological molecules. The name carbohydrates, which literally means "carbon hydrate" stems from their chemical composition which is roughly $(CH_2O)_n$ where $n \geq 3$. These also are defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols or compounds which yield these derivative on hydrolysis.

Q. What are Monosaccharides.

Ans. Monosaccharides consist of single polyhydroxy aldehyde or ketone unit which cannot be broken down into simpler substances. Their general formula is $C_nH_{2n}O_n$. They are also called as simple sugars. They are again divided into 2 groups depending upon whether aldehyde (–CHO) or ketone (–CO) group is present.

Table 4.1 Classification of Monosaccharides

General formula	*Aldo sugars*	*Keto sugars*
Trioses ($C_3H_6O_3$)	Glyceraldehyde	Dihydroxy acetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentose ($C_5H_{10}O_5$)	Ribose	Ribulose
Hexoses ($C_6H_{12}O_6$)	Glucose	Fructose

Q. What are Aldoses and Ketoses?

Ans.

Aldose—The monosaccharides containing the aldehydic group as the functional groups are called as aldoses. They are classified according to the number of carbon atoms present. Aldoses containing three to seven carbon atoms are called as Trioses, Tetroses, Pentoses, Hexoses and Heptoses respectively.

Ketoses—These are the monosaccharides, which contain ketone group as the functional group. These are also classified according to the number of carbon atoms in them as trioses, tetroses, hexoses and heptoses.

Q. Write a note about the isomerism and its types existing in the carbohydrate molecule.

Ans. The term isomer was first used by J.J.Berzelius to different compounds with same molecular formula. The isomerism is of two type, Structural isomerism and Stereoisomerism.

Structural isomerism

The structural isomers have similar molecular formulae, but different structural formulae e.g. D-glucose and D-fructose have same molecular formula but differ in their structural formulae.

The structural isomers are again of three types

Chain isomers: They have different arrangement of carbon atoms generally in the form of a chain or link.

Positional isomers: A substituent group in two compounds is at different positions, but their chain is the same.

Functional isomers: Isomers in which the compounds have different functional groups e.g. compounds having formula (C_2H_6O) may be ethyl alchol ($CH_3–CH_2OH$) or a dimethyl ether ($CH_3–O–CH_3$), and D-glucose and D-fructose.

Stereoisomerism

Stereoisomers have the same structural and molecular formula ,but differ in spatial arrangement of atoms or groups in the molecule. The arrangement of the groups in different patterns always takes place around the asymmetric carbon atoms i.e. carbon atoms in which their 4 valancies are completely satisfied by different kinds of atoms, e.g. D-glucose and D-mannose have change in spatial arrangement of hydrogen and hydroxyl groups.

In the L- forms the –OH group of the same carbon is shown on the left. The L-form of glucose and mannose would be;

The structural formulae of L-glucose and L-mannose would be the mirror image of D-glucose and D-mannose respectively. This is also known as optical isomerism which is a kind of stereoisomerism.

Ring structure

The molecules in sugars may exist in two different ring forms. The two forms differ from each other in their stability and reactivity. The two ring forms are-

Pyranose ring

It is more stable than furanose ring. Here C-1 and C-5 are linked by an oxygen atom, thus form a large sized stable ring. The ring structure is exhibited by pentose and hexose sugars which are generally written as closed hexagons or pentagons.

Furanose ring

It is less stable. Here C-1 and C-4 are linked by an oxygen atom, thus forming a small sized less stable ring. The ring structure is exhibited by pentoses and hexose sugars. They may be written as closed carbon rings or as chain carbon rings.

Each of the hexoses exists in the α and β forms depending upon the position of the –H and –OH groups to C-1 which is also asymmetric in the pyranose and furanose rings. In the β form the –OH group is attached to C-1 on the left side, while in the α form towards the right side.

Q. Write Classification of Monosaccharides.

Ans. The monosaccharides are simple type of sugars and they cannot be broken down further. Structurally they are made up of either aldoses or ketoses. But they are further classified on the basis of their carbon skeleton. The classification is as follows-

Trioses—These are D – Glyceraldehyde and dihydroxy acetone, they both occur in the form of phosphate esters and are called as Glyceraldehyde 3 phosphate and Dihydroxy acetone phosphate. They are the intermediates of glycolysis cycle in the body. They are also the precursor of glycerol, which the organism synthesize and incorporates into the various types of lipids.

Tetroses—The commonly occuring tetrose is erythrose 4 phosphate which occurs as an intermediate in hexosemonophoshate shunt (HMP shunt) which is also an alternative pathway for oxidation of glucose.

Pentose—D Ribose is a constituent of nucleic acid RNA and is also constituent of certain coenzymes e.g. FAD, NAD and Coenzyme A. L xylulose is a metabolite of D glucuronic acid and is excreted in urine of humans affected with a hereditary abnormality in metabolism called as **pentosuria.**

Hexoses—The most commonly occuring hexoses are D-Galactose, D-Fructose, D- Mannose, D-Galactose. D-Glucose is the chief physiological sugar present in normal blood. All tissues utilize glucose for energy. Erythrocytes and brain cells are solely dependent on glucose for energy purposes. The glucose occurs as a constituent of disaccharide and polysaccharides.

D-Fructose—It is a ketohexose and commonly called as fruit sugar as it occurs free in fruits. It occurs as a constituent of sucrose and inulin. It is laevorotatory and hence is also called as **laevulose.**

D-Galactose—It is seldomyl found free in nature. It occurs both in plants and animals. It occurs as a constituent of milk sugar lactose and also occur in tissues as a constituent of galactolipid and glycoproteins. It is an epimer of glucose and differs in orientation of H and OH at 4^{th} carbon. It is formed in body from glucose by epimerization by enzyme epimerase in liver.

D-Mannose—It does not occur free in nature but is widely distributed in combination as the polysaccharide mannan e.g. in ivory nut. In body it is found as a constituent of glycoproteins.

Heptoses—Sedoheptulose is a ketoheptose found in plants. Its phosphate form is an important intermediate in the HMP shunt and has been identified as a product of photosynthesis.

Q. What are Disaccharides? Briefly give an account of their classifcation.

Ans. The disaccharides are composed of two monosaccharide units joined by a glycosidic linkage. The physiologically important disaccharides are maltose, lactose and sucrose. Their general formula is $C_nH_{2n}O_{n-1}$ and they are hydrolyzed by hot acids or corresponding enzymes as

$$\underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + H_2O \longrightarrow \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Glucose}}{C_6H_{12}O_6}$$

On hydrolysis maltose gives 2 glucose units, lactose gives glucose and galactose and sucrose give glucose and fructose. When both aldoses and ketoses are involved in the linkage the disaccharide sugar will not exhibit reducing properties and will not be able to form osazones. For example sucrose is made up of glucose (aldose) and fructose (ketose), and is unable to form osazone, so sucrose is also called as **invert sugar.**

Maltose

Maltose is an intermediate in acid hydrolysis of starch and also can be obtained by enzyme hydrolysis of starch. This is not found free in the body. Since it has one free aldehyde it shows reducing properties and forms characteristic osazones which has characteristic appearance of sunflower. It can form α and β forms and exhibit mutarotaion. On hydrolysis it gives two molecules of glucose.

Lactose

Lactose is also called as milk sugar as it is found largely in milk. At body temperature it shows an equilibrium of α and β forms in 2:3 ratio. Neither it is very soluble nor so sweet. The lactase enzyme present in intestinal juice is able to hydrolyze it into glucose and galactose. Because it contains galactose as one of its units it is able to produce mucic acid, when treated with conc. HNO_3 after hydrolysis. It also shows the reducing properties and can form osazones. Lactosazone crystals have typical hedge hog shape or powder puff appearance.

Sucrose

This is also called as table sugar, sugarcane sugar or invert sugar. It does not exist in the body but occurs in cane sugar, pineapple, carrot roots, honey and sweet potato. It is hydrolysed to glucose and fructose by enzyme invertase (sucrase) in the alimentary canal. The products of hydrolysis are absorbed. It has no free aldehyde or keto group because the linkage between the aldehyde group of glucose and keto group of fructose. Hence it is a non reducing sugar. It does not exhibit mutarotation and cannot exist in α or β forms. It cannot form osazone with phenyl hydrazine. The specific rotation of sucrose is +66.5° (dextrorotatory), but its hydrolytic products are laevorotatory because fructose has a greater specific laevo rotation than the dextrorotation of glucose. As the hydrolytic products invert the rotation, it is called as **invert sugar** and the process is called as **inversion.** Honey is largely invert sugar and due to presence of fructose honey is more sweeter.

Q. What are Oligosaccharides?

Ans. Oligosaccharides yield 3 to 6 monosaccharide units on hydrolysis. These carbohydrates are attached to either the side chain oxygen atom of serine or threonine residues by O-glycosidic linkages or to the side chain nitrogen of asparagine residues by N-glycosidic linkages. The N-linked oligosaccharides contain a "common penta saccharide core" consisting of three mannose and two N acetyl glycosamine residues. Additional sugars are attached to this common core in many different ways to form the great variety of oligosaccharide patterns found in glycoproteins.

The oligosaccharides are closely associated with the integral membrane proteins, many secreted proteins such as antibodies and coagulation factors. The diversity and complexity of the oligosaccharide units of glycoprotein suggests that they are rich in information and are functionally important. Carbohydrates participates in molecular targeting and cell-cell recognition. Many newly synthesized glycoproteins such as Immunoglobulins and peptide hormones contain oligosaccharide carbohydrate units with terminal sialic acid residues.

Q. What are Polysaccharides? How they are classified?

Ans. Polysaccharides are more complex substances. These are polymers of single monosaccharide and are termed as Homopolysaccharides or Heteropolysaccharides.The homopolysaccharides contain sme monosaccharide units while heteropolysaccharides may contain same or some other groups i.e.other than carbohydrates such as hexuronic acid . Most of the polysaccharides are white amorphous compounds of which starch is a typical example. They are not sweet and when pure do not reduce or give other characteristic aldose or ketose reactions.

Homopolysaccharides

Starch. It is the store carbohydrate of chlorophyll containing plants. In plants starch is laid down in the cells in granules. The microscopic form of the granules is characteristic or the source of starch. Starch granules under microscope appears to be particles made up of concentric layers of materials.

They differ in shape, size and markings according to the source. Starchy foods are mainstay of our diet. Large amounts are present in cereals, such as wheat, rye, rice, corn and barley, in potatoes in legumes and in nuts.

The composition of starch granule consists of two polymeric units of glucose called **Amylose** and **Amylopectin.** They differ in molecular architecture and in certain properties.Starch granules are insoluble in cold water but when their suspension is heated, water is taken up and swelling occurs, at first to a slight degree but later to a large extent and starch gels or pastes are formed.

Glycogen

Glycogen is the reserve carbohydrate of the animal hence it is called as animal starch. In higher animals, it is deposited in the liver and muscle as storage material which are readily available as immediate source of energy. It is also found in plants which have no chlorophyll system e.g. fungi and yeast but not in green plants. The molecular weight of glycogen obtained from different sources may range from 10^5 to 10^8 and each molecule contains from 5,000 to 10,000 glucose molecules. Glycogen have a complex structure of highly branched chains. It is a polymer of D glucose units and resembles amylopectin. Glucose unit in main stem are joined by α 1$\rightarrow$4 glucosidic linkages and branching occurs at a branch points by α 1$\rightarrow$6 glucosidic linkage. A branch point occurs for every 12 to 18 glucose units.

Heteropolysaccharides

These are usually composed of amino sugar and uronic acid units as the principal components although some are chiefly made up of amino sugars and monosaccharide units without the presence of uronic acids. They are essential components of tissues, where they are present either in free form or in combination with proteins. There is variation in their carbohydrate content. When the carbohydrate content is greater than 4%, they are called as **Mucoproteins** and when it is less than 4%, they are called as **Glycoproteins.**

Q. Write a note on Biological significance of carbohydrates.

Ans. The carbohydrates are of great importance to plants as well as to animals and human beings.Carbohydrates are the structural materials of plants for example, cellulose is found in plant fibres and in wood.They are widespread and act as a reserve materials in tubers, grains and roots. Sucrose is present in the nectar of flowers, in roots and in fruits. Glucose, fructose and simple sugars are also found in small amounts in plants as reserve food materials.The carbohydrates such as starches and sugars are the main food for human beings.They are easily digestible and are easily oxidized to provide energy for various physiological proceses. These are present in cereals.The carbohydrate derivatives such as glucosides, form important drugs and other medicines for various diseases.The carbohydrates, particularly cellulose and its derivatives are used in the production of artificial silk, paper, plastics, cinema films and explosives.All animal tissues, blood, milk and tissue fluids contain carbohydrates and their derivatives as important constituents, e.g. blood contains glucose as sugar.Muscles and other tissues remove glucose from blood and form glycogen which provides energy on oxidation. Many tissues are formed by combinations of sugars or sugar derivatives and proteins.

5

LIPIDS

Q. What are lipids?

Ans. The lipids are heterogeneous group of compounds related to the fatty acids and are insoluble in water but soluble in solvents such as ether, chloroform and benzene. The lipids occur widely in plant and animal kingdom. The lipids include fats, oils, waxes and related compounds. Oils are liquids at 20°C but fats are solid at 20°C. The fats and oils used almost universally as stored form of energy in living organisms are highly reduced compounds which are derivatives of fatty acids.

Q. What are Simple Lipids?

Ans. Simple lipids are the esters of fatty acids with various alcohols. If the alcohol is glycerol, then they are called as **fats** or **neutral fats.** If the fat is liquid at ordinary temperature it is called oil. But if their is alcohol of high molecular weight instead of glycerol than they are called as **waxes.**

Q. What are fats?

Ans. Fats are the esters of fatty acids with glycerol.

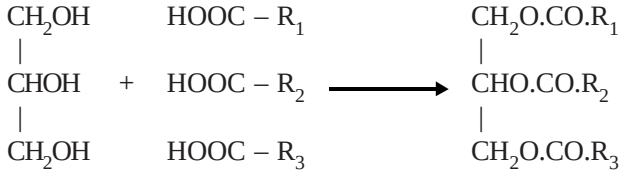


Fig. 5.1. Fat molecule (triglyceride).

The chemical structure of fat consists of three different molecules of fatty acids with one molecule of glycerol. The three different fatty acids (R_1, R_2, R_3) are esterified with the three hydroxyl groups of glycerol because the polar hydroxyls of glycerols and the polar carboxylate of the fatty acids are bound in ester linkages, triacyl glycerols are non-polar, hydrophobic molecules insoluble in water.

Q. List different properties of fats.

Ans. The different physical and chemical properties of fats are as follows—

Physical properties

Fats are insoluble in water but readily soluble in ether, chloroform, benzene, carbon tetrachloride. They are themselves good solvents for other fats, fatty acids etc. and are tasteless, odourless, colourless and neutral in reaction.They spread uniformly over the surface of water, so their spreading

effect is to lower surface tension. Their melting points are low.

Chemical properties

Hydrolysis—Hydrolysis of triacylglycerol takes place by lipases producing fatty acids and glycerols. Phospholipases attack the ester linkage of phospholipids.

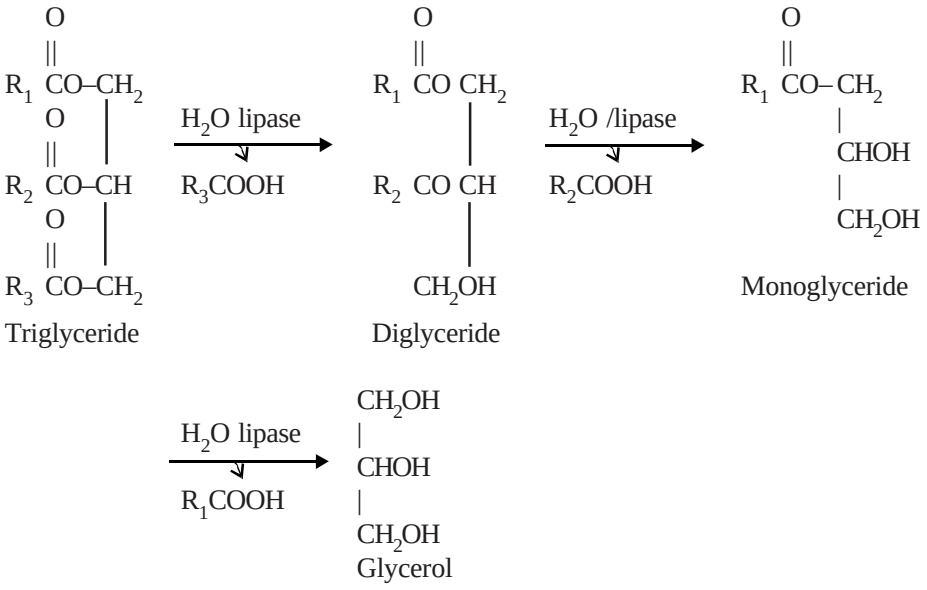


Fig. 5.2. Hydrolysis of fat.

Saponification—Saponification is the hydrolysis of a fat by alkali and the products formed are glycerol and the alkali salts of fatty acids which are called as **soaps.**

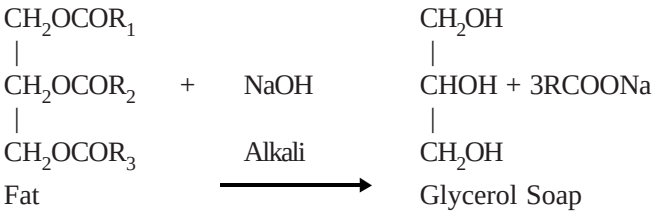


Fig. 5.3. Saponification of fat.

The number of milligrams of KOH required to saponify 1 gram of fat or oil is called as its **saponification number.**

Acid number—The number of milligrams of KOH required to neutralize the free fatty acids of 1 gram of fat.

Iodine number—It is the amount in grams of iodine absorbed by 100 grams of fat. Thus it is the measure of the degree of unsaturation of a fat.

Acetyl number—The number of milligram of KOH required to neutralize the acetic acid obtained by saponification of gram of fat after it had been acetylated. This is a measure of the number of hydroxy acid groups in the fat.

Halogenation—The halogen atoms i.e. chlorine, bromine and iodine may be added to the double bonds of unsaturated fatty acids containing fat.

Rancidity—Nearly all-natural fats are oxidized when exposed to air, light and moisture. They develop an unpleasant odour and taste. This happens so due to the formation of peroxides at the double bonds of unsaturated fatty acids. Vitamin E is an important natural antioxidant.

Hydrogenation—Unsaturated plant fats are converted into more saturated and solid fats by catalytic hydrogenation. This is usually done over finely divided nickel. In the production of margarine and vegetable shortening, this property is exploited commercially.

$$\text{Vegetable oil} \xrightarrow[\text{Ni powder/Pressure}]{H_2 \text{ gas}} \text{Solid fat}$$

$$\begin{array}{l} CH_2O.CO.C_{17}H_{33} \\ | \\ CHO.CO.C_{17}H_{33} \\ | \\ CH_2O.CO.C_{17}H_{33} \\ \text{Triolein (oil)} \end{array} \quad + 6H \longrightarrow \quad \begin{array}{l} CH_2O.CO.C_{17}H_{35} \\ | \\ CHO.CO.C_{17}H_{35} \\ | \\ CH_2O.CO.C_{17}H_{35} \\ \text{Tristaerin (fat)} \end{array}$$

Q. Define Waxes.

Ans. Waxes are the esters of fatty acids with higher alcohols and are formed as secretions, which are mostly protective in functions in many animals. They resemble the fat and are usually solids. In the human body the commonest waxes are esters of cholesterol. Their constituent acids and alcohols have usually 24 to 36 carbon atoms.

Q. What are compound lipids? Mention brief classification of compound lipids.

Ans. Compound lipids are the esters of fatty acids containing groups, other than and in addition to an alcohol and fatty acids. They are further subdivided into

A. Phospholipids
B. Glycolipids
C. Sulpholipids
D. Lipoproteins
E. Aminolipids (Proteolipids)

Phospholipids

These are the compound lipids, which are the esters of fatty acids with glycerol containing an esterified phosphoric acid and a nitrogen base. They are present in large amounts in nerve tissues, brain, liver, kidney, pancreas and heart. They form the structures of membranes, matrix of cell wall, myelin sheath, microsomes and mitochondria. They also act as carriers of inorganic ions across the membranes and helps in blood clotting. They act as prosthetic group to certain enzymes and increases the rate of fatty acid oxidation.

Glycolipids

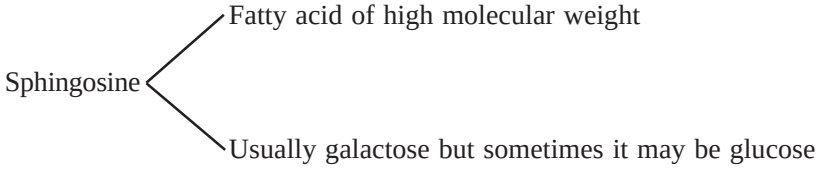
These contain an amino alcohol (sphingosine or isosphingosine) attached with an amide linkage to a fatty acid glycosidically to a carbohydrate moiety (sugars, amino sugar, sialic acid). They are

further classified into

1. Cerebroside
2. Ganglioside

1. *Cerebrosides*

Cerebrosides occur in large amounts in the white matter of brain and myelin sheath of nerve. A cerebroside is considered to be built on the following lines.



There is no glycerol, no phosphoric acid and no nitrogenous base. Thus a cerebroside on hydrolysis yields

a. A sugar usually galactose.
b. A high molecular weight fatty acid.
c. The alcohol, sphingosine or dihydrosphingosine.

Thus they contain nitrogen although there is no nitrogenous base.

Individual cerebrosides are differentiated by the kinds of fatty acids in the molecule. Four types of cerebrosides have been isolated and their fatty acids have been identified.

(a) Kerasin—It contains lignoceric acid $C_{23}H_{47}COOH$ as fatty acid.
(b) Cerebron or Phrenosin—It contains hydroxy lignoceric acid and also called as cerebronic acid.
(c) Nervon—It contains an unsaturated homologue of lignoceric acid called nervonic acid $C_{23}H_{45}COOH$.
(d) Oxynervon—It contains hydroxy derivative of nervonic acid.

2. *Gangliosides*

Gangliosides have been isolated from ganglion cells, neuronal bodies and dendrites, spleen and RBC stroma. The highest concentrations are found in grey matter of brain. Although the exact structures of gangliosides are not definitely established but they contain ceramide (sphingosine fatty acids) glucose or galactose, N-acetyl galactosamine and sialic acid. Some gangliosides also contains dihydrosphingosine or gangliosine in place of sphingosine. The gangliosides can serve as specific membrane binding sites (receptor sites) for circulating hormones and thereby influences various biochemical processes in the cell.

Sulpholipids

These lipids are characterized by possessing sulphate groups. In general they appear to be sulfate esters of glycolipids, the sulphate group is esterified with OH group of hexose moiety of the molecule.

Lipoproteins

These are the lipid molecules conjugated with the protein molecules. They contain triacylglycerol (45%) phospholipids (35%) cholesterol and cholesteryl esters (15%) free fatty acids (less than 5%) and also proteins in combination. The density of lipoproteins increases as the protein content rises and the lipid content falls and the size of the particle becomes smaller. Lipoproteins may be separated on

the basis of their electrophretic properties and may be identified more accurately by means of immunoelectrophoresis. Four major groups of lipoproteins have been identified which are important physiologically and in chemical diagnosis in some metabolic disorders of fat metabolism. These are

(*a*) Chylomicrons
(*b*) Very low density lipoproteins (VLDL)
(*c*) Low density lipoproteins (LDL)
(*d*) High density lipoproteins (HDL)
(*e*) Chylomicrons and VLDL

The predominant lipid is triacyl glycerol (50%) and cholesterol (23%). The concentrations of these are increased in artherosclerosis and coronary thrombosis etc. The protein moiety in lipoprotein is known as apoprotein, which constitues nearly 60% of some HDL and 1% of chylomicrons. Many lipoproteins contain more than one type of apoprotein polypeptide. The larger lipoproteins such as chylomicrons and VLDL consist of lipid core of non-polar triacylglycerol and cholesteryl ester surrounded by more polar phospholipid, cholesterol and apoproteins.

The lipoproteins help in transfer of lipids to tissues. They also maintain the structural integrity of cell surface and subcellular particles like mitochondria and microsomes.

Q. Write a note on derived lipids.

Ans. Derived lipids are the derivatives of simple and compound lipids, which still possess the general characteristics of lipids. There are further classified into

(a) Fatty acids
(b) Steroids and sterols

(a) Fatty acids

The fatty acids are carboxylic acids with hydrocarbon chain of 4 – 36 carbons. In some fatty acids, this chain is fully saturated i.e. contains no double bond, and unbranched, others contain one or more double bonds. A few contains 3 carbon rings or hydroxyl groups.

The physical properties of the fatty acids, and of compounds that contain them are largely determined by the length and degree of unsaturation of the hydrocarbon chain. The nonpolar hydrocarbon chain accounts for the poor solubility of fatty acids in water. The longer the fatty acyl chain and the fewer the double bonds, the lower the solubility in water. The carboxylic acid group is polar (and ionized at neutral pH), and accounts for the slight solubility of short chain fatty acids in water.

There are 2 types of fatty acids.

Saturated fatty acids

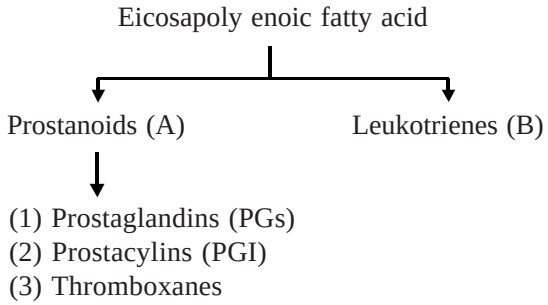
They contain no double bond and have general formula $C_nH_{2n+1}COOH$. Saturated fatty acids having 10 carbon or less number of carbon atoms are called as lower fatty acids for e.g. acetic acid, butyric acid. Saturated fatty acids having more than 10 carbon atoms are called as higher fatty acids presents some saturated fatty acids occurring widely in nature.

Unsaturated fatty acids

These are the fatty acids which posses one or more than one double bonds in them. The common examples of unsaturated fatty acids are palmitoleic acid, oleic acid and Eicosanoids

Q. What are Eicosanoids?

Ans. Eicosanoids are derived from eicosapoly enoic fatty acid.



Prostanoids

These include prostacyclins (PGI) and thrombxanes (TX). These prostanoids virtually exists in all mammalian tissues and acts as local hormones. They have important physiological and pharmacological activites.

Prostaglandins

The prostaglandins are relative derivatives of hypothetical molecule C_{20} (Prostanoic acid). All prostaglandin are 20C fatty acid containing a cyclopentane ring on parent saturated acid called prostanoic acid and have OH group at 15 position and trans double bond at 13 position.

Prostacyclins (PGI)

They are formed in vascular endothelium and continuously formed in heart and kidney. They are formed from cyclic endoperoxide PGH_2 by the reaction of micosomal prostacyclin synthetase. They decrease blood pressure and protect coronary arteries. They increase renal blood flow and stimulate rennin production.

Thromboxanes

They contract smooth muscles of blood vessels, GI tract, uterus, bronchioles.

Q. What are Leukotrienes?

Ans. The Leukotrienes are the second group of eicosanoid derivatives formed via lipoxygenase pathway rather than cyclization of the fatty acid chain. They are characteized by the presence of three conjugated double bond. They are stimulators of mucus secretion and are responsible for vasoconstriction of bronchial muscles. The most common example of leukotrine is prostaglandin E_2 (PGE_2).

Q. Define Essential Fatty Acids.

Ans. The essential fatty acids are the polyunsaturated fatty acids, which are not synthesized in the body but are taken from the natural sources. They are essential for the growth and health. The linoleic acid, linolenic acid and arachidonic acid are the essential fatty acids. The essential fatty acids of vegetable oils have low melting points and iodine number. They become saturated fatty acids on hydrogenation and thus oils become solid fats. These along with the lipids constitute the structural elements of the tissues. The deficiency of these acids in the diet causes eczema in babies. They retard artheroclerosis. They also affect the prolongation of clotting time and increase the fibrinolytic activity.

Q. What are steroids and sterols?

Ans. The steroids are often associated with fat. All of the steroids have a similar cyclic nucleus

which resembles phenanthrene to which cyclopentane ring is attached. It is referred to as cyclopentano perhydrophenathrene nucleus.

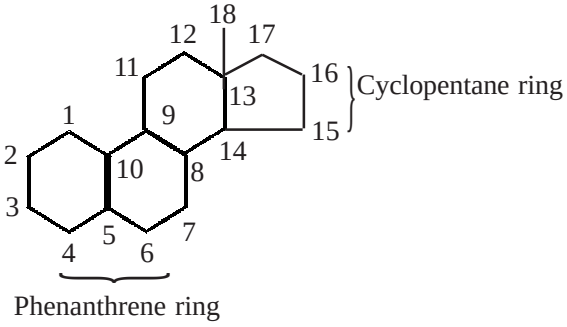
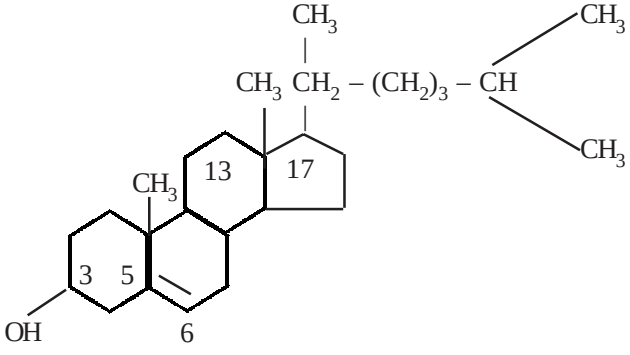


Fig. 5.4. Cyclopentanoperhydrophenathrene nucleus.

A side chain at position 17 is usual. If the compound has one or more OH groups and no carbonyl or carboxy groups it is called as **sterol.** Most important sterol in human body is cholesterol.

Q. Write in brief the structure, properties and functions of Cholesterol.

Ans. The cholesterol is the most important sterol in the body. Its molecular formula in $C_{27}H_{45}OH$. It possesses the cyclopentanoperhydrophenathrene nucleus with OH group at C_3



Cholesterol

It has an unsaturated double bond between C_5 and C_6 with two methyl groups at C_{10} and C_{13}. It also has 8-carbon side chain attached to C_{17}. Cholesterol occur both in free form and in ester form in which it is esterified with fatty acids at –OH group at C_3 position. The ester form of cholesterol is also referred as bound form while free cholesterol is equally distributed between plasma and red blood cells but the latter do not contain esters.

Q. Write a note on the biological significance of lipids.

Ans. The lipids have several important biological functions.

1. Lipids serve, as the reservoir of energy because of their high-energy content (9 kcal/gm).
2. Lipids form the structural components of cell membranes.

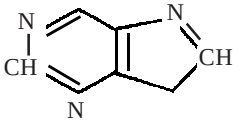
3. Lipids forms the protective coating on the surface of many organs such as kidney. They facilitate absorption of the fat soluble vitamin i.e. A, D and K.
4. Lipoproteins and glycolipids are essential for maintaining cellular integrity.They produce metabolites through oxidation in the tissues, which are used in the interconversion of substance.

6

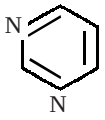
NUCLEIC ACIDS

Q. What are the bases in the nucleic acid?

Ans. The bases in the nucleic acid are of two kinds, purines and pyrimdine



Purine

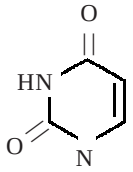


Pyrimidine

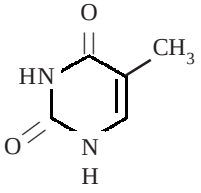
Pyrimidine Bases

There are three main pyrimidine bases. These are

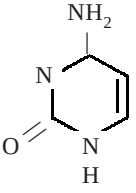
(*a*) **Uracil**—It is 2, 4 dioxypyrimidine.



(*b*) **Thymine**—It is 2, 4 dioxy 5 methyl pyrimidine.



(*c*) **Cytosine**—It is 2 dioxy 4 amino pyrimidine.

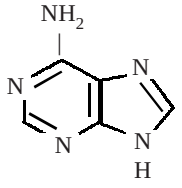


The oxypyridine and oxypurines exist in enol and keto form, enol form is called lactim and keto form is called lactum form. Lactum form is predominant in purines and pyrimidines.

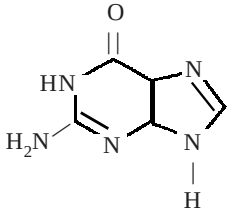
Purine Bases

The purine bases are of two types

(*a*) **Adenine—**It is 6 amino purine

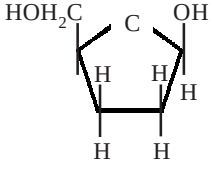


(*b*) **Guanine**—It is 2 amino 6 oxypurine



Q. What are the sugars present in the nucleic acids?

Ans. The sugars present in the nucleic acids are the pentose sugars. The pentose sugars present are D-ribose and D-2 deoxyribose. Both sugars occur as furanose form in the nucleotides.



D–ribose

Q. Differentiate between Nucleosides and Nucleotides.

Ans. A nucleoside is composed of a purine or a pyrimidine base and a ribose or a deoxyribose sugar.

Nucleoside = Base + Sugar

The purine bases are attached at N-9 position to sugar moiety whereas pyrimidine bases are attached at N – 1 position to sugar moiety by glycosidic linkage. Nucleotides are phosphorylated nucleosides, and are represented by base sugar phosphate unit.

Thus

Nucleotides = Base + Sugar + Phosphoric acid

Adenosine monophosphate (AMP) = Adenine + Ribose + Phosphate

Thymidylic acid (TMP) = Thymine + 2 Deoxyribose + Phosphate

Uridylic acid (UMP) = Uracil + Ribose + Phosphate

Cytidylic acid (CMP) = Cytosine + Ribose + Phosphate

Guanylic acid (GMP) = Guanine + 2 Deoxyribose + Phosphate

Q. Write in detail the structure of DNA.

Ans. DNA is a double stranded molecule held together by hydrogen bonds between the purine and pyrimidine bases. It consist of two polynucleotide strands that wind about a common axis with a right handed twist to form an ~20A° diameter double helix. The two sugar phosphate back bones wind around the outside of the bases like the banisters of a spiral staircase and are exposed to the aqueous solution. The phosphodiester bonds in the two interwoven strands run in opposite directions. Thus, DNA is a polymer of deoxyribonucleotides and is found in chromosomes, mitochondria and chloroplasts. DNA is present in every nucleated cell and carries the genetic information. The two strands are antiparallel (run in opposite directions). One strand runs in the 5' to 3' direction and the other in the 3' to 5' direction. The information resides in the sequence of nucleotides on one strand. The opposite strand is considered as antisense i.e. the complement of the sense strand.

The planes of the bases are nearly perpendicular to the helix axis. Each base is hydrogen bonded to a base on the opposite strand to form a planar base pair. It is these hydrogen bonding interactions,

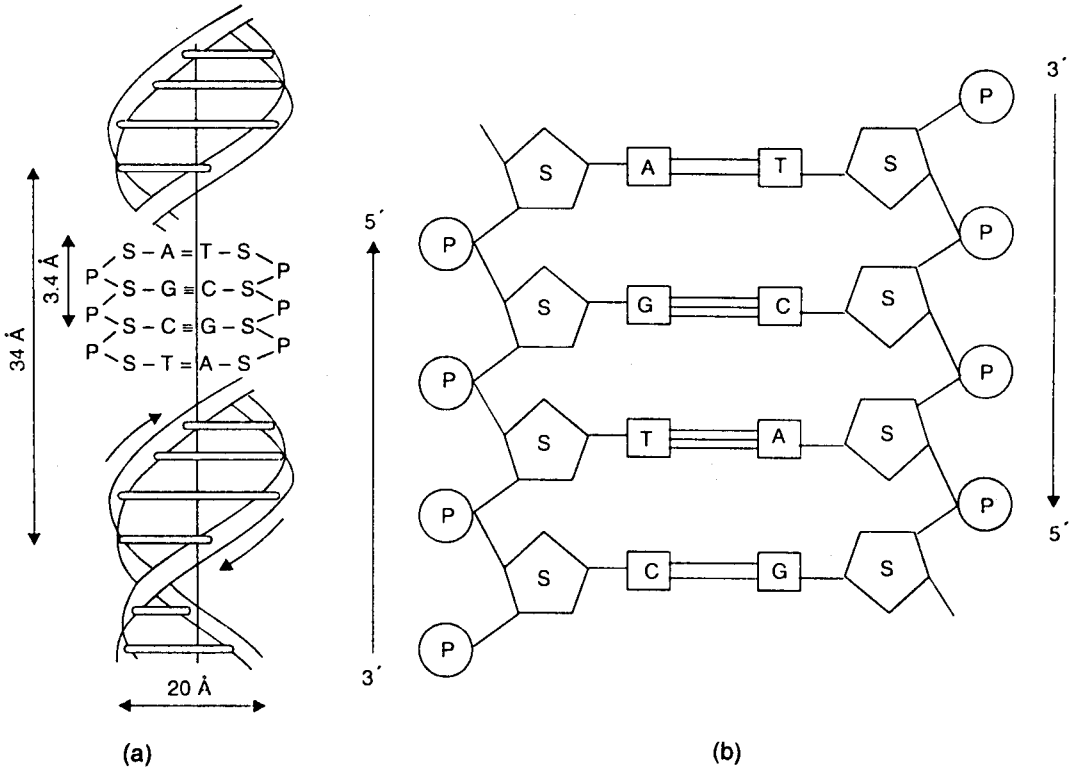


Fig. 6.1. (a) Diagrammatic representation of DNA molecule as proposed by Watson & Crick. (b) Base pairing and binding of nucleotides in DNA.

a phenomenon known as complementary base pairing, that results in the specific association of the two chains of the double helix.

Adenine base of one strand of DNA is hydrogen bonded to a thymine in the opposite strand. While the guanine is hydrogen bonded to a cytosine.

The ratio of purine to pyrimidine bases in the DNA molecule is always around 1 (i.e. G+A/T+C ≈ 1). This relationship is known as **Chargaff's rule.** The purine and pyrimidine bases carry genetic information whereas sugar and phosphate groups perform a structural role.

Q. What is B DNA?

Ans. The B DNA is the normal DNA found normally in every cell. It is a double helix in which the adjacent nucleotides in each chain are rotated by 34.6°C relative to each other. Double helix completes one turn approximately every 10.4 base pairs. One turn of the double helix spans a distance of 3.4 nM. This distance is the pitch of the helix. Each base pairs therefore increases the length of the double helix by 0.33nM, when B DNA crystals are dried or when salt content of the crystal is lowered, the long thin. B DNA molecule becomes short, stubby molecule and is called as A DNA.

Q. What is Z DNA?

Ans. Alexander Rich and his coworkers elucidated the occurrence of Z DNA in 1979; although the existence of such a DNA molecule was demonstrated earlier also, especially in the tumor cells. In a given DNA molecule a part may be in the configuration of B-DNA type while other part may be of Z DNA type. Z DNA formation has been detected in plasmids, simian virus 40, protozoan and in many other organisms. In general, prokaryotic DNA seems to form Z DNA configuration more readily than the eukaryotic DNA. This is possibly because prokaryotic DNA have abundant C - G sequences which favour Z - DNA formation.

Q. Differentiate between B DNA and Z DNA.

Ans. Some important configurational differences between B and Z DNA are given below

(1) B – DNA has right handed coiling while Z DNA has left handed coiling of the helix.
(2) In stereoscopic view, Z – DNA is seen possessing only one groove, while B DNA has two grooves a minor and a major groove. The single groove of Z DNA corresponds to the minor groove of B DNA.
(3) B – DNA has 10.5 base pairs per helical turn with a pitch of 34A° and diameter of 20A°. The Z DNA has 12 base pairs per helical turn with a pitch of 44.6A° and a diameter of 18A°.
(4) In B – DNA, all the nucleotides have anti conformation. In Z DNA containing C – G sequences, the deoxy cytidines have anti configuration and deoxy guanosine have syn configuration.

The biological role of Z DNA is not clearly understood. However it is believed that such DNA segments are involved in transcription enhancer in Simian virus 40. The involvement in chromatin structure as or binding sites for certain carcinogens is also suggested.

Q. Write a note on Replication of DNA.

Ans. DNA is semi conservatively replicated and it directs its own replication. Each polynucleotide strand acts as a template for the formation of its complementary strand through base pairing in reactions. The two strands of the parent molecule must therefore be separated, so that a complementary daughter strand may be enzymatically synthesized on the surface of each parent

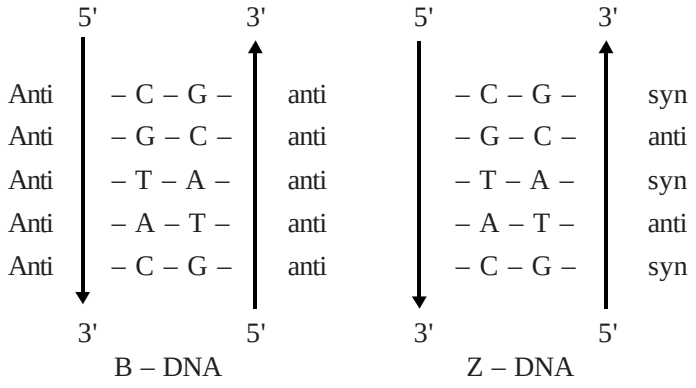


Fig. 6.2. Conformation of nucleotides in B and Z DNA.

strand. This results in two molecules of duplex (double stranded) DNA, each consisting of one polypeptide strand from the parent molecule and a newly synthesized complementary strand . Such a mode of replication is termed as **semiconservative replication.**

DNA replication is a complex process. The salient features of replication are summarized as:—

The nucleases (of which endonucleases break internal bonds while exonucleases clip off terminal nucleotide) produce a break in one strand of the DNA molecule to relieve the twist or torque of the helix. DNA replication then process from the given point either in one direction (unidirectional) or both directions (bi-directional). In this way either one or two forks are produced. In the case of bidirectional replicating fork, which is the most common the movement of two forks away from each other generates an eye or bubble. The latter may be formed at one or at several points on the replicating DNA. Replication at the level of short fragments is initiated by production of a short segment of RNA to serve as a primer for DNA polymerase. This RNA region in later excised by nuclease action or DNA polymerase I and the gap thus created is filled with DNA. The RNA primer is initiated by an enzyme RNA polymerase. This forms an essential step for the start of DNA synthesis. Certain proteins, known as unwinding proteins, are involved in converting a duplex DNA to single strands. On the other hand histone proteins stablize DNA in the duplex state.

In general therefore, the proteins required during replication of DNA are RNA polymerase, DNA polymerase III, unwinding protein, DNA polymerase I and ligase.

Q. What are single stranded and circular DNA?

Ans. Although most of the organisms have regular double helical DNA, some bacteriophages and animal viruses contain single stranded DNA. Further DNA of most phages is not linear but is in the form of a circle. The DNA of $\Phi \times 174$ phage and of polyoma virus, which causes cancer in monkeys, are circular. DNA from the mitochondria of higher organisms is also circular.

The invitro origin of circular DNA in phage has been studied. DNA isolated from some viruses is infact linear. When it is heated the two strands of DNA separate and it is denatured. Controlled recooling brings the two strands together and some normal stranded DNA is obtained. In this recooling some portions of DNA may assume circular form also. Probably because the sticky ends of DNA are exposed and the two ends join to form a circle. DNA in by observing under electron microscope, it has been established that about 20% of the renatured polyoma virus assumes circular shape during renaturation

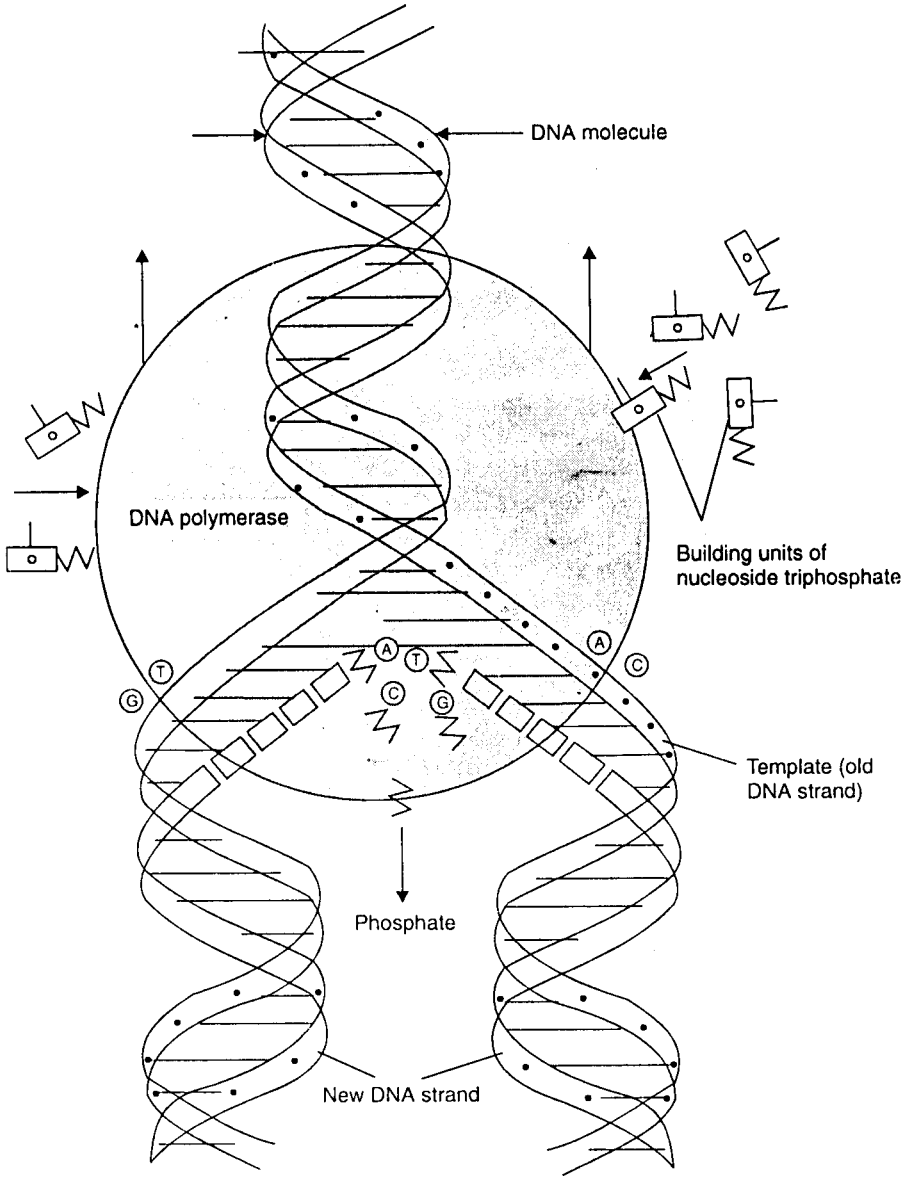


Fig. 6.3. Semiconservative replication of DNA.

Q. What are RNA?

Ans. The RNA is a polymer of purine and pyrimidine ribonucleotides linked together by 3', 5' phosphodiester bridges. The sugar moiety is ribose. RNA contains uracil instead of thymine. As RNA is a single stranded molecule, its guanine content does not necessarily equals to its cytosine content and its adenine content does not necessarily equal its uracil content.

Q. Discuss the Structural organization of RNA.

Ans. There are 3 main classes of RNA molecule, which exists, in all prokaryotic and eukaryotic organisms. They are

(a) Messenger RNA (mRNA)
(b) Transfer RNA (tRNA)
(c) Ribosomal RNA (rRNA)

(a) The messenger RNA (mRNA)

It is single stranded and complimentary to the sense strand of DNA. mRNA is synthesized in the nucleus during transcription in which sequence of bases in one strand of DNA are transcribed in the form of a single strand of mRNA. The sequence of bases in mRNA strand is complimentary to that of DNA.

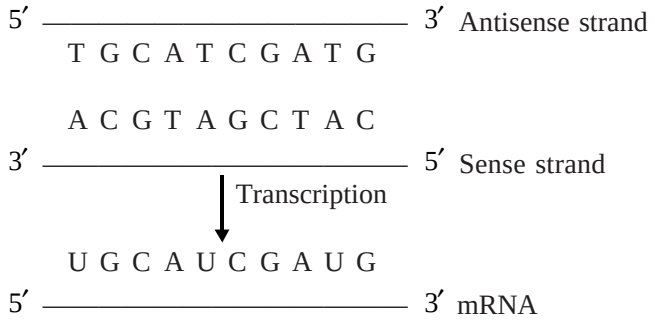


Fig. 6.4. Relationship between the sequence of an RNA transcription.

After transcription mRNA passes into cytoplasm and then on to ribosomes where it serves as template for the sequence of amino acids during biosynthesis of proteins.

(b) The transfer RNA (tRNA) or soluble RNA (sRNA)

The tRNA accounts 10 to 20 percent of total cellular RNA molecules. It consists of about 75 nucleotides and has molecualr weight of 25,000. There are at least 20 tRNA molecules in every cell, one corresponds to each of the 20 amino acids required for protein synthesis. It has a clover leaf like structure and possess a specific triplet nucleotide known as anticodon which is complimentary to the 3 bases on the mRNA called **codon.**

(c) Ribosomal RNA (rRNA)

It comprises 50 to 80 percent of total cellular RNA. Ribosomes carry ribosomal RNA. It has a homogenous base composition on the ribosomes, the mRNA and the tRNA molecules interact to translate into a specific protein molecule, the information transcribed from the gene. The ribosomal particles are very complex. The mammalian ribosome contains 2 major nucleoprotein subunits - a larger one (60S) and a smaller one (40S).

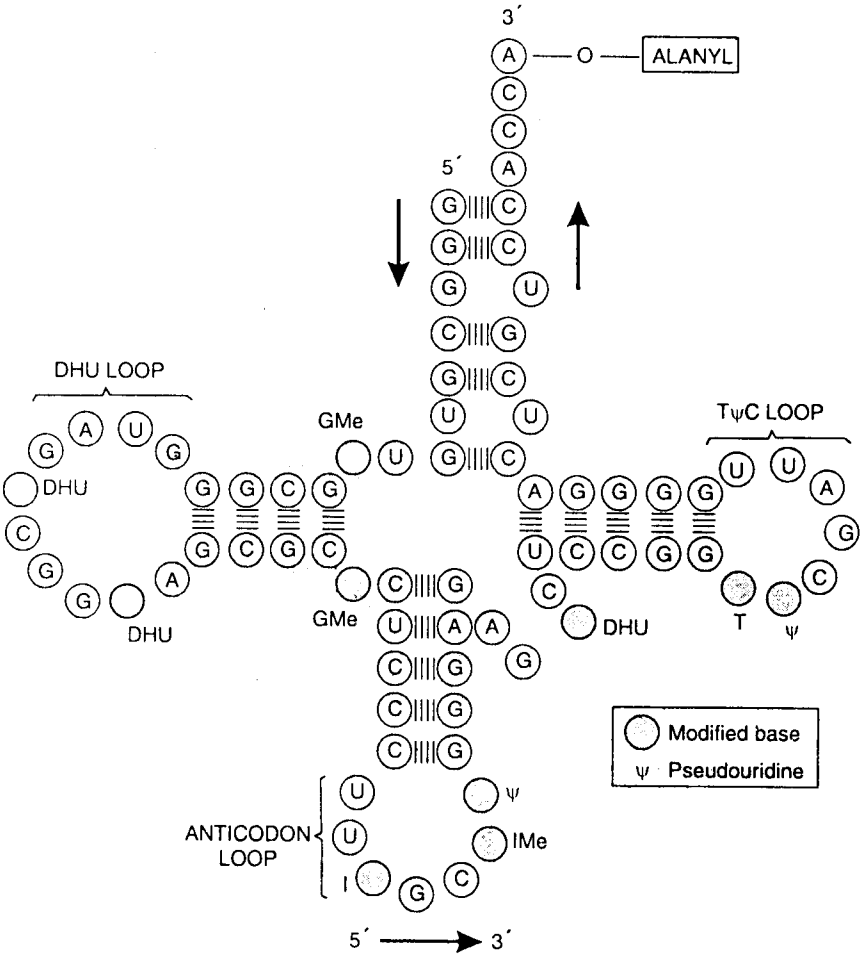


Fig. 6.5. Structure of tRNA.

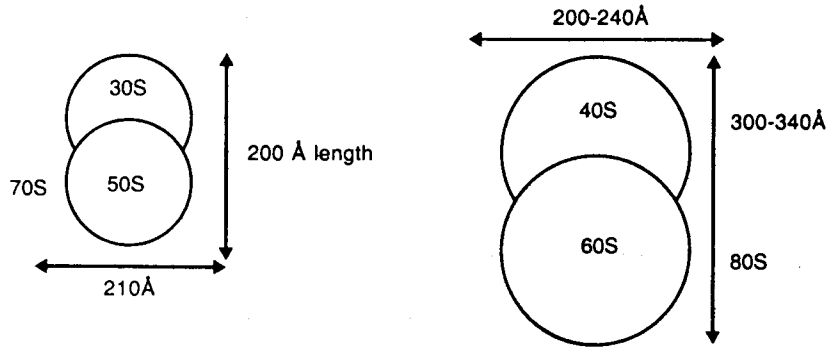


Fig. 6.6. rRNA (70S and 80S).

Q. Highlight the important differences between DNA and RNA.

DNA	*RNA*
1. DNA is present in nucleus, probably in the chromosomes.	1. RNA is present in cytoplasm and nucleolus.
2. They carry genetic information from one generation to another and can also undergo mutation.	2. They carry no genetic information and do not undergo mutation.
3. They contain 1,600 to 9000 nucleotides. DNA molecule is long and thread like having a length of about 250 times greater that their breadth. Their structure is highly complex.	3. They contain 60 to 6,000 nucleotides. The molecules are unbranched. Their structure is less rigid.
4. DNA is double helical structure having 2 strands.	4. RNA exists as single stranded molecule.
5. DNA is concerned with protein synthesis.	5. RNA is also concerned with protein synthesis.
6. DNA is formed by 4 types of monomeric units i.e. adenylate, guanylate, cytidylate and thymidylate.	6. RNA is formed by 4 types of monomeric units i.e. adenylate, guanylate, cytidylate and uracidylate.
7. The sugar moiety in DNA is deoxyribose.	7. The sugar moiety in RNA is D-ribose.

Q. List some of the important properties of Nucleic acid.

Ans. The important properties of the nucleic acids are—

Molecular weight and sedimentation behavior

Molecular weight of nucleic acids depends upon the source and the method of isolation. The molecular weight of DNA ranges from a few million in viruses to several billion in eukaryotes. Native DNA are in fact so large that it is often difficult to isolate them in intact form, since they are easily broken by mechanical shear forces. RNA are also variable in their sizes and molecular weight. Sedimentation coefficient value is often used to denote the size and molecular weight of biological macromolecules. The relationship between sedimentation coefficient and molecular weight can be expressed by the following equation-

$$MW = \frac{SRT}{D\left(1 - V_\rho\right)}$$ where S is the sedimentation coefficient.

R is gas constant
T is absolute temperature
D is diffusion coefficient
V is the specific partial volume of the nucleic acid and
ρ is the density of solvent

The expression D, V and ρ relate to the properties of nucleic acid in a solution, which are used to determine its sedimentation coefficient. The sedimentation coefficient can be determined by centrifuging the nucleic acid sample at a very high speed (in a ultra centrifuge) in a $CsCl_2$ gradient. The nucleic acids are separated in the $CsCl_2$ gradient according to their sedimentation coefficients.

$$\text{Sedimentation coefficient or } S = \frac{V}{\omega^2 X}$$

Where,

V is the sedimentation velocity $\frac{dx}{dt}$

ω is the angular velocity (sec^{-1})
X is the distance between boundary to the center of the rotor.

Sedimentation coefficient is often expressed as **Swedberg unit** (S, capital s). The relationship between sedimentation coefficient and Swedberg unit is as follows

Swedberg unit (S) = 1013 × s

Equilibrium sedimentation in $CsCl_2$ can also be used for determining the buoyant density of nucleic acids. When a concentrated solution (8.0 M) of $CsCl_2$ is centrifuged at high speed, it forms a linear gradient in the centrifuge tube. The density of top layer is about 1.55 g cm^{-3} while the bottom layer has a density of 1.80 g cm^{-3}. The density of the nucleic acid can be determined by knowing its layering in a $CsCl_2$ gradient.

Viscosity

Because of the rigidity of the double helix and immense length of DNA in relation to its small diameter, even very dilute solutions are viscous. Further, when DNA samples are dissolved in water, they sweep away large amount of water with them, because of enormous size. For this reason DNA shows ideal behavior as a solute only in very dilute solutions.

Denaturation and Renaturation

The structure of DNA is stabilized by hydrogen bonds. Heat, pH, alcohols, ketones, urea, amides and similar solutes can break these bonds. This rupturing of hydrogen bonds and irregular arrangement of polynucleotide chains is called denaturation. If heated denatured DNA is cooled slowly, the original structure is often restored. This is called renaturation.

The denaturation of DNA involves a transition from a double helix to a random coil conformation. During this transition the absorption of UV light (at 260 nm) by the DNA sample increases. This increase is because of unstacking of bases in native duplex DNA, and the process is called **hyperchronism.** If we plot a graph between the absorbance of UV light at 260 nm and temperature by DNA sample, a sigmoid curve is obtained.

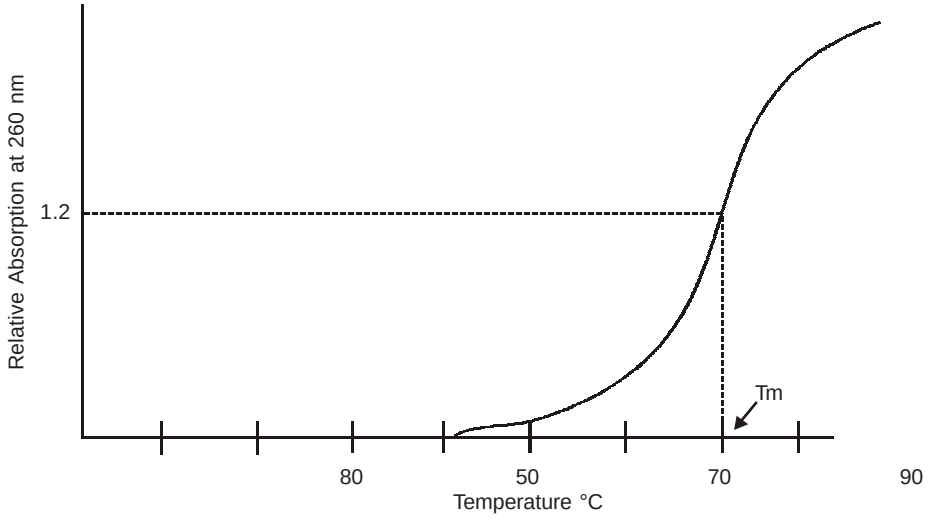


Fig. 6.7. Typical melting curve of DNA

The temperature at which the DNA is half denatured is called the **melting temperature (Tm).** Tm differs from the species to species and according to the G + C or A + T content of the sample. The guanine – cytosine (G – C) base pair having three hydrogen bond is more stable than adenine – thymine (A – T) base pair, which has only two hydrogen bonds. Thus DNA samples with a higher G – C content have a higher melting point. The careful estimate of Tm of DNA sample can give an estimate of base composition of the nucleic acids.

Denaturation of DNA can be viewed directly in an electron microscope. A first A–T rich region forms a loop or bubble in DNA molecule because they dissociate at lower temperatures.

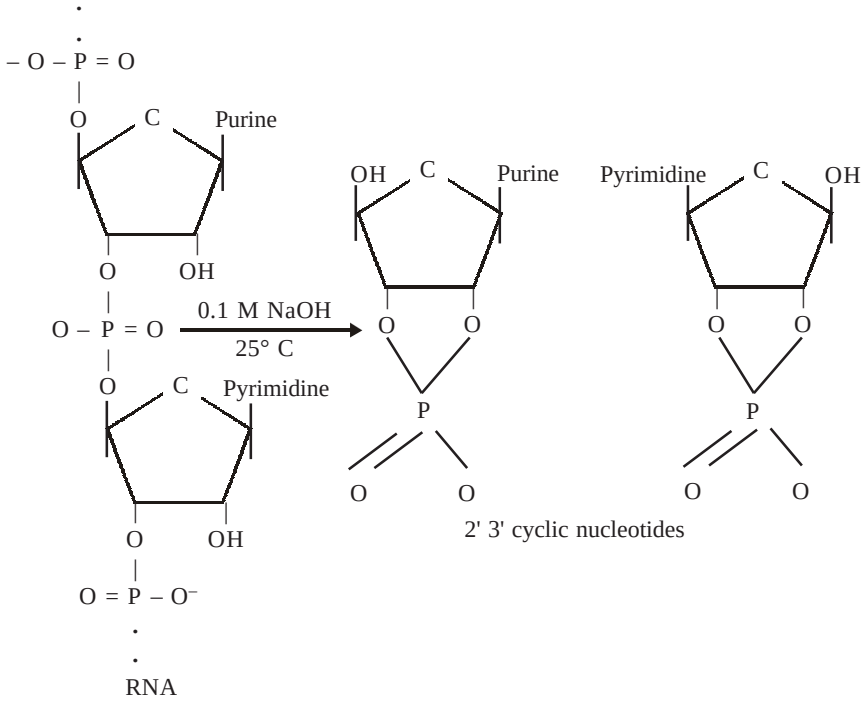
Acid Base properties

The nucleotides in nucleic acids are joined together through phosphate bonds. These bonds have a low pka (dissociation constant) and ionize at any pH above 4.0 to produce phosphoric acid. Thus nucleic acid solutions are acidic. Basic properties are also exhibited by nucleic acids in solutions because of free purine and pyrimidine bases.

Hydrolysis of nucleic acids

There are several enzymes, which can hydrolyze nucleic acids into nucleotides, nucleosides and phosphates. During assimilation of nucleosides they are further hydrolyzed into bases and sugars. Acids and bases can bring about hydrolysis of nucleic acids also. Mild acid hydrolysis of DNA at pH 3.0 causes hydrolytic removal of purine bases without affecting pyrimidine nucleotides. The resulting DNA sample, which is devoid of purine bases is called apurinic acid. RNA does not give **apurinic acid** under these conditions. Selective removal of the pyrimidine bases accomplished by some different chemical means produces apyrimidinic acid.

DNA is not hydrolyzed by dilute alkali, whereas RNA undergoes hydrolysis, because it contains a $2'$ OH group in the sugar moiety. Dilute NaOH produces $2' - 3'$ nucleotides from RNA.



Further degradation of $2' - 3'$ cyclic nucleotides by bases produces a mixture of 2' and 3' nucleotides.

Q. Write a note on biological significance of nucleic acids.

Ans. DNA is found in all biological systems, with an exception of certain viruses. In higher organism, it confined to the nucleus, chloroplasts and mitochondria. DNA is genetically active component of chromosomes and functions as basic carrier of genetic information. Many of the enzymes present in mitochondria and chloroplasts are synthesized under the direction of DNA present in those organelles. In some plant viruses RNA performs the genetic function. DNA also works as a template for the synthesis of various types of RNA's present in the cell. RNA's in turn perform other functions like structural organization of ribosomes, transfer of amino acids from cytoplasm to the ribosomes and carrying the message for protein synthesis. Besides this, many nucleotides and bases are important metabolites. Vitamin B (thiamine) is a pyrimidine derivative. In recent years, certain pyrimidine derivatives have been found to be of important biological applications. Alloxan (2, 4, 5, 6 tetra-oxypyrimidine) produces experimental diabetes in animals. Further tRNA from yeasts, bacteria, mammals and higher plants, when degraded by enzymes or acids, yield active cytokinins.

7

VITAMINS

Q. What are vitamins?

Ans. In addition to water, oxygen, proteins, fats, carbohydrates and inorganic salts a number of organic compounds are also necessary for proper growth and maintenance of life. These organic compounds are also known as **accessory dietary factors** or **vitamins** and are only required in very small amounts.

Definition

Vitamins are "accessory food factors", which are organic in nature and necessary for the normal functioning of the tissues i.e. growth, maintenance and reproduction. They differ from other organic food stuffs as they do not enter into tissue structure, do not undergoes degradation for providing energy like carbohydrates and lipids. They also differ from hormones in not being produced within the organism and most of them have to be provided in the diet.

Q. Write in brief the classification of vitamins.

Ans. All vitamins are broadly classified into two groups according to their solubility.

(A) Fat soluble vitamins.

(B) Water-soluble vitamins.

Fat soluble vitamins—These are soluble in fats and are generally stored in liver. Example—Vitamin A, D, E and K.

Water soluble vitamins—These are soluble in water and are easily absorbed. They are not stored in the body except B_{12}. Example: Vitamin C, B-complex (B_1, B_2, B_6, Niacin, Pantothenic acid, Biotin, Lipoic acid, Folic acid, B_{12}, Inositol).

Q. Give an account of Vitamin A.

Ans. Vitamin A, or retinal is growth promoting, anti-infective and antixerophthalemic vitamin.

Occurrence

Plant sources: All pigmented particularly yellow vegetables and fruits e.g. sweet potatoes, papayas, tomatoes, apricot and leaf green vegetables supply provitamin A (carotene) in the diet. Cereals also contain carotene.

Animal sources: Preformed vitamin A (retinal) is supplied by foods of animal origin, they are liver, milk, eggs, butter, fat of muscle meats and fish liver oil.

Daily requirements

Adults	5000 I.U
During pregnancy and lactation	6000 - 8000 I.U
Children	2000 - 3000 I.U
Infants	1500 I.U

1 I.U = 0.3 g of retinol, 0.6 ug of retinal.

Physiological functions

Vitamin A helps in preservation of the structural integrity and the normal permeability of the cell membrane as well as of membrane of subcellular particles as lysosomes and mitochondria.It also helps in maintaining the integrity of epithelial tissues such as epithelial layer of skin, respiratory mucosa, oesophagus and genitourinary tract.It acclerates the normal formation of teeth and bones. It plays an important role in vision (Visual Cycle of Wald).

Biochemical function

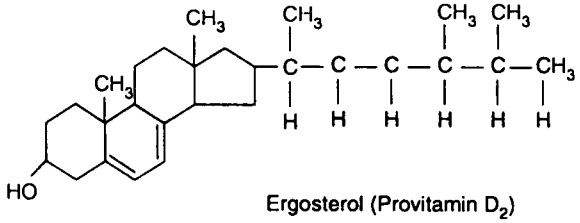
Retinal (vitamin A_1) and its aldehyde retinal are reactants in chemical changes that occur during the visual process in the rods of the eye. The retina of the human eyes and of most animal eyes, contain two types of light receptor cells, the rods are used for seeing at low intensities of light i.e. scotopic vision; shades of grey whereas the colour vision i.e. photobic vision is located in the cones.

Q. Write a note on deficiency of Vitamin A.

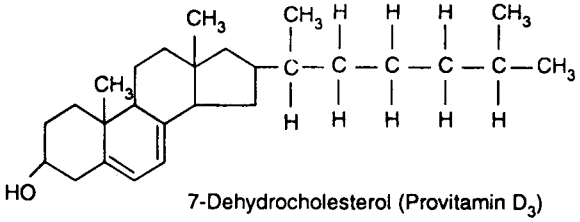
Ans. The deficiency of Vitamin A results in Xerophthalmia In this deficiency disease there is thickening and loss of transparency of the bulbar conjunctiva with yellowish pigmentation. Due to keratinization of ocular tissue, the blindness occurs.and is a major cause of blindness in childhood, which is more prevalent in Manila, Hongkong etc.

Q. Briefly write a note on Vitamin D.

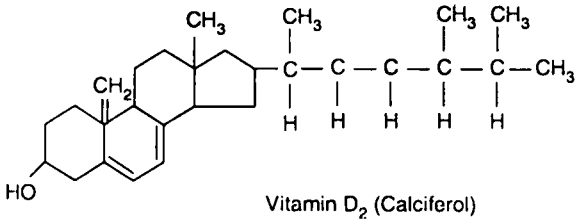
Ans. The vitamin D is a group of compounds. All are steroids occurring chiefly in animals. Provitamin D possesses the property of curing or preventing rickets when subjected to long-wave UV light. Vitamin D is a steroid hormone. It is represented by a group of steroids.



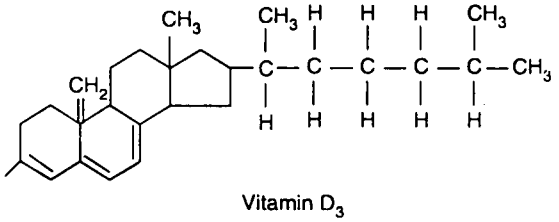
Ergosterol (Provitamin D_2)



7-Dehydrocholesterol (Provitamin D_3)



Vitamin D_2 (Calciferol)



Vitamin D_3

Fig. 7.1. Structures of Vitamin D.

Occurrence

In the active form vitamin D is not well distributed in nature. The rich sources are liver and viscera of fish, eggs and butter. The cheapest source is sunlight, which forms D_3 from 7-dehydrocholesterol in the skin.

Daily requirements

Adults	200 I.U.
During pregnancy and lactation	400 I.U.
Infants and children	400 I.U.

1 I.U = 0.025 µgm of cholecalciferol.

Physiological functions

Vitamin D directly stimulates the intestinal absorption of calcium by stimulating transcription of mRNA for calcium transport protein. Thus it is concerned with intestinal absorption, renal excretion and bone metabolism of calcium. Its ultimate effect is very much related to the concurrent activity of parathormone.It increases the excretion of phosphate by kidney and decreases the concentration of serum phosphate.It decreases the pH in the lower intestinal tract which aids in increasing the absorption of Ca and P. It increases the citrate level of blood, bone, kidney and heart tissues and also the excretion of citric acid.It stimulates the activity of phytase which catalyze the hydrolysis of phytic acid in intestine.

Biochemical functions

Vitamin D_3 induces the appearance of a specific calcium binding protein (CaBP) in the intestinal mucosa of a number of animals. This protein , has molecular weight of 24,000 and binds one atom of calcium per molecule of protein .

Vitamin D behaves more like a hormone than as the cofactor of an enzyme. That is, its effect is in controlling the production of a specific calcium binding protein rather than influencing directly the activity of a specific enzyme.

Q. Write a note on Vitamin E (Tocopherols).

Ans. Vitamin E refers to a group of compounds, which are known as tocopherol. These tocopherols are soluble in fat solvents and destroyed in alkaline medium. These are largely methyl derivative of the parent compound tocol and they are yellow oily substances. Four tocopherols i.e. α, β, γ and δ have been isolated. β and γ tocopherols have two methyl groups in the aromatic nucleus whereas a tocopherols has three methyl groups in the aromatic nucleus and δ has only one methyl group.

Occurrence

Tocopherols (Vitamin E) are richly present in eggs, meats, fish, chicken, corn oil, oatmeal and cottonseed oil.

Daily requirements

Adults	25-30 mg
Normal concentration in blood	10 mg

Physiological function

Vitamin E protects the polyunsaturated fatty acid from oxidation by molecular oxygen in the formation of peroxides. Vitamin E along with vitamin C is important in inhibiting the damage to lung

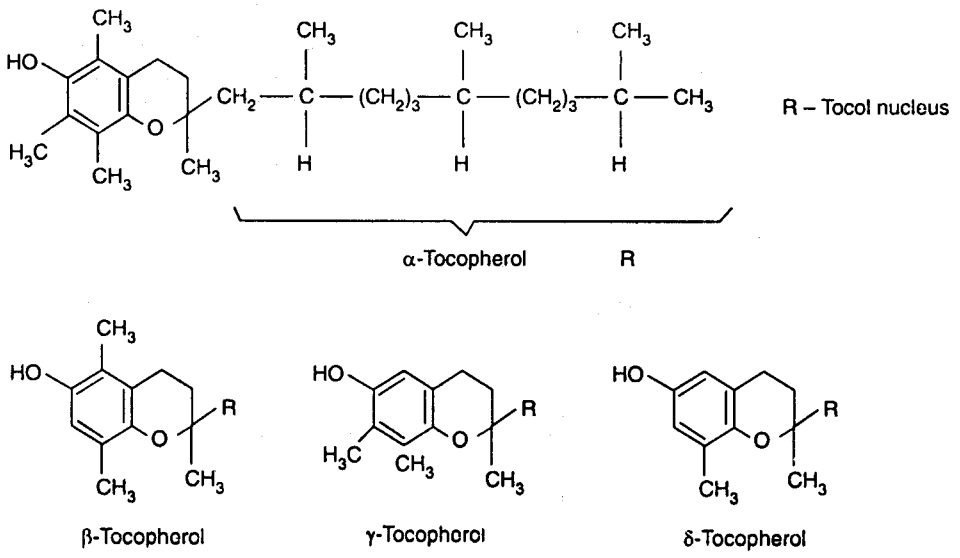


Fig. 7.2. Structure of Vitamin E.

tissue from oxidants in the air. It also protects enzymes in muscle, nerves or gonads from destruction. It prevents the development of cerebral disorder.

Biochemical functions

Vitamin E or tocopherols provides strong antioxidant activity in invitro systems. It has been suggested that the biochemical activity of tocopherol is its capacity to protect sensitive mitochondrial systems from irreversible inhibition of lipid peroxides. Thus, in mitochondria prepared from tocopherol-deficient animals there is a prefound deterioration of mitochondrial activity because of haematin catalyzed peroxidation of highly unsaturated fatty acids normally present in these particles.

Deficiency of Vitamin E

Its deficiency causes neurologic disorder and hepatic necrosis. Anaemia occurs in pregnant and lactating women and in new born infants. Decreased erythrocyte life span, hemolysis, creatinuria and ceriod deposition in malabsorption syndromes in children occur.

Q. Write a note on Vitamin K.

Ans. There are Vitamin K_1 and K_2 which are two naturally occurring vitamins. Vitamin K_1 was isolated originally from alfalfa and possesses a phytyl radical on position 3, which occurs in plants. Vitamin K_2 was originally isolated from putrid fish meal and possesses a difarnesyl radical which occurs in bacteria. The synthetic vitamin K is obtained from menadione and these vitamins lack the long hydrocarbon chain and hence are soluble in water.

Occurrence

Most bacteria present in the human intestine produce vitamin K_1 if it is not supplied in the diet. The sources to obtain this vitamin are green leafy vegetables i.e. (alfalfa, spinach, cabbage, cauliflower, and carrots) milk, meat and fish.

Daily requirements

The average diet contains adequate amounts of vitamin K_1 and K_2 being synthesized by bacteria present in the intestine. So, the vitamin K deficiency has not been reported in healthy individuals except in newborn infants feeding on mothers milk when mothers diet has low vitamin K contents.

Physiological functions

Vitamin K catalyzes the synthesis of prothrombin by the liver after transcription from information carried on mRNA. It reduces the prothrombin time and regulates the synthesis of plasma clotting factors (Factor VII, IX and X). It also acts as a cofactor of the carboxylase that forms γ-carboxyglutamate residues in the precursor proteins.

Biochemical functions

No clear-cut role has been found for vitamin K in any enzyme system. On the other hand, the fundamental importance of vitamin K in blood clotting process is well established. This process is highly complex and is affected in that a deficiency of vitamin K results in a decreased level of prothrombin in the blood. The vitamin may also influence the overall process at the level of another factor i.e. proconvertin, since this protein is also administered in vitamin D deficient states.

Deficiency of Vit K

The deficiency of vitamin K leads to a lowering of prothrombin level and increased clotting time of blood. This may lead to haemorrhagic conditions. Vitamin K deficiency is caused by fat malabsorption which may be associated with pancreatic dysfunction, biliary disease, atrophy of the intestinal mucosa or any causes of steatorrhea.

Q. Write a note on Vitamin C.

Ans. Vitamin C is also called as ascorbic acid owing to its antiscorbutic properties. Vitamin C is a white crystalline water soluble substances with sour taste. It is chemically an enediol-lactone, which is oxidized to dehydroascorbic acid (ascorbone) and both these forms are biologically active. It is a powerful reducing agent, which can reduce Fehling solution in cold. It is also readily destroyed by cooking.

Occurrence

The richest source of vitamin C is amla. Besides this, citrus fruits, tomatoes, green peppers, raw cabbage, guava, and cauliflowers are also good sources of vitamin C. The artificial food or sterilized milk is devoid of vitamin C.

Daily requirements

Adults	45 mg
Children	40 mg
Pregnant women	60 mg
Lactating women	80 mg

Normal concentration in blood plasma is 0.6-1.5 mg.

Physiological functions

Vitamin C is very sensitive to reversible oxidation-reduction reactions and is involved in redox reactions of the cell. It is required for the absorption of iron and incorporation of plasma iron in ferritin. Vitamin C is also essential for the normal regulation of the colloidal condition of intercellular

substances including the fibrils and collagen of connective tissue, osteoid tissue, dentine, the intracellular 'Cement substance' of the capillaries. It has an inhibiting effect on the hyaluronidase-hyaluronic acid system. It is also required in the metabolism of tyrosine, phenylalanine and tryptophan.

Biochemical function

The absence of ascorbic acid in the human diet gives rise to **scurvy.** The biochemical role which ascorbic acid plays is undoubtedly related to it being a good reducing agent. Its oxidized form, dehydro – ascorbic acid is capable of being reduced again by various reductants including glutathione (GSH) and the two forms of ascorbate constitute a reversible oxidation-reduction systems.

In case of collagen formation, ascorbic acid can function as the external reductant that is required in the conversion of proline to hydroxyproline. Ascorbic acid can function as an external reductant in the liver and in the conversion of dopamine to norepinephrine that occurs in the adrenals. Thus it appears that the biochemical role of ascorbic acid is related to its involvement in hydroxylation reactions in the cell.

Deficiency symptoms

Severe deficiency of vitamin C causes **scurvy.** The signs of this deficiency are entirely confined to supporting tissues of mesenchymal origin i.e. bone, dentine, cartilage and connective tissue. Scurvy is characterize by failure in the formation and maintenance of intercellular materials which results in internal haemorrhage, loosening of the teeth, poor healing of wounds swelling, sponginess, tenderness and bleeding of gums.

Q. List different members of Vitamin B – Complex.

Ans. The vitamins of B group includes following vitamins.

1. Thiamine (Vitamin B_1, Antiberiberi substance, Antineuritic vitamin)
2. Riboflavin (Vitamin B_2, Lactoflavin)
3. Niacin (Pellagra preventive factor, Nicotinic acid)
4. Pyridoxine (Vitamin B_6)
5. Pantothenic acid (Vitamin B_5,Filtrate factor)
6. Vitamin B_{12} (Cyanocobalmin, Cobamide)
7. Folic acid group (Vitamin, M)
8. Biotin (Vitamin H, Anti egg white injury factor)
9. Lipoic acid (Thioctic acid, Protogen)
10. Inositol (Mouse anti-alopecia factor)

Q. Write a note on Thiamine (Vitamin B_1).

Ans. Thiamine contains a pyrimidine ring and thiazole ring. Thiamine is readily soluble in water, and stable in acid medium. It is when oxidized with potassium ferricyanide in alkaline solution, is converted into thiochrome which has a strong fluorescence in UV light.

Occurrence

Thiamine is practically present in all plants and animal tissues, which are commonly used as food. Rice polishing, wheat germ, yeast, cereals and pulses are good sources of vitamin B_1.

Daily requirements

Adults	1.0 – 1.2 mg
Children	0.7 – 1.2 mg
Infants	0.3 – 0.5 mg

The requirement of thiamine is increased when metabolism is elevated in fever, hyperthyroidism, increased muscular activity, pregnancy and lactation.

Physiological functions

Thiamine in the form of pyrophosphate (TPP) is involved in oxidative decarboxylation of certain intermediates in carbohydrate metabolism e.g. pyruvic acid and α keto glutaric acid. It is therefore also called as co-carboxylase. It also acts as an coenzyme for transketose activity in the hexose monophosphate shunt. It is also essential for maintaining the nerve in normal condition.

Biochemical functions

Thiamine pyrophosphate participates as a coenzyme in α-keto acid dehydrogenases, pyruvic decarboxylase, transketose, and phosphoketose, an enzyme concerned with the metabolism of pentose in certain bacteria, for example;

$$\text{D-Xylulose} - 5 - \text{Phosphate} + \text{Pi} \xrightarrow[\text{Co carboxylase}]{\text{Phosphoketolase}} \text{Acetyl phosphate} + \text{Glyceraldehyde 3 phosphate}$$

Deficiency of Vitamin B_1

Deficiency of vitamin B_1 causes beriberi and neurological manifestations. In beriberi the muscles become wasted and weak and difficult to walk. The subject needs stick to stand and walk and finally becomes bedridden. **Oedema** is the important feature along with palpitation and breathlessness.

Q. Write occurrence, daily requirenment, sources, functions and deficiency symptoms of Riboflavin (Vitamin B_2).

Ans. Riboflavin is also called as lactoflavin, as it was first isolated from milk.

Occurrence

Riboflavin is widely distributed throughout the plant and animal kingdoms. Anaerobic fermenting bacteria, milk, cereals, roots, germinating wheat and barley are rich sources of riboflavin.

Daily requirement

Adults	1.1 - 1.8 m
Children	0.8 - 1.2 mg
Infants	0.4 - 0.6 mg

Physiological functions

It is involved in the regulatory functions of some hormones connected with carbohydrate metabolism. It is a constituent of enzymes, which are called as **flavoproteins.** FMN and FAD are concerned in oxidation-reduction reactions. Riboflavin is also the prosthetic group of acyl CoA dehydrogenase which is involved in the oxidation of fatty acids.

Biochemical functions

Riboflavin functions as a coenzyme because of its ability to undergo oxidation-reduction reactions. On reduction the yellow colour disappears since the reduced flavin is colourless. On exposure to air, the yellow colour of the oxidized form reappears.

It is difficult to generalize on the types of enzyme reactions in which flavoproteins participate. They do accept hydride ions (from NADH) together with proton from the environment and/or pairs of hydrogen atoms from a wide variety of organic metabolites such as amino acids, thioesters of fatty acids, pyrimidines, aldehydes, α hydroxy acids.

Deficiency of Vitamin B_2

The deficiency of vitamin B_2 results in dermatitis of the face, lesions in lips, fissure at the angles of the mouth and there are certain functional and organic disorders of the eyes.

Q. Write occurrence, daily requirenment, sources, functions and deficiency symptoms of Niacin or Vitamin B_3

Ans. Niacin is pyridine 3-carboxylic acid and occurs in tissues as niacinamide. It is soluble in water, stable to heat and not destroyed by autoclaving. When treated with strong alkali or acid it is converted into nicotinic acid. Nicotinamide exists in human and animal tissues as coenzyme 1(NAD) and coenzyme 11(NADP).

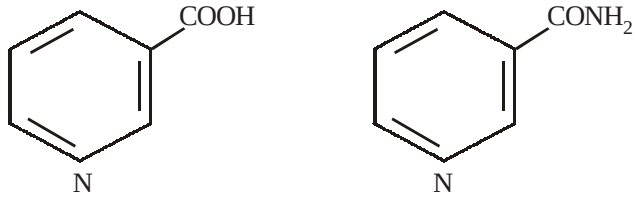


Fig. 7.3. Structure of Niacin.

Occurrence

The richest source of vitamin B_3 are yeast rice polishing. Meat, liver, milk, eggs and leafy vegetables are also good sources of this vitamin.

The amino acid tryptophan present in the dietary proteins is converted into niacin. In the body 60 mg tryptophan produce 1 mg of niacin, so tryptophan present in food stuff also provides additional niacin.

Daily requirements

Adults	12 – 20 mg
Children	9 – 16 mg
Infants	5 – 8 mg

The normal concentration of niacin in the whole blood is 0.5 - 0.8 mg/100 ml.

Physiological functions

Nicotinic acid is essential for the normal functioning of the skin, intestinal tract and nervous system. It is used therapeutically for lowering plasma cholesterol. Nicotinic acid principally occurs as nicotinamide or niacinamide. This niacinamide is component of two coenzymes NAD and NADP. These coenzymes play an important role in metabolism by acting as hydrogen and electron transfer agents by means of reversible oxidation and reduction. Hence the great importance of niacin in human nutrition as well as in the requirements of many other organisms including bacteria and yeast is well established.

Biochemical functions

The nicotinamide nucleotides are coenzymes for enzymes known as dehydrogenases that catalyze oxidation-reduction reaction. For example alcohol dehydrogenase, an enzyme widely distributed in

nature catalyzes the oxidation of ethanol with the concomitant reduction of NAD^+.

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + NADPH + H^+$$

The second manner in which the nicotinamide nucleotides function is in the reduction of flavin coenzymes. Since the flavin coenzymes are the prosthetic groups of enzymes, which accomplish the oxidation, or reduction of organic substrates, reduction provides a link for reaction between nicotinamide nucleotides and their substrates. As an example we may cite the reduction of the disulphite compound oxidized glutathione by glutathione reductase. The glutathione reductases are the enzymes that contain flavin adenine dinucleotide (FAD) as prosthetic group. In presence of NADH the flavin is first reduced, and the resulting FDD - H_2 in turn accomplishes the reduction of GSSG.

$$NADH + H^+ + FAD \rightarrow NAD^+ + FAD - H_2$$
$$FADH_2 + GSSG \rightarrow FAD + 2GSH$$

Where G stands for the tripeptide moiety of the glutathione molecule. A third function which the nicotinamide nucleotide performs is as a source of electrons for the hydroxylation and desaturation of aromatic and aliphatic compounds.

Deficiency of Vitamin B_3

The deficiency of niacin cause the disease pellagra. The clinical feature of the disease include three D'S-dermatitis, (lesions of skin face skin) diarrhoea and dementia, (headache depression, anxiety, insomnia and forgetfulness).

Q. Write occurrence, daily requirenment, sources, functions and deficiency symptoms of Pyridoxine or Vitamin B_6

Ans. In nature pyridoxine is a mixture of three compounds i.e. pyridoxine, pyridoxal and pyridoxamine. The more active derivatives are pyridoxal and pyridoxamine phosphates.

Occurrence

The richest sources of pyridoxine are yeast, rice polishing, wheat, milk, leafy vegetables, fish, fruits and some intestinal bacteria are also capable to synthesize this vitamin.

Daily requirements

Adults	1.6 - 2.0 mg
Children	0.6 - 1.2 mg
Infants	0.3 mg

Physiological functions

Pyridoxine is essential for growth of infants. Its phosphate form i.e. pyridoxal phosphate shows different coenzymic activities. Pyridoxal phosphate functions as co decarboxylase in the decarboxylation of tyrosine and arginine. It also acts as cotransaminase in the transamination reactions. It also acts as coenzyme in transulfuration, desulphuration, deamination reactions.

Biochemical functions

Pyridoxal phosphate is versatile vitamin derivative, which participates in the catalysis of several important reactions of amino acid metabolism known as transamination, decarboxylation and racemization. Each reaction is catalyzed by a different specific apoenzyme but in each case pyridoxal functions as a coenzyme.

Deficiency of Vitamin B_6

Deficiency of vitamin B_6 is rare because of its easy availability in most foodstuffs. But its deficiency gives rises to irritability and depression. In some cases there are lymphopenia and peripheral neuropathy. In deficiency states, there are inborn errors of metabolism including cystathioninuria some pyridoxal responsive anaemia's and familial xanthurenic aciduria.

Q. Write occurrence, daily requirenment, sources, functions and deficiency symptoms of Pantothenic acid or Vitamin B_5

Ans. Pantothenic acid exists in the tissues in the active form as coenzyme A which consists pantothenic acid, β–mercaptoethylamine, adenine, ribose and phsphoric acid. It consits of β–alanine and pantoic acid joined through a peptide bond. It is soluble in water and not obtained in crystalline form but its sodium, potassium or calcium salts crystallize readily.

$$\underbrace{HOCH_2 . C(CH_3)_2 . CH(OH) . CO}_{\text{Pantoic acid}} . \underbrace{NH . CH_2 . CH_2 . COOH}_{\beta - \text{alanine}}$$

Fig. 7.4. Structure of pantothenic acid.

Occurrence

Pantothenic acid is widely distributed in jelly, yeast, liver, rice polishing, wheat germ, milk, meat, eggs and leafy vegetables.

Daily requirements

Adults	5 – 10 mg
Children	4 – 5 mg
Infants	1 – 2 mg

Physiological functions

Pantothenic acid is essential for the growth of infants and children as it is a constituent of coenzyme A, which is required for several fundamental reactions in metabolism.

Biochemical functions

Thioesters formed from coenzyme A and carboxylic acids possess unique properties that accounts for the role the coenzyme plays in biochemistry.

Deficiency of Vitamin B_5

Deficiency of this vitamin in man results in nausea, vomiting, and certain gastrointestinal disorders, irritability and anaemia. It also causes burning foot syndrome in prisoners of war and is associated with reduced capacity for acetylation.

Q. Write a note on Vitamin B_{12}.

Ans. Vitamin B_{12} is a complex structured compound, which has molecular weight of 1355 and an emperical formula of $C_{63}H_{88}N_{14}O_{14}PCo$.

Occurrence

The rich sources of vitamin B_{12} are liver, kidney, meat, fish, eggs, milk and cheese. It is not present in foods of vegetable origin. Bacterial synthesis of cobalamin occurs in the human colon but it is not absorbed.

Daily requirements

Adults	3.0 µg
Children	1–2 µg
Infants	0.3 µg

Physiological functions

Vitamin B_{12} with folic acid is required for the development of red blood cells beyond megaloblast stage. It stimulates the appetite and general health of the subject. It cures the neurological symptoms of pernicious anaemia.

Biochemical functions

Vitamin B_{12} acts as a coenzyme and is involved with tetrahydrofolate in the synthesis of labile methyl groups, which can be transferred to homocysteine to form methionine. It is also involved in the maintenance of sulfhydryl groups in the reduced state. It is involved in the biosynthesis of proteins.

Deficiency of Vitamin B_{12}

Deficiency of vitamin B_{12} results in megaloblastic macrocytic anaemia, mucosal atrophy and inflammation of tongue and mouth. It may also lead to severe diseases of nervous system. A severe form of acidosis in children due to excessive production of methylmalonate may occur.

Q. What are Folic Acids? Give an account of them.

Ans. Folic acid was first isolated in a crystalline form from liver in 1947 by Pifiner et al. It is a compound made up of pteridine nucleus, P-aminobenzoic acid and glutamic acid. It is soluble in water, stable to heat at neutral pH. Its activity is not lost if it is heated at 120°C for 30 min at neutral pH .

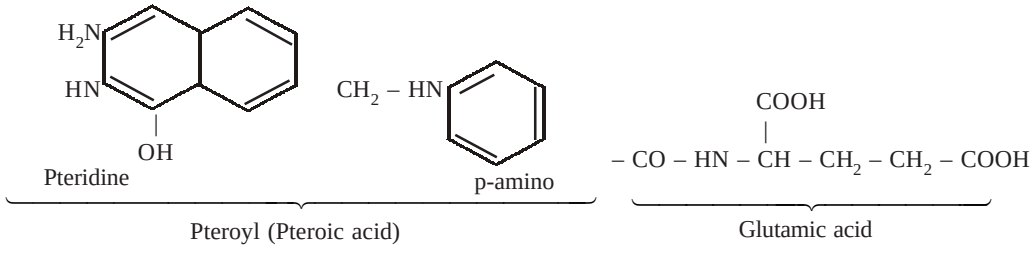


Fig 7.5. Folic Acid.

Occurrence

The richest source of folic acid are yeast, liver, kidney, meat, fish, green leafy vegetables, milk and fruits.

Daily requirements

Adults	400 µg
Children	100 – 300 µg
Infants	50 µg

Physiological functions

Folic acid attains clinical application in the treatment of malignant disease and confirms the action of folic acid in cell growth.

Biochemical functions

Folic acid as a coenzyme is involved in the transfer and utilization of the single carbon (C – 1) moiety. Before functioning as C – 1 carrier, folic acid is first reduced to 7, 8 dihydrofolic acid and then to tetrahydro compound. The folic acid coenzymes takes part in the synthesis of purines and thymine, they are so fundamentally involved in growth and reproduction of cells. These coenzymes are not only confined to the hematopoietic system but also generalized throughout the body.

Deficiency of folic acid

The deficiency of folic acid give rise to **megaloblastic anaemia.** The nuclei of the neutrophil, polymorphonuclear leukocytes contain more than normal number of lobes. Other deficiency manifestations include retardation of growth, weakness, infertility inadequate lactation in females and increased output of formiminoglutamic acid (FIGLU) in the urine after histidine loading.

Q. Write a note on Biotin.

Ans. Biotin is also called as anti egg white injury factor. It is highly soluble in hot water, forms salt with alkali hydroxides. The carboxyl group of biotin combines with the terminal nitrogen of lysine residue of enzyme protein forming biocytin (Lysine - biotin conjugate).

Occurrence

Biotin is widely distributed in natural foods. Intestinal bacteria also supply biotin in large proportions. Besides these egg yolk, liver, kidney, yeast, milk, tomatoes, fruits, vegetables and human milk are good sources of biotin.

Daily requirements

Adults	50 - 60 µg
Children	20 - 40 µg
Infants	10 - 15 µg

Physiological functions

Biotin is concerned with the carboxylation reactions (CO_2 fixation). The biotin coenzyme apoenzyme complex attaches CO_2, which is afterward transferred to other substances.

Biochemical functions

Biotin bound to its specific enzyme protein is intimately associated with carboxylation reactions. The overall reaction catalyzed by biotin - dependent carboxylases can be divided into two discrete steps. The general term, carboxylase, includes two activities; the carboxylation of a biotinyl carboxyl carrier protein and then the subsequent transfer to an acceptor by a transcarboxylase. The first step involves the formation of carboxyl biotinyl enzyme; the second step involves carboxyl transfer to an appropriate acceptor substrate depending upon the specific transcarboxylase that is involved. Pyruvic carboxylase is an example of an enzyme employing α keto acid as an acceptor, while acetyl CoA carboxylase and propionyl CoA carboxylase are examples of an acyl CoA serving as the specific acceptor.

Deficiency of biotin

The deficiency of biotin may result from the destruction of intestinal bacteria by sulfonamide drugs or from the inadequate intake of raw egg white, which contains the protein avidin. The deficiency develops nausea, anorexia, anemia, muscular pain and dermatitis of extremities.

Q. What are Lipoic acid?

Ans. Lipoic acid is a crystalline substance, isolated from the insoluble residue of liver. It is a sulphur containing fatty acid called 6, 8 dithiooctanoic acid (a lipoic acid or thioctic acid). It contains 8 carbon and 2 sulphur atoms. Oxidized and reduced forms of the compound are shown as

$$\begin{array}{l} CH_2 - CH_2 - CH\,(CH_2)_4 - COOH \\ \quad | \qquad\qquad\quad | \\ SH \qquad\quad SH \end{array}$$

α Lipoic acid (reduced form)

$$\begin{array}{l} CH_2 - CH_2 - CH\,(CH_2)_4 - COOH \\ \quad | \qquad\qquad\quad | \\ S \qquad\quad S \end{array}$$

α Lipoic acid (oxidized form)

Occurrence

It occurs in a wide variety of natural materials.

Daily requirements

Its requirements in the diet of higher animals has not yet been demonstrated.

Physiological functions

It is recognized as an essential component in metabolism, although it is active in extremely minute amounts.

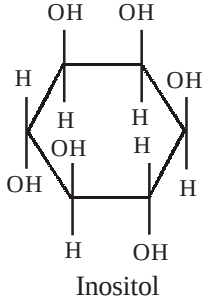
Biochemical functions

It function as a coenzyme of pyruvate dehydrogenase complex and α oxoglutarate dehydrogenase complex. Lipoic acid is also required for the action of the enzyme sulphate oxidase required for conversion of SO_2 to SO_4^{2-}. Hypoxanthine is also required for the action.

$$\text{Lipoic acid, Hypoxanthine} \quad \begin{array}{c} SO_2^- \\ \downarrow \\ SO_4^{2-} \end{array} \quad \text{Sulfide oxidase}$$

Q. Give an account of Inositol.

Ans. Inositol is also called as Meso-inositol or Myo-inositol and is hexahydroxy cyclohexane. It is crystalline compound, highly soluble in water, stable to heat in neutral acid and alkaline medium.



Inositol

Occurrence

It is present in animal tissues such as muscles, brain, red blood cells. It is also widely distributed in plants i.e. fruits, vegetables, whole grain and yeasts.

Daily requirements

Not yet reported.

Physiological functions

Inositol plays an important role in different physiological activities. It is found in large concentrations in heart muscles and increase the amplitude and rate of contraction. It also causes an increase in nerve chronaxie in rats. It also increase peristalsis of the small intestine.

Biochemical functions

It function as antiketogenic compound. In its presence, blood cholesterol level is not increased as expected on high cholesterol diet in experimental animals.

Q. Write short notes on—

1. Beri–Beri

Beriberi is caused by the deficiency of vitamin B_1 (thiamine). There are three types of beriberi.

(a) **Wet beriberi**—The symptoms of wet beriberi include edema. It is rapidly developed and not only the legs but also the face, trunk, serous cavities are involved. Palpitation and breathlessness appear, diastolic blood pressure is low while cystolic is high. Pulse is high and death occurs as a result of heart failure.

(b) **Dry beriberi**—The muscles are progressively wasted and weak and difficult to walk. The affected individual takes the help of stick to stand and walk and ultimately becomes bed ridden and dies if untreated.

(c) **Infantile beriberi**—Those infants are suffered of this type of beriberi, whose mother's breast milk contains low thiamine. The infants feel restlessness and sleeplessness. Anorexia, vomiting and breathlessness develop but most of the symptoms are due to cardiac eliation and failure. Sudden death occurs if not treated with thiamine immediately.

Beriberi can be treated by the administration of thiamine by intramuscular injection.

2. Pellagra

Pellagra is caused by the deficiency of niacin. It occurs whenever corn i.e. maize is the main diet. The corn protein is deficient in some of the essential amino acids, notably tryptophan and lysine. Pellagra is characterized by the three D's – dermatitis, diarrhea and dementia. In dermatitis there is bright red erythema resembling sunburns, which occurs over the exposed parts of the body. The commonest sites are the back of the fingers and hands, the forearms, dorsum of the feet and ankles and the neck. Secondary infection is always present. In most cases of diarrhea nausea and vomiting are found along with few to several loose stools a day with blood and mucus. Dementia occurs more frequently in chronic cases. Irritability, changes in disposition, depression, inability to concentrate are found in milder mental disturbances. In chronic cases, spasticity, ataxia, the involvement of the bladder and rectal sphincters are seen.

Taking niacinamide in doses of-15–25 mg three times a day can prevent pellagra. Adequate quantities of meat eggs, milk and vitamin B complex should also be given. Any accompanying infection should be treated with proper antibiotic.

3. Osteomalacia

Deficiency of vitamin D in adults causes osteomalacia in adults. It generally occurs in pregnant women of low-income groups who consume a diet devoid of vitamin D and calcium. The symptoms of osteomalacia include changes in the blood and bones similar to rickets. There is progressive decalcification of bones and bones become soft. Bone deformities occur in pelvis, leg, ribs, sacrum and lower lumbar vertebrae. This disease can be prevented by the administration of vitamin D and calcium.

4. Pernicious anaemia

Pernicious anaemia occurs due to the lack of intrinsic factor in the stomach resulting in the failure of absorption of vitamin B_{12}. The RBC count is low i.e. 1.5 to 2.5 million per cubic mm. There is excessive destruction of the abnormal circulating red cells, which results in increased serum bilirubin. The cells of the stomach responsible for acid and enzyme secretions are atrophied. So the gastric secretions are devoid of acid, pepsin and intrinsic factor (I.F). The tongue is sored and inflamed. In advanced cases, demyelation of the white fibers of spinal cord occurs but it can be prevented by administration of vitamin B_{12}.

5. Rickets

Rickets is caused by deficiency of vitamin D. It is characterized by bone deformities. The bones become soft due to the non - deposition of calcium salts and hence they are easily bent by the weight of the body. In case the rickets continue for two to three years, there is serious bone deformities such as bowlegs, deformities of the spine and pelvis. There is extension and widening of epiphysis at the growing points. Renal rickets is caused by defective transport of phosphate by renal tubules.

Rickets can be prevented by the administration of vitamin D and calcium. Vitamin D in ordinary dose cannot cure the renal rickets and so renal rickets are sometimes also called as vitamin D resistant rickets.

6. Scurvy

Severe deficiency of ascorbic acid leads to scurvy. It may be in adults as well as in infants. The first symptoms are weakness, easy fatigue and listlessness; which are followed quickly by pain in bones, joints and muscles of the extremities, shortness in breath. Haemorrhages in muscle occur particularly in calf, thigh and forearm. As deficiency advances the gums become swollen blue red and spongy. Bacteria may also infect the gums. There is loss of teeth also. In infants, in the beginning of disease, the infant lies with legs drawn up on the abdomen. The infant's cries when touched especially when its legs or arms are moved of lifted. Extreme tender swellings may be felt at the end of long bones. If treatment is delayed dyspnea, cyanosis, convulsions and death may occur.

The disease can be prevented by the administration of high dose of ascorbic acid. Citrus fruits are to be taken regularly.

8

PROTEIN METABOLISM

Q. Present a brief outline sketch of metabolism of protein in general.

Ans. All body proteins continually undergo degradation and synthesis. More than half of the protein of the liver and intestinal mucosa are broken down and resynthesized in ten days. The rate is slower in muscles and erythrocytes. The balance between the rate of synthesis and degradation of its constituent is maintained in adult organisms. The proteins are hydrolyzed to amino acids by enzymes in the gastrointestinal tract, and these are absorbed into the blood and transported to the liver. This organ removes a portion of amino acids for specific biosynthetic tasks, while the remainder pass on to extrahepatic tissues where they can be synthesized into proteins. The liver is the site of synthesis of several blood proteins (plasma albumin, the globulin's, fibrinogen, and prothrombin). It also metabolizes any amino acid present in excess either by protein synthesis or by converting nitrogen atoms into urea and the carbon skeleton into different metabolic intermediates.

Metabolic pool of proteins

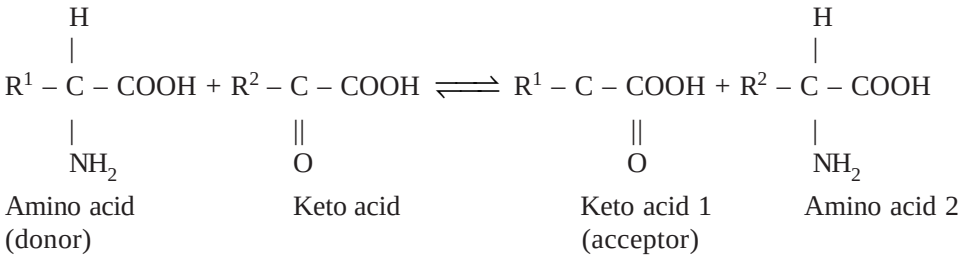
The mixture of endogenous and exogenous proteins constitutes as a reservoir or metabolic pool of the compound. It is derived from the catabolism of protein in the tissues by the stimulation of the excessive amounts of thyroid hormones and 11-oxygenated adrenocorticoid hormones and substance absorbed from the intestine. The metabolism of protein is integrated with that of carbohydrate and fat through pyruvate acetate, oxaloacetate and α ketoglutarate etc. and the members of one pool are metabolically equilibrated with other pools e.g alanine is deaminated to pyruvic acid and hence the carbon skeleton of this amino acid is involved in the pyruvate pool which is directly connected to the carbohydrate pool.

Q. Mention the important general reactions of amino acids.

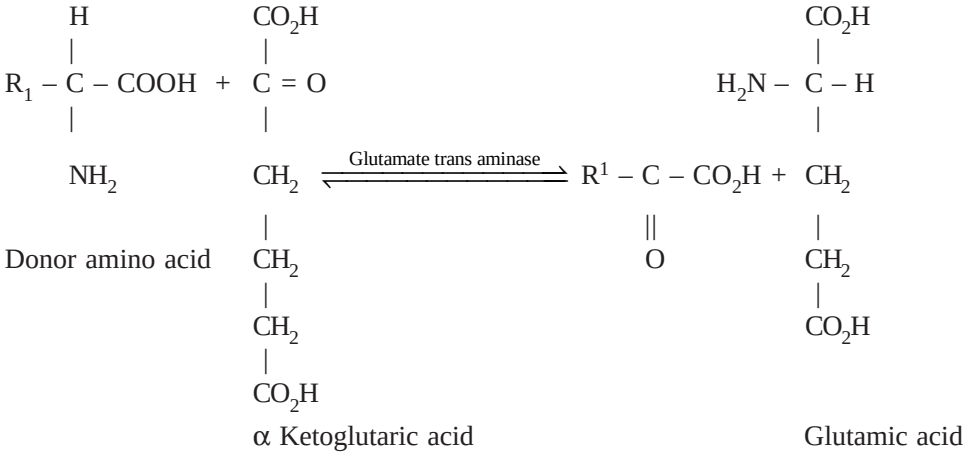
Ans. The amino acids in the body undergo various chemical reactions so as to actively participate in the metabolism. The important reactions are-

Transamination

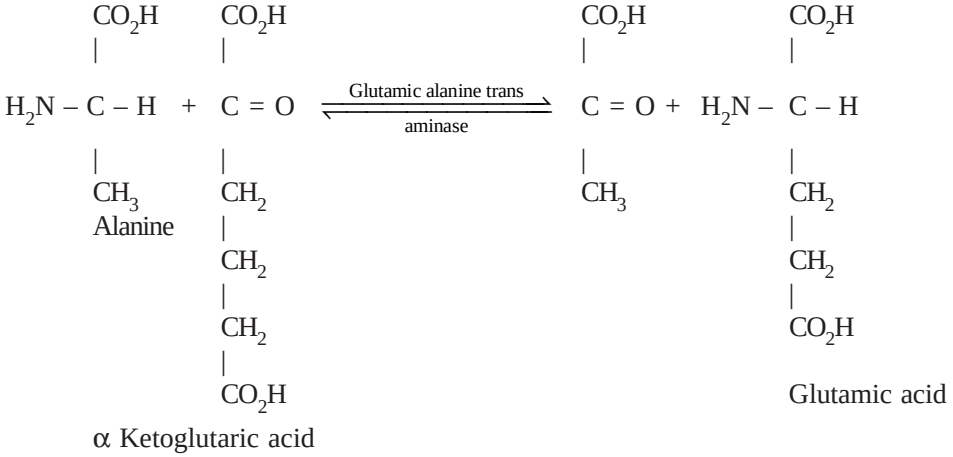
The reaction of transamination involves the transfer of the amino group from an amino acid to a keto acid (the carbon atom) to form the analogous amino acid to produce the keto acid (the carbon skeleton) of the original donor.



Transaminases capable of reacting with nearly all the amino acids have been reported, but especially important are glutamic transaminase and alanine transaminase. Glutamate transaminase is specific for glutamic and α ketoglutaric acid as one of its two substrate pairs.



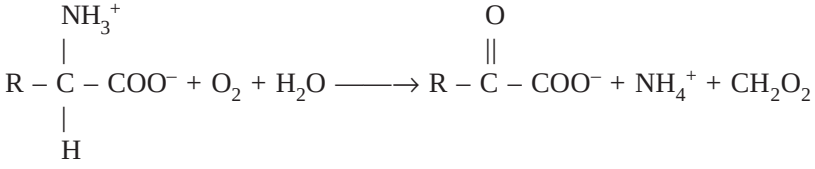
Similarly alanine transaminase is specific for alanine and pyruvic acid as one of its substrate pairs but reacts with almost any other amino acids.



The transaminase reactions occur primarily in the cytoplasm and it is the glutamic acid which being specifically permeable to the inner mitochondrial membrane, enters the matrix of mitochondria. There it can transaminate again with a mitochondrial asparate transaminase or alternatively be oxidatively eliminated by the mitochondrial glutamic dehydrogenase. Transaminase therefore are found both in the cytoplasm and the mitochondria of eukaryotic cells, the enzymes in each region of the cell having characteristic properties.

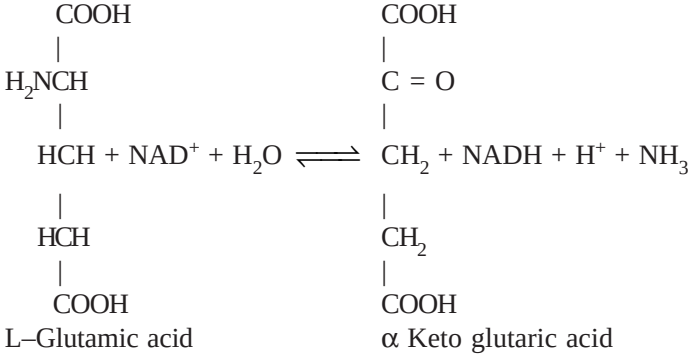
Deamination

Oxidative deamination involves the removal of α amino group of amino acids to their corresponding keto acids. The enzyme is amino acid oxidase. In the amino acid oxidase reaction, the amino acid is first dehydrogenated by the flavoprotein of oxidase forming an α keto acid. This spontaneously adds water to decompose the corresponding a keto acid with the loss of a amino nitrogen as ammonia. The overall reaction is

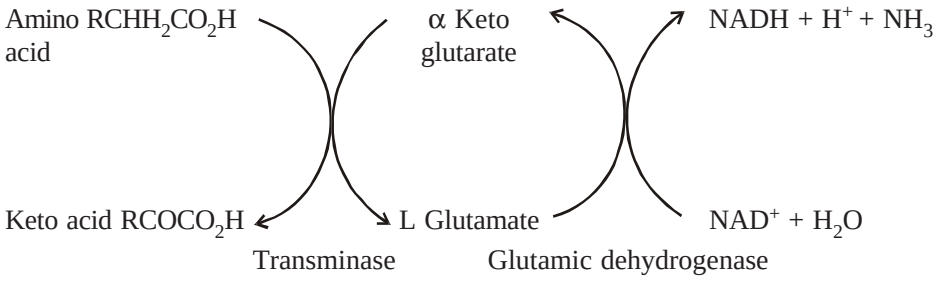


Deamination by glutamic dehydrogenase

L glutamic acid plays a key role in the metabolism of amino acids because of the widespread occurrence of the enzyme glutamic dehydrogenase. This enzyme catalyzes the reversible oxidative deamination by NAD^+ of L – glutamate to form α ketoglutaric acid, NH_3 and NADH.



The coupling of this reaction with transamination of glutamic acid creates a mechanism for deaminating all other amino acids. NH_3 produced in this way is toxic and must be disposed of. In animals, elaborate mechanisms for detoxification exists i..e. urea cycle. In plants, the NH_3 is converted to nontoxic amides, glutamine and asparagine and remarkably high concentration of these compound accumulate.



Transmination of Glutamic acid

Because of its importance in the metabolism of other amino acids as well as the fact that glutamic acid serves as precursor of proline and ornithine, it is obvious to note that glutamic dehydrogenase is an allosteric enzyme.

Decarboxylation

A third type of general enzymatic reaction, which many amino acids undergo, is decarboxylation.

$$R-\underset{\displaystyle NH_3^+}{\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}}-COO^- \longrightarrow R-\underset{\displaystyle NH_2}{\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}}-H + CO_2$$

Q. What are the important ways by which the metabolic fate of amino acids remains in the body?

Ans. The proteins and amino acids are not usually degraded for energy production, if carbohydrates or lipids are available to the organism, instead the amino acids are used.

(1) in the synthesis of peptides and proteins.

(2) as a source of nitrogen atoms (transamination) for the synthesis of other amino acids.

(3) in the synthesis of other nitrogenous and non nitrogenous compounds.

Any amino acids in excess of the amounts required for these three activities will be degraded by deamination and the resultant carbon skeleton is metabolized. The NH_3 produced, if in excess, will be eliminated as a nitrogenous waste product. Table (8.1) lists the catabolic end product of the twenty amino acids. It may be seen that nearly all of the amino acids yields on breakdown either an intermediate of the tricarboxylic acid cycle, pyruvate or acetyl CoA.

Table 8.1 End products of Amino acid Metabolism

Amino acids	*End products*
1. Alanine, Serine, Cysteine, Glycine, Threonine	Pyruvic acid
2. Leucine	Acetyl CoA
3. Phenylalanine, Tyrosine Leucine, Lysine, Tryptophan	Aceto acetic acid
4. Arginine, Proline, Histidine, Glutamine, Glutamic acid	α Ketoglutaric acid
5. Methionine, Isoleucine valine.	Succinyl CoA
6. Phenylalanine, Tyrosine	Fumarate
7. Asparagine, Aspartic acid	Oxaloacetate

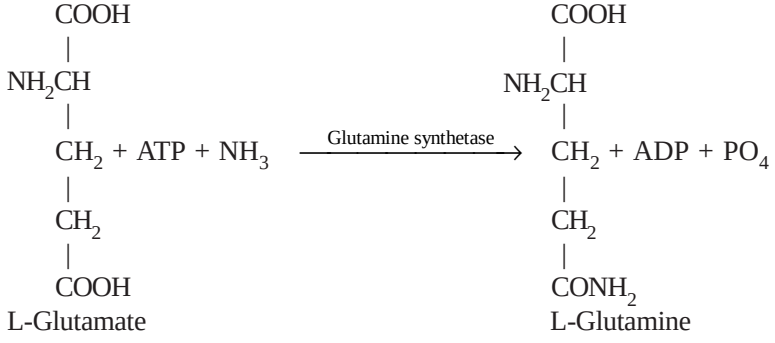
The exceptions are the five amino acids, which give rise to acetoacetic acid. Since, however, this compound also forms acetyl CoA, carbon skeletons of all of the amino acids are ultimately oxidized via TCA cycle. Those amino acids which give rise to an intermediate of the cycle (or to pyruvic acid) can in turn be converted to glucose. For this reason, those amino acids are called as **glucogenic amino acids.** On the other hand, those which on degradation produce acetyl CoA or acetoacetic acid will under certain conditions will give rise to ketone bodies in the animals and they have therefore been described as **ketogenic amino acids.** Some amino acids such as phenylalanine and tyrosine, are both glucogenic and ketogenic because part of their carbon atoms are converted to fumarate while the remainder are converted to acetoacetate.

Q. How Urea is formed in the body? OR Biefly descibe the formation of urea in the body.

Ans. The formation of urea in the body is a complex process and involves different processes.

Assimilation of NH_3

A major reaction in the assimilation of NH_3 is catalyzed by glutamine synthetase, an enzyme that is ubiquitous in nature.



The reaction catalyzed by glutamate synthase can be coupled with glutamine synthetase and transamination to accomplish the synthesis of amino acids ($RCHNH_2COOH$) from ketoacids (RCOCOOH) by a process that is unidirectional and driven by the hydrolysis of ATP.

$$\text{L-Glutamic acid} + ATP + NH_3 \xrightarrow{\text{Glutamine synthetase}} \text{L-Glutamine} + ADP + H_3PO_4$$

$$\alpha \text{ Ketoglutarate} + \text{L-Glutamine} + NADPH + H^+ \longrightarrow 2\ \text{L-Glutamic acid} + NADP^+ + H_2O$$

$$\text{L-Glutamic acid} + RCOCOOH \longrightarrow RCHNH_2COOH + \alpha \text{ Ketoglutarate (transamination)}$$

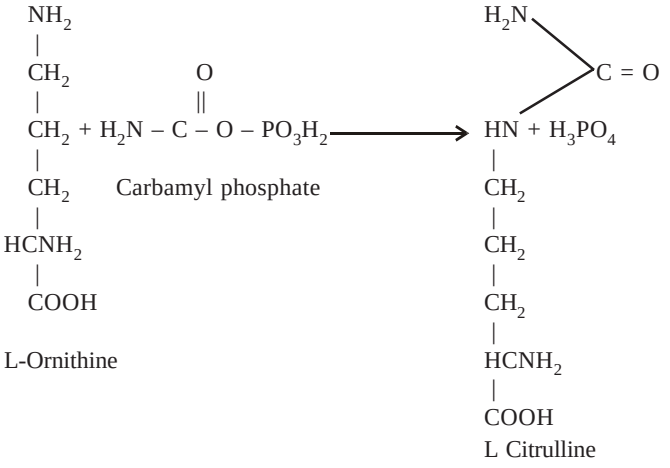
$$RCOCOOH + ATP + NH_3 + NADPH + H^+ \longrightarrow RCHNH_2COOH + ADP + H_3PO_4 + NADP + H_2O$$

Coupling reaction of glutamate synthase and glutamine synthetase

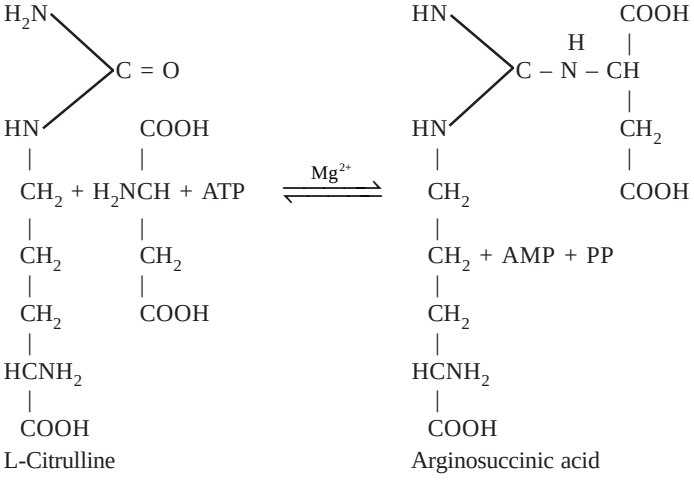
Urea Cycle

Sir Hans Kreb and K. Henselit were among the first to study the formation of urea in animal tissues.

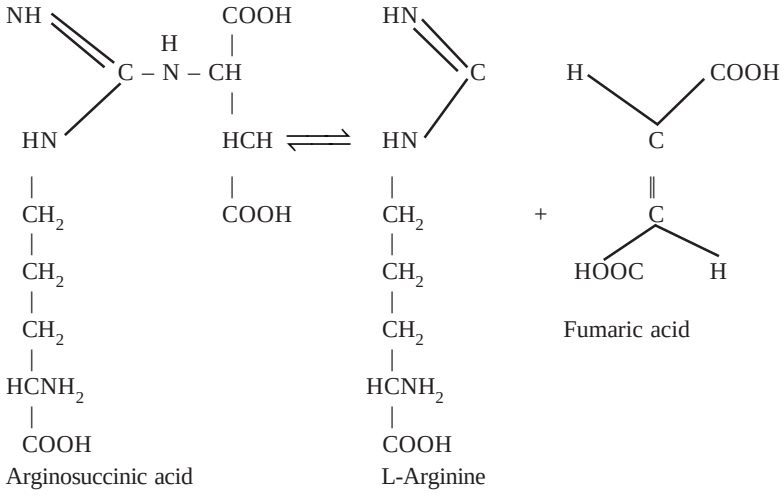
In the initial step, carbamyl phosphate reacts with ornithine to form citrulline in the presence of the enzyme ornithine transcarbamylase. This enzyme requires no factors and exhibit extreme substrate specificity.



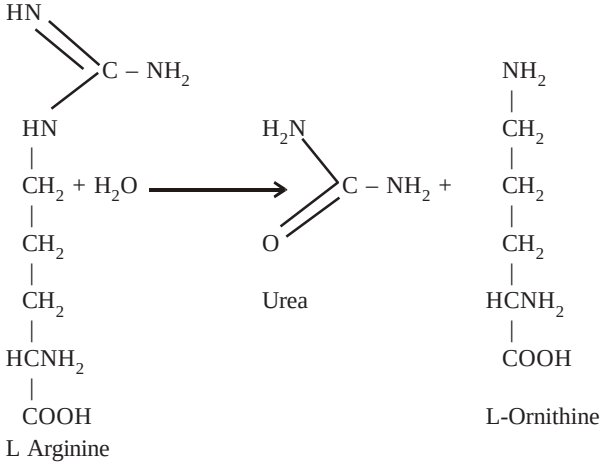
In the next step of the cycle, arginosuccinic acid is formed by the combination of citrulline and aspartic acid in presence of argino succinic acid synthetase and ATP.



Arginosuccinic acid is cleaved to arginine and fumaric acid by argino succinase which is present in mammalian liver and kidney. The fumarate formed is converted to oxaloacetate via the fumarase and malate dehydrogenase reactions and then transaminated to regenerate asparate.



Arginase, which catalyzes the irreversible hydrolysis of L-arginine to ornithine and urea, is the enzyme, which converts the unidirectional sequence for biosynthesis of arginine into a cyclic process for making urea.



Thus all the above reactions accomplish the formation of arginine, a widely occuring amino acid, from ornithine, NH_3 and CO_2. The enzymes catalyzing these reactions presumably occur in a wide number of tissues in animals, plants and microorganisms. Liver is the major site of urea formation in mammals although some urea synthesis can occur in brain and kidney.

The sequence of reactions just discussed is shown in Fig 8.1. The cycle accounts for the formation of urea from NH_3, CO_2 and the amino group of aspartic acid. The requirement for the oxidizable substrates reported by Krebs is explained by the participation of ATP in the formation of carbamyl phosphate and arginosuccinic acid. By the eventual conversion of fumaric acid back to aspartic acid, another mole of amino nitrogen can be brought to the point of reaction in the cycle.

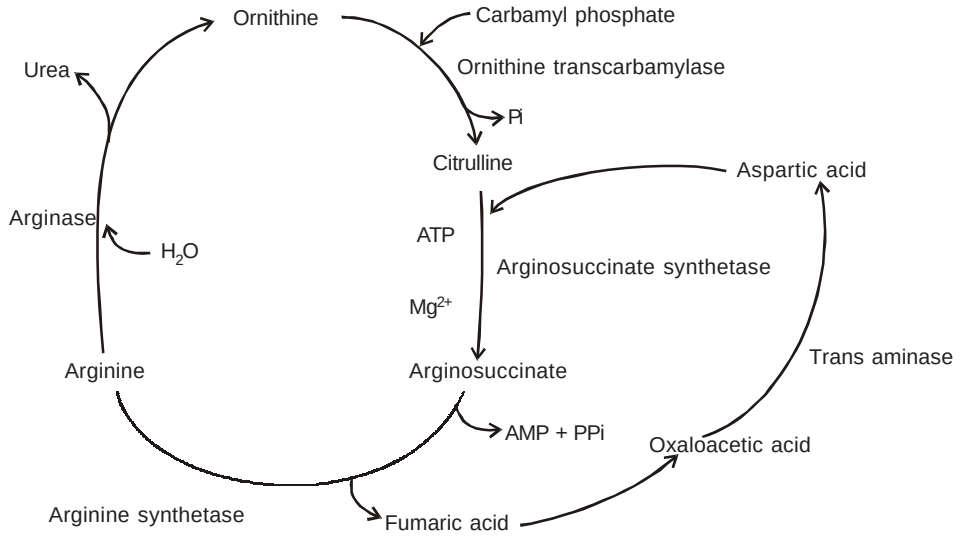


Fig. 8.1. Krebs Henseleit cycle for urea formation.

Q. How Uric Acids are formed in the body?

Ans. Uric acid is the form in which birds and terrestrial reptiles excrete the NH_3 produced in protein metabolism. It is also the chief end product of metabolism of purines in man and other primates. Thus, the birds and reptiles which have uric acid as their chief nitrogen waste product first must convert NH_3 into purines.

The free purine bases are converted to uric acid as shown in Fig 8.2. Xanthine oxidases, which catalyze the formation of uric acid is found in the peroxisomes of the kidney along with the oxidases. Mammals other than the primates and most reptiles produce allantoin as their end product of purine metabolism. Such organisms contain the enzyme uricase, which converts uric acid to allantoin. The teleost fish convert allantoin on to allantoic acid, while most fish and the amphybia degrade allantoic acid further to urea and glyoxylic acids.

Q. Describe in brief the biosynthesis of S containing amino acids.

Ans. The sulphur containing amino acids are cysteine and methionine. Their synthesis is considered briefly due to their unusual nature.

The primary reaction for incorporation of sulphur into organic compounds is the formation of cysteine by cysteine synthase, a reaction that occurs in bacteria and higher plants but not in animals.

$$\begin{array}{lcl} CH_2 - O - \text{Acetyl} & & CH_2SH \\ | & & | \\ CHNH_2 + H_2S & \longrightarrow & CHNH_2 + CH_3COOH \\ | & & | \\ COOH & & COOH \\ \text{O – Acetyl serine} & & \text{Cysteine} \quad \text{Acetic acid} \end{array}$$

Bacteria and higher plants can utilize the cysteine as a sulfur source to form methionine, the process known as **trans sulfurylation.** In the presence of the enzyme cystathionine synthase I, a sulfur containing addition product known as cystathionine is formed.

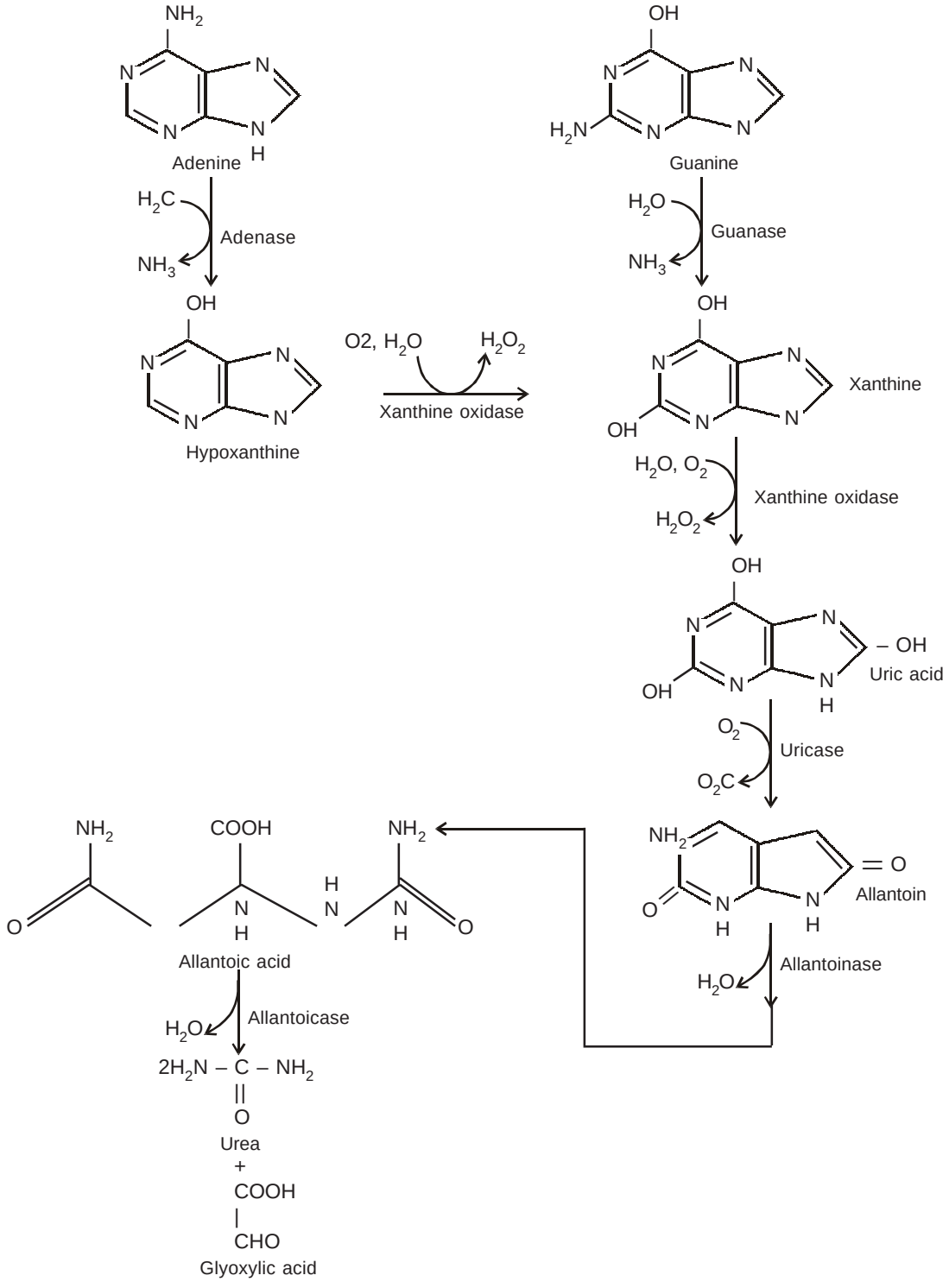
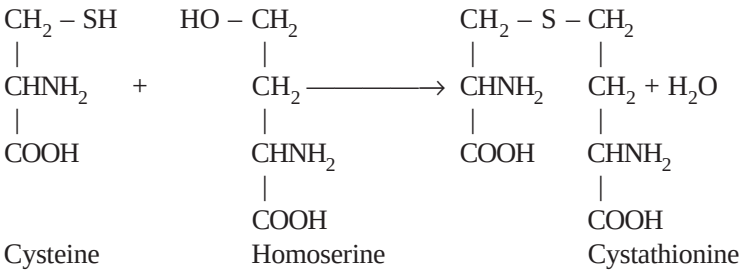
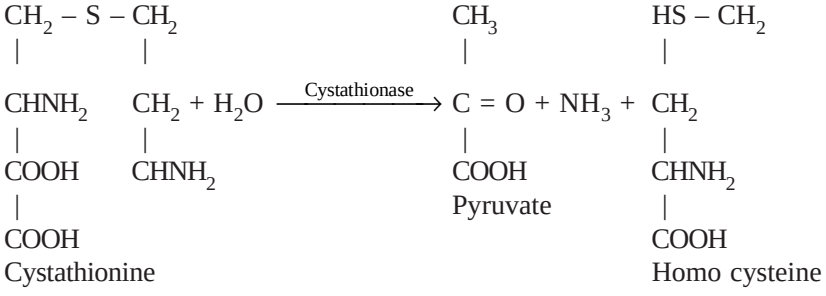


Fig. 8.2. Formation of Uric acid.



The three carbon unit is contributed by cysteine and the four carbon unit by homoserine (derived from aspartic acid). In the presence of cystathionase the cystathionine is hydrolytically cleaved on the opposite side of the sulfur atom to produce homo cysteine, pyruvic acid and NH_3.



The homocysteine is subsequently methylated to form methionine. The reaction and relationship just described for bacteria and plants are almost reversed in animals, which cannot make cysteine (or homocysteine) from H_2S and SO_4^{2-}. Instead, animals synthesize their cysteine from methionine, which is an indispensible amino acid. Indeed, the essentiality of methionine is due to this inability. Since, the sulfur of methionine can be utilized to make cysteine however, the latter is not essential amino acid. Cystathionine encountered above is again an intermediate in this process. In the presence of mammalian cystathioine synthase II, homo cysteine (derived from the demethylation of methionine) reacts with serine to produce cystathionine, which is then hydrolyzed to yield cysteine, α ketobutyric acid and NH_3.

In this reaction, the three carbon atoms of cysteine originate in serine while the sulfur atom comes from homocysteine and indirectly from methionine. The four enzymes just described that are involved in the formation and hydrolysis of cystathione are enzymes that contain pyridoxal phosphate as cofactor.

Q. By which means the Porphyrin are synthesized.

Ans. The biochemically important compounds chlorophyll, haemoglobin, and the cytochrome have in common a cyclic tetrapyrrole structure called a **porphyrin.** The parent compound, **porphin** contains four pyrrole rings linked by methine bridges (–CH=). In the figure, rings I, II, III and IV are connected by methine bridges α, β, γ and δ. The double bond system is conjugated. In actual fact, the double bonds are not definitely assigned, since the structure is a resonating system with several possible structures. Protoporphyrin IX is one of fifteen possible isomers and is the most common in nature. The porphyrin ring is sterically a flat structure with a specific metal, very firmly chelated by electron pairs of the nitrogen atoms of the four pyrrole residues. The only metals found in the

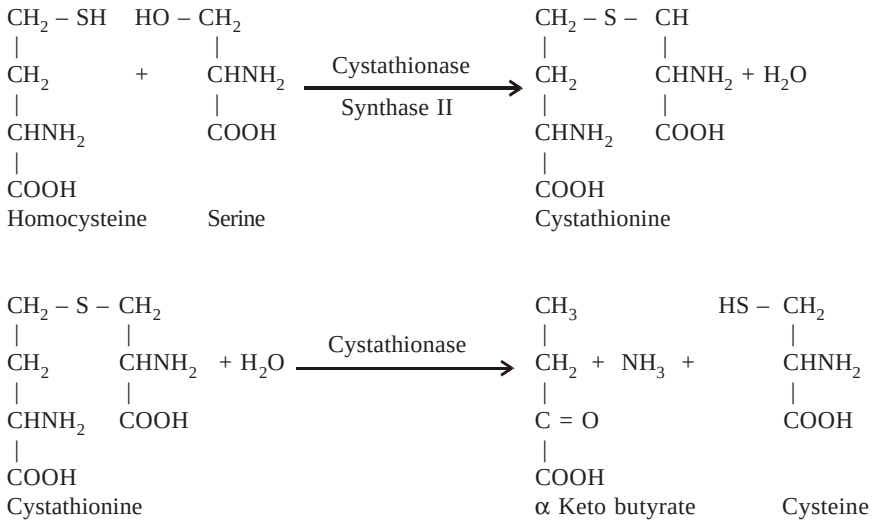


Fig. 8.3. Synthesis of Cysteine.

biologically functional tetrapyrroles are magnesium (in cholorophyll) iron (in heme, the cytochromes, peroxidases and catalases) and cobalt (in the cobalamines, modified tetrapyrroles).

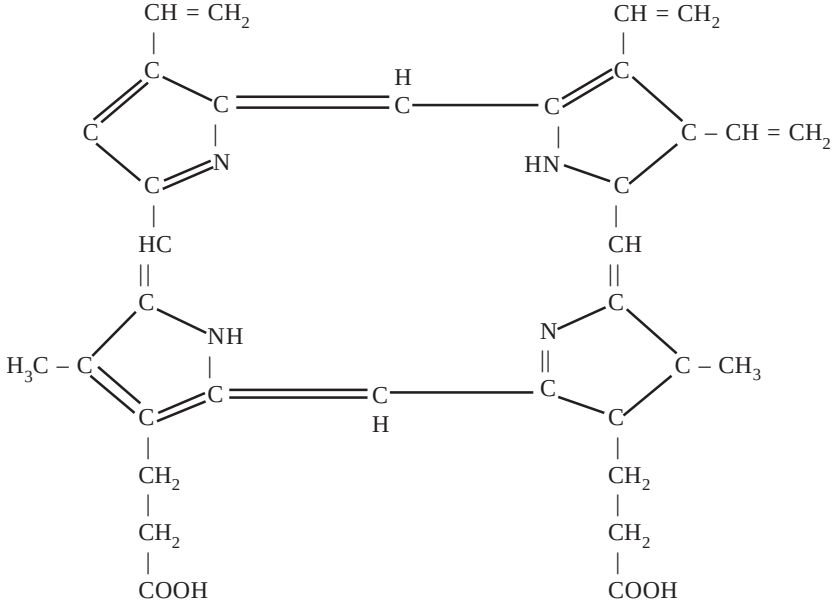


Fig. 8.4. Protoporphyrin IX.

9

CARBOHYDRATE METABOLISM

Q. List some of the important means or methods of utilization of glucose.

Ans. The glucose is utilized within the body for different purposes, some are mentioned below:

1. **Storage:** In the absence of urgent demand of glucose i.e. energy in the body, the excess glucose may be deposited as glycogen in the liver via process called as **glycogenesis.** Since the amount of glucose stored in the liver is limited, excess of glucose is also converted to fatty acids and stored as triglycerides in the fat depots.
2. **Oxidation:** Glucose is completely oxidized in all tissues to produce CO_2 and H_2O for physiological demands of energy. But under special circumstances in muscle (anaerobic conditions) there is only partial degradation of glucose forming lactic acid.
3. **Conversion to fat:** Glucose is converted to fatty acids when glycogen storage is exceeded. The conversion of glucose to fatty acid is irreversible but transformation of glucose to glycerol is reversible.
4. **Conversion to amino acids:** Glucose also produce certain amino acids during metabolic processes. These amino acids are said to be **glucogenic amino acids** i.e. derived from glucose.

Q. Write a note on Glycolysis, OR Describe in brief how the body gets the energy from glucose.

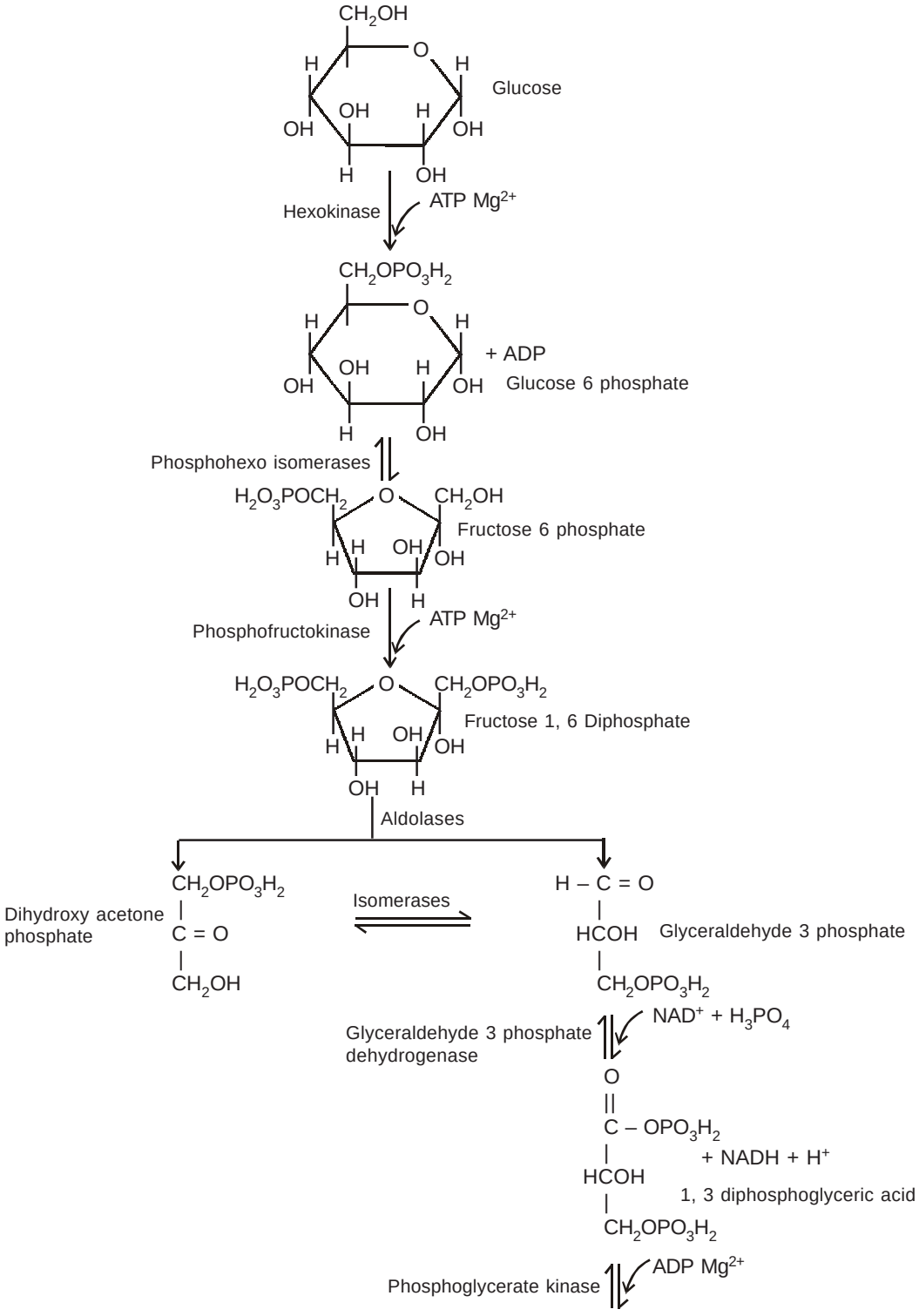
Ans. D-glucose is the major fuel of most organisms and occupies a central position in metabolism. The sequence of reactions by which glucose is degraded anaerobically is called as glycolysis or glycolytic sequence. This refers to the production of two molecules of lactic acid from one mole of glucose.

$$C_6H_{12}O_6 \xrightarrow{\text{Glycolysis}} 2CH_3CHOHCOOH$$

Monosaccharides other than glucose can be broken down by glycolysis provided they can be converted into an intermediate in that sequence. Energy is released in the form of ATP as the monosaccharide is degraded and several important metabolites are produced for use elsewhere in the intermediary metabolism.

Reactions of the glycolytic sequence

Ten reactions are involved in the conversion of glucose to pyruvic acid ; these are divided into two groups. The first four reactions are concerned with converting glucose into a compound D – glyceraldehyde 3 – phosphate, whose oxidation subsequently releases energy to the cell. In contrast, the four reactions of the preparative phase require an expenditure of energy as the glucose molecule is phosphorylated prior to the formation of the glyceraldehyde 3 phosphate.


CH2OH
O
H
H
OH
H
OH
H
OH
H
OH
Glucose
Hexokinase
ATP Mg2+
CH2OPO3H2
+ ADP
Glucose 6 phosphate
Phosphohexo isomerases
H2O3POCH2
CH2OH
Fructose 6 phosphate
Phosphofructokinase
ATP Mg2+
H2O3POCH2
CH2OPO3H2
Fructose 1, 6 Diphosphate
Aldolases
CH2OPO3H2
Dihydroxy acetone phosphate
C = O
CH2OH
Isomerases
H – C = O
HCOH
Glyceraldehyde 3 phosphate
CH2OPO3H2
Glyceraldehyde 3 phosphate dehydrogenase
NAD+ + H3PO4
O
C – OPO3H2
+ NADH + H+
HCOH
1, 3 diphosphoglyceric acid
CH2OPO3H2
Phosphoglycerate kinase
ADP Mg2+

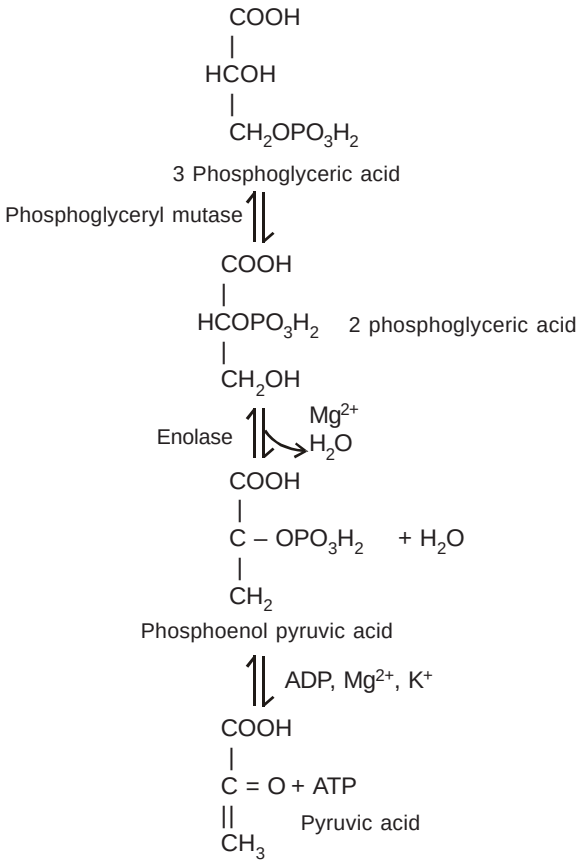


Fig. 9.1. Steps of glycolysis.

1. The initial step in the utilization of glucose in glycolysis is its phosphorylation by ATP to yield glucose 6 phosphate in the presence of hexokinase.
   In liver, instead of hexokinase another enzyme glucokinase is present. The activity of glucokinase is affected by nutritional status. This step is irreversible.
2. The next reaction in glycolysis is the isomerization of glucose 6 phosphate catalyzed by phosphoglucoisomerase.
3. Fructose 6 phosphate is phosphorylated with ATP by phosphofructokinase to form fructose1, 6 diphosphate. The enzyme require Mg^{2+} and is a irreversible reaction.
4. The next reaction in the glycolytic sequence involves the cleavage of fructose 1, 6 diphosphate to form the two triose phosphate sugars i.e. dihydroxy acetone phosphate and D-glyceraldehyde 3 phosphate. The finding of this enzyme in high concentration in a particular tissue is indicative of a functioning glycolysis pathway.
5. The production of D-glyceraldehyde 3 phosphate in the aldolase reaction technically completes the preparative phase of glycolysis. The second or energy yielding phase involves the phase involves the oxidation of glyceraldehyde 3 phosphate. Only half of the glucose molecule has been converted to D – glyceraldehyde 3 phosphates in above reactions.
   If cells were unable to convert dihydroxyacetone phosphate to glyceraldehyde 3 phosphate, half

of the glucose molecule would accumulate in the cells as the ketose phosphate or be disposed of by other reactions.

6. This next reaction is the first in energy yielding or second phase of glycolysis, and is also the first reaction in the glycolytic sequence to involve oxidation - reduction. It is also the first reaction in which a high energy phosphate compound has been formed.
7. The following reaction accomplishes the transfer of the phosphate from the acyl phosphate formed in the preceding reaction to ADP to form ATP. In this reaction, the acyl phosphate group has been utilized to drive the phosphorylation of ADP and make ATP.
8. Phosphoglyceryl mutase catalyze the interconversion of the two phosphoglyceric acids.
9. The next reaction in the degradation of glucose involves the dehydration of 2 phosphoglyceric acid to produce phosphoenol pyruvic acid, a compound with a high energy phosphate group.
10. Pyruvate kinase catalyze the transfer of phosphate from phosphoenol pyruvic acid to ADP produce ATP and pyruvic acid.

Production of ATP

There is a net formation of high energy phosphate in the form of ATP in glycolysis (Fig. 9.2)

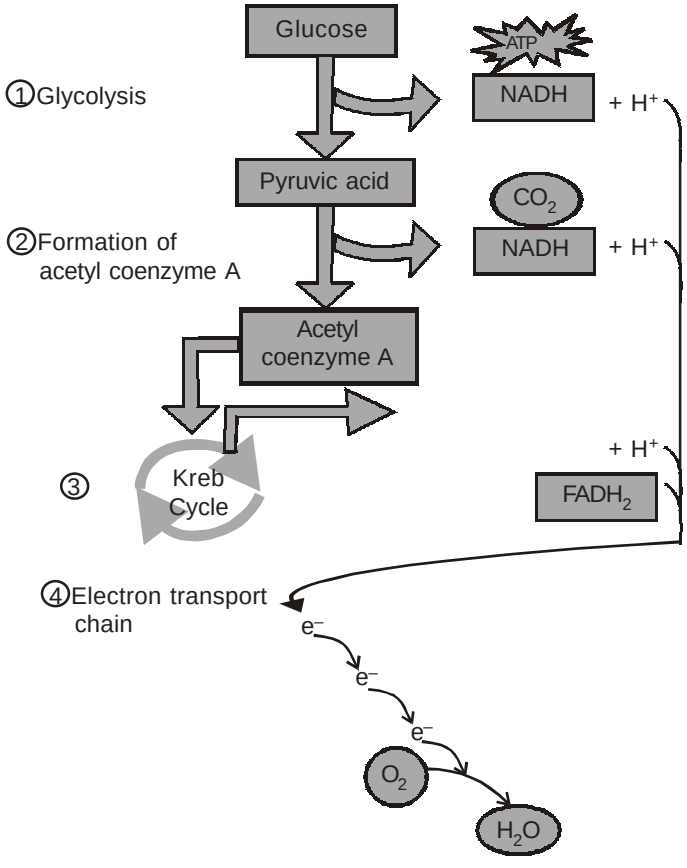


Fig. 9.2. Net gain of two ATP and NADH + $2H^+$

The total number of ATP formed are as follows—

Reactions	*ATP formed*
Glyceraldehyde 3 phosphate → 1, 3 Diphosphoglycerate	6
1, 3 Diphosphoglycerate → 3 Phosphoglycerate	2
Phosphoenol pyruvate → Enol pyruvate	2
Total	10

But in glycolysis there is net expenditure of 2 ATP molecules.

Reactions	*ATP expenditure*
Glucose → Glucose 6 phosphate	1
Fructose 6 phosphate → Fructose 1, 6 diphosphate	1
Net ATP synthesized	10 – 2 = 8 ATP

Q. Mention the factors which act as inhibitors of glycolysis.

Ans. There are different inhibitors which inhibit the enzymes involved in the glycolytic sequence or interfere with the mechanism of reactions. For example, Iodoacetate is the inhibitor of glyceraldehyde 3 phosphate dehydrogenase involved in the conversion of glyceraldehyde 3 phosphate to 1, 3 diphosphoglycerate. Arsenate inhibits the synthesis of ATP by accomplishing incoupling of oxidation and phosphorylation in the conversion of 1, 3 diphosphoglycerate to 3 phosphoglycerate. Fluoride inhibit enolase involved in the conversion of 3–phosphoglycerate to 2 phosphoglycerate.

Q. Enumerate the effect of hormones in glycolysis.

Ans. Insulin and glucagon hormones are two important hormones, which greatly influences the overall carbohydrate metabolism. Insulin stimulates hexokinase and glucokinase, which catalyze the conversion of glucose-to-glucose 6 phosphate. It also stimulates phosphofructokinases, which catalyze the conversion of fructose 6 phosphate to fructose 1, 6 diphosphate. Glucagon stimulates liver glucose 6 phosphatase which is involved in the conversion of glucose 6 phosphate to glucose and also fructose 1, 6 diphosphatase involved in the conversion of fructose 1, 6 diphosphate to fructose 6 phosphate.

Q. Describe how the process of glycolysis is regulated.

Ans. Glycolysis is regulated in all cells so that energy is released from carbohydrates only as it is needed by those cells. Some tissues that possess not only the capacity to convert glucose to lactate by glycolysis, but also can oxidize pyruvic acid completely to CO_2 and H_2O via the Krebs cycle. Such tissues utilize glucose much more rapidly in the absence of O_2 than when they do when O_2 is present. The functional significance of this inhibition of glucose consumption by oxygen is known as **Pasteur effect.** This is appreciated when much more energy is made available as ATP when glucose is oxidized aerobically to CO_2 and H_2O than when it is anaerobically converted only to lactic acid or alcohol and CO_2. Since more ATP is formed under aerobic conditions, less glucose is needed to be consumed to do the same amount of work in the cell.

Glycolysis in muscle is regulated at the reactions catalyzed by the following enzymes.

(a) Phosphorylase (for glycogen) and hexokinase (for glucose).

(b) Phosphofructokinase and pyruvate kinase.

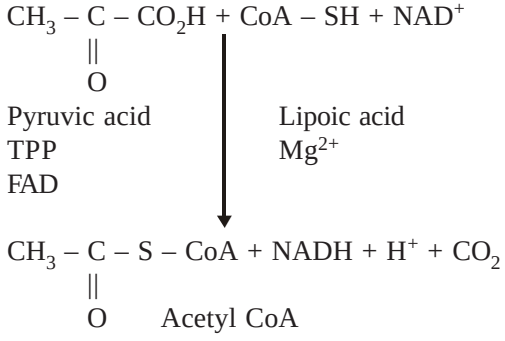
In a reciprocal manner, **gluconeogenesis** in which carbon flows back into carbohydrate from lactate or pyruvate, is regulated at the steps catalyzed by pyruvic carboxylase, fructose 1, 6 diphosphatase, glucose 6 phosphatase and glycogen synthetase. The regulation occurs at those reactions in glycolysis and gluconeogenesis that are unidirectional. Thus, glycolysis is not only the main pathway for glucose metabolism leading to the formation of acetyl CoA and oxidation in the citric acid cycle, but it also provides the principal pathway for the metabolism of fructose and galactose derived from the diet. It produces ATP in absence of oxygen because this allows skeletal muscle to perform efficiently when aerobic oxidation becomes insufficient and it allows tissues with significant glycolytic ability.

Q. Describe the fate of Pyruvate under aerobic conditions and in anaerobic conditions.

Ans. The pyruvic acid is not an intermediate in the tricarboxylic acid cycle. After completion of glycolysis, the pyruvic acid formed is converted to acetyl Co A under aerobic conditions. The process is enzymatic. But if there is no oxygen available this pyruvic acid ids converted to lactic acid.

The conversion of pyruvate to acetyl Co A is described as—

The α keto acid is first converted to acetyl CoA by the multienzyme complex known as pyruvic dehydrogenase complex. This conversion which is known as α - oxidative decarboxylation is carried on in the mitochondria following the formation of pyruvic acid in the cytosol during glycolysis. The reaction involves six cofactors coenzyme A, NAD^+, lipoic acid, FAD, Mg^{2+} and thiamine pyrophosphate.



This is an irreversible step. The pyruvate dehydrogenase complex is constituted of three enzymes:

(1) Pyruvate dehydrogenase: It brings about decarboxylation of pyruvic acid.
(2) Dihydrolipoyl transacetylase: S acetyl lipoate reacts with coenzyme A to form acetyl CoA and reduced lipoate in presence of this enzyme.
(3) Dihydrolipoyl dehydrogenases: The reduced lipoate is reoxidized by FAD in presence of this enzyme.

Q. Describe the Tricarboxylic Acid Cycle.

Ans. The tricarboxylic acid or citric acid cycle is the process in which acetate (in the form of acetyl CoA) is oxidized completely to CO_2 and water. Since acetyl CoA is readily produced from pyruvate, the cycle is also the process in which the oxidation of glucose to CO_2 and H_2O is completed. The electrons are removed from the substrates as they are oxidized and transferred eventually to molecular oxygen; thus the process is aerobic one.

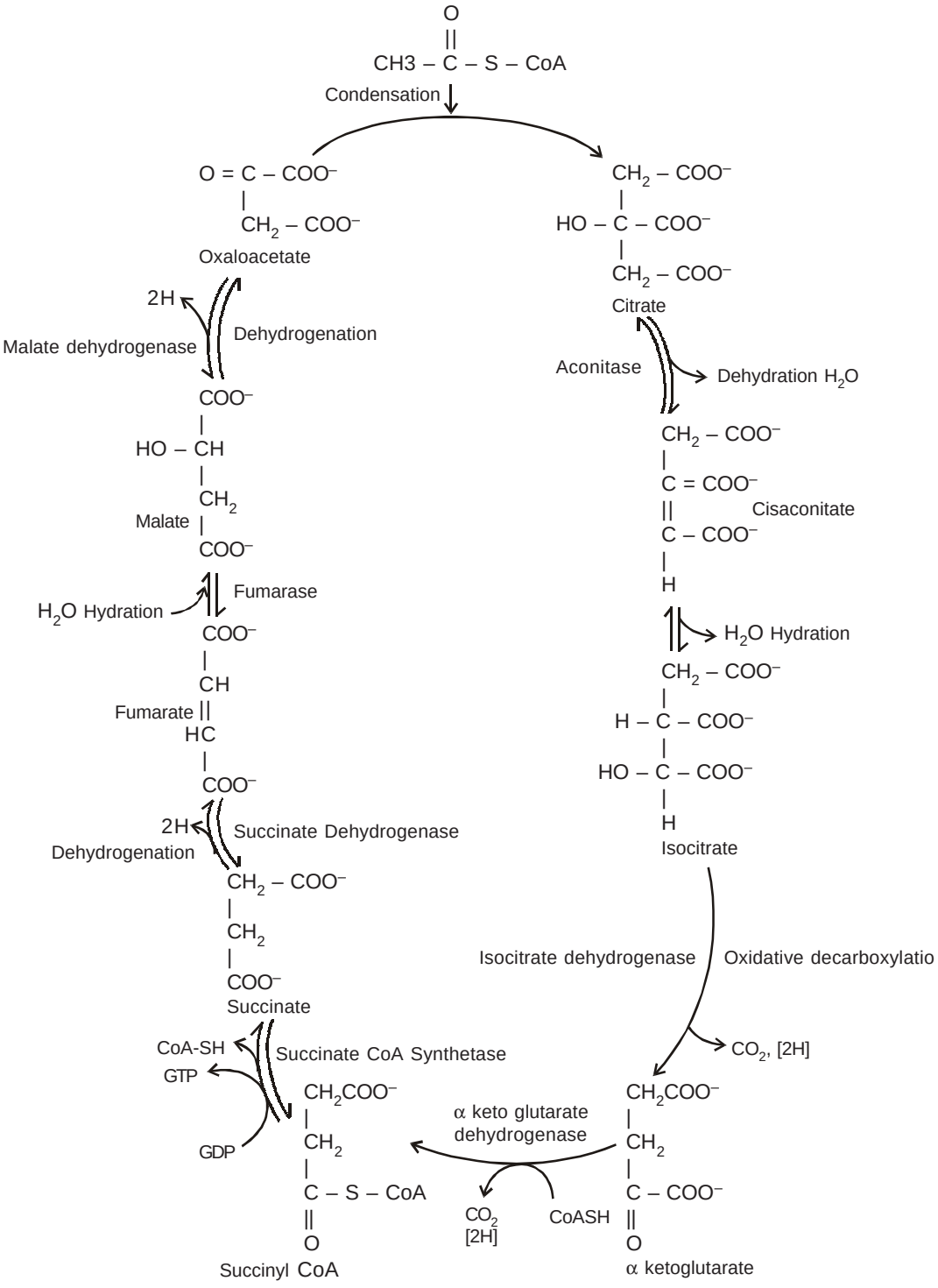


Fig. 9.3. The Tricarboxylic acid cycle.

The reactions of cycle, which accounted for the oxidation of pyruvic acid to CO_2 and H_2O were contributed by distinguished English biochemist Sir Hans Kreb, and so the cycle is also called as Kreb cycle.

Reactions of Tricarboxylic acid cycle

1. The synthesis of citric acid from acetyl CoA is the first reaction in the Krebs cycle. This reaction is catalyzed by citrate synthetase, and it is found in the matrix of mitochondrion.
2. The reaction of interest that is catalyzed by aconitase is the interconversion of citric acid to isocitric acid.
3. Isocitric acid dehydrogenase catalyze the oxidative β - decarboxylation of isocitric acid to β - ketoglutaric acid and CO_2 in the presence of a divalent cation (Mg^{2+} or Mn^{2+}), and a nicotinamide nucleotide as the oxidant.
   The evidence, however indicates that oxalosuccinate, if formed is firmly bound to the surface of the enzyme and is not released as a free intermediate in either the oxidative decarboxylation of isocitrate or the reverse reaction, the reductive carboxylation of α - ketoglutarate.
4. The next step of tricarboxylic acid cycle involves the formation of succinyl CoA by the oxidative α - decarboxylation of α ketoglutaric acid. This reaction is catalyzed by the a ketoglutaric dehydrogenase complex which require TPP, Mg^{2+}, NAD^+, FAD, lipoic acid and coenzyme A as cofactors.
   The reaction as a whole is not readily reversible because of the decarboxylation step. Succinyl CoA and NADH, produced in the reaction, are inhibitory to the enzyme that produces them.
5. In the preceding reaction the high energy bond of thioester has been formed as the result of an oxidative decarboxylation. The enzyme succinic thiokinase catalyzes the formation of a high energy phosphate structure at the expense of the thioester.
6. Succinic dehydrogenase catalyzes the removal of two hydrogen atoms from succinic acid to fumaric acid
7. The next reaction is the addition of H_2O to fumaric acid to form L – malic acid.
8. The tricarboxylic acid cycle is completed with the oxidation of L-malic acid to oxaloacetic acid which is accomplished by the enzyme malic dehydrogenase. The reaction is the fourth oxidation reduction reaction to be encountered in the cycle.

Features of the Tricarboxylic acid cycle

The balanced equation for the complete oxidation of pyruvic to CO_2 and H_2O may be written as

$$CH_3 - \underset{\underset{O}{\|}}{C} - COOH + 2\,½ \xrightarrow{O_2} 3CO_2 + 2H_2O$$

(*a*) In the overall cycle there are five oxidation steps. In each of these a pair of hydrogen atoms are removed from the substrate and transferred to either a nicotinamide coenzyme or a flavin coenzyme. The reoxidation of these reduced coenzymes five in all, by means of the cytochrome electron transport system results in the reduction of five atoms or 2 ½ moles of oxygen.

(*b*) When the five electron pairs are used to reduce O_2, 5 moles of H_2O are formed.

$$½\ O_2 + 2H^+ + 2e^- \longrightarrow H_2O$$

(*c*) Finally 3 moles of CO_2 are produced in the tricarboxylic acid cycle. These are equivalent to the three carbon atoms in the pyruvic acid. The CO_2 produced in reaction arises directly from the pyruvic acid, the other two CO_2 have as their origin the two carboxylic groups of oxaloacetic acid.

Production of ATP

The following steps summarize the net production of ATP in the TCA cycle:

Reaction	*No. of ATP formed*
Isocitrate → Oxalosuccinate	3
α Ketoglutarate → Succinyl CoA	3
Succinyl CoA → Succinate	1
Succinate → Fumarate	2
Malate → Oxaloacetate	3
Total	12

Total number of ATP produced in the complete oxidation of one molecule of glucose. One molecule of glucose forms 2 molecules of pyruvic acid by glycolysis.

(a) Number of ATP formed in glycolysis	8
(c) Number of ATP formed in the oxidation of pyruvate to acetyl CoA	3 × 2 = 6
(d) Number of ATP formed in the citric acid cycle	12 × 2 = 24
Total	38 ATP mol

Thus, therefore the overall reaction of aerobic respiration is

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 + 36 \text{ or ADP} + 36 \text{ or } 38\ \textcircled{P} \longrightarrow 6CO_2 + 6H_2O + 36 \text{ or } 38 \text{ ATPs}$$

Q. What are tbe factors which act as inhibitors of TCA cycle?

Ans. The inhibitors either inhibit the reactions or inhibit the enzymes involved in the TCA cycle. For example, Fluoroacetate inhibits the enzyme aconitase and prevents the conversion of citrate to isocitrate. Arsenite – inhibits α ketoglutarate dehydrogenase and causes α ketoglutarate to accumulate. Malonate or oxaloacetate inhibits succinate dehydrogenase competitively resulting in succinate accumulation.

Q. Why the TCA cycle is considered to be an important one in metabolism?

Ans. The major significance of the citric acid cycle is that it acts as the common metabolic pathway for the oxidation of carbohydrate, lipids and protein's because glucose, fatty acids and many amino acids are metabolized to acetyl CoA which is finally oxidized in the citric acid cycle. The reducing equivalents in the form of hydrogen or electrons are formed by the activity of specific dehydrogenases during the oxidation of acetyl CoA in the cycle. These reducing equivalents then enter

the respiratory chain, where large amounts of high energy phosphate bonds are generated by the oxidative phosphorylation. The enzymes of the citric acid cycle are located in the mitochondrial matrix either free or attached to the inner surface of the inner mitochondrial membrane which facilitates the transfer of reducing equivalents to the adjacent enzymes of the respiratory chain which is also situated in the inner mitochondrial membrane.

Thus citric acid cycle is amphibolic (dual) in nature which is the source for anabolic processes such as fatty acid and amino acid synthesis and gluconeogenesis.

Q. Write a note on the regulation of TCA cycle.

Ans. The regulation of kreb cycle is a complicated process. A continuous supply of oxidized NAD^+ is required to permit the Kreb cycle to operate. The enzymes of the electron transport chain carry out this vital activity and the concomitant process of oxidative phosphorylation. Where these process are inhibited, the Kreb cycle cannot function. Pyruvic dehydrogenase, which provides a supply of acetyl CoA for oxidation via the Krebs cycle is inhibited when the level of NADH or acetyl CoA builds up. Additional control may also be exerted by cyclic AMP produced when the concentration of ATP increases. Citrate synthase is under fine control . ATP and NADH can inhibit this initial reaction of the Kreb cycle. The inhibition of ATP and NADH on isocitric dehydrogenase have also been noted. Thus the energy charge of the cell can readily affect the rate at which the tricarboxylic acid cycle operates.

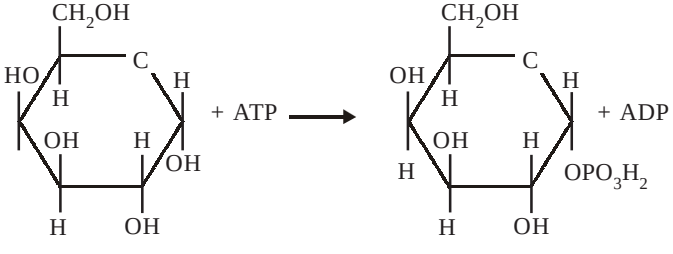
Q. Give a brief account of how the other carbohydrates are utilized in the body.

Ans. Sugars other than glucose are metabolized in the glycolytic sequence following their conversion by auxillary enzymes to intermediates in that sequence. Thus, fructose and mannose can be phosphorylated by ATP in presence of hexokinase and can be converted into fructose 6 phosphate and mannose 6 phosphate. The former is an intermediate in glycolysis, mannose 6 – phosphate is converted to fructose 6 phosphate and then enter glycolysis.

Dissacharides such as lactose and sucrose are extremely common sources of carbohydrate in the diet of animals. The initial step in their utilization involve hydrolysis to the component monosaccharides by specific glycosidases, lactase and invertase found in animals digestive tract.

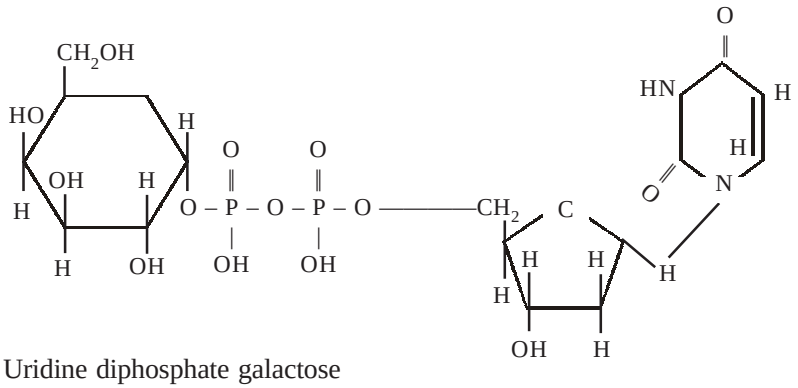
Q. Describe in brief how the galactose is metabolized in the body.

Ans. The metabolism of galactose in the body occurs by its conversion to glucose–1- phosphate, which then enters the glycolytic sequence in the body. The initial reaction with galactose involves phosphorylation by ATP in the presence of a specific galactokinase that produces galactose 1 phosphate.



Galactose Galactose 1 phosphate

Further metabolism of galactose 1 phosphate involves uridine triphosphate and a uracil derivative of that sugar known as uridine diphosphate galactose (UDP galactose)



Uridine diphosphate galactose
(UDP-galactose)

The galactose 1 phosphate formed is converted to UDP galactose by the enzyme UDP – galactose pyrophosphorylase

$$\underset{\text{phosphate}}{\text{Gal – P Galactose l}} + \underset{\text{UTP}}{\text{U – R – P – P – P}} \rightleftharpoons \underset{\text{UDP galactose}}{\text{U – R – P – P – }\boxed{\text{Gal}}} + \underset{\text{Pyrophosphate}}{\text{P – P}}$$

In the next step the galactose moiety in UDP – galactose is isomerized to a glucose moiety, thereby forming UDP glucose. The enzyme that catalyze this reaction is known as UDP – glucose epimerase.

$$\text{UDP – Galactose} \rightleftharpoons \text{UDP glucose}$$

Finally, the action of third enzyme UDP glucose pyrophosphorylase liberates the glucose (formerly the galctose) moiety from UDP – glucose as glucose 1 phosphate.

$$\underset{\text{UDP – glucose}}{\text{U – R – P – P – Glu}} + \underset{\text{UTP}}{\text{P – P}} \rightleftharpoons \text{U – R – P – P – P} + \underset{\text{Glucose 1, phosphate}}{\text{Glu – P}}$$

Thus, there is conversion of galactose to glucose 1 phosphate and it is the normal route for galactose metabolism in infants. This series of reactions has attracted much attention because of a hereditary disorder known as **galactosemia.** Infants that have this defect cannot metabolize galactose and they exihibit a high level of galactose in the blood. The sugar is excreted in the urine and if the condition is not attended to, the infants can develop catracts and may become mentally retarded. The simple remedy, once the condition is identified, is to remove the source of galactose, usually the milk in the infants diet and supply a galactose free diet.

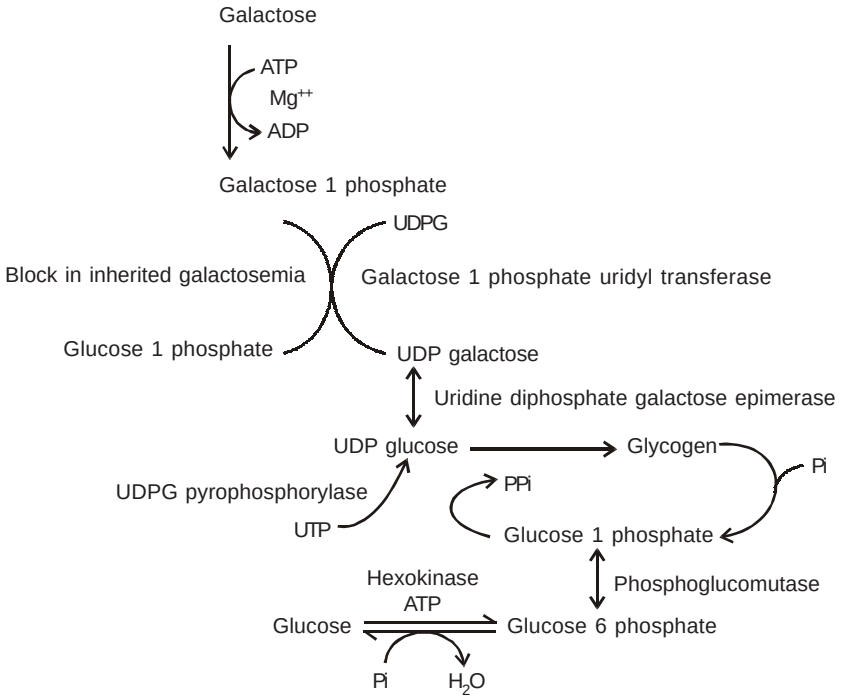
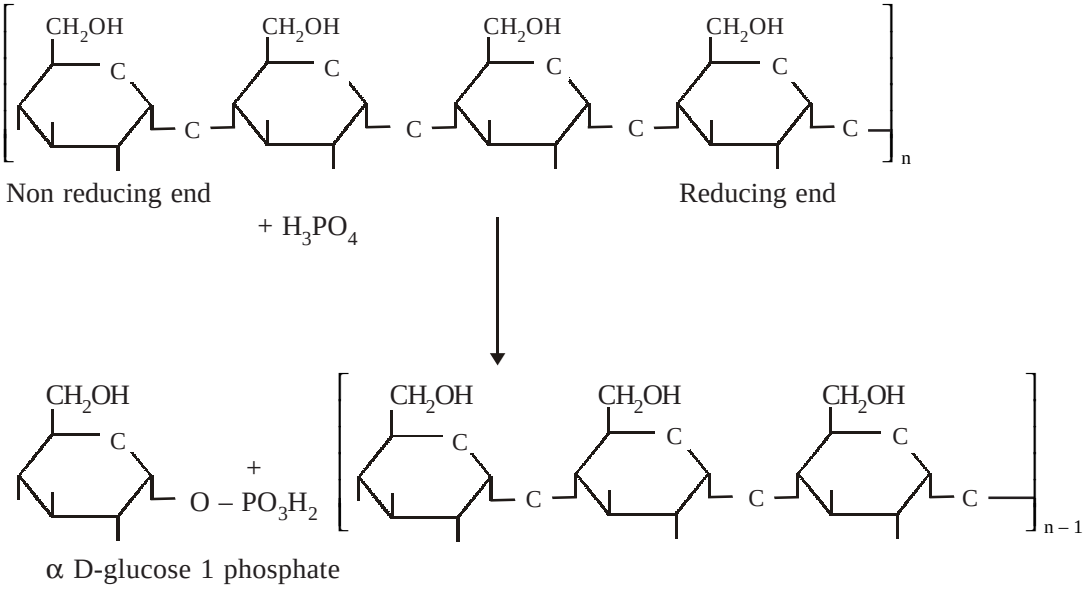


Fig. 9.4. Metabolism of galactose.

Q. Briefly describe how the different polysaccharides are metabolized in the body.

Ans. The polysaccharides, starch and glycogen are important fuel molecules. These are polymers of glucose and can be broken down to glucose by two different processes in order that they can be accomodated in the glycolytic sequence. In one process, the polysaccharide is hydrolyzed to produce ultimately D – glucose, which can be phosphorylated and metabolized in the glycolytic sequence. This pathway is used in the digestive tract where food polysaccharides are broken down via dextrins to maltose, isomaltose and glucose. These fragments can enter the mucosal cells where the disaccharides are split by maltose and isomaltase to glucose. The monosaccharides can be absorbed into the portal blood and thus be admitted to the cells. In the cell the glucose is phosphorylated and can be catabolized in the glycolytic sequence.

The other process is phosphorylytic cleavage by which the fuel polysaccharides can be degraded through the action of phosphorylase enzymes. These enzymes catalyze the phosphorylytic cleavage of the α 1, 4 glucosidic linkages at the non-reducing end of the starch or glycogen chain. The reaction is reversible and is represented as



Phosphorylation will catalyze the stepwise removal of glucose units from a linear portion of a starch or glycogen molecule until it approaches within 4 units of an α–1–6 branch point. This branch point constitutes an area in which the enzyme is inactive.

Glycogenolysis

The breakdown of glycogen to glucose in the liver and pyruvate and lactate in the muscle is called as **glycogenolysis.** This is initiated by the enzyme phosphorylase. In liver phosphorylase exists in both active and inactive form. But physiologically it is active. It is a tetramer containing 4 mol. of pyridoxal phosphate. It is hydrolytically converted to phosphorylase b, a dimer by phosphorylase phosphatase. Phosphorylase b contains 2 mol of pyridoxal phosphate.

Phosphorylase a + $4H_2O$
(active)
↓ Phosphorylase phosphatases
2 phosphorylase b + 4Pi
(inactive)

Phosphorylase is specific for the phosphorylytic breaking of the α 1-4 linkages of glycogen to produce glucose 1 phosphate. The removal of 1-4 glucosyl residues remain on either side of α 1-6 branch.

The debranching enzyme causes the hydrolytic splitting of 1 – 6 linkages. By the combine action of these enzymes glycogen is converted to glucose 1 phosphate. The action of phosphoglucomutase is reversible and glucose 6 phosphate is formed from glucose 1 phosphate. In liver and kidney (but not in muscle) exists a specific enzyme, glucose 6 – phosphatase which removes phosphate from glucose 6 phosphate enabling the free glucose to diffuse from the cell into the surrounding extracellular spaces including the blood. The overall reaction is shown in Fig 9.5.

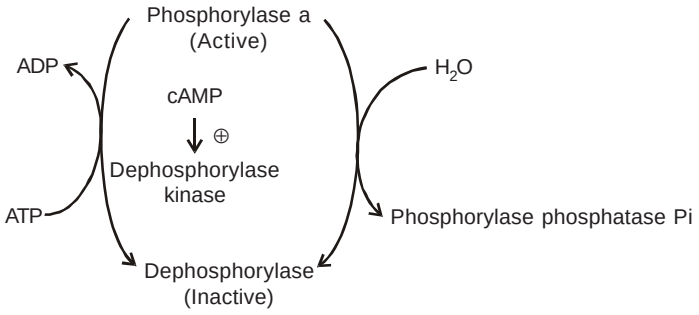


Fig. 9.5. Inactivation and reactivation of liver phosphorylase.

Q. Describe in brief the regulation of Glycogen Metabolism.

Ans. The control of glycogen metabolism by the enzyme phosphorylase and glycogen synthase is effected in animals tissues mainly through the interconversion of the active and inactive forms of these enzymes. The active phosphorylase can be inactivated by phosphorylase phosphataase to dephosphorylase. Reactivation takes place by the enzyme phosphorylase b kinase or dephosphorylase kinase in presence of ATP.

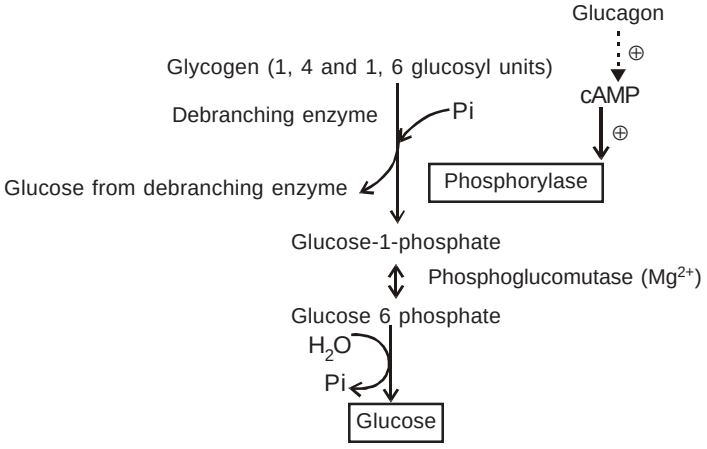


Fig. 9.6. Glycogenolysis in Liver.

Protein kinase exists in an inactive form, which in turn is activated by cyclic AMP. It has 4 subunits, two regulatory units (R) and two catalytic (C) units. Cyclic AMP binds directly with the R subunits, releasing C units, which now catalyze the phosphorylation of phosphorylase b kinase.

$$\underset{\text{Inactive kinase}}{R_2C_2} + 2cAMP \xrightarrow{Mg^{2+}} \underset{\text{Active kinase}}{R_2(cAMP)_2} + 2C$$

A similar cascade phenomenon exist for regulation of glycogen synthetase in muscle except that protein kinase is the enzyme that phosphorylates glycogen synthase I converting it to glycogen synthase D.

Q. Describe the main events in which there exist the reversal of the glycolytic sequence.

Ans. The main reactions where the reversal events of the glycolytic sequence occurs are as follows—

Reversal in photosynthesis

Organisms are capable of synthesizing carbohydrates from simple precursors by, in effect, reversing the glycolytic sequence. The most important example of reversal is that part of photosynthesis in which CO_2 is utilized to make the storage polysaccharides of plants. The reactions of reduction phase and several reactions of the regeneration phase of the CO_2 reduction cycle are identical with some reversible reactions of glycolysis.

Conversion of propionate to succinyl CoA

Propionate in ruminants enters the main glucogenic pathway via the citric acid cycle being converted to succinyl CoA This includes following steps.

(1) Propionate is first activated by thiokinase with ATP and CoA to form propionyl CoA.
(2) Propionyl CoA undergoes CO_2 fixation reaction to form D methyl malonyl CoA. This reaction is catalyzed by propionyl CoA carboxylase and biotin as coenzyme.
(3) D – methyl malonyl CoA is converted to L methylmalonyl CoA by enzyme methyl malonyl CoA racemase.
(4) L – methyl malonyl CoA is isomerised to succinyl CoA by methyl malonyl CoA isomerase which require vitamin B_{12} as a coenzyme.

Conversion of fatty acids to glucose

Fatty acids are metabolized to acetyl CoA by β – oxidation. Acetyl CoA enters the citric acid cycle and then converted to malate. Malate is diffused from the mitochondria to the extramitochondrial portion of the cell where it is finally converted to glucose by the enzymes involved in gluconeogenesis. Acetyl CoA is not permeable to pass from the mitochondria to the cytosol through the mitochondrial membrane. But citrate is permeable through mitochondrial membrane to pass to cytosol where it is splitted to acetyl CoA and oxaloacetate.

Conversion of aminoacids to glucose

The glucogenic amino acids are converted to the intermediates of the citric acid cycle either by transamination or deamination. These intermediates are converted to malate and finally converted to glucose by the enzymes involved in gluconeogenesis.

Conversion of glycerol

Glycerol is first converted to glycerol 3 phosphate by glycerokinase with ATP in liver and kidney. Glycerol 3 phosphate is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol 3 phosphate dehydrogenase in presence of NAD^+. DHAP is then converted to glucose.

Q. Describe Gluconeogenesis.

Ans. Gluconeogenesis is the formation of new glucose molecule from a non carbohydrate source. It may also be defined as the regeneration of glucose and glycogen from non carbohydrate sources, such as lactic acid, amino acids and glycerols. A continuous supply of glucose is necessary as a source of energy and glucose is the only fuel which supplies energy to skeletal muscles in anaerobic conditions. When the carbohydrate is insufficient in the diet, gluconeogenesis meets the needs of the body for glucose. In mammals, the liver and kidney are the principal organs responsible for gluconeogenesis.

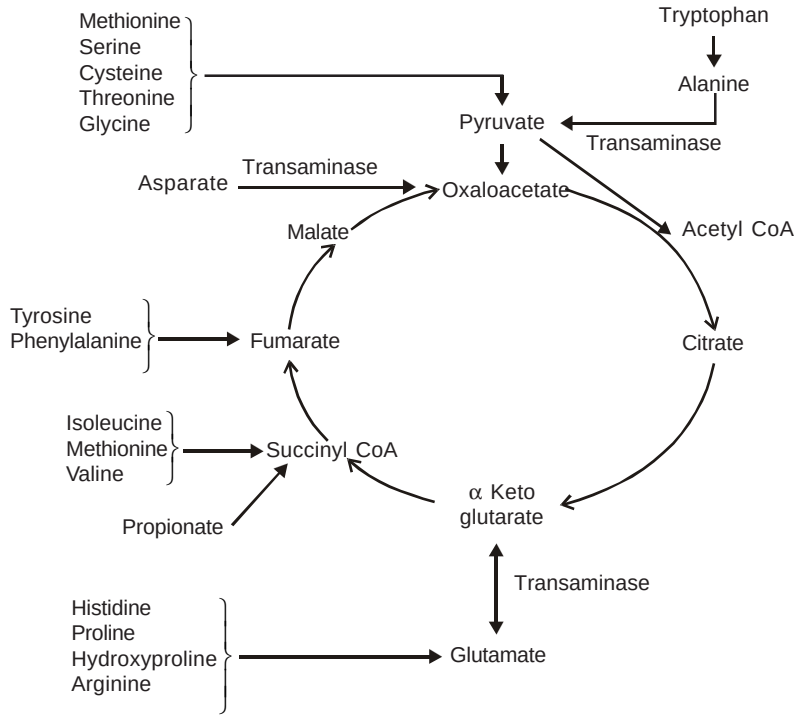


Fig. 9.7. Conversion of amino acids to the intermediates of TCA cycle by transamination or deamination.

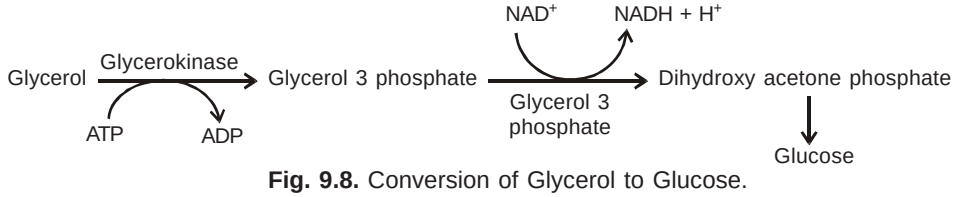


Fig. 9.8. Conversion of Glycerol to Glucose.

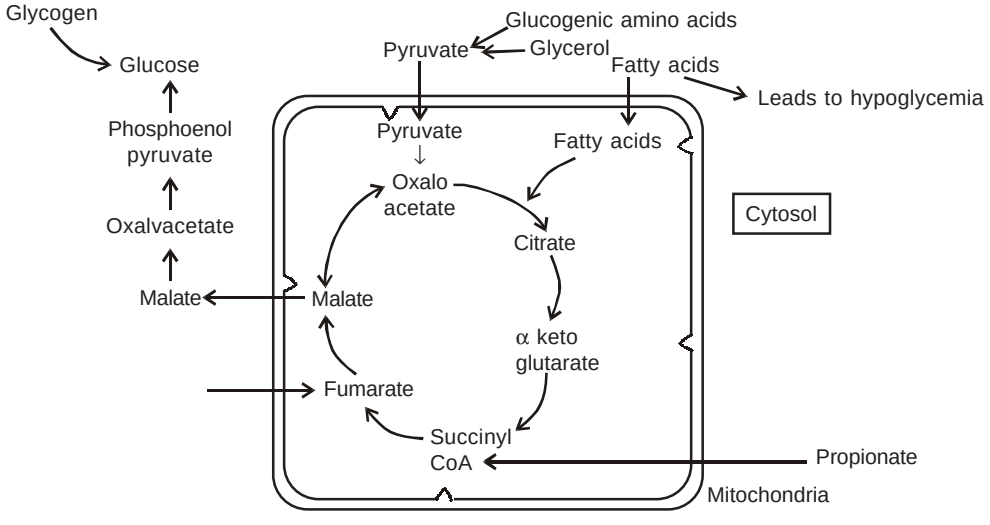


Fig. 9.9. Gluconeogenesis.

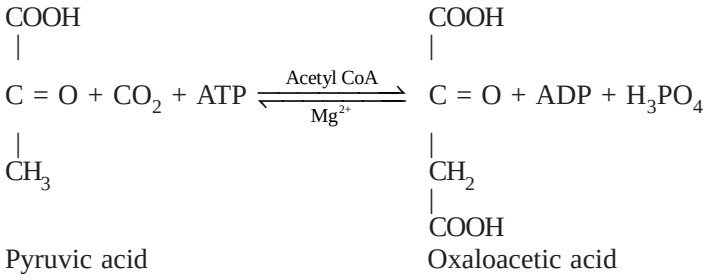
Metabolic pathways in gluconeogenesis

The metabolic pathways in connection with gluconeogenesis are the modifications of the Embden – Meyerhof pathway and the citric acid cycle. They are concerned with the conversion of glucogenic amino acids, lactate, glycerol, propionate to glucose or glycogen. Gluconeogenesis can also occur from compounds other than lactic acid. Pyruvic acid and oxaloacetate an intermediate of the tricarboxylic acid cycle, can be converted to glucose. This occurs however in animals only during starvation or under other fasting conditions, when an organism urgently requires glucose and has no other source of carbon than proteins. Regardless of the precursors, all of these compounds must 'by pass' the energy barriers which obstruct a simple reversal of glycolysis. These are

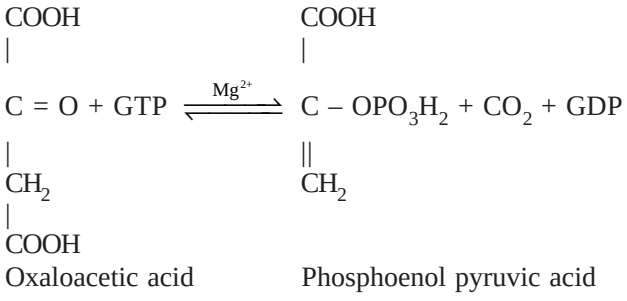
(1) between pyruvate and phosphoenol pyruvate
(2) between fructose 1, 6 diphosphate and fructose 6 phosphate
(3) between glucose 6 phosphate and glucose
(4) between glucose 1 phosphate and glycogen

These barriers are overcome by some enzymes during the following reactions.

(1) The enzyme pyruvate carboxylase present in mitochondria converts pyruvate to oxaloacetate in presence of ATP, biotin and CO_2.

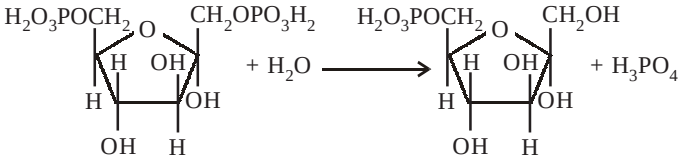


The second enzyme involved in reversing this part of glycolytic sequence is known as phosphoenol pyruvate carboxykinase (PEP). In this reaction oxaloacetate produced in above equation is converted to phosphoenol pyruvic acid, by a reaction involving little change in free energy, but one in which CO_2 is produced, a reverse CO_2 fixation.



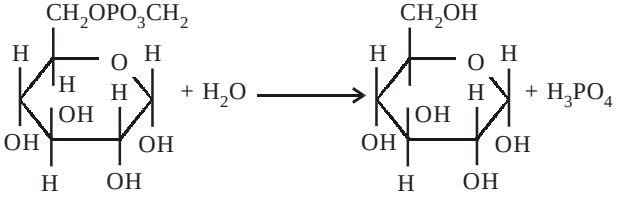
Lactate with the help of these two enzymes and dehydrogenases is converted to PEP. But oxaloacetic acid does not diffuses readily from mitochondria. Alternative means are applied to convert oxaloacetate to malate, which is readily diffused from mitochondria. Malate is then converted to oxaloacetate in the extramitochondrial portion of the cell.

1. The energy barriers between fructose 1, 6 diphosphate to fructose 6 phosphate is catalyzed by fructose 1, 6 diphosphatases. This enzyme is present in liver, kidney and striated muscle but absent from adipose tissue, heart muscle and smooth muscle.



Fructose 1, 6 diphosphate Fructose 6 phosphate

2. The glucose 6 phosphate is converted to glucose by glucose 6 phosphatases which is present in liver, intestine, kidney but absent from muscle and adipose tissue.



Glucose 6 phosphate Glucose

3. The conversion of glucose 1 phosphate to glycogen is mediated by UDPG and glycogen synthetase.

Starting with 2 molecules of lactate and proceeding through the reactions, we can write the overall equation accounting for the reversal of glycolysis.

$$2\text{Lactate} + 4\text{ATP} + 2\text{GTP} + 6H_2O \longrightarrow \text{Glucose} + 4\text{ADP} + 2\text{GDP} + 6H_3PO_4$$

From this it is apparent that a total of six energy rich phosphates are required to make glucose. This equation is not clearly the reverse of glycolysis in which glucose was converted to 2 moles of lactate and serves again to emphasize the energy relationship of the glycotic sequence.

Q. Define Cori cycle.

Ans. Lactic acid is the major end product in muscle in anaerobic glycolysis. Muscle tissue is incapable of resynthesizing glucose from lactate. The conversion takes place entirely in the liver. Muscle lactate is transported to the liver via blood. In the liver, it is converted to glucose and glycogen by the enzymes concerned in gluconeogenesis. Liver glycogen is converted to glucose, which is carried back to muscle by blood. This conversion of muscle lactate to glucose in liver and its reentry into muscle is called as Cori Cycle.

Q. Describe HMP Shunt.

Ans. The Embden meyerhof pathway is a mechanism for partially degrading glucose and obtaining energy as ATP for the cell. This sequence undoubtedly was the first of several metabolic processes to arise and meet the needs of evolving life forms. As organisms become more complex,

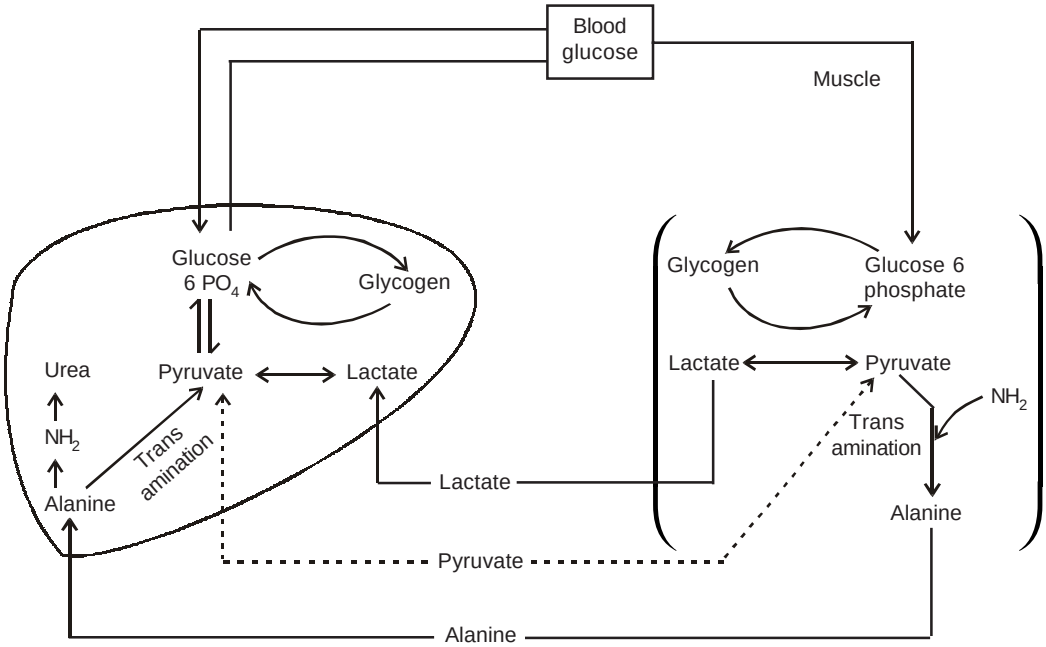


Fig. 9.10. Cori cycle.

there developed a need for biosynthetic capacities beyond those represented by intermediates of the glycolytic sequence and specifically the need for a source of reducing power in biosynthesis developed. Since the reducing agent NADH produced in one part of the glycolytic scheme is consumed in another part, reactions that were capable of producing a different reductant were presumably selected. The pentose phosphate pathway contains two reactions capable of producing the reductant NADPH. This pathway also produces a number of different sugar phosphates.

Thus, this is an alternate aerobic pathway for the oxidation of glucose in the liver, lactating mammary gland and adipose tissues. The enzymes of this pathway are present in the extramitochondrial portion of the cell. The reaction takes place in two phases.

(1) Glucose 6 phosphate by dehydrogenation and decarboxylation give rise to ribulose 5 phosphate.
(2) Ribulose 5 phosphate is converted back to glucose 6 phosphate by transkelotase and transaldolase.

Significance of the Pentose Phosphate Pathway

CO_2 is the characteristic product in this pathway and it is utilized for the synthesis of fatty acids and purine bases etc. The reduced NADP, (NADPH) formed in this pathway are used in the synthesis of fatty acids, cholesterol, steroids and amino acids. The reduced NADP is also required in uronic acid pathway for the synthesis of ascorbic acid and xylulose 5 phosphate. The reduced NADP also function in the operation of the shunt pathway in red blood cells and it has direct correlation between the glucose 6 phosphate dehydrogenase and fragility of red cells when the cells are subjected to certain drugs as sulfonamide and primaquine. The pentose sugar produced in this pathway are utilized for the synthesis of nucleic acids and nucleotides.

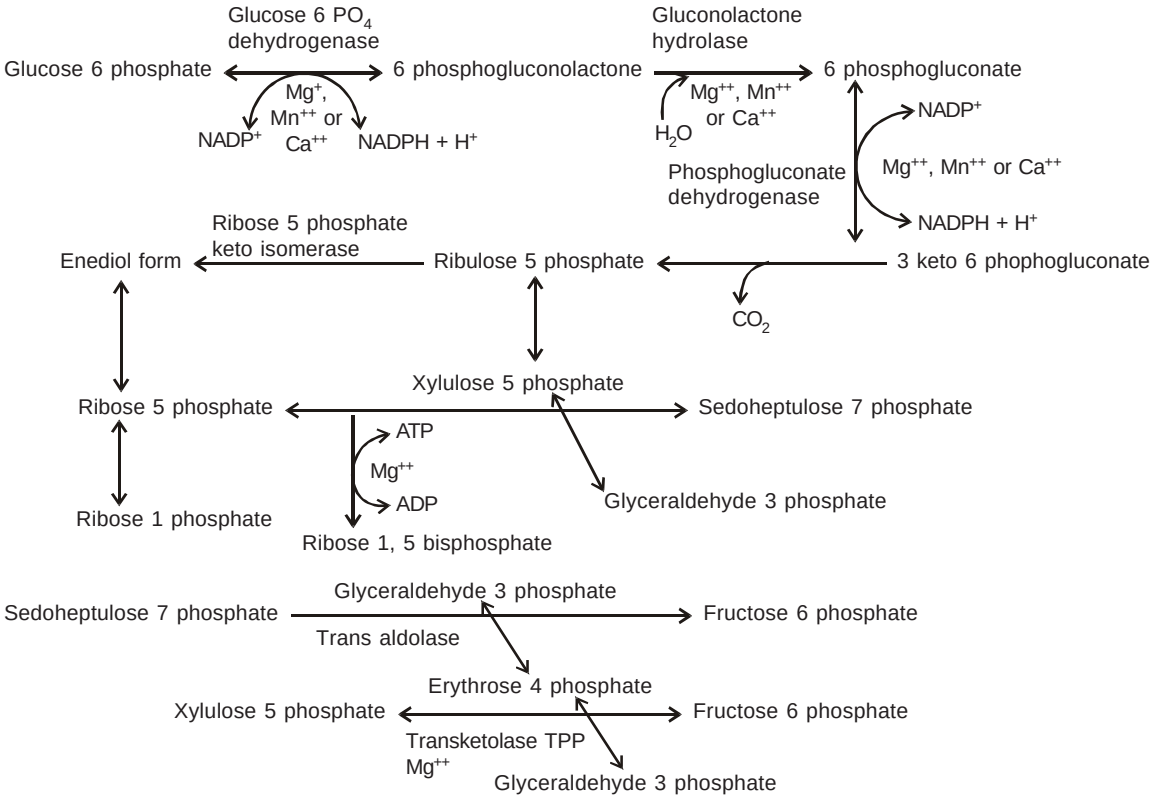


Fig. 9.11. Flow chart of HMP Shunt or Pentose phosphate pathway.

Q. Describe the Uronic Acid Pathway.

Ans. This pathway is the alternative oxidative pathway for glucose. Glucose is converted to glucuronic acid, ascorbic acid and pentoses.

The metabolic reaction involves the following steps.

1. Glucose 6 phosphate is converted to glucose 1 phosphate by phosphoglucomutase. The product then reacts with UTP to form UDPG by the help of enzyme UDPG pyrophosphorylase.
2. UDPG is oxidized to uridine diphosphoglucuronic acid by UDPG dehydrogenase in presence of NAD^+. UDP glucuronic acid is the active form of glucuronic acid for reactions involving incorporation of glucuronic acid into chondroitin sulfate.
3. Glucuronic acid is reduced to L – gluonic in the presence of reduced NADP. This L – gluonic acid is the precursor of ascorbic acid.
4. In man and other primates, L – gluonic acid is oxidized to 3 keto L – xylulose acid which is then decarboxylated to L – xylulose. L – xylulose is converted to D – xylulose in presence of reduced NADP. D – xylulose being converted to D xylulose 5 phophate by ATP is further metabolized in the HMP shunt.

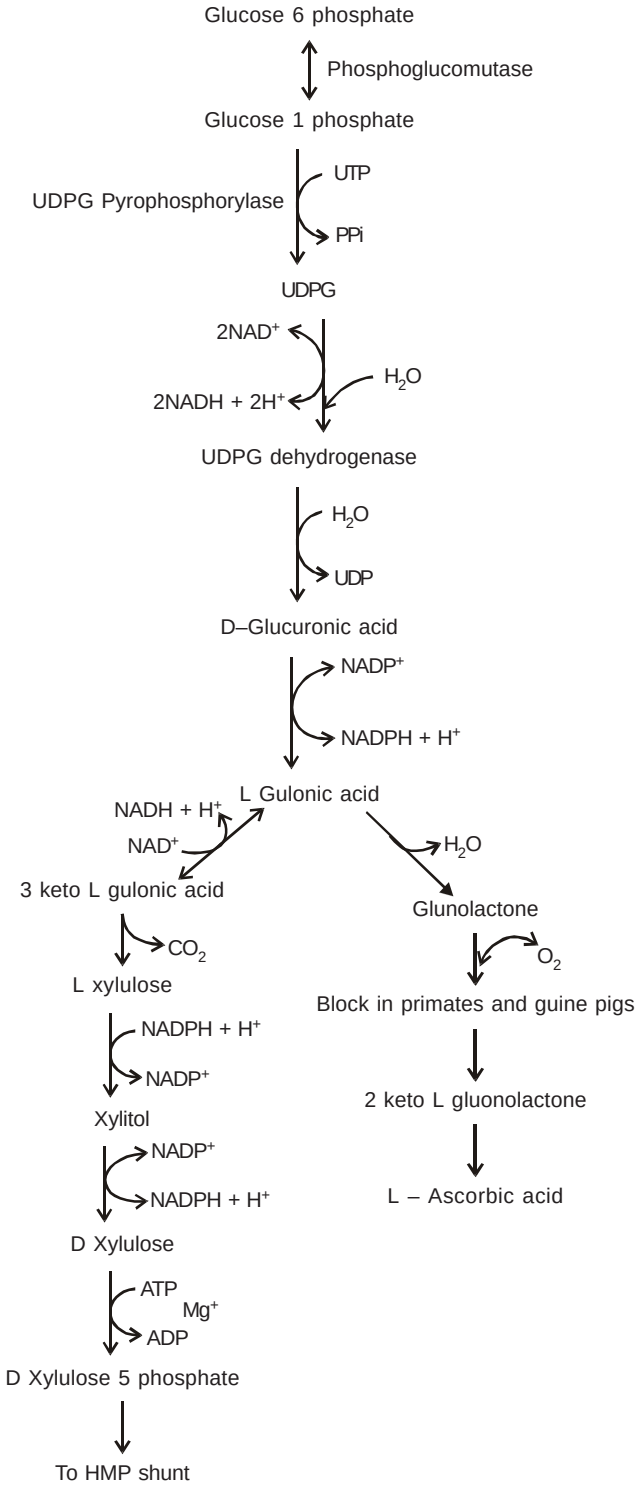


Fig. 9.12. Flow chart of uronic acid pathway.

Q. Describe the regulation of Glucose Concentration in Body. OR How the blood glucose concentration in the body is regulated.

Ans. In the resting state, the true glucose present in the blood is 60 – 100 mg/100 ml but this value is higher i.e. 80 – 120 mg/100 ml in non resting state. The condition in which the blood sugar is raised above the normal range is called **hyperglycemia** and when it goes below the normal level it is called **hypoglycemia.** The concentration of glucose in the blood depends on two general factors:

(1) The rate of its entrance into the blood.

(2) The rate of its removal from the blood stream.

Sources of blood glucose

Mainly the dietary food is responsible for high blood glucose level. Most carbohydrates in the diet after digestion form glucose, galactose or fructose, which are absorbed in the portal vein. Different glucogenic compounds are also responsible for increasing the blood glucose concentration in the body For example lactic acid from the skeletal muscle is transported by blood to the liver and kidney where glucose is reformed under the process called as Cori cycle.

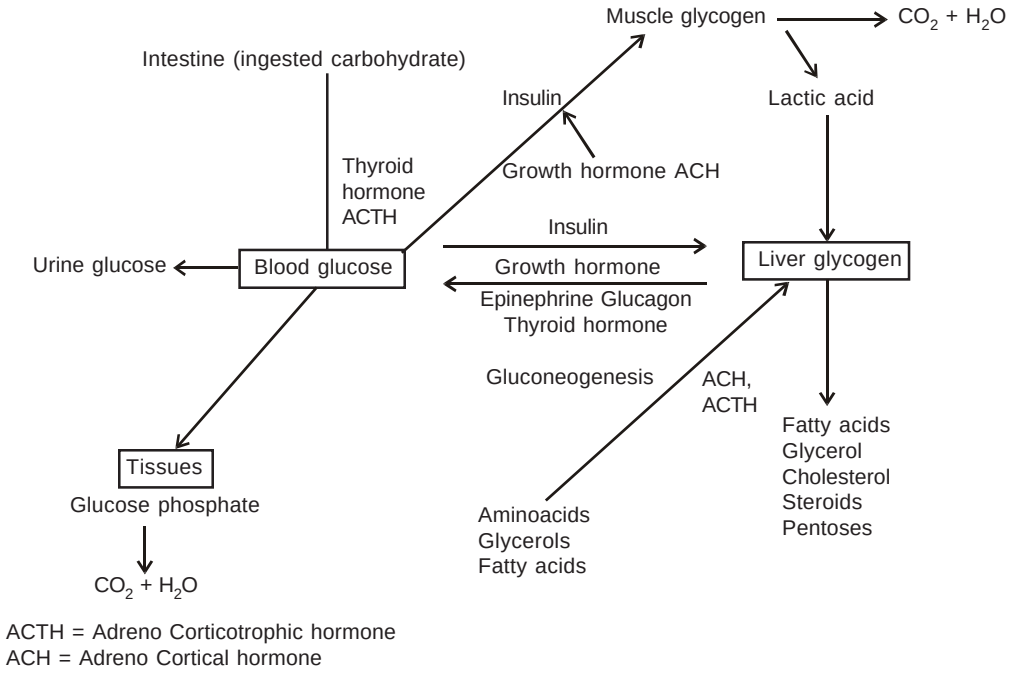


Fig. 9.13. Factors affecting blood glucose level in body.

In the adipose tissues, the synthesis of triacyl glycerol takes place from glycerol, which is derived initially from blood glucose. Acylglycerols of adipose tissue continually undergo hydrolysis to form free glycerol, which diffuses out of the tissue into the blood. This is converted back to glucose in the liver and kidney by gluconeogenesis. Hence a continuous cycle exists in which glucose is transported to adipose tissue from the liver and kidney and glycerol is returned to be synthesized into glucose by the liver and kidney. Glucose is also formed in the liver from glycogen by process of glycogenolysis. These all factors are mainly responsible for increasing the blood glucose concentration in the body.

But the stable glucose level is maintained by the liver, kidney, muscles and hormones.

Q. Describe the role of liver, kidney, muscles and different hormones in the regulation of blood sugar level.

Ans. ***Role of liver***

Liver is the chief organ in the body to maintain the normal glucose concentration. The glucose 6 phosphatases converts glucose 6 phosphate to glucose, which diffuses into the blood stream to form the constant and the only source of blood glucose unless and until glucose is available from the intestine from carbohydrate diet. The lactic acid of the muscle is transported via blood to liver and gets converted to glucose via Cori cycle. The liver cells, unlike other cells, require the oxidation of organic substances to maintain their own vital functioning. In the absence of fuel i.e. glucose, glycogen is diminished, the oxidation of fat occurs which in turn forms keto acids. Some of the keto acids are utilized for cellular energy. During glycogen crisis, the liver cells also utilize amino acids and protein to meet the energy demand of body via gluconeogenesis.

Role of kidney

The kidneys are able to form glucose by a number of carbohydrate intermediates as well as via gluconeogenesis. But its capacity to produce is less as compared to that of liver. When the blood glucose level exceeds the renal threshold level i.e. 160 – 180 mg/100 ml, the renal tubules are incapable of reabsorbing all the filtered sugar in glomeruli and the excess glucose is excreted in urine. This results in the decrease of blood glucose concentration.

Role of muscles

The extrahepatic tissues are relatively impermeable to glucose, therefore insulin is required for the uptake of glucose to these cells. Increased blood glucose promotes glycogenesis and oxidation of glucose in muscles. Muscle glycogen does not serve directly as a source of glucose during hypoglycemia. But glucose is supplied to the blood from muscle glycogen by Cori cycle or lactic acid cycle. However, muscular exercise promotes the entry of glucose into muscle cells and the glucose is utilized by the muscles. Thus it lowers the blood glucose level.

Role of Hormones

Different hormones play vital role in the maintenance of blood sugar level. They directly or indirectly helps in carbohydrate metabolism and thus affect the glucose level in the body.

Insulin

It is produced by the ß cells of the islets of Langerhans in the pancreas and is liberated into the blood by the direct response to hyperglycemia. It increases the rate of uptake of glucose to tissues and promotes glucogenesis by stimulating hexokinase and glycogen synthetase along with oxidation of glucose by stimulating phosphofructokinase. It also stimulates lipogenesis and protein synthesis. But it decreases hepatic glycogenolysis and gluconeogenesis and inhibit ketogenesis. Insulin is released by amino acids, free fatty acids, ketone bodies, glucagon, secretin and tolbutamide. Epinephrine and norepinephrine block the release of insulin.

Glucagon

It is produced by the a cells of the islets of Langerhans of pancreas being stimulated by hypoglycemia. It causes glycogenolysis by activating phosphorylase in the liver. It stimulates glucose 6 phosphatase in the liver to form glucose 6 phosphate.

Anterior pituitary hormones

The anterior pituitary gland secretes hormones that elevate the blood sugar level by antagonizing the action of insulin. They are growth hormones and ACTH. The growth hormone secretion is stimulated by hypoglycemia. It decreases glucose uptake in muscles and also mobilize free fatty acids from adipose tissue which themselves inhibit glucose utilization. They also produce hyperglycemia, which stimulates secretion of insulin causing ß cells to get exhausted.

ACTH enhances the release of free fatty acids from adipose tissue and inhibits glucose utilization. It also increases blood glucose level by stimulating the secretion of adrenal cortex hormones. The glucocorticoids (11-oxysteroids) are important in carbohydrate metabolism. They lead to gluconeogenesis by the increased protein catabolism in tissue, increased hepatic uptake of amino acids and increased activity of transaminases in the liver. They also inhibit the utilization of glucose in extrahepatic tissues. They are antagonist to insulin. They increase the formation of glucose in the liver by stimulating the activity of glucose 6 phosphatase and fructose 1, 6 diphosphatase.

Epinephrine

The epinephrine is secreted by the adrenal medula, it stimulates glycogen breakdown in the muscle by increasing phosphorylase activity. In muscle, glycogen is converted to lactic acid instead of glucose due to the lack of glucose 6 phosphatase. This lactic acid is converted to glucose in the liver by Cori cycle and diffuse into the blood.

Thyroid hormone

The thyroxine has diabetogenic action and increases the rate of absorption of hexoses and accelerate gluconeogenesis. It also stimulates hepatic glycogenolysis with consequent rise in blood sugar. This is due to increased sensitivity to epinephrine.

Q. What is Renal threshold for glucose?

Ans. Glucose is continually filtered by the glomeruli when the blood sugar rises to a high level. But it is completely returned to the blood by the renal tubular reabsorption. The reabsorption is influenced by phosphorylation by enzymes. The capacity of renal tubules to reabsorb glucose is limited to the rate of about 350 mg/minute. If the filtrate contains more glucose that can be reabsorbed, the excess passes into the urine to produce glycosuria. It occurs in the individuals when the venous blood sugar exceeds 160 – 180 mg/100 ml. This level of the venous blood sugar is said to be renal threshold for glucose.

Q. What is Glycosuria? OR Define glycosuria.

Ans. Glycosuria is the condition in which abnormal quantities of glucose are excreted in urine. Normal urine contains traces of glucose, which cannot be detected by Benedict's test. Beyond the renal threshold value (160 – 180 mg/100 ml) the tubules cannot reabsorb glucose which escapes reabsorption and is excreted in urine. Glycosuria occurs in two conditions:

(1) In hyperglycemia.

(2) In normal blood glucose level.

Glycosuria in hyperglycemia: It occurs due to increased blood glucose level in diabetes mellitus. The blood glucose level in this condition become very high and glucose is excreted in urine.

Glycosuria in normal blood glucose level: When the blood glucose level is normal in the body, glycosuria can be due to the following reasons:

Alimentary glycosuria: Some individuals excrete glucose in urine after the intake of large

amounts of sugars or carbohydrate rich meal inspite of their normal blood glucose level. They have normal renal threshold value for glucose but their blood glucose level shoots upto 200 – 220 mg/100 ml for a short period. Hence a transitory glycosuria occurs.

Renal glycosuria (Hereditary glycosuria): Some individuals have low renal threshold value for glucose which may be below 150 mg/100 ml. This condition is harmless and is known as **"diabetes innocens"** or **benign glycosuria.** These individuals have an impaired tubular reabsorption for glucose, it is a hereditary defect.

Glycosuria in pregnancy and lactation: Glycosuria occurs in normal pregnant women, particularly in later months, due to temporary reduction in maximum tubular reabsorption capacity of glucose and partly due to decreased glucose tolerance caused by temporary hypertrophy of the pituitary gland. Lactose is also excreted in urine during late pregnancy and lactation.

Emotional glycosuria: Glycosuria also occurs during periods of excessive nervous strains and emotional excitement such as intense fear, anger and severe anxiety due to increased secretion of epinephrine. This condition is termed as **emotional** or **pshychic** glycosuria. This has been observed in college students sitting for the final examination, worried athletes and candidates for competitive examinations. This results in the use of ether and chloroform as anaesthetics due to hypersecretion of epinephrine.

Q. Write a note on Diabetes mellitus.

Ans. Diabetes mellitus is a group of disorders that all lead to an elevation of glucose in the blood (hyperglycemia). As hyperglycemia increases glucose appears in the urine (glycosuria). Hallmark of diabetes meelitus are three polys: an inability to reabsorb water, resulting in excessive urine production (polyuria), excessive thirst (polydipsia) and excessive eating (polyphagia).

Types of diabetes

There are two major types of diabetes:

(1) Type I

(2) Type II

Type I: In type I diabetes there is an absolute deficiency of insulin. It is called insulin dependent diabetes mellitus (IDDM). It was previously known as juvenile onset diabetes because it most commonly develops in people younger than age 20, although it persists throughout life. IDDM appears to be an autoimmune disorder, one in which a persons immune system destroys the pancreatic beta cells, that occurs in genetically susceptible people.

The cellular metabolism of an untreated type I diabetes is similar to that of a starving person. Since insulin is not present to aid the entry of glucose into body cells, most cells use fatty acids to produce ATP. Organic acids called ketones (ketonic bodies) are by products of fatty acid catabolism. As they accumulate, they causes a form of acidosis called as keto acidosis, which lowers the pH of blood and result in death. As lipids are transported by the blood from storage depots to cells, lipid particles are deposited on the walls of blood vessels. The deposition leads to artherosclerosis and a multitude of cardiovascular problems including cerebro vascular insufficiency, ischemic heart disease, peripheral vascular disease and gangrene. One of the major complications of diabetes is loss of vision due to cataracts. (excessive glucose attaches to lens proteins causing cloudiness) or damage to blood vessels of the retina. Severe kidney problems also may result from damage to renal blood vessels.

Type II: It represents more than 90% of all cases. It is more common in people who are over 40 and over weight. Since type II diabetes usually occurs later in life, it previously was called maturity onset diabetes. Clinical symptoms are mild and the high glucose levels in the blood usually can be

controlled by diet, exercise and weight loss. Many type II diabetics however have a sufficient amount or even a surplus of insulin in the blood. For these people, diabetes arises not from a shortage of insulin but because target cells become less sensitive to it, probably through down regulation of insulin receptors. Type II diabetes is therefore called as non insulin dependent diabetes mellitus (NIDDM). However some NIDDM patients do not need insulin.

Gestational diabetes

Gestational diabetes refers to diabetes that occur during pregnancy and then disappears immediately following delivery. It is due to change in glucose metabolism during pregnancy. Although the condition may be mild and produce no symptoms in the mother, it presents many of the some hazards to the fetus as other types of diabetes.

Treatment of diabetes mellitus

The patient suffering from diabetes mellitus should have high protein and low carbohydrate and fat diet. High protein meals serves as a prolonged source of carbohydrate without rapid hyperglycemic effect and it has protective effect on liver. The patient should have normal psychological affairs. The physiological stress and strain stimulate the a cells of pancreas and adrenal medulla causing the liberation of glucagon and epinephrine respectively which have glycogenolytic effect resulting in increased blood sugar (hyperglycemia). Exercise also lowers the blood sugar level stimulating the β cells of pancreas to liberate more insulin to act on blood sugar for glycogenesis and glycolysis. Administration of oral antidiabetic drugs like diabenese can be taken. But a diabetic patient requiring more than 200 units/day of insulin is regarded as insulin resistant which is due to antigenic effect of insulin.

10

LIPID METABOLISM

Q. Give a brief representation about the fate of dietary lipid in the body.

Ans. The flow of lipids in the body is briefly given in figure. There are three important compartments in the body i.e. liver, blood and adipose tissue. Both liver and adipose tissue are the principal sites of metabolic activity while the blood serves as a transport system. Other compartments, which include cardiac and skeletal muscle, are important utilizers of fatty acid and ketone bodies.

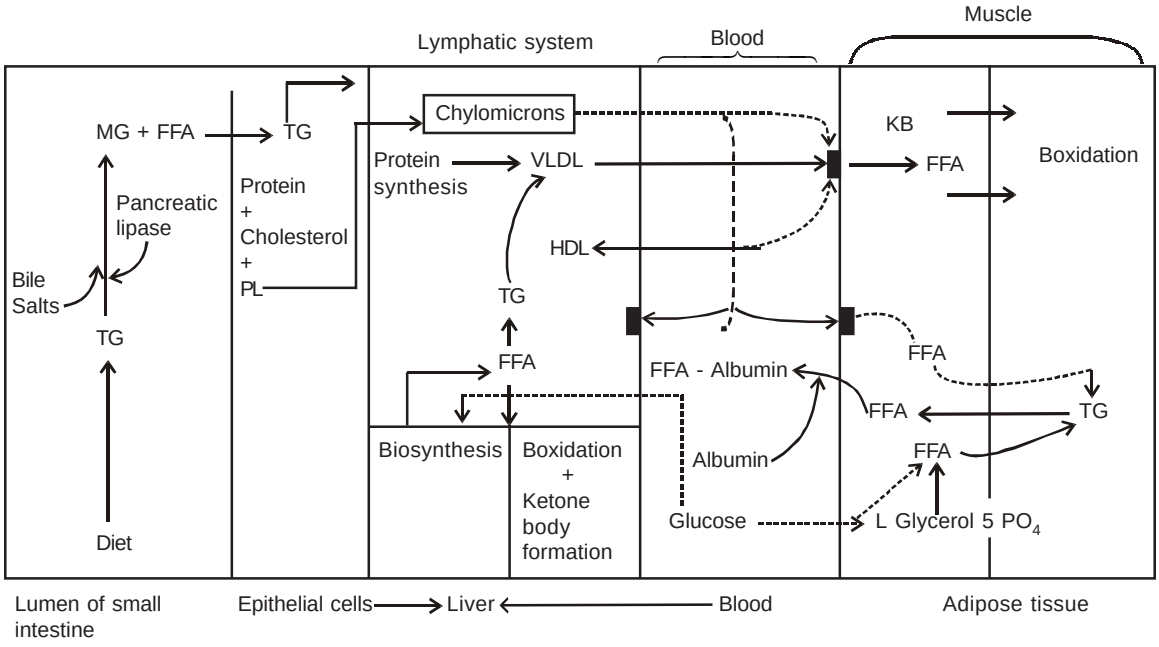


Fig. 10.1. Figure showing role of different compartments in the utilization of lipids in animals.

TG	=	Triacyl glycerol	KB	=	Ketone bodies
MG	=	Monoacyl glycerol	VLDL	=	Very light density lipoproteins
FFA	=	Free fatty acids	HDL	=	High density lipoproteins
PL	=	Phospholipids			

Lumen of small intestine

In the lumen of the small intestine triacylglycerols are degraded to free fatty acids (FFA) and monoacyl glycerols in the presence of conjugated bile acids and pancreatic lipase. Conjugated bile acids are detergents that consist of a lipid soluble (steroid) and a polar (taurine, glycine) part. The bile acids, fatty acids and monoglycerides forms micelles. In the micelles, the nonpolar fraction is located centrally and the polar fraction on the surface. Micelles are also the absorption vehicle for fat soluble vitamins and cholesterol. However the bile acids are not absorbed via the lymphatic route through the portal blood to the liver and are recycled back to the lumen via the gall bladder.

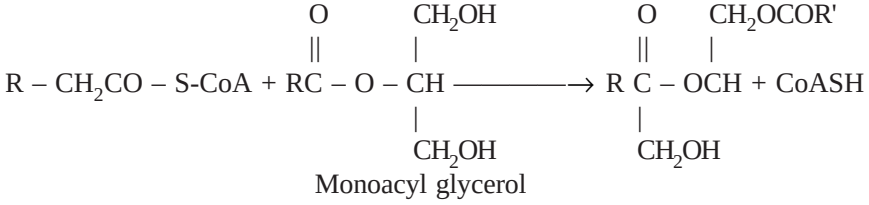
Epithelial cells and chylomicrons

The free fatty acids, monoacylglycerols, and the remaining triacylglycerols are absorbed as micelles into the epithetial cells of the small intestine where the following reactions are catalyzed by the enzymes in the endoplasmic reticulum.

(a) Acyl CoA synthetase:

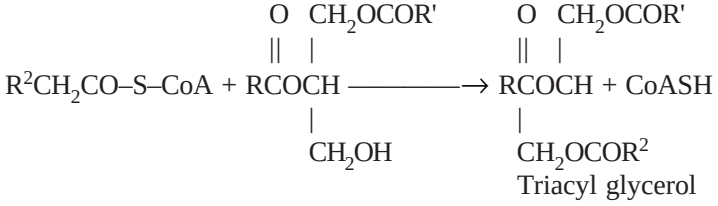
$$RCH_2COOH + ATP + CoASH \xrightarrow{Mg^{2+}} RCH_2CO\text{–}S\text{–}CoA + AMP + PPi$$

(b) Monoacyl glycerol acyl transferase:



Monoacyl glycerol

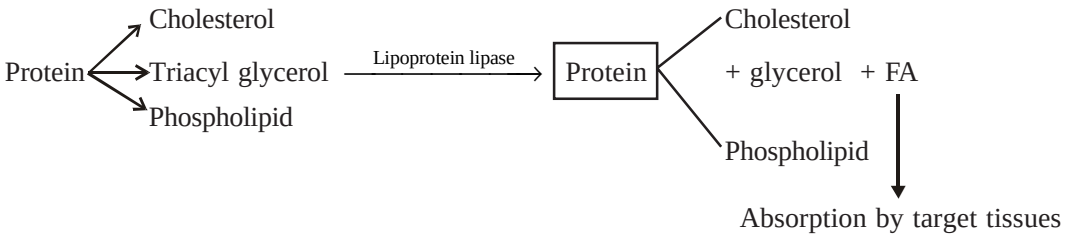
(c) Diacyl glycerol acyl transferase:



Triacyl glycerol

The newly synthesized triacyl glycerol, dietary cholesterol, and newly synthesized proteins are combined in the endoplasmic reticulum of the epithelial cells and are excreted into lacteals as **chylomicrons.** These particles pass from the intestinal lacteals into the lymphatic system and then finally into the thoracic duct to be discharged into the blood system at the left subclavion vein as a milky suspension.

Under isocaloric conditions most of the chylomicrons are transported to adipose tissue for fat storage. However under starvation conditions, when fat storage would be considerable disadvantage to the animal, chylomicrons are utilized primarily by red skeletal muscle, cardiac muscle, and the liver for energy demands.



Adipose tissue

As free fatty acid enter the fat cells of adipose tissue from the action of lipoprotein lipase in the adjacent capillary walls, these acids are rapidly converted to triacylglycerols (Fig. 10.3). The mature fat cell consists of a thin envelop of cytoplasm stretched over a large droplet of triacylglycerol that occupies upto 99% of total cell volume. The main fat depots in humans are the subcutaneous tissue, the muscle and mesenteric tissues. Fat depots are not stationary, that is lipids are continuously being mobilized and deposited. Normally the quantity of body lipids is kept constant over long periods of time possibly by regulation of appetite by an unknown mechanism. When stress conditions develop in the animals such as starvation, prolonged exercise, or rapid fear responses in terms of violent exercise adrenalin from the blood stream binds to a specific receptor in the fat cell surface and triggers a response. A hormone sensitive lipase is activated, rapidly converting triacylglycerols to diacylglycerols and FFA. The FFA are ultimately transferred to the blood where they combine with serum albumin to form soluble, stable FFA albumin complexes. Serum albumin makes up about 50% of the total plasma proteins. With a molecular weight of 69,000, this protein is principally concerned with osmotic regulation in blood. Because of its high solubility and its unique binding sites for fatty acids, it plays in addition a very important transport role for fatty acid that would otherwise be highly insoluble and toxic. Once bound to serum albumin, the FFA albumin complex is highly soluble, nontoxic and is rapidly transported to the liver for further utilization. Once the FFA albumin complex enters the liver, a rapid transfer of the FFA into liver cells takes place with a simultaneous return of the fatty acid free albumin into the blood stream.

Q. Describe the β–oxidation in the Liver.

Ans. All enzymes associated with the β – oxidation system are localized in the inner membranes and the matrix of liver and other tissue mitochondria. When acetyl CoA is produced in the breakdown of fatty acids, it may be subsequently oxidized to CO_2 and H_2O by means of tricarboxylic acid cycle enzymes which are localized as soluble enzymes in the matrix. An unusual property of liver and other tissue mitochondria is their inability to oxidize fatty acids or fatty acyl CoA unless carnitine (3 hydroxy – 4 trimethyl ammonium butyrate) is added in catalytic amounts. Evidently free fatty acids or fatty acyl CoA's cannot penetrate the inner membranes of liver and other tissue mitochondria, whereas acyl carnitine readily passes through the membrane and is then converted to acyl CoA in the matix. Fig.10.4 outlines the translocation of acyl CoA from outside the mitochondrion to internal site of the β-oxidation system. The key enzyme is carnitine acyl CoA transferase.

The overall β – oxidation is presented in Fig 10.5. One molecule of ATP is required to activate a fatty acid for its complete degradation to acetyl CoA, regardless of the number of carbon atoms in its hydrocarbon chain. Five enzymes are required for this cycle in following steps:

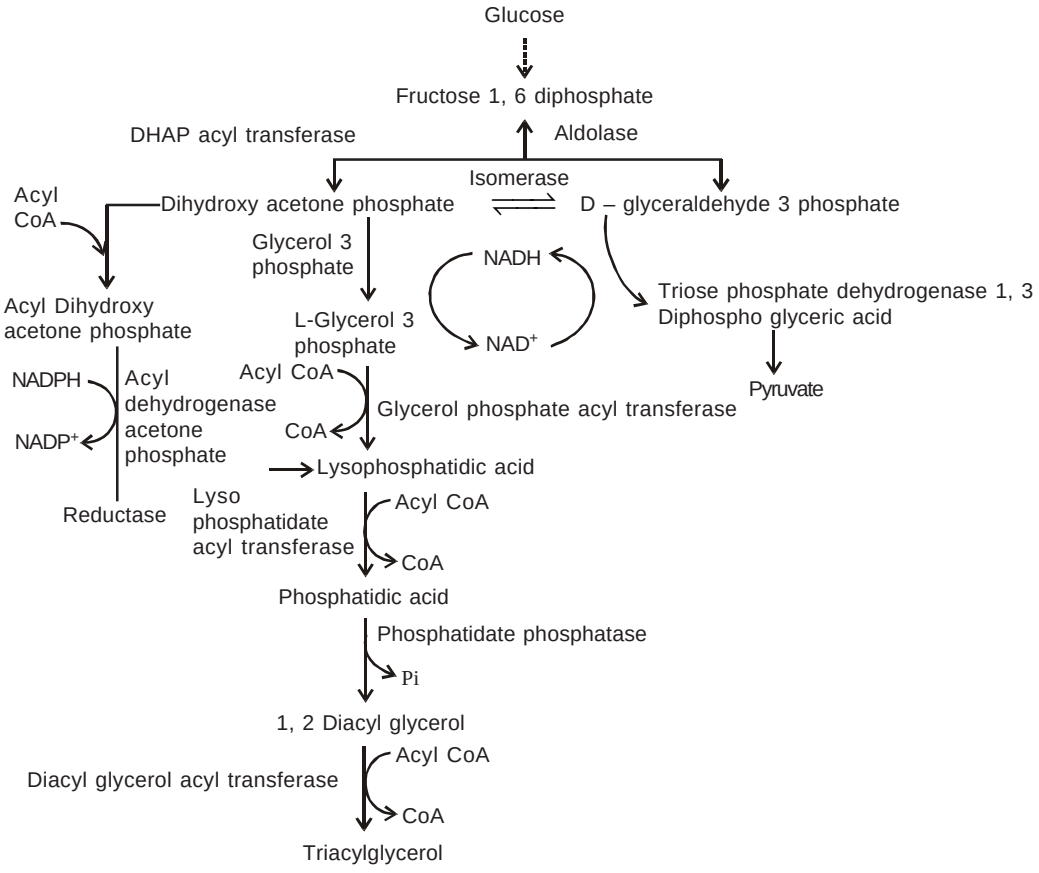


Fig. 10.2. Synthesis of triacyl glycerols.

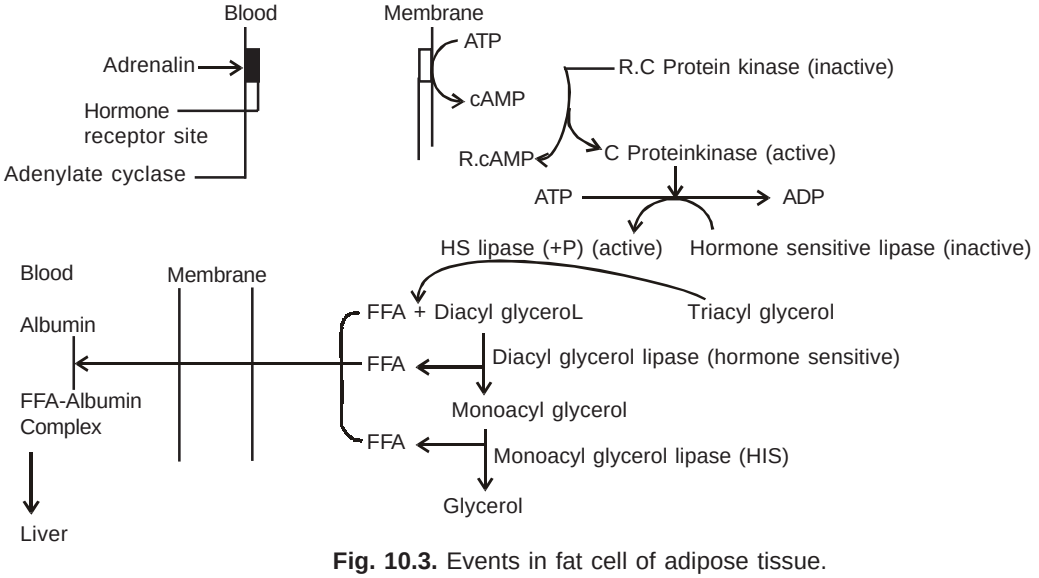


Fig. 10.3. Events in fat cell of adipose tissue.

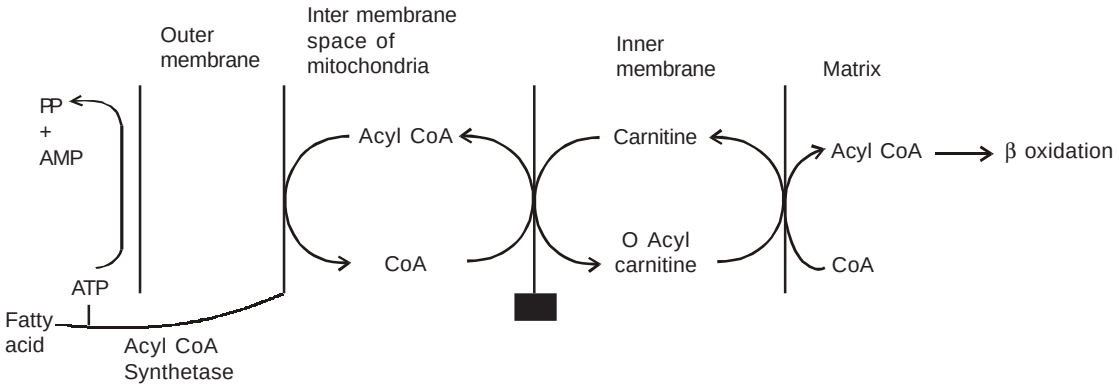


Fig. 10.4. Transport mechanism for fatty acids from the cytosol to the β – oxidation site in the mitochondria.

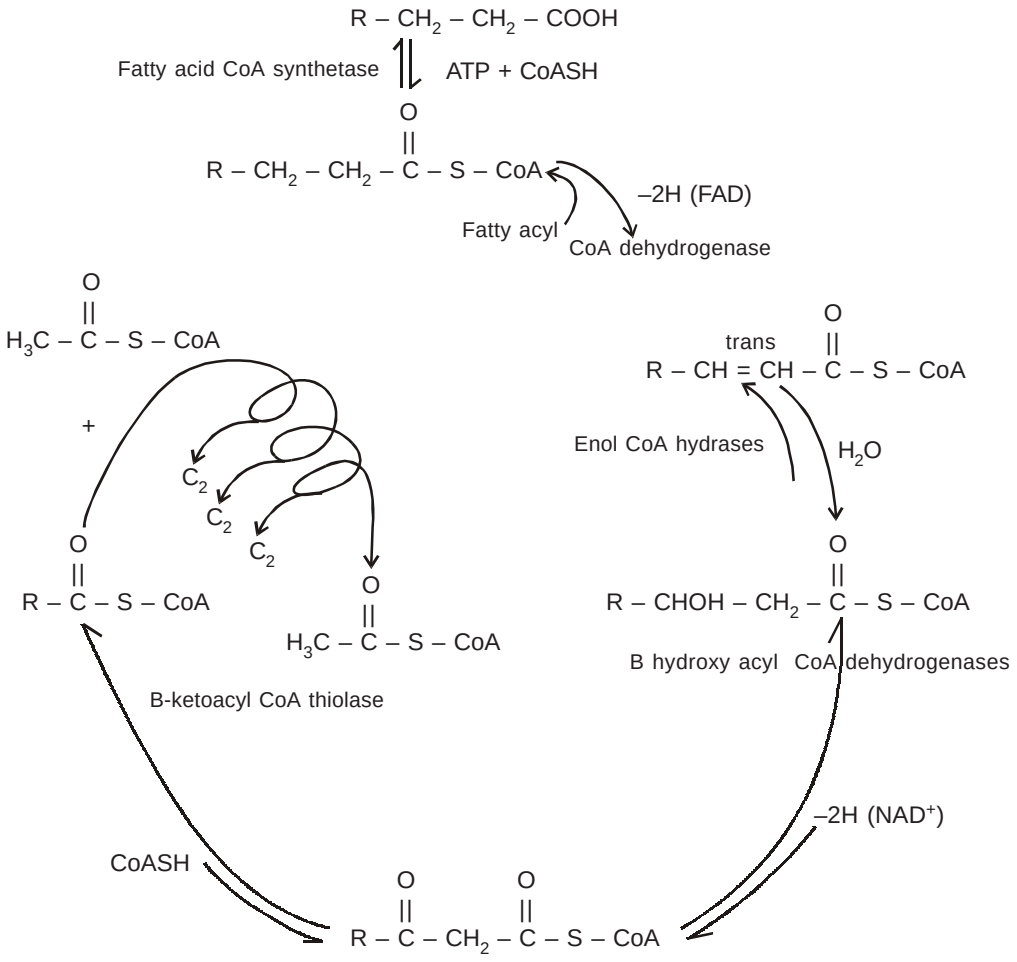


Fig. 10.5. The β – oxidation helical scheme.

Formation of acyl SCoA's by acyl CoA synthetase

The overall type reaction is depicted as:

$$RCOOH + ATP + CoASH \overset{Mg^{2+}}{\rightleftharpoons} RCO - S - CoA + AMP + PPi$$

α, β – dehydrogenation of acyl CoA

$$RCH_2CH_2 - \overset{O}{\overset{\|}{C}} - S - CoA + FAD \longrightarrow RCH = CH - \overset{O}{\overset{\|}{C}} - S - CoA + FADH_2$$

Three acyl CoA dehydrogenases are found in the matrix of mitochondria. They all have FAD as a prosthetic group. The first has a specificity ranging from C_2 to C_6 acyl CoAs, the second from C_6 to C_{14} and the third from C_6 to C_{18}. The $FADH_2$ is not directly oxidized by oxygen

Hydration of α, β – unsaturated acyl CoAs

$$RCH = \overset{O}{\overset{\|}{C}}HC - S - CoA + H_2O \rightleftharpoons \underset{\underset{L\ (+)\ \beta - \text{hydroxy acyl CoA}}{}}{RCH(OH) - CH_2\overset{O}{\overset{\|}{C}} - S - CoA}$$

The enzyme enoyl CoA hydrase, catalyzes this reaction, it possesses broad specificity.

Oxidation of β – hydroxyl CoA

$$L(+)\ RCHOHCH_2CO - S - CoA + NAD^+ \rightleftharpoons \underset{\underset{\beta \text{ keto acyl CoA}}{}}{RC(=O) - CH_2\overset{O}{\overset{\|}{C}} - S - CoA} + NADH + H^+$$

A broadly specific L β hydroxyacyl CoA dehydrogenase catalyzes this reaction and it is specific for the L form.

Thiolysis

The enzyme thiolase, carries out a thiolytic cleavage of the β – ketoacyl CoA.

$$RC(=O)CH_2\overset{O}{\overset{\|}{C}} - SCoA + CoASH \rightleftharpoons RCOSCoA + CH_3COCoA$$

The enzyme protein has a reactive SH group on a cysteinyl residue that is involved in the following series of reactions.

$$RCOCH_2CO - S - CoA + Enz\text{-}SH \rightleftharpoons RCO - S - Enz + CH_3CO\text{-}SCoA$$

β keto acyl CoA — Thiolase — Acyl S – Enz — Acetyl CoA

$$RCO - S - Enz + CoA - SH \rightleftharpoons RCO - S - CoA + Enz - SH$$

Acyl CoA

In β – oxidation there is shortening of an acyl CoA by two carbon atoms, acetyl CoA, the net ΔG' is – 8.45 Kcal per mole. The universality of the β oxidative system implies the prime importance of this sequence as a means of degrading fatty acid.

Energetics of β–oxidation

In the total combustion of palmitic acid, considerable energy is released

$$C_{16}H_{32}O_2 + 23O_2 \longrightarrow 16CO_2 + 16H_2O$$

$\Delta G' = -2340$ Kcal/mole.

Palmitic acid

$$C_{15}H_{32}COOH + 8CoASH + ATP + 7FAD + 7NAD+ + 7H_2O$$

$$\downarrow$$

$$8CH_3CO \sim SCoA + AMP + PPi + 7FADH_2 + 7NADH + H^+$$

Acetyl CoA

$$8CH_3CO \sim SCoA + 16O_2 \xrightarrow[\text{Cycle}]{\text{TCA}} 16CO_2 + 16H_2O + 8CoASH$$

When palmitic acid is degraded enzymatically, one ATP is required for the primary activation and eight energy rich acetyl CoA thioesters are formed. Each time the helical cycle is traversed, 1 mole of $FAD–H_2$ and 1 mole of NADH are formed, they may be reoxidized by the electron transport chain. Since in the final turn of the helix, 2 moles of acetyl CoA are produced, the helical scheme must be traversed only 7 times to degrade palmitic acid completely. In this process 7 moles each of reduced flavin and pyridine nucleotide are formed. The sequence can be divided into two steps.

Step 1:

Palmitic acid $\longrightarrow$ β acetyl SCoA + 14 e^- pair

7 e^- pairs via flavin system at 2 ~ P/one electron pair	= 14 ~ P
7 e^- pairs via NAD^+ system at 3 ~ P/one electron pair	= 21 ~ P
Total	= 35 ~ P
Net	= 35 ~ P – 1 ~ P
	= 34 ~ P

Step 2:

$$\beta \text{ Acetyl CoA} + 16O_2 \xrightarrow[\text{Cycle}]{\text{TCA}} 16CO_2 + 16H_2O + 8\ CoA - SH$$

Assuming that for each oxygen atom consumed 3 ~ P are formed during oxidative phosphorylation then 32 × 3 = 96 ~ P.

Thus step 1 (34 ~ P) and step 2 (96 ~ P) = 130 ~ P and

$$\frac{130 \times 8000 \times 100}{2,340,00} = 48\%$$

In the complete oxidation of palmitic acid to CO_2 and H_2O, 48% of the available energy can theoretically be conserved in a form (ATP) that is utilized by the cell for work. The remaining energy is lost, probably as heat. It hence becomes clear why as a food fat is an effective source of available energy.

Q. Describe the α oxidation.

Ans. This system was first observed in seed and leaf tissues of plants and is also found in brain and liver cells. In this system only free fatty acids, serve as substrates and molecular oxygen is indirectly involved. The products may be either a D – α hydroxy fatty acid or a fatty acid containing one less carbon atom. This mechanism explains the occurrence of a hydroxy fatty acids. The latter may, in nature also be synthesized de novo from propionate. The α oxidation system has been shown to play a key role in the capacity of mammalian tissue to oxidize phytanic acid, the oxidation product of phytol, to CO_2 and water.

Q. Describe the ω oxidation.

Ans. The microsomes in hepatic cells rapidly catalyze the oxidation of hexanoic, octanoic, decanoic and lauric acids to corresponding dicarboxylic acids via a cytochrome $P_{450}\omega$ oxidation system. In addition, a number of aerobic bacteria have been isolated from oil soaked soil, these rapidly degrade hydrocarbons or fatty acids to water soluble products. The reaction involve an initial hydroxylation of the terminal methyl group to a primary alcohol and subsequent oxidation to carboxylic acid. Thus straight chain hydrocarbon are oxidized to fatty acids and fatty acids in turn are β–oxidized to acetyl CoA. The mechanism of the oxidation of oils is primarily by the ω oxidation mechanism.

Q. Describe the process by which the ketone bodies are formed.

Ans. The free fatty acids enter liver cells from chylomicrons and from FFA – albumin complexes originating from adipose tissue fat cells. Fatty acids formed de novo from glucose in the liver also are major contributors to this dynamic pool. Under normal nutritional conditions, these fatty acids have several fates.

1. The acids are esterified to triacyl glycerols. However, the liver has a limited capacity for triacyl glycerol storage, any excess combines with HDL, cholesterol esters, and phospholipids to form VLDL particles. These are now excreted into the blood system and are transported via the vascular system to target tissues such as muscle and adipose tissue. Here lipoprotein lipase removes and converts triacylglycerol to free fatty acids, which are then absorbed by the tissues and utilized. The residual VLDL particles in the meantime convert to HDL particles that presumably return to the liver via the blood system to pick up excess triacylglycerols and repeat the cycle.
2. The free fatty acids enter the mitochondria to be β – oxidized and then converted by the TCA cycle to CO_2 and H_2O.

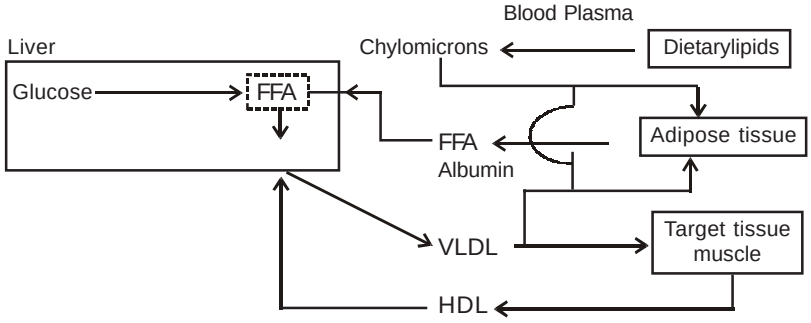


Fig. 10.6. Route of fatty acids in animals.

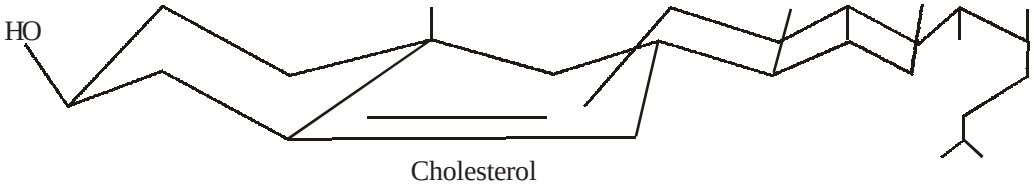
3. The free fatty acids are converted to ketone bodies in the mitochondrion and then transported from the liver to target tissues like red muscle, brain and cardiac muscles to be burned to CO_2 and H_2O. Recent evidence strongly suggest that ketone bodies are major fuels for peripheral muscles and become important sources of energy in muscles involved in prolonged muscle exercises such as long distance running etc.

Ketone bodies are D – α hydroxy butyric acid, acetoacetic acid and acetone. They are formed by a series of unique reactions, primarily in the liver and kidney mitochondria. The enzymes involved in the synthesis of ketone bodies are localized primarily in liver and kidney mitochondria. Ketone bodies cannot be utilized in the liver since the key utilizing enzyme 3 oxoacid CoA transferase is absent in the tissue but is present in all tissue metabolizing ketone bodies, namely red muscle, cardiac muscle, brain and kidney.

Thus, ketone bodies are alternative substrates to glucose for energy sources in muscle and brain. The precursor of ketone bodies, namely free fatty acids are toxic in high concentrations, have a very little solubility and readily saturate the carrying capacity of the plasma albumin. Ketone bodies on the other, are very soluble, are low in toxicity, are tolerated at high concentrations diffuse rapidly through membranes, and are rapidly metabolized to CO_2 and H_2O.

Q. Describe briefly the biosynthesis of Cholesterol.

Ans. Cholesterol has a ring structure and is planar. It can be written as



Cholesterol

All the carbon atoms of cholesterol are derived directly from acetate. Its biosynthesis can be divided into three groups of reactions:

(1) Formation of mevalonic acid.

(2) Conversion of mevalonic acid to squalene.

(3) Conversion of squalene into lanosterol and then to cholesterol.

Fig. 10.8 Illustrates the sequence of events in the synthesis of cholesterol.

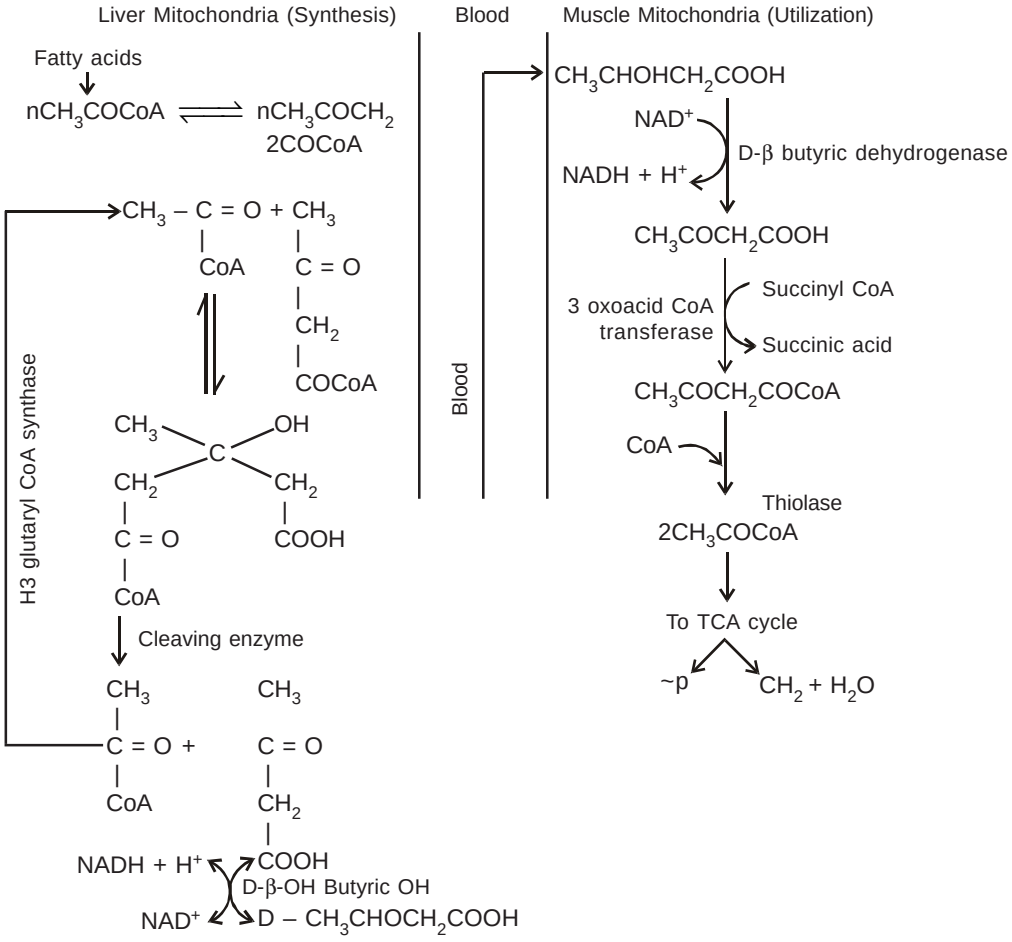
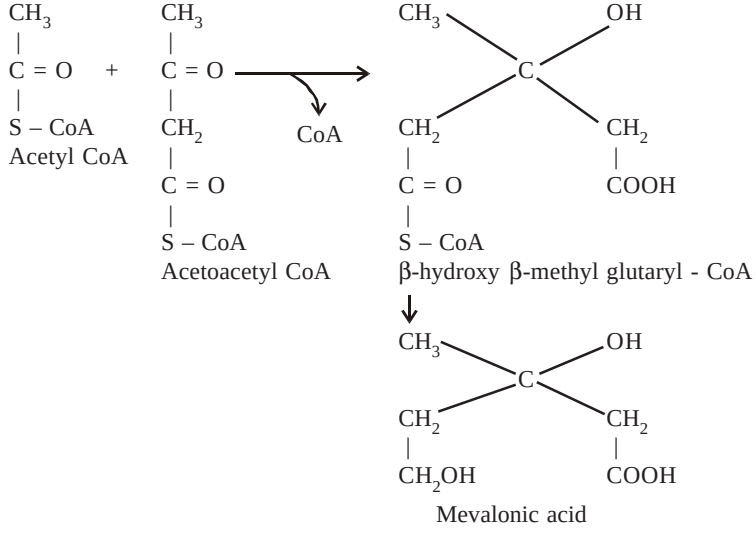
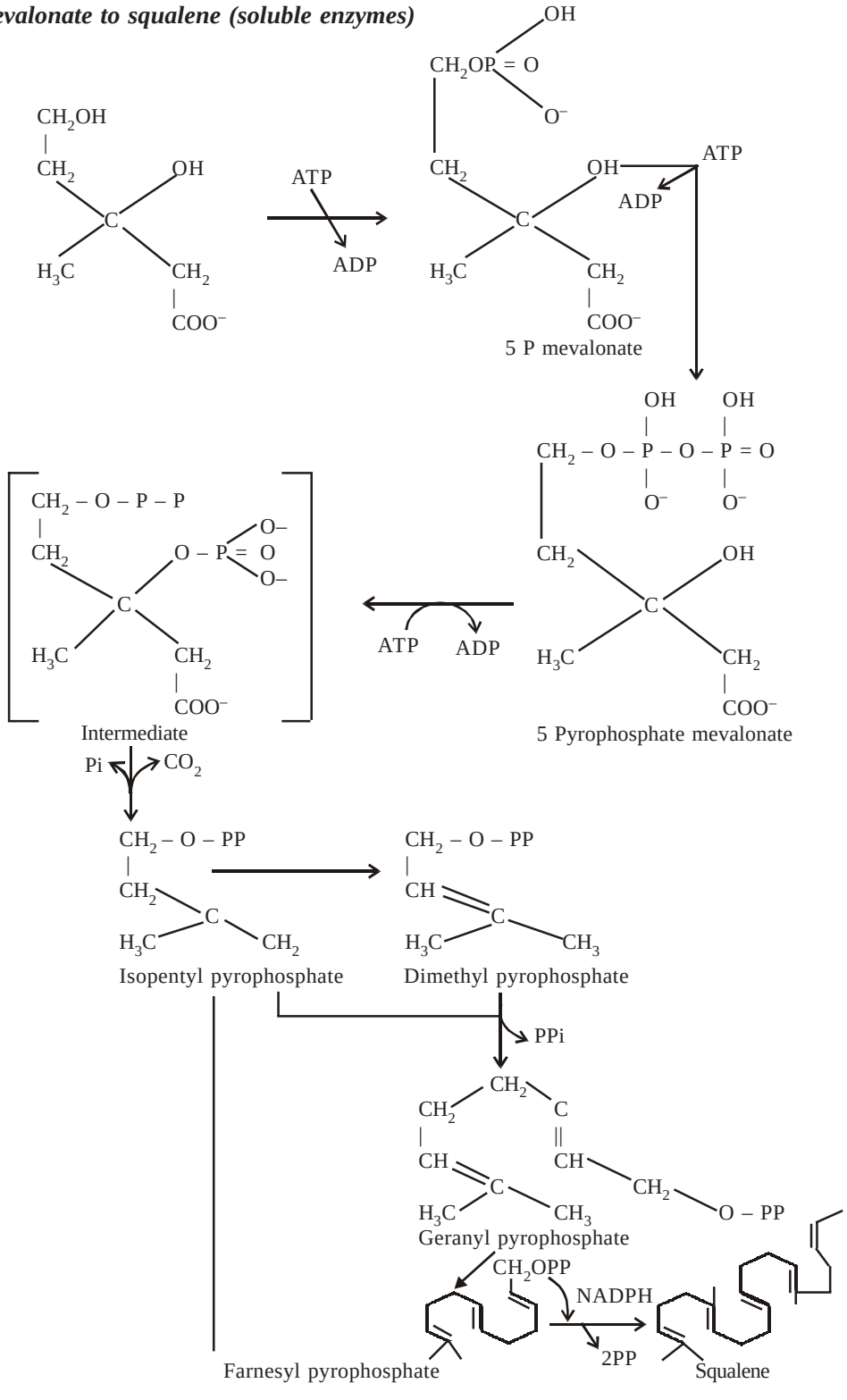


Fig. 10.7 Biosynthesis of ketone bodies and their utilization

(1) Acetate ⟶ Mevalonate (micosomal enzymes)



(2) Mevalonate to squalene (soluble enzymes)



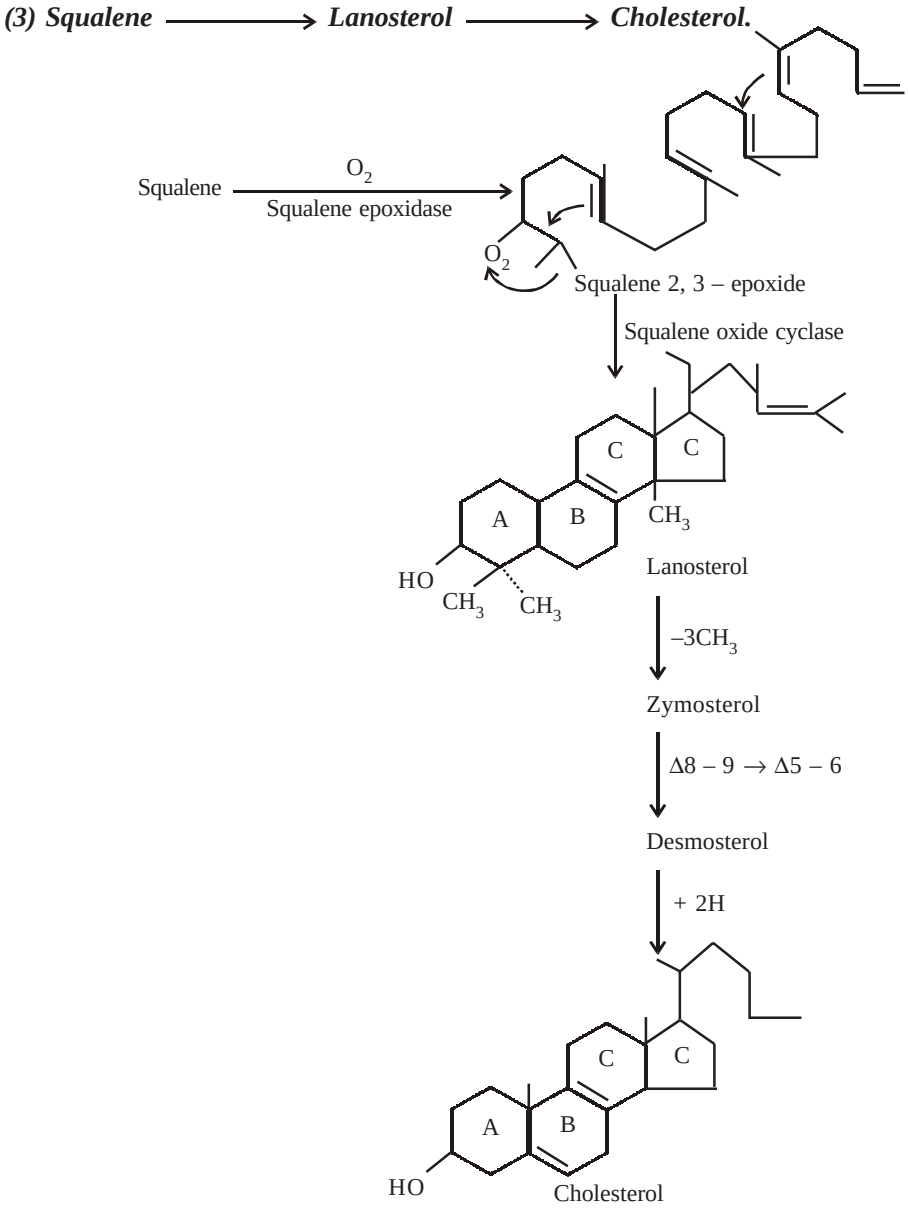


Fig. 10.8. Biosynthesis of cholesterol (step 1, 2 and 3).

Regulation of cholesterol synthesis

Cholesterol is synthesized in all animal tissues. When the diet is rich in cholesterol, denovo synthesis is markedly inhibited. When deficient, denovo synthesis occurs. Since there is no evidence that cholesterol inhibits the enzyme responsible for the conversion of β – hydroxy β methyl glutaryl CoA to mevalonic acid, the nutritional results cannot at present be explained in biochemical terms.

β hydroxy β methyl glutaryl CoA $\xrightarrow[\text{reductase}]{\text{NADPH}}$ Mevalonate + CoA

Fasting also inhibit cholesterol synthesis, although details of this inhibition are not known. In vertebrates, cholesterol is the substrate for a complex of modifications of the side chains and the ring system to form progesterone, androgens, estrogens and cortiosteroid, all extremely important mammalian hormones.

11

NUCLEIC ACID METABOLISM

Q. Give an account of different nitrogenous bases present in nucleic acids.

Ans. Nitrogenous bases are of common occurrence in nucleic acids and are five in number.The purine bases are attached at N-9 position to sugar moiety whereas pyrimidine bases are attached at N – 1 position to sugar moiety by glycosidic linkage. Nucleotides are phosphorylated nucleosides, and are represented by base sugar phosphate unit.

Purine bases

Adenine and guanine comprise the purine base and occur in both DNA and RNA .In RNA a large number of bases are methylated and occur as methyladenine, methylguanine or methylcytosine.A purine ring is formed by the fusion of a 6- membered pyrimidine ring and a 5- membered imidazole ring.In a purine the positions are designated by the numbers 1 to 9.

Pyrimidine bases

Pyrimidine bases are all derivatives of the parent compound pyrimidine which shows a six membered ring. The derivatives are found in the nucleic acids. Cytosine is found in both DNA and RNA, uracil in RNA and thymine in DNA only.

Q. What are Nucleosides?

Ans. A nucleoside is composed of a purine or a pyrimidine base and a ribose or a deoxyribose sugar.

Nucleoside = Base – Sugar

Thus

Nucleotides = Base + Sugar + Phosphoric acid

Adenosine monophosphate (AMP) = Adenine + Ribose + Phosphate

Thymidylic acid (TMP) = Thymine + 2 Deoxyribose + Phosphate

Uridylic acid (UMP) = Uracil + Ribose + Phosphate

Cytidylic acid (CMP) = Cytosine + Ribose + Phosphate

Guanylic acid (GMP) = Guanine + 2 Deoxyribose + Phosphate

Q. Give an account of Purine Biosynthesis.

Ans. The nine atoms of purine nucleus are derived from five different precursors, each precursor contributing the atoms indicated in Fig 11.1.

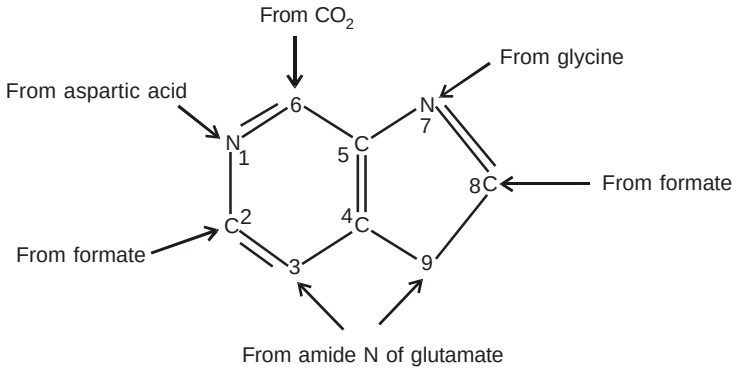


Fig. 11.1. Precursors of N and C atom of purine nucleus.

Reaction of purine biosynthesis

Step 1:

The starting point of purine biosynthesis is the compound α 5-phosphoribosyl 1-pyrophosphate (PRPP) which is obtained from ATP and ribose -5 phosphate.The enzyme, a kinase, catalyzes the transfer of the pyrophosphate moiety of ATP rather than the terminal phosphate group to the acceptor molecule ribose 5 phosphate.

Step 2:

α – 5-phosphoribosyl – 1 – pyrophosphate then participates in the initial step in purine biosynthesis by reacting with glutamine to form 5 phosphoribosyl – 1 amine, glutamic acid and pyrophosphate. This is a reaction in which glutamine donates its amide nitrogen atom into organic combination. The enzyme (glutamine phosphoribosyl pyrophosphate amido transferase) which catalyzes this reaction is inhibited by purine nucleotides produced in the later steps of the biosynthetic pathway. For this reason this reaction is then the site of feed back inhibition by the later products of the pathway.

Step 3:

In the next step the amino acid glycine is linked to the ribosylamine in an amide linkage. The reaction requires energy supplied by ATP.

Step 4:

The glycinamide ribonucleotide formed in above reaction then reacts in the presence of a transformylase which catalyzes the transfer of a formyl group from the formyl transfer coenzyme, methenyl N5-10 tetrahydrofolic acid to produce formylglycinamide ribonucleotide.

Step 5:

The next reaction involves the addition of the nitrogen atom located at position 3 of the purine structure. It is predicted that the nitrogen is provided by the amide group of glutamine in the presence of an energy source, ATP.

Step 6:

The α N-formyl glycinamide ribonucleotide then undergoes ring closure by a poorly understood dehydration reaction, which requires ATP. In this step the imidazole ring of purine nucleus is formed, and ATP is hydrolyzed to ADP and H_3PO_4.

Step 7:

In the next step the imidazole nucleus react with CO_2 by process of carboxylation.

Step 8:

The next step in the pathway is one of the several reactions in intermediary metabolism where a nitrogen atom is contributed by aspartic acid. The process is quite analogus to the synthesis of arginosuccinic acid in the urea cycle where ATP is required as an energy source.

Step 9:

The succinocarboxamide derivative is cleaved to form fumaric acid.

Step 10:

The final carbon atom is acquired before the six membered ring of purine can be formed by ring closure. This atom is provided through the one carbon metabolism of the folic acid system in the form of a formyl group.

Step 11:

Ring closure then occurs in the presence of an enzyme, which catalyzes the removal of H_2O in a reversible reaction. The product is **inosinic acid,** which does not occur free in biological materials, but of course is not a component of RNA or DNA.

The overall steps can be written as

$$2NH_3 + 2\ \text{Formic acid} + CO_2 + \text{Glycine} + \text{Aspartic acid} + \text{Ribose 5 phosphate}$$
$$\downarrow$$
$$\text{Inosinic acid} + \text{Fumaric acid} + 9H_2O$$

The energy required to accomplish this process, provided by ATP molecules, all but one of which are cleaved to produce ADP and H_3PO_4.

$$9ATP + 9H_2O \longrightarrow 8ADP + 8H_3PO_4 + AMP + PPi$$

Q. Describe briefly the biosynthesis of pyrimidine ring.

Ans. The atoms of the pyrimidine nucleus are derived from three simple precursors, CO_2, NH_3 and aspartic acid.

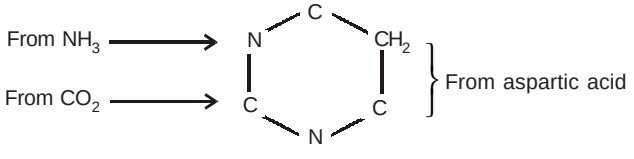


Fig. 11.2. Precursors of N and C atom of pyrimidine nucleus.

Reactions of pyrimidine biosynthesis

Step 1:

The biosynthetic pathway commences with the transfer of a carbamyl group from carbamyl phosphate to aspartic acid to form N – carbamyl aspartic acid (ureidosuccinic acid).

Step 2:

The enzyme which catalyzes this reaction is aspartic transcarbamylase. Ring closure of N-carbamyl aspartic acid catalyzed by the enzyme dihydrorotase leads to the formation of dihydroorotic acid.

Step 3:

In the next step, a flavin enzyme dihydroorotic acid dehydrogenase catalyses the formation of orotic acid by removing two hydrogen atoms from adjacent carbon atoms to form a carbon - carbon double bond. The reduced flavin in turn is reoxidized by a NAD dependent dehydrogenase.

Step 4:

Orotic acid reacts with phosphoribosyl pyrophosphate (PRPP) to acquire the 5' phosphoribosyl moiety and become the nucleotide orotidine 5 phosphate. In this process the ring nitrogen of orotic acid reacts as nucleophile to displace the pyrophosphate group of PRPP and form the β – N glycosyl bond.

Step5:

Finally, the orotidylic acid is decarboxylated in the presence of specific decarboxylase to yield uridine 5' phosphate (UMP), the starting point for synthesis of the cytidine and thymidine nucleotides.

Q. Write the synthesis of the Diphosphates and Triphosphates.

Ans. Once the synthesis of the monophosphates of purines and pyrimidine nucleotides is achieved, formation of di and triphosphates is readily accomplished. There are specific kinases that will catalyze the transfer of phosphate from ATP to the specific nucleoside monophosphates NMP.

$$NMP + ATP \overset{Mg^{2+}}{\rightleftharpoons} NDP + ADP$$

These kinases are highly specific for the individual bases but utilize either the riboside or deoxyriboside. The synthesis of the nucleoside diphosphate is favoured because of the removal of ADP and its subsequent phosphorylation to ATP by oxidative phosphorylation.

The triphosphate can in turn, be formed by the phosphorylation of the nucleoside diphosphate in the presence of nucleoside diphosphate kinase.

$$NDP + XTP \rightleftharpoons NTP + XDP$$

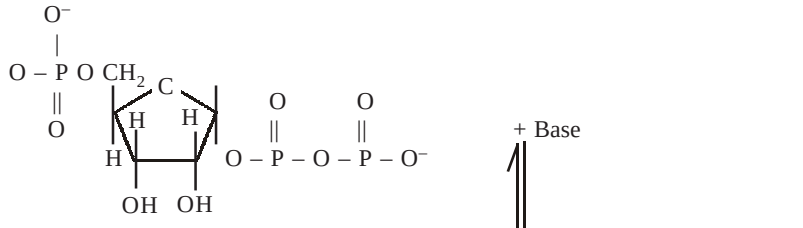
$$dNDP + XTP \rightleftharpoons dNTP + XDP$$

This enzyme is ubiquitous in nature and quite nonspecific, showing no preference either for any particular base or for ribose instead of deoxyribose. Again, the donor (XTP) is usually ATP and the synthesis of the other triphosphate (NTP) is driven by the ability of the cell to reform ATP from ADP by energy yielding phosphorylation processes.

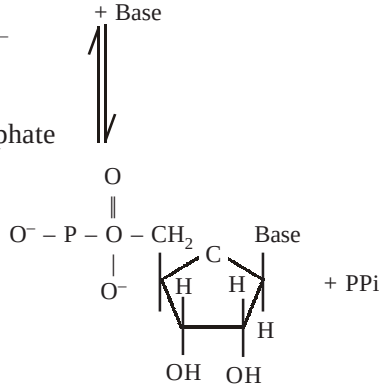
Q. Define the Salvage Pathway for Purine and Pyrimidine Nucleotides.

Ans. The synthesis of purine and pyrimidine nucleotides requires significant quantites of energy

because of its denovo nature, that is, synthesis from the simple monomers NH_3, CO_2, formic acid, glycine and aspartic acid. The organisms have evolved a means for rescuring or salvaging these complex nitrogen bases if they are formed during breakdown of DNA and RNA. There are several reactions that serve to rescue these bases from further breakdown. One is the reaction catalyzed by an enzyme called nucleotide pyrophosphorylase.



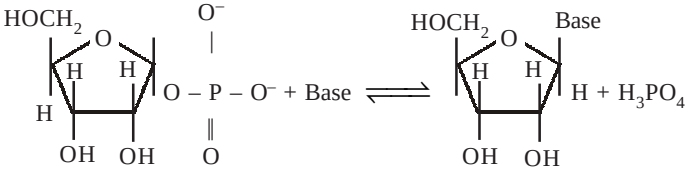
α – 5 Phosphoribosyl - 1 Pyrophosphate (PRPP)



Nucleotide

The reaction is readily reversible but in practice operates from left to right because of the action of the ubiquitous pyrophosphate that hydrolyzes the pyrophosphate formed. The enzyme utilizes the purine bases, adenosine and guanosine and in some bacteria uracil.

A second salvage reaction is catalyzed by nucleoside phosphorylase.



Ribose 1 phosphate Nucleoside

A third salvage reaction is catalyzed by a nucleoside kinase that is relatively specific for thymidine and ATP. In this process, which is formally analogus to hexokinase an energy rich phosphate is used to produce a low energy phosphate ester.

Q. How the biosynthesis of Nucleic acid occurs in the body?

Ans. The nucleic acids in the body are the DNA and the RNA. In the synthesis of informational biopolymers, the mechanisms which the cell employs for the flow of information from DNA, the primary carrier, to proteins, the ultimate products of that information, and the means by which the cell can regulate its metabolism by control of specific proteins, namely enzymes.

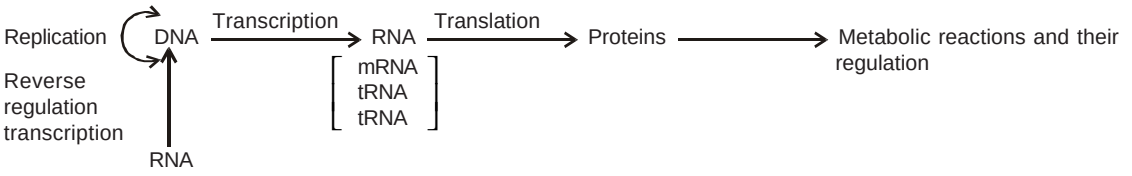


Fig. 11.3. Metabolism of Informational molecules.

The terms that describe these above reactions are as follows:

(1) **Replication** — It is the process by which each strand of the parental DNA duplex is copied precisely by base pairing with complementary nucleotides. The product is two duplexes identical to the parent duplex.

(2) **Transcription** — It is a complex process by which the information contained in DNA is copied, by base pairing to form a complementary sequence of ribonucleotides, a RNA chain.

(3) **Translation** — It is a complex process by which the information is transcribed from DNA into a special type of RNA i.e. mRNA, which directs the ordered polymerization of specific amino acids for the synthesis of proteins.

Reverse transcription — It involves the use of RNA as the genetic information, in place of DNA, for the synthesis of new duplex DNA.

Template — It refers to the DNA (or RNA) chain that provides precise information for the synthesis of a complementary strand of nucleic acid.

Primer — It refers to the initial terminus of a molecule onto which additional units are added to produce the final product. For example, in glycogen biosynthesis, the primer is a small polysaccharide onto which glucosyl units are added.

Q. How the DNA Replication occurs?

Ans. Replication is the process by which each strand of the parental DNA duplex is copied precisely by base pairing with complementary nucleotides. The product is two duplexes identical to the parent duplex. In this process, strands of DNA separates and new complimentary strands are assembled from the four available deoxyribonucleotide triphosphates on each of the two separate parent strands. Assuming the base pairing to be precise, the two new DNA molecules should be identical to the parent molecule.

Initation of Replication

Replication is discontinuous and always occurs in 5' $\rightarrow$ 3' direction on both strands of a duplex DNA. The process begins at several points along the duplex strand, however for initation to begin, the duplex strand must first be separated into single strand for the polymerase to function. Presumably at the several points, unique proteins of low molecular weights (~35,000) in both prokaryotic and eukaryotic organisms bind specifically to one of the two strands. The binding appears to be related to those regions of the duplex which are rich in A-T base pairing. Since A-T base pairs have a lower energy of hydrogen bonding than G-C base pairs, these regions appear to be more susceptible to melting or conversion from duplex to single stranded DNA. These proteins are called **unwinding proteins,** and are essential for initation as well as for the continuation of replication.

Elongation

After initation, elongation now occurs in the presence of holoenzyme, DNA polymerase III, a multisubunit protein. A component of this enzyme, copolymerase III and ATP are required to form the

active complex with the primer template. Unwinding protein must also be present. Elongation now begins, ATP is cleaved to ADP + Pi, deoxyribonucleotides are properly positioned for a nucleophilic attack by the 3' OH terminus end of the growing RNA – DNA chain. Once begun, copolymerase III is no longer required, it dissociates, and now DNA polymerase III catalyze the further elongation in a 5' → 3' direction until about 500 to 1000 deoxyribonucleotide residues have been added. The formation of daughter strands is discontinuous and the fragments of RNA-DNA are laid down in a sequential manner.

The RNA-DNA fragments are called **Okazaki fragments,** after the Japanese biochemist that first isolated them, and proposed in 1968 the discontinuous DNA replication mechanism to explain these observations.

Termination

As the DNA chain grow and approach each other, that is, as the 3' OH terminus approaches the 5' pp terminus (A') three events must occur.

(1) Excision of the RNA primer fragment.
(2) Filling in of the remaining gaps with deoxyribonucleotide residues.
(3) Fusion of the DNA fragments by a phosphate diester bond to form a continuous daughter strand.

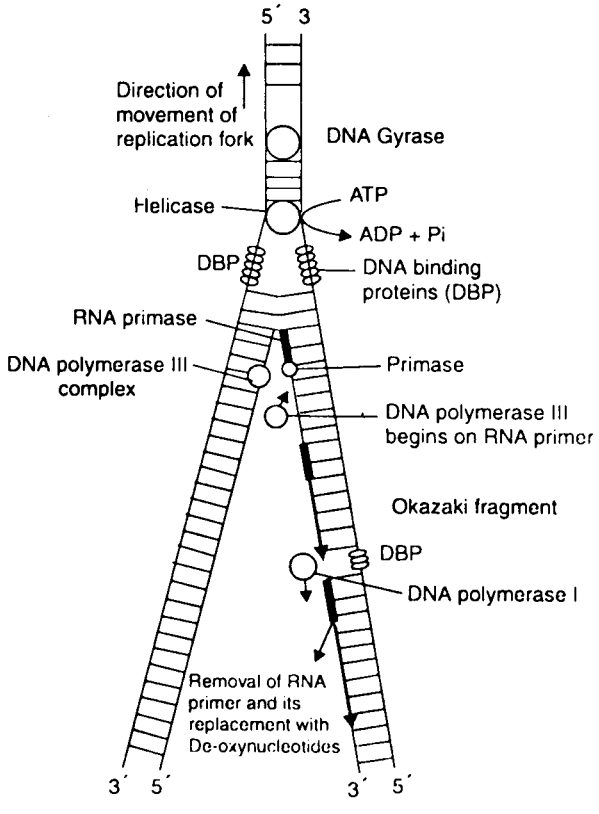


Fig. 11.4. Summary of major steps in DNA replication.

DNA polymerase I, the enzyme that Kornberg discovered in 1955 in E coli and whose function had remained undefined in recent years, turns out to be the unique enzyme which by possesing the 5' → 3' exonuclease and polymerase activites, fulfills the (1) and (2) requirements.

This enzyme is widely distributed in both prokaryotic and eukaryotic organisms. The two key activities of DNA polymerase (a) the removal of the RNA fragment the original primer-template duplex by 5' → 3' exonuclease activity and (b) the filling in the gap by its polymerase activity prepares the almost completed replicated sequence for the final step, i.e. the fusion of the 3' OH terminus with the 5' ppp terminus by a special enzyme, DNA ligase. In E coil, this enzyme requires NAD^+, the products being 5' adenylate and nicotinamide mononucleotide whereas in animal cell ATP is required and 5' adenylate and pyrophosphate are the products.

Q. Describe the process of Transcription.

Ans. In eukaryotic cells the site of DNA dependent RNA biosynthesis (transcription) is nucleus. While the nucleolus appears to contain the enzymes and genes for ribosomal RNA biosynthesis. The enzymes responsible for the synthesis of messenger and transfer RNA's are localized in the nucleoplasm.

One gene codes for only one protein, only one DNA strand functions as a template, while the other strand does not function as a template. The term **asymmetric transcription** is employed for the copying of one strand of DNA and **symmetrical transcription** for the copying of both template strands. The core enzyme will transcribe a DNA template symmetrically, that is both strands of DNA can serve as templates. However, the transcription reaction is slow and nonspecific with the s factor, the holoenzyme transcribes DNA asymmetrically initating RNA chains at specific promotor sites.

Association with DNA template

The RNA polymerase need only a duplex template and no primer . With RNA polymerase, the transcription begins at specific promoter sites in the DNA template and terminates at the end of a defined genetic sequence. Presumably RNA polymerase repeatedly associate and disassociate with DNA until a promoter site is found, promoter sites must have specific sequences of bases that are recognized by the holo RNA polymerase as suitable for bonding. In the process of binding the promoter site, about 6 to 10 base pairs are converted to an open complex by localized melting of the

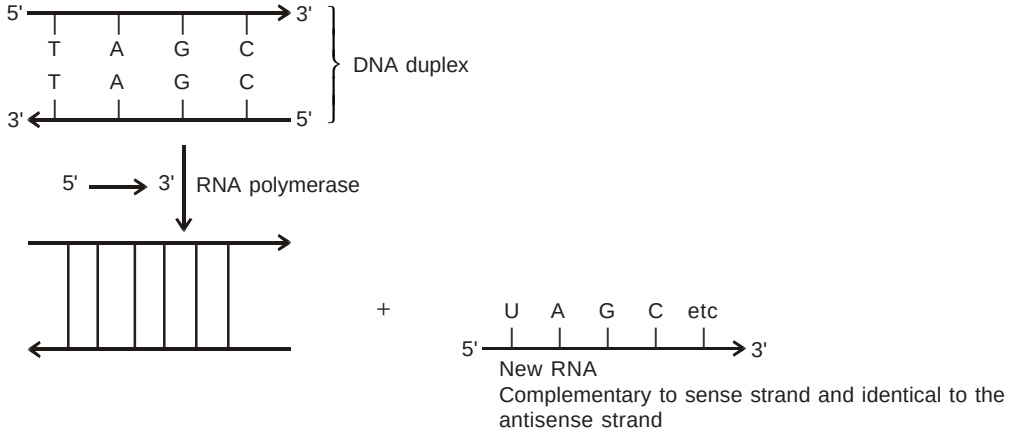


Fig. 11.5. Activity of RNA polymerase.

duplex and thereby allow the polymerase to select the appropriate DNA strand as template. An essential component of binding at the promoter site is the sigma factor (σ). The sigma factor is probably involved in the opening of the duplex DNA at its promoter site or its immediate region. Without the σ factor, the core polymerase will read indiscriminately both the DNA strands; with the σ factor only the sense strand will be recognized and correctly read.

Initation and Elongation

Since the 5' end of many RNA's has either pppA or pppG and ATP or GTP is probably bounded initially at the promoter site and becomes the initial 5' terminal nucleotide residue. It is at this initial stage of binding, of either ATP or GTP at the promoter site that rifampicin blocks. The open or melted complex in the presence of a nucleoside triphosphate now initiates transcription at the initiation site that is adjacent to the promoter site. Once transcription begins, the σ factor dissociates, and the core polymerase completes the transcription. The rate of RNA synthesis apparently is controlled by the rate of initation and not by the rate of elongation. As soon as the polymerase has moved away from the initation site to continue and complete the transcription, a second polymerase molecule can bind to the same promoter site and then will move to the open initation site to begin a second transcript etc.

Termination

At the end of a gene, a sequence of bases must signal the completion of transcription. A release factor, rho (ρ), an oligomeric protein, with a molecular weight of 200,000 presumably attaches to the RNA polymerases blocking further transcription and the RNA product is released.

Transcription in Eukaryotes

In eukaryotic cells, nuclear transcription is a process of great complexity. The mass of nuclear DNA is extremely large and contains a great complexity of nucleotide sequences. Unlike the bacterial systems with a single species of DNA dependent RNA polymerases, eukaryotic cells contain at least three different nuclear RNA polymerases. The RNA polymerases is associated with the nucleolus, requires either Mn^{2+} or Mg^{2+}, has a molecular weight of 500,000-700,000, and appears to be oligomeric. It is presumably responsible for the synthesis of ribosomal RNA's. The RNA polymerase II and III are in the nucleoplasm. Enzyme II, which has been extensively purified, requires Mn^{2+} and has a molecular weight of 700,000, and unlike enzyme I is highly sensitive to the bicyclic peptide toxin from a toad stool, α – amanitin. It appears to be also oligomeric and is responsible for mRNA synthesis. Enzyme III appears to be involved in tRNA and 5 sRNA synthesis. Polymerase IV is localized in the inner mitochondrial membrane and is concerned with the asymmetric transcription of mitochondrial DNA. The product appears to become associated with the mitochondrial ribosomes. Finally, in chloroplast containing plants, a chloroplast DNA dependent RNA polymerase has also been characterized. All these polymerases may have σ like initation factors associated with them, although highly purified. E coil σ factor is completely inactive in cross reactions with eukaryotic RNA polymerase. The terminating DNA sequence is not too well understood.

12

PROTEINS SYNTHESIS

Q. Write a brief note on translation.

Ans. The process of protein synthesis is also called as translation, because the language consisting of four base pairing letters nucleic acid is converted into that comprising the twenty letters of amino acids in the proteins. It is a very complex process which requires more than 100 macromolecules. The transfer RNA molecules, activating enzymes, soluble factors and m-RNA are required ,in addition to ribosomes.Proteins are synthesized in the amino acid to carboxyl direction by the sequential addition of amino acids to the carboxyl end of the growing peptide chain. The site of protein synthesis is ribosomes.The protein synthesis takes place in four steps—Activation, Initiation, Elongation and termination.

Q. Mention the components of Protein Synthesis.

Ans. The genetic information from DNA is programmed in RNA for the orderly synthesis of new proteins as indicated

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{Translation}} \text{Protein}$$

The mechanism of transcription, is the process whereby the genetic information in DNA is employed to order a complementary sequence of bases in a new RNA chain. In this process three key RNA's are synthesized (1) messenger RNA, which carries the genetic message from DNA for the orderly and specific sequence of amino acids for the new protein (2) ribosomal RNA's which serve as important structural component in ribosome and (3) transfer RNA's which carry activated amino acids to specific recognition sites on the mRNA template.

The machinery of protein synthesis thus consists of a large number of components, the important ones are mRNA, the ribosomes, which are the actual sites of protein synthesis, amino acyl tRNA, and a number of enzymes and cofactors. The whole machinery operates in the cytoplasm of prokaryotic and eukaryotic organisms. It is closely connected to the endoplasmic reticulum. Mitochondria and chloroplasts have somewhat limited but rather complete machinery for protein synthesis.

Q. Write in brief the process of activation of Amino acid before protein synthesis occurs.

Ans. The twenty amino acids commonly found in protein structure undergo an initial activation step, which also involves a selection and preliminary screening of amino acids. Thus D isomers and certain amino acids such as ornithine, citrulline, β alanine and diaminopimelic acid, which are used for other purposes in the cell are rejected at this stage. Each amino acid of twenty normally found in

proteins has its own specific activation enzyme system called amino acyl tRNA synthetase. The step involves the following—

$$\text{ATP + Amino acid} \underset{Mn^{2+}}{\overset{\text{Enzyme}}{\rightleftharpoons}} \text{aa – AMP-Enz Amino acid – Adenylate – Enzyme complex + PPi}$$

Amino acyl adenylates are extremely reactive but are stabilized by remaining associated with the parent enzyme. The activation step, namely the formation of an amino acid - adenylate enzyme complex, has considerable specificity built into it some activating enzymes have a limited capacity for activating a number of amino acids. Thus specificity is exercised at two level, (a) the activation step and (b) the transfer step to tRNA suggesting that the synthetase protein must have had at least two recognition sites, one for its specific amino acid and the other for its specific tRNA. The mechanism of transfer of the amino acid-adenylate -enzyme complex to its specific tRNA.

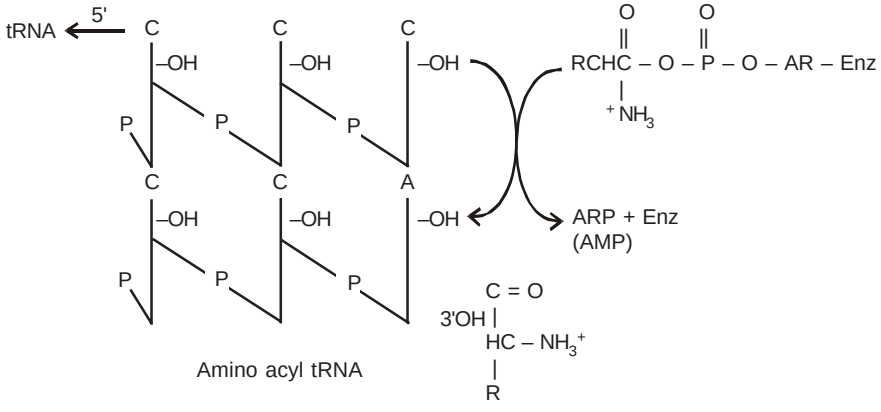


Fig. 12.1. The amino acylation of tRNA.

(2) Transfer RNA

The special acceptors of activated amino acids are the specific transfer RNAs (tRNAs). Transfer RNA's have three specific functions

(*a*) Recognition of specific amino acid t RNA synthetase in order to accept the correct activated amino acid.

(*b*) Making possible recognition of the correct codon in the mRNA sequence for a specific amino acid with its own specific anticodon and thereby insure that the correct amino acid will be placed in its proper sequence in the growing polypeptide chain.

tRNA genes (DNA)
↓ Transcription
Precursor tRNA (120-130 bases)
↓ Translocation
tRNA (-CCA)
$2CTP^{+}$ | tRNA –C-C-A
\ATP ↓ pyrophosphorylase
Functional tRNA (+CCA)
+ 3 Pyrophosphates

Fig. 12.2. Eukaryotic biosynthesis of tRNA species.

(*c*) Binding the growing peptide chain to the ribosome participating in the translation process. Thus the transfer RNA's serve as adaptors in directing the proper placement of amino acids according to the nucleotide sequence of mRNA. Transfer RNA must have several recognition sites in its structure, namely (i) the anticodon site, that is, the three base site responsible for the recognition of the complementary triplet encoded in mRNA (ii) the synthetase site by, which the specific amino acyl tRNA synthetase recognizes and charges the specific amino acid with the tRNA (iii) amino acid attachment site, which in all tRNA's is the 3' terminal –CCA nucleoside sequence and (iv) the ribosome recognition site.

Q. Write a note on the role played by the mRNA and ribosomes during protein synthesis.

Ans. mRNA is the key component of the translation process and it comprises only a few percent of the total RNA of a cell. It carries the genetic message from DNA to the site of protein synthesis, i.e. the ribosome and hence is called as messenger RNA. mRNA varies greatly in chain length and thus in molecular weight. This great variation might be related to the heterogenous nature of protein chain lengths. Most eukaryotic mRNAs are monocistronic that is, code for only one polypeptide.

Ribosomes are larger ribonucleoprotein particles on which the actual process of translation occurs. In prokaryotic cells they occur in the free form as **monosomes** or are associated with mRNA as **polysomes.** An average bacterial cell contains about 107 ribosomes. In eukaryotic cell they occur in forms similar to those found in the prokaryotic cells and also are associated with membranes of the rough endoplasmic reticulum.

Specific ribosomal proteins are directly involved in binding mRNA and tRNA. rRNA apparently does not participate directly in these binding sites but serve as a structural polymer holding the multiprotein particle in a compact configuration.

Diagram showing transcription and transport of a nuclear RNA in a eukaryotic cell and participation of tRNA and mRNA in protein synthesis.

Q. Discuss the process of Protein Synthesis.

Ans. The translation of coded information from DNA via mRNA into the amino acid sequences of proteins involves the orderly interactions of over 100 different macromolecules. There are four broad steps in the synthesis of a protein:

(*a*) activation of amino acids (mentioned in earlier question)
(*b*) initiation of the synthesis of the polypeptide chain
(*c*) its elongation
(*d*) termination

1. Initiation

The first reaction in the E coli system is the binding of mRNA to the 30S subunit in the presence of IF3 to yield a mRNA–30S-IF3 complex with a ratio of 1:1:1. IF1 and IF2 now participate in the binding of 6 MET–tRNA and GTP to the 30S mRNA IF3 complex to form the initiation complex of 30S mRNA-fMET-tRNA-GTP with the release of IF3. Now the 50S ribosomal subunit enters into the picture. GTP is hydrolyzed to GDP + Pi and both IF1 and IF2 are released. The final product is a 70S complex containing fMET-tRNA-mRNA with fMET-tRNA occupying the peptidyl site of the 70S ribosome.

2. Elongation step

This step involves three stages:

(1) The codon directed binding of a new amino acyl tRNA to site A of the 70S ribosome.

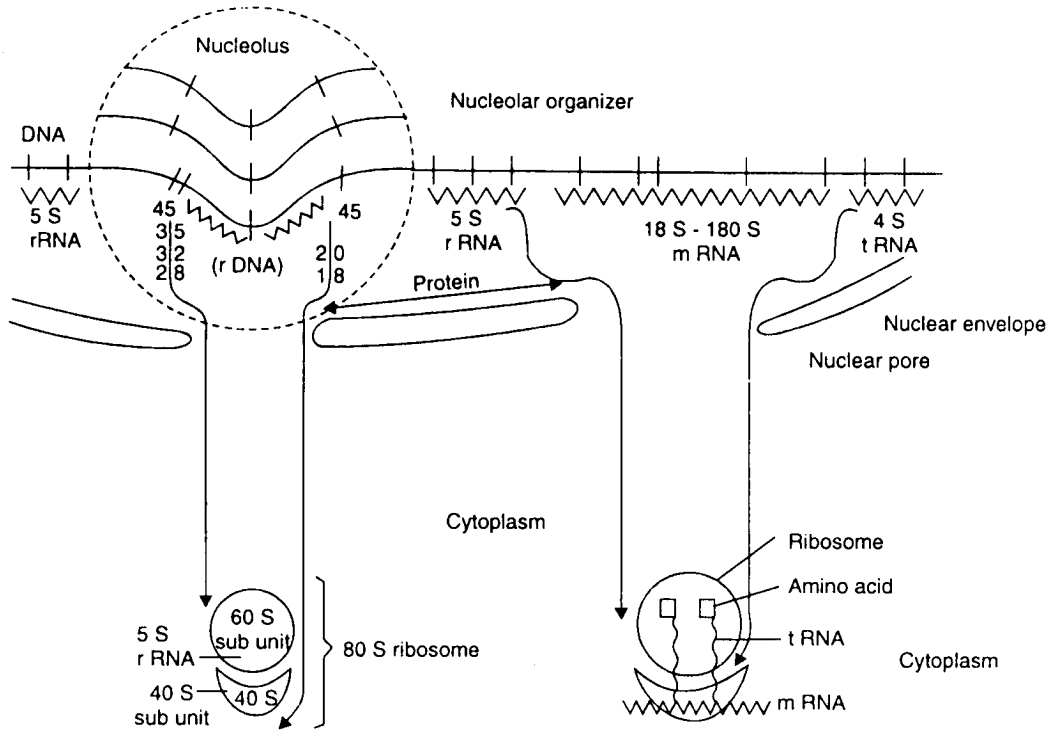


Fig. 12.2

(2) Peptidyl transfer from the peptidyl residue of the tRNA bound to the P site, to the newly bound amino acyl tRNA on site A thereby forming a new peptide and

(3) Translocation of the newly formed peptidyl (n+1) tRNA from site A to site P on the 70S ribosome by a movement of the 70S ribosome in a 5' → 3' direction on the mRNA.

Step 1:

A new and specific amino acyl tRNA is bound to the A site of the 70S ribosome as determined by the codon on the mRNA at the A site. GTP and two elongation factors, EFTu and EFTs, are involved. EFTu in a complex with GTP, namely EFTu. GTP interacts with an amino acyl tRNA to form a ternary complex. The following event then occurs:

→ EFTu – GTP + Amino acyl + RNA ⟶ Amino acyl + RNA . EFTu . GTP

Amino acyl tRNA . EFTu . GTP + mRNA Ribosome . FMET. tRNA

↓

Amino acyl tRNA . mRNA . fMET . tRNA Ribosome + EFTu . GDP + Pi
(A site) (P site)

EFTu . GDP + EFTs ⟷ EFTu . EFTs . GDP

EFTu . EFTs + GTP ⟷ EFTu . GTP + EFTs

Of some importance all amino acyl tRNAs must react with EFTu . GTP in order to bind at the A site of the 70S ribosome. The significant exception is fMET – tRNA. Since this initiator amino acyl tRNA does not react with EFTu . GTP, its insertion into an internal position in the elongation polypeptide is avoided.

Step 2:

The formation of the new peptide bond is catalyzed by specific proteins associated with the 50S ribosome subunit. The peptidyl moiety associated with its tRNA on the P site is transferred to the amino group of the amino acyl tRNA on the A site to form a new peptide bond, leaving a deacylated tRNA on the P site. Relatively high concentrations of K^+ cations are required for this reaction. The antibiotic, puromycin, inhibits protein synthesis at this step. The peptidyl transferase can transfer the peptidyl residue from the charged tRNA, bound to the P site to puromycin to form a peptidyl puromycin that is released from the ribosome and is inactive.

Step 3:

The translocation process involves the shift of the new peptidyl tRNA from the A site to the P site with the deacylated tRNA on the P site being released from the ribosome. In this shift, the peptidyl tRNA remains bound to its codon mRNA but the ribosomes move relative to the peptidyl tRNA in a 5' $\rightarrow$ 3' direction, thereby positioning it's A site over the next codon on the mRNA. For this translocation to occur, a new factor, EFG is required as well as GTP, which is hydrolyzed to GDP + Pi.

3. Termination

The termination reaction consists of two steps:

(1) The recognition of a termination signal in the mRNA and

(2) The hydrolysis of the final peptidyl tRNA ester linkage to release the nascent protein.

The termination codons are UAA, UAG and UGA. Three protein factors are required R1, R2 and R3. The R1 factor is required for the recognition of the codons UAA and UAG, and R2 is required for the recognition of UAA and UGA. The third protein R3 has no release activity but appears to aid in terminator codon recognition. The picture that is emerging suggests that the termination step can be divided into a terminator codon dependent R1 or R2 factor binding reaction in which either R1 or R2 converts the peptidyl transferase activity at site P into a hydrolytic reaction with the transfer of the peptidyl tRNA to water rather than to another amino acyl tRNA. A final factor TR, may be involved in discharging the residual tRNA from site P, once the tRNA, is removed, the 70S ribosome dissociates from the mRNA into a 30S and a 50S subunit and is ready to reenter. The ribosomal cycle for the synthesis of another protein molecule IF3 combines with the 30S subunit thereby preventing a reassociation of the 50S and 30S units and also prepares the 30S unit for recycling .

The nascent protein presumably has formyl methioyl NH_2 terminus that must be removed before the protein completes its folding sequence. Two enzymes may participate at this final stage:

1. A specific deformylase
   Formyl methionyl peptide $\rightarrow$ Formic acid + Methionyl peptide
2. A specific aminopeptidase
   Methionyl peptide $\rightarrow$ Methionine + Peptide

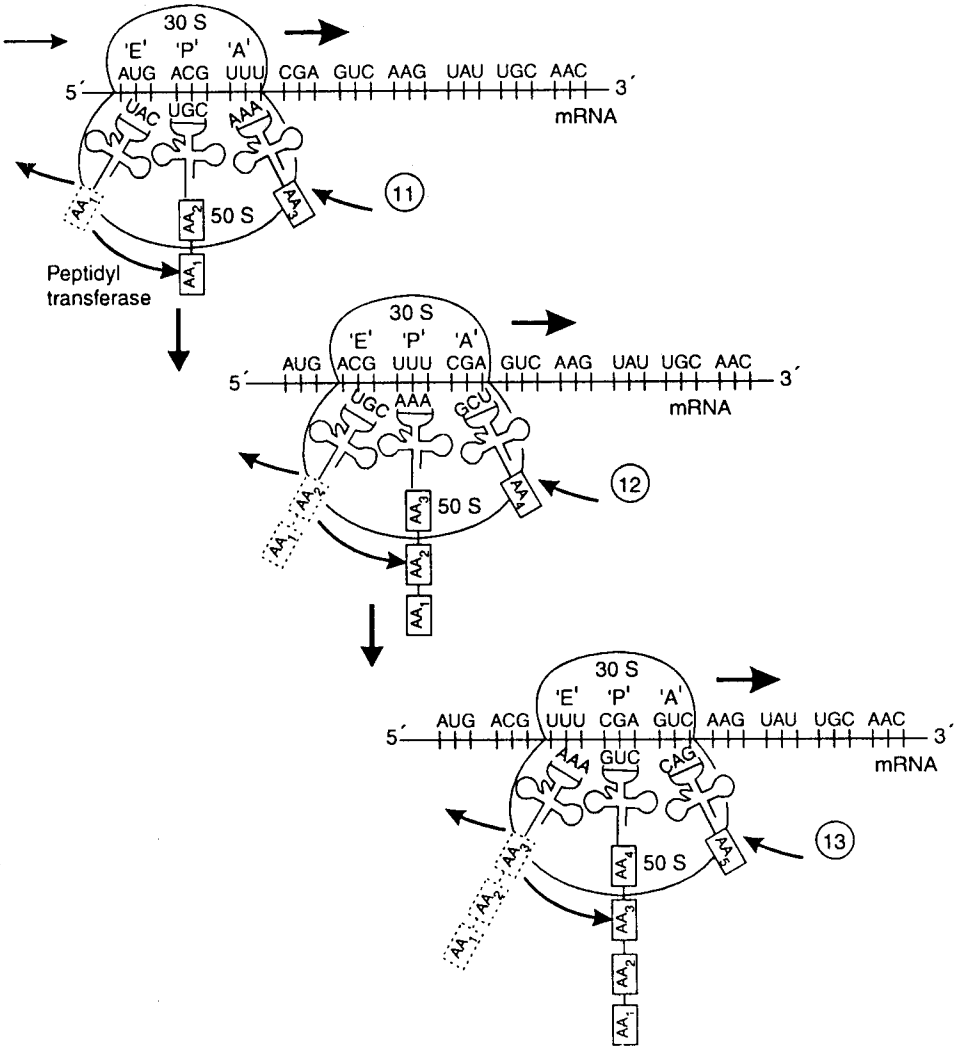


Fig. 12.3. Diagrammatic representation of various steps of protein synthesis.

Q. Mention the energy requirements during protein synthesis.

Ans. The reactions to occur require the energy. The energy requirements during protein synthesis are as follows—

2 ATP bonds are required in the activation of amino acid.

1 GTP is hydrolyzed in the binding of amino-acyl-t-RNA to A site.

1 GTP is hydrolyzed in the translocation ribosomes.

Total of 4 high energy bond_4 × 4.3 _29.2Kcal, for each peptide bond is synthesized. And each bond gives about 50 K Cal to generate peptide bond. Infact, it is the most expensive process in cells which is biosynthetic.

Q. What are the inhibitors of Protein synthesis?

Ans. Many inhibitors which are used in human beings for treating infections act by inhibiting the protein synthesis in the prokaryotes.

Inhibitors	*Site of action*
Chloramphenicol	Block peptidyl transfer in 70 S ribosome.
Streptomycin	Binds to 30 S subunit to affect initation
Tetracycline	Inhibits binding of incoming amino acyl –t- RNA to A site.
Puromycin	Reacts with peptidyl t-RNA to give puromycin –peptidyl- t-RNA.
Fusidic acid	Inhibits translocation.

Q. What is Genetic Code?

Ans. The sequence of bases on the mRNA directs the precise synthesis of amino acid sequence of a protein. The codon, the unit that codes for a given amino acid, consists of a group of three adjacent nucleotide residues on mRNA, the next three nucleotide residues on the mRNA code for the next amino acid etc. The evidence for these conclusions is based on considerable data. Some generalization considering the code are as follows:

(1) The code is **universal,** that is all prokaryotic and eukaryotic organisms use the same codons to specify each amino acid.

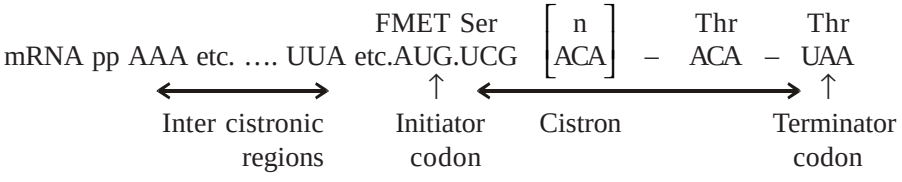
(2) The code is **degenerate,** that is more than one arrangement of nucleotide triplets specify the same amino acid. Thus, UUA, UUG, CUU, CUC, CUA and CUG are codons for leucine.

(3) The code is **nonoverlapping,** that is, adjacent codons do not overlap.

(4) The code is **commaless,** that is, there are no special signals or commas between codons.

(5) Of the 64 possible triplet codons, 61 are employed for encoding amino acids. Three UAA, UAG, and UGA had been orginally called nonsense codons, but now are recognized as specific chain termination codons for the COOH end of a peptide chain.

(6) The codon AUG is of considerable intrest, since it is the only codon for methionine regardless of whether f M-E-T - tRNAfmet or MET-tRNAfmet are employed as methionyl carriers. It serves as the extremely important initator codon as well as for internal methionyl insertion. Presumably, the initation factors IF1, IF2 and IF3 or the secondary structure of the mRNA discriminates as to the correct use of AUG. The roles of the initator and terminator codons are depicted in Figure.



(7) In general, amino acids with hydrocarbon residues have U or C as the second base, those with branched methyl groups have U as the second base. Basic and acidic amino acid have A or G as the second base.

Q. Explain how the regulation of gene expression occurs.

Ans. Genes, carrying the genetic information, expresses itself by leading to the formation of a specific protein through the process of transcription and translation. Hence, the regulation of gene expression in effect means the regulation of protein synthesis. This regulation can therefore, takes place at the following two levels.

1. Transcription control i.e., the formation of m-RNA from the gene is the point of regulation.
2. Translational control,i.e., the synthesis of the proteins from m- RNA is the point of regulation.

However, in the most bacteria and prokaryotes transcriptional control is the main regulatory method.

Transcriptional control

The basic process of protein synthesis is regulated by induction and or repression. Depending upon the metabolic state of the organism, certain enzymes i.e., the proteins are either induced or repressed.

Jacob and Monard proposed the concept of Operon to explain the phenomenon of induction and repression as the means of transcription control of protein synthesis. Initially, this hypothesis named as operon model was postulated with particular reference to lactose metabolism regulation in E.coli by the genetic pathway.

This hypothesis postulates the existence of an operon as the group of functionally related structural genes lying contiguous to each other in the chromosomes which can be turned off and on coordinately by the same regulatory gene. Since they explained the protein synthesis with particular reference to lactose, they proposed the lactose operon as the model for regulatory mechanism .

This explained the induction of three protein brought about by the lactose and the repression of these proteins by the presence of glucose in the following way.

There are present structural genes which are responsible for transcription m- RNA for three proteins i.e., β – galactosidase, permease and transacetylase. These three functional genes are under the regulation of an inhibitory locus- now called as regulatory gene. This is then the operator locus on the chromosome of DNA adjacent to the structural genes. The regulatory gene normally exerts an inhibitory in the structural genes preventing them from transcripting the various m- RNAs by means of a protein molecule called repressor. This repressor then binds with the operator to inhibit the transcription under normal circumstances.

The binding of the repressor, which is reversible to the operator interferes with the bindings of RNA polymerase on to the promotor, another locus present in the vicinity of operator. There appears to be some overlapping in the limits of the promotor and operator. To initate the protein synthesis and when the repressor molecule is not bound to the operator, the transcription is carried out uninterrupted by the structural gene. Thus according to this operon model, the repressor controls the rate of transcription of DNA into RNA.

13

BIOCHEMISTRY OF HORMONES

Q. What are exocrine glands?

Ans. Most glands of the body deliver their secretions by means of ducts. These are called exocrine glands.

Q. What are endocrine glands and hormones?

Ans. There are few glands that produce chemical substance that they directly secrete into the blood stream for transmission to various target tissues. These are ductless or **endocrine glands.** The secretions of endocrine glands are called as **hormones.** Therefore, it is a chemical substance which is produced in one part of the body, enters the circulation and is carried to distant target organs and tissues to modify their structures and functions.

Q. Define hormones.

Ans. Hormones are stimulating substances and act as body catalysts. The hormones catalyze and control diverse metabolic processes. Despite their varying actions and different specificity's depending on the target organ, the hormones have several characteristics in common with enzymes.

They act as body catalysts resembling enzymes in some aspect. They are required only in small quantities. They are not used up during the reaction. They differ from enzymes because they are produced in an organ other than that in which they ultimately perform their action.

Q. List the major hormone secreting glands.

Ans. The major hormone secreting glands are as follows—

Pituitary
Thyroid
Parathyroid
Adrenal
Pancreas
Ovaries
Testes

Q. How the hormones are chemically classified?

Ans. The hormones are classified into following main classes according to the chemical structures.

Table Chemical classes of hormones

Chemical class	*Examples*	*Where produced*
Steroids	Aldosterone, cortisol	Adrenal cortex
	Androgens (Male sex hormone)	
	Calcitrol	Kidneys
	Testosterone	Testes
	Estrogens, Progesterone	Ovaries
	(Female sex hormones)	
Biogenic amines	T_3, T_4 (Thyroid hormones)	Thyroid gland.
	Epinephrine and	(Follicular cell)
	Norepinephrine	Adrenal medulla
	Histamine	Mast cells
	Serotonin	Platelets in blood
	Melatonin	Pineal gland
Peptides and protein	All hypothalamic releasing and inhibiting hormone	Hypothalamus (neuro secretory cells)
Oxytocin	Oxytocin; ADH	Hypothalamus
	Insulin, glucagon, somatostatin, pancreatic	Pancreas
	polypeptide	Parathyroid hormone
	Calcitonin	Thyroid gland
	Hormones regulating digestion (Gastrin, secretin	Stomach and intestine
	cholecystokinin)	
	Erythropoietin	Kidneys
Eicosanoids	Prostaglandins leukotrienes	All cells except RBC
Steroids	All sex hormones	Testes and ovaries

Q. Describe few important properties of hormones.

Ans. The few important properties of hormones are as follows—

Action in low concentration

Hormones act in a very low conc. like vitamins.

Storage destruction and excretion

Hormones are not ordinarily stored, except in the gland of origin. They do not have any cumulative action, because they are destroyed and excreted as soon as their functions are over. Some hormones work quickly and are destroyed quickly e.g. epinephrine, other perform their work slowly and are also disposed of slowly e.g. Thyroxine.

Mode of action

Role of c-AMP and Hormone Action

3'-5' c-AMP plays a unique role in the action of many protein hormones. Its level may be decreased or increased by hormonal action as the effect varies depending on the tissue. The hormones such as glucagon, catecholamines, PTH, etc. act by influencing a change in intracellular c-AMP concentration through the adenylate cyclase- c-AMP system. The hormone binds to a specific

membrane receptor. Different types of these receptors remain associated with either Gs or Gi type of GTP dependent trimeric nucleotide regulatory complexes of the membrane. Both Gs and Gi are made up of 3 subunits: Gs contains $\alpha_s\beta\gamma$ while Gi contains $\alpha_i\beta\gamma$. Formation of the receptor-hormone complex promotes the binding of GTP to the α subunit of either Gs or Gi. When α_s-GTP is released it binds to adenylate cyclase located on the cytoplasmic surface of the membrane and changes its conformation to activate it. However, in some cells calmodulin – 4 Ca^{++} is also required for activation. Adenylate cyclase catalyzes the conversion of ATP to c-AMP thus increasing the intracellular concentration of the latter. On the other hand α_I-GTP inhibits adenylate cyclase by binding with it. This lowers the intracellular concentration of c-AMP. The action of c-AMP is mainly to activate some protein kinase allosterically.

Role of Calcium in Hormone Action

The action of most protein hormones is inhibited in absence of calcium even though ability to increase or decrease c-AMP is comparatively unimpaired. Thus calcium may be more terminal signal for hormone action than c-AMP. It is suggested that ionized calcium of the cytosol is the important signal. The source of this calcium may be extracellular fluid or it may arise from mobilization of intracellular tissue bound calcium. The hormone receptor binding may directly inhibit the Ca^{++}-ATPase. It may also directly open up voltage-independent Ca^{++} channels in the membrane to increase the diffusion of Ca^{++} into the cell down its inward concentration which then acts as a second messenger to affect cellular activities. The receptor-hormone complex may produce ITP which in turn can increase cytosolic Ca^{++} concentration by enhancing the mobilization of Ca^{++} from mitochondrial and endoplasmic reticular pools. Calcium is involved in the regulation of several enzymes such as phospholipase A_2 Ca^{++}-phosphatidyl serine dependent protein kinases, guanylate cyclase, adenylate cyclase and glycogen synthetase. All these enzymes have special biochemical metabolic roles. Ca^{++} also changes membrane permeability. Many of its effects are mediated through its binding to Ca^{++}-dependent regulatory proteins like calmodulin and troponin.

Q. Explain the regulation of hormone secretion.

Ans. Hormone secretion is strictly under control of several mechanisms. They are—

Neuroendocrinal Control mechanism

Nerve impulses control some endocrine secretions. Cholinergic sympathetic fibres stimulate catecholamine secretion from adrenal medulla. Centres in the midbrain, brainstem, hippocampus, etc. can send nerve impulses which react the hypothalamus through cholinergic and bioaminergic neurons. At the terminations of these neurons they release acetylcholine and biogenic amines to regulate the secretions of hypophysiotropic peptide hormones from Hypothalamic peptidergic neurons. Some of the endocrine releases are controlled by either stimulatory or inhibitory hormones from a controlling gland, e.g., corticosteroids are controlled by corticotropin and thyroid hormones are controlled by thyrotopin from anterior pituitary. The tropins are further regulated by Hypothalamic releasing hormones.

Feedback Control Mechanism

It is due mainly to negative feedback that such control is brought about. When there is a high blood level of a target gland hormones, it may inhibit the secretion of the tropic hormones stimulating that gland. Adrenal cortex secretes a hormone called cortisol which bring about the inhibition of secretion of corticotropin from anterior pituitary and corticotropin releasing hormone from the hypothalamus by a long-loop feedback. This leads reduction in cortisol secretion.

Q. Enumerate the details of major endocrine glands and their hormones.

Ans. The release of hormones into the blood stream and spread of depolarization through nerves transmit signals, which allow an animal to respond to changes in its environment. Either nerve impulse or a change in the concentration of some substance into the blood which perfuses the gland may trigger such release. Such a substance called hormone is produced by a gland or specialized neuro secretory cell, is often stored in this cell, and is released into the blood stream in response to some specific stimulus. As soon as the hormone reaches its target cells, it modifies the function of the cell to remove the stimulus that primarily caused the release of the hormone. This negative feedback mechanism operates to maintain homeostasis within the organism. Signals that are transmitted through nerve possess the advantage of localization and speed to single cells within a large group of similar cells.

In the human body the different endocrine glands and their secretion/hormones are given in table

Table Different endocrine glands and their hormones

	Gland/organs	*Hormones*
I.	Pituitary gland	(1) Growth (GH) or STH
		(2) Thyroid Stimulating Hormone (TSH) or Thyrotrophin
		(3) Adrenocorticotrophin (ACTH)
		(4) Leuteinising (LH) or Interstitial Cell Stimulating Hormone (ICSH)
		(5) Follicle Stimulating Hormone (FSH)
		(6) Prolactin or Leuteotrophic (LTH)
		(7) Melanocyte Stimulating (MSH) or Intermedian
	(Posterior Pituitary)	(1) Vasopressin or Antidiuretic hormone (ADH)
		(2) Oxytocin
II.	Thyroid gland	(1) Thyroxine
		(2) Triiodothyroxine
		(3) Calcitonin
III.	Parathyroid gland	(1) Parathyroid hormone
IV.	Adrenal gland	(1) Glucocorticoids
		(2) Mineralocorticoids
		(3) Epinephrine
		(4) Nor epinephrine
V.	Ovary	(1) Oestrogen
		(2) Progesterone
VI.	Testes	(1) Testosterone
VII.	Pancreas	(1) Insulin
		(2) Glucagon
VIII.	Pineal body	(1) Melatonin

Q. Write a note on Pituitary gland and its hormones.

Ans. The pituitary gland consist of two parts, anterior and posterior, which are separated by pars intermedia.

Hormones of Anterior pituitary gland

(1) Growth hormone
(2) Thyroid stimulating hormone
(3) Follicle stimulating hormone

(4) Prolactin
(5) Melanocyte stimulating hormone

(1) Growth Hormone (Somatotropin)

Growth hormone from all mammalian species consist of a single polypeptide with a molecular weight of about 21500. It consists of 191 amino acids. There are two disulfide bridges between the adjacent cysteine residues (53 and 165 and 182 and 189).

Metabolic functions

Growth hormone has a variety of effects on different tissues. The hormone acts slowly requiring from 1-2 hours to several days before its biological effects are detectable. This slow action and its stimulatory effects on RNA synthesis suggest that it is involved in protein synthesis. The hormone acts by binding to specific membrane receptors on its target cells. But its exact mechanism of action and the second messenger are not yet known.

Growth hormone brings about **positive nitrogen balance** by retaining nitrogen. It stimulates overall protein synthesis with an associated retention of phosphorus probably by increasing tubular reabsorption. Blood amino acid and urea level are decreased. It facilitates the entry of amino acids into the cell. It also brings about lipolysis in a mild way by mobilizing fatty acids from adipose tissue by activating the hormone sensitive triacylglycerol lipase. Thus it increases circulating free fatty acids.

Growth hormone is a diabetogenic hormone, antagonizes the effect of insulin. Hypersecretion of GH can result in hyperglycemia, poor sugar tolerance and glycosuria Growth hormone when secreted in abnormally high concentration prolongs the growth of epiphyseal cartilages to cause overgrowth of long bones. Acromegaly is found in adults. Hyposecretion causes stunted stature due to premature cessation of growth of the epiphyseal cartilages and consequently of long bones. Growth hormones has a sequence homology with prolactin. Growth hormone binds to membrane receptors for prolactin and stimulates the growth and enlargement of mammary glands.

It is observed that the intestinal absorption of calcium is increased by GH, since the bone growth and development is stimulated by growth hormone. Growth hormone retains Na, Ca, K, Mg and PO_4^{-3}.

(2) Thyrotropic Hormone or Thyroid Stimulating Hormone (TSH)

This is produced by basophil cells of anterior pituitary and is glycoprotein in nature. Its molecular weight is approximately 30,000. This consists of a α and β subunits.

The α-subunit of TSH, LH, HCG and FSH are nearly identical. The biological specificity of thyrotropin must therefore be in β-subunit.

Metabolic function

There are glycoprotein receptors on the thyroid cell membrane which bind to the receptor binding site on β-subunit of TSH. The complex then activates adenylate cyclase which catalyzes the formation of c-AMP which acts as the second messenger for most TSH actions as follows:

The TSH stimulates the synthesis thyroid hormones at all stages such as iodine uptake, organification and coupling. It enhances the release of stored thyroid hormones. It increases DNA content, RNA and translation of proteins, cell size. It stimulates glycolysis, TCA cycle, HMP and phospholipid synthesis. Stimulation of last two does not involve c-AMP. It activates adipose tissue lipase to enhance the release of fatty acids (lipolysis).

(3) Adrenocorticotropic Hormone (ACTH) or Corticotropin

It is a single polypeptide containing 39 amino acids in its structure with a molecular weight of 4500. Two forms have been isolated, α-corticotropin and β-corticotropin.

Metabolic function

The principal actions of corticotropin are exerted on the adrenal cortex and extra adrenal tissue. ACTH increases the synthesis of corticosteroids by the adrenal cortex and also stimulates their release from the gland. Profound changes in the adrenal structure, chemical composition and enzymatic activity are observed as a response to ACTH. ACTH also stimulates the synthesis and secretion of glucocorticoids.It is found to increase the transfer of cholesterol from plasma lipoproteins into the fasciculata cells. It induces rise in c-AMP, brings about phosphorylation and activation of cholesterol esterase. The enzyme action ultimately makes a large pool of free cholesterol.It leads to increased ketogenesis and decreased R.Q.The direct effects of ACTH on carbohydrate metabolism include, lowering of blood glucose, increase in glucose tolerance; deposition of glycogen in adipose tissue is increased, regarded as due to stimulation of insulin secretion.

(4) Pituitary Gonadotrophins

These tropic hormone influence the function and maturation of the testes and ovary. These are—

Follicle Stimulating Hormone (FSH)

Leutenizing Hormone (LH)

Both of them are glycoproteins with sialic acid, hexose and hexosamine as the carbohydrate moiety (16%). Molecular weight of FSH is 25000 and that of LH is 40000.

Leutenizing Hormone (LH)

Metabolic function of LH

This hormone is also known as interstitial cells stimulating hormone (ICSH).

In females it causes the final maturation of Graffian follicle and stimulates ovulation. It also stimulates secretion of estrogen by the theca and granulosa cells. It helps in the formation and development of corpus luteum for leutinization of cells. In conjunction with leuteotropic hormone (LTH), it is concerned with the production of estrogen and progesterone by the corpus luteum. In the ovary it can stimulate the non germinal elements, which contain the interstitial cells to produce the androgens, andro stenedione, DHEA and testosterone.

It is necessary for final follicular growth and ovulation. Without this hormone, even though large quantities of FSH are available, the follicle will not progress to the stage of ovulation. LH acts synergistically with FSH to cause rapid swelling of the follicle shortly before ovulation. It is worth noting that especially large amount of LH called ‘ovulatory surge’ is secreted by the pituitary during the day immediately preceding ovulation.

Follicle Stimulating Hormone (FSH)

Metabolic function of FSH

It brings about its action by specific receptor binding and C-AMP.

In females it promotes follicular growth and prepares the Graffian follicle for the action of LH and enhances the release of estrogen induced by LH.

In males it stimulates seminal tubule and testicular growth, and plays an important role in maturation of spermatozoa.

Role of FSH in Spermatogenesis

The conversion of primary spermatocytes into secondary spermatocytes in the seminiferous tubules is stimulated by FSH. In absence of FSH spermatogenesis cannot proceed. However, FSH by

itself cannot cause complete formation of spermatozoa. For its completion testosterone is also required. Thus, FSH seems to initiate the proliferation process of spermatogenesis, and testosterone is apparently necessary for final maturation of spermatozoa. Since the testosterone is secreted under the influence of LH, both FSH and LH must be secreted for normal spermatogenesis.

(5) Prolactin

This is a simple monomeric protein. It contains 199 amino acids with three -S-S linkages.

Metabolic functions

The main functions of prolactin is to stimulate mammary growth and secretion of milk. By acting through specific glycoprotein receptors on plasma membrane of mammary gland cells, it stimulates m RNA synthesis. This ultimately leads to the enlargement of breasts during pregnancy. The synthesis of milk proteins such as lactalbumin and casein takes place after parturition such an effect is called as lactogenic action.

Hormones of middle lobe of pituitary

The hormones secreted by intermediate lobe or middle lobe of pituitary gland are called melanocyte-stimulating hormones or MSH. MSH darkens the skin and is involved in skin pigmentation by deposition of melanin by melanocytes.

Hormones of Posterior Pituitary

The hormones of posterior pituitary gland are—

(1) Vasopressin (Pitressin) or ADH

(2) Oxytocin

These are small peptides containing nine amino acids. Oxytocin differs from Vasopressin with respect to 3rd and 8th amino acid residues. Their biological activities depend on C-terminal glycinamide, the side chain amide groups of glutamine and asparagine,the hydroxy phenyl group of tyrosine, and the intra-chain – S – S – linkage between cysteine Ist and 6th amino acid.

Metabolic function of Vasopressin

Antidiuretic action— Antidiuretic effect is its main function. It reabsorbs water from the kidneys by distal tubules and collecting ducts. It is found to be mediated through formation of c–AMP. It is released due to rise in plasma osmolarity. This leads to formation of hypertonic urine having low volume, high sp. gr. and high conc. of Na^+, Cl^-, phosphate and urea. Halothane, colchicines and vinblastine inhibit antidiuretic effect of vasopressin.

Inappropriate vasopressin secretion is characterized by a persistently hypertonic urine, progressive renal loss of Na^+ with low plasma levels of Na^+, symptoms of water intoxication like drowsiness, irritability, nausea, vomiting, convulsions, stupor and coma.

Metabolic functions of Oxytocin

Contraction of smooth muscle is the primary function of oxytocin. There are basically two effects, one on mammary glands called as galactabolic effect and the other on uterus called as uterine effect.

Q. Describe the hormones of thyroid gland.

Ans. The principal hormones secreted by the follicular cells of thyroid are:

(1) Thyroxine (T_4)

(2) Tri-iodo thyronine (T_3) and

(3) 'Reverse' T_3

The hormones T_4, T_3 and "reverse" T_3 are iodinated amino acid tyrosine. The iodine in thyroxine accounts for 80% of the organically bound iodine in thyroid venous blood. Small amounts of 'reverse' tri-iodo thyronine, Monoiodotyrosine (MIT) and other compound are also liberated.

Metabolic functions of thyroid hormones

The hormones of thyroid gland plays an active role in the metabolism. Their main affects are on protein and carbohydrate metabolism.

Effect on protein metabolism:

In moderate concentrations the thyroid hormone has an anabolic effect, causing an increase in RNA and protein synthesis, an action which precedes increased BMR not only is RNA synthesis increased, but there is increased protein synthesis only. In hypothyroidism, the hormone has an anabolic effect, on plasma and high tissue protein but catabolic action on extracellular proteins. In high concentrations, negative nitrogen balance is observed and protein synthesis is depressed.

Effects on carbohydrate metabolism:

It increases the blood sugar and causes glycosuria. There is increase in glucose utilization, and decrease in glucose tolerance. Thyroid hormones are, therefore, antagonistic to insulin. They increases the rate of absorption of glucose from intestine. Decreased glucose tolerance may be contributed to also by acceleration of degradation of insulin.

Effects on lipid metabolism:

The thyroid hormones increases lipolysis in adipose tissue thus increasing plasma free fatty acids. This effect is rather indirect in the sense it increases sensitivity to catecholamines, potentiates the lipolytic effect of epinephrine, by increasing the β–adrenergic receptors on adipocyte cell membrane. They may stimulate, at the same time, lipogenesis by increasing the activities of malic enzyme, ATP citrate lyase and Glucose-6-Phosphatedehydrogenase.

Q. Mention the hormones of Parathyroid gland.

Ans. The hormones of parathyroid glands are—

(1) Parathormone (PTH)

(2) Calcitonin.

Parathormone is a linear polypeptide consisting of 84 amino acids. N-terminal amino acid is alanine and C-terminal is glutamine. Bovine PTH has molecular wt of 9500. Parathormone from different species differ only slightly in structure. The parathyroid gland is the principal target organ for bone and kidneys. It increases the rate of bone reabsorption with consequent mobilization of calcium and phosphate. An early effect on osteocytes leads to release of calcium from crystals of mature bone into blood, occurs within 15-30 minutes and does not initially depend upon the synthesis of RNA by osteocytes.

Metabolic functions of parathyroid

It directly increases renal tubular reabsorption of calcium and direct renal excretion of phosphate. It may increases the rate of calcium ion absorption in the gut and may also decrease the rate of calcium ion secretion by lactating mammary gland. The parathyroid glands are intimately concerned with regulation of the concentration of Ca and PO_4 ions in the blood plasma. This is accomplished by secretion of a hormone, parathormone (PTH) by the chief cells, the net effect of which is to increase

the concentration of Ca and decrease the PO_4.In addition to its effects on plasma ionized Ca via its action on bone, parathormone controls renal excretion of Ca and PO_4.

Calcitonin

Calcitonin is a calcium regulating hormone. It is proved that calcitonin originates from special cells, called "C-cells", parafollicular cells. C-cells constitute an endocrine system which has only been appreciated recently. These cells are derived from "neutral crest" and are found in thyroid, parathyroid and in thymus. Calcitonin is a single chain lipophilic polypeptide, having a mol. wt. of 3600. It contains 32 amino acids, N-terminal amino acid is cysteine, and C-terminal prolinamide. An interchain disulfide bridge joins two cystine residues between position 1 and 7. Low number of ionizable groups are present, 5 of 6 possible –COOH groups being amidated.

Mechanism of Action

Calcitonin binds to specific calcitonin receptors on the plasma membrane of bone osteoclasts and renal tubular epithelial cells, activates adenyl cyclase which increase c-AMP level and mediates the cellular effects of the hormone. This is the principal method by which calcitonin acts. Calcitonin may directly affect the relative distribution of bone cells. The hormone both in vitro and in vivo produce a cellular shift, in which the number of osteoclasts are decreased. Calcitonin may regulate pH at cellular level producing more alkaline medium which diminishes resorption.

Metabolic functions of calcitonin

Calcitonin acts both on bone and kidneys.

Indirectly, the effects of these two organ systems account for Hypocalcaemia and Hypophosphataemia produced by the hormone. Calcitonin inhibits the resorption of bones by osteoclasts and thereby reduced mobilization of Ca and inorganic PO_4 from bones into the blood. It is also stimulates influx of phosphates in bones. There is decrease in activities of lysosomal hydrolases, pyrophosphatase and alkaline phosphatase in bones. The hormone acts on the distal tubule and ascending limb of loop of Henle and decreases tubular reabsorption of both calcium and inorganic PO_4 thus producing calcinuria and phosphaturia.

Q. Describe the important functions of adrenal hormones.

Ans. The adrenal gland is composed of an outer cortex and inner medulla. The cortex is responsible for secretion of steroid hormones. The medulla is responsible for secretion of epinephrine.

The adrenal cortex hormones are of two types

(1) Glucocorticoids

(2) Mineralocorticoids

Glucocorticoids

They primarily affect metabolism of carbohydrates, proteins and lipids and relatively have minor effects on electrolytes and water metabolism, e.g. cortisol, cortisone and corticosterone.

Mineralo-corticoids

Mineralo-corticoids are those which primarily affect the reabsorption of Na^+ and excretion K^+ (Mineral metabolism) and distribution of water in tissues, e.g. Aldosterone.

Metabolic functions of Glucocorticoids

In general, glucocorticoids have anti-insulin effect. They are catabolic to peripheral tissues and anabolic to liver. Over-all effect increases blood glucose level (Hyperglycaemia).

Metabolic functions of Mineralo corticoids

The mineralocorticoids (Aldosterone) stimulates renal absorption of sodium, hydrogen, ammonium and magnesium ions. Due to its pronounced sodium retention property it increases water absorption.

Adrenal medullary hormones

Two biologically active compounds have been isolated from the adrenal medulla and synthesized. They are:

(1) Epinephrine

(2) Norepinephrine (Nor adrenaline)

The naturally occuring forms are laevorotatory, the synthetic are racemic, the former being almost twice as active as the latter. The above two hormones are called catecholamines and are closely related to tyrosine and synthesized in body from tyrosine. Epinephrine is primarily synthesized and stored in adrenal medulla. Norepinephrine is primarily synthesized in sympathetic nervous system and acts locally as a 'neurotransmitter' at the post synaptic cell. Norepinephrine is also synthesized and stored in adrenal medulla.

Metabolic functions of Catecholamines

Epinephrine stimulates rapid breakdown of glycogen of liver (glycogenolysis) producing hyperglycaemia. In muscles, epinephrine also cause breakdown of glycogen (glycogenolysis) by increasing cyclic AMP level (β-effect), but in this tissue it is more active than glucagon. Glucagon has very little effect or no effect due to lack of specific receptors. An increases in cyclic AMP after epinephrine administration is seen in 2 to 4 seconds, the effect of epinephrine on cardiac output (ionotropic effect) is seen shortly afterwards, whereas activation of phosphorylase is not detectable In vivo, actually epinephrine action can result in an increase in heart glycogen. Both epinephrine and norepinephrine increases breakdown of triacyl glycerol in adipose tissue by increasing cyclic-AMP level (β_1 effect). Net effect of lipolysis is rapid release of FFA and glycerol from adipose tissue to blood. Epinephrine increases hepatic gluconeogenesis (β_2 effect).

Norepinephrine and epinephrine are almost equally potent in their calorigenic action. They produce a prompt rise in the metabolic rate which is independent of the liver, and a smaller delayed rise which is abolished by hepatectomy.

Q. Write a note on the important functions of Gonadal Hormones.

Ans. The sex hormones or the gonadal hormones are elaborated by the testes, ovary and corpus luteum mostly, and also in small quantities by the placenta and adrenal cortex. They are all steroid compounds related to cholesterol and are synthesized from that precursor. Sex hormones are also related to the adrenal cortical hormones both in the chemical nature and in the common biosynthetic pathway and inter conversions.

Sex hormones are of 3 types:

Androgens or male hormones

Oestrogens or female hormones

Gestrogens or progestational hormones.

Androgens

Androgens are hormones capable of producing certain characteristic musculinizing effects, i.e. they maintain the normal structure and function of the prostate and seminal vesicles and influence the development of secondary male sex characteristics, such as hair distribution and voice. The naturally occuring androgens in man are:

Testosterone
Epiandrosterone(3β - androsterone)
Androsterone
Dehydro epiandrosterone (DHEA)

Metabolic functions of Androgens

Both testosterone and dihydrotestosterone are protein anabolic and growth promoting hormone. Their dominant general metabolic effect is stimulation of protein anabolism. This is reflected in, a decreased urinary nitrogen without an increase in blood NPN. There is decrease in hepatic arginine synthetase activity, the enzyme that catalyzes conversion of citrulline to arginine. Androgen promote protein synthesis in male accessory glands. It causes increased RNA synthesis and RNA polymerase activity in the nucleus, and increased amino acyl transferase at the ribosomal level. Androgens increase the fructose production by seminal vesicles and utilization of this sugar by the seminal plasma by enhancing the activity of both aldose reductase as well as keto reductase.

Female sex hormones

Two main types of female hormones are secreted by the ovary:
The follicular or Estrogenic hormones
The Pro gestational hormone

Estrogen

Estrogens are hormones capable of producing certain biological effects, the most characteristic of which are the changes which occur in mammals of estrus. They include growth of female genital organs and the appearance of female secondary sex characteristics. The growth of the mammary duct system and numerous other phenomena which vary somewhat in different species.

The naturally occurring estrogens in humans are:

β–Estradiol
Estrone
Estriol

The principal estrogenic hormone in circulations and the most active form of the estrogen in β – estradiol, which is in the metabolic equilibrium with estrone. Estriol is the principal estrogen found in the urine of pregnant women and in the placenta.

Metabolic functions

After administration of estrogens their is proliferation of vaginal epithelium and endometrium, an increase in glycogen contents of the cells, and increase in alkaline phosphatase activity in endometrium is observed. Glycogen also increases in vaginal epithelial cells. There is increased rate of glycolysis with accumulation of lactic acid (LA). The vaginal glycogen is probably the source of L.A, which, by increasing the acidity of the vaginal secretion (pH 4.0 to 5.0), favours a homogeneous flora of acid bacteria.

Luteal hormones
Progestrone

Progesterone is the hormone of the corpus luteum, the structure of which develops in the ovary from the ruptured Graffian follicle. It is also formed by the placenta, which secretes progesterone, during the later part of pregnancy. Progesterone is also formed in the adrenal cortex, as a precursor of both C19 and C21 corticosteroids. It is also formed in the testes.

Metabolic functions

In the humans, progesterone produces characteristic changes (progestational) in the estrogen primed endometrium. This hormone appears after ovulation and causes extensive development of the endometrium preparing the uterus for the embedding of the embryo and for its nutrition.

Relaxin

Relaxin is a hormone concerned with the relaxation of pelvic tissues and cavity operating in conjunction with other factors. Relaxin is produced, during pregnancy, in tissues of the reproductive system, e.g., principally by corpus luteum and also by placenta. Its production is stimulated by progesterone, pregnenotone and related adrenocortical steroids, e.g. deoxy corticosterone.

Metabolic functions

It increases the vascularity of the connective tissue of the symphysis.

Q. What are Placental hormones? Mention their important functions.

Ans. Pregnancy activates the placental hormones. The implanted blastocyst forms the trophoblast which is subsequently organized into the placenta. The placenta provides the nutritional connection between the embryo and the maternal circulation.

Human placenta produces and secretes two peptide hormones and two steroid hormones.

Human chorionic gonadotropin hormone (hcG)
Chorionic somatomammotropin (CS)
Progestins
Estrogens

Human Chorionic Gonadotropin

It is a glycoprotein .This binds to specific receptor on the cell membranes of the target tissues like ovaries and testes.

Metabolic functions

Luteotrophic effect

The hormone produces enlargement of corpus luteum and stimulates its secretion. It maintains a secretory corpus luteum in first three months of pregnancy.

Testosterone secretion

Like LH, the hormone stimulates the growth of interstitial cells (Leydig cells) of embryonic testes and produces testosterone. This helps in virilization of the reproductive system of male embryo.

Chorionic somatomammotropin(Placental Lactogen):

This hormone has biologic properties of prolactin and growth hormone of pituitary gland. It is a peptide hormone and amino acid sequences are similar to that of growth hormone and prolactin.

Metabolic functions

The exact role of this hormone is not clear, because pregnant women lacking this hormone have normal pregnancies and deliver normal babies.

14

DIET AND NUTRITION

Q. What is diet and balanced diet? What are their essential elements.

Ans. A correct diet provide the nutrients for the maintenance of the body as well as energy requirements, for growth and reproduction. The more important factors are

1. Energy value
2. Quality and quantity of primary foods, minerals and vitamins.
3. Variation in the diet
4. Digestibility
5. Cooking
6. Psychological factors
7. Cost

Energy value

The average caloric requirement of the adult male is 3,000 daily.The figures of the daily caloric requirement are given below:

Workhouse	3,702 Calories
Person (Class B)	3,038 Calories
Person (hard labour)	4,159 Calories

Quality and Quantity of the constituents of food

Primary foods (Protein, fat and carbohydrate):

Protein, fat and carbohydrate are consumed in the ratio of 1:1:4 in our country. 3,000 calories are provided by 1,00 grams of proteins, 100 grams of fat and 400 grams of carbohydrate. Although the 1:1:4 ratio is prevalent in this country still wide variations of carbohydrate and fat may occur without harm. It is advisable that 10%-15% of the total calories should be obtained from protein, 20%-35% from fat and 50%-66% from carbohydrate.

Balanced diet

Definition

A balanced diet is one which contains all the food constituents in proper proportions to meet the energy and nutritional requirements of the individual.The proportions of proteins, fat and carbohydrate should approximately be 1:1:4 respectively. The balanced diet for an adult male of 70 kg requiring 3,000 calories per day should contain the following:

Protein	…….	70 gms
Fats	…….	50 gms

Carbohydrate	…….	440 gms
Calcium	…….	0.8 gms
Phosphorus	…….	1.4 gms
Iron	…….	40 gms
Vitamin A	…….	7,300 I.U
Vitamin B_1	…….	1.8 mg
Vitamin C	…….	200 mg

Values in calories of some common foods

Foods		*Calories*
Banana	…….	132/one full
Desi ghee	…….	830-890/100 gms
Butter	…….	720/100 gms
Egg	…….	165/100 gms
Buffalo's milk	…….	109/100 ml
Meats	…….	100-450/100 gms
Potato	…….	83/one medium size
Cow's milk	…….	69/100 ml
Human milk	…….	67/100 ml
Apple	…….	66/one
Peas (Cooked)	…….	56/half cup
Fishes	…….	50-150/100 gms
Tomato (raw)	…….	20/one medium size

Principles in planning a balanced diet

In planning a balanced diet it is to be aimed that the diet must contain various groups of foodstuffs such as energy yielding food, body building foods and protective foods in the correct proportions. The constituents of balanced diet differ according to age, sex, physical activity, economic status and the physiological condition.

Q. What are energy yielding foods? Give an account of them.

Ans. The energy yielding foods contains high carbohydrates and also pre fats and carbohydrates. They are divided into two groups: (a) cereals, roots and tubers and (b) pure carbohydrates and fats. Cereals provide proteins, certain minerals and vitamins in addition to energy in the diets of the low-income groups.Roots and tubers provide some amounts of proteins, minerals and vitamins.Pure carbohydrates and fats provide only energy.

Q. What are body building foods?

Ans. These contain high protein and are divided into two groups: (a) Milk, egg, meat and fish. (b) Pulses, oilseeds and nuts.

Q. What are protective foods?

Ans. The protective foods are rich in proteins, vitamins and minerals. These are classified into two groups:

(*a*) Foods rich in vitamins, minerals and proteins of high biologic value e.g. milk, eggs, fish and liver.

(*b*) Foods rich in certain vitamins and minerals only e.g. green leafy vegetables and some fruits.

Q. Give an account of Food Toxins and Additives.

Ans. A good number of potentially harmful compounds are present in foods. Some occur naturally. They include harmful substances such as the neurotoxins from shellfish or mushrooms, goitrogens from plants of the cabbage family, bean compounds that interfere with collagen formation. Pesticides and packaging materials are added to food through inadvertent contamination. Mainly compounds are added to foods in order to preserve them or to add colour, flavour or texture. Some of the additives may be harmful. Consumption of fresh and unprocessed foods are advised in order to minimize intake of natural toxins and additives.

Q. Write a note on Proteins in terms of diet.

Ans. The expected amount of protein is 100 g per day, less amount can also maintain health and nitrogen equilibrium. Even 1g per kg body weight may be ample. Larger amounts are necessary for growth, pregnancy and lactation. Increased amounts may be required in wasting diseases. The protein requirements vary with the net protein utilization (NPU) of the dietary proteins. If the NPU is low, the requirements are high and if the NPU is high, the requirements are low.

The requirement of protein in the diet is not only quantitative; the qualitative aspect is also important since the metabolism of protein is connected with that of its constituent amino acids. Certain amino acids are called 'essential' because they must be obtained preformed and cannot be synthesized by the animal organism. The remainder, the so called non-essential, are also required by the organisms since they are found in the protein of the tissues, but they can be synthesized from α-keto acids by amination. Histidine in the diet is also necessary to maintain growth during childhood. Such amino acids are said to be relatively essential. The nutritive value of a protein depends on its content of essential amino acids.

Some proteins, if they are the sole source of nitrogen, will not support life, whereas others are sufficient. Hence, proteins are of two types. First class or good protein and second class or poor protein. First class protein can support life in the absence of other forms of protein. Proteins of animal origin (meat, milk and eggs) are almost completely utilized if taken alone; whereas, 10% to 40% of vegetable proteins (peas, beans, potatoes) may remain unabsorbed and excreted in the feces. The absorption of vegetable proteins is improved if taken with other foodstuffs. Animal proteins are associated with fat, but very little carbohydrate (except milk); whereas vegetable proteins are almost associated with a large amount of carbohydrate.

It has been observed that the dietary requirement for protein is influenced markedly by the level in the diet of fat and carbohydrate. These latter foodstuffs have a “Protein-sparing” effect. Fat, primarily, functions as a fuel (9 Cal/g), carbohydrate also serves as a fuel (4 Cal/g), but is required for the synthesis of certain catalytic compounds of metabolic cycles (e.g. oxaloacetate in the TCA cycle) and provides the carbon skeletons for the synthesis of the non essential amino acids. At least 5 g of carbohydrate per 100 calories must be supplied if nitrogen equilibrium is to be maintained. Protein has a more catalytic function in the form of enzymes. In the absence of fat and carbohydrate from the diet, protein is degraded to provide fuel (4 Cal/g) and catalytic compounds of metabolic cycles. Essential amino acids may be broken down to supply the materials for the synthesis of non essential amino acids. For these increased burdens, the protein intake is ultimately increased. The protein requirement is decreased when fats and carbohydrates are taken alongwith.

Proteins differ in "biologic value" depending on their content of essential amino acids. The proteins of eggs, daily products, kidney and liver have high biologic value since they contain all of the essential amino acids. Good quality proteins include soya beans, peanuts, potatoes and the muscle tissue of meats, poultry and fish. Fair proteins are cereals and most root vegetables. Poor biologic value includes nuts and legumes. The mixture of poor and fair can provide good biologic value.

Proteins are also the important sources of nitrogen, sulphur, and phosphorus for the body. Many amino acids have specific functions in metabolism such as methionine as a methyl donor, cystine as a source of –SH groups, the dicarboxylic acids (Aspartic acid, glutamic acid) in transamination, tryptophan as precursor of niacin, arginine in the urea cycle etc.

The protective substances in a diet are those which are essential for specific purposes other than energy production, i.e. first class proteins, mineral elements, and vitamins.

Q. Write a note on Fats in relation to its nutritive value.

Ans. Fat has high fuel value. It increases the palatability of foods. It has got high capacity to be stored as energy in the body. Fat provides the essential fatty acids such as linoleic, linolenic and arachidonic acids. Fats are essential for the absorption of fat-soluble vitamins. Excess of saturated fat and cholesterol consumption is associated with hypercholesterolemia and atherosclerosis. A lack of fat causes a feeling of hunger shortly after a meal due to rapid digestion in its absence. Fat reduces the bulk of the food.

The consumption of fat usually is over 80g per day. It may increase up to 150 g. with an increase of family income.

As a source of energy there is little difference between animal and vegetable fats. Animal fats have a better biologic value because they contain vitamins A and D in particular. Animal fats are most used when the incidence of sunlight is low.

Fats provide fat-soluble vitamins and the essential fatty acid linoleic acid which is required for the synthesis of arachidonic acid from which protaglandins are synthesized in the body. The major symptoms of essential fatty acid deficiency are a scaly dermatitis, hair loss, and poor wound healing. Linoleic acid is widely distributed in the lipid portion of both plant and animal foods; vegetable seed oils are especially rich sources.

In societies where fat is the principal food for energy intake, the population tends to develop coronary heart disease, obesity, and cancer of the bowel and breast.

Blood cholesterol level can be reduced significantly by changing the diet containing less saturated fatty acids or cholesterol. The serum cholesterol level is the major factor for the development of artherosclerosis.

Q. Why carbohydrates are called energy yielding foods?

Ans. Carbohydrate is the cheapest source of food. It can be readily digested, absorbed and utilized for producing energy. It is most efficient source of energy for vital processes. It can furnish 60-80% of the total caloric intake in the scarcity of proteins and fats. It is bulky and liable to undergo fermentation producing acid (e.g. lactic acid) if digestion is delayed. Hence, it is not wise entirely to replace fat by carbohydrate.

In gluconeogenesis, carbohydrate can be supplied from most amino acids as well as form the glycerol moiety of fats. A minimum of 5 g of carbohydrate per 100 KCal of total diet is necessary to prevent the development of ketosis. Sufficient carbohydrate should be included in the diet to have complete oxidation of fat. The amount is near about 400 g per day.

The chief carbohydrates are starch and sugars (from milk, fruits and vegetables as well as sucrose). Glycogen is only consumed in living tissues such as oyesters. Carbohydrates are almost entirely derived from vegetable sources. Cellulose stimulates peristalsis. Some cereals, e.g. oatmeal, contain much phytin as to interfere with the absorption of calcium.

Galactose is synthesized in the body and lactose is also synthesized from galactose in the body. Hence, no need of giving lactose to lactating women. Pentoses are absorbed and excreted unchanged except ribose or deoxyribose Sugar is valuable for muscular work in minimizing fatigue.

Sucrose (common table sugar) is one of the major etiologic factors in dental caries. This sugar is not the cause of diabetes, heart disease, or obesity.

Q. Give an account of minerals in relation to diet and nutrition.

Ans. Some minerals are always required since small amounts of inorganic salts are always excreted. Fruits, vegetables and cereals are the chief sources of the mineral elements in the diet. Milk products supply the majority of the calcium and phosphorus in the diet.

Calcium:

Increased amounts of calcium are required by children, pregnant and lactating women.Children, whose normal intake is low, can absorb a higher percentage of calcium ingested than those with a high intake.In many Eastern countries, where little milk is taken, the average consumption by adults is about 400 mg daily but this low intake does not show any signs of calcium deficiency. The average intake should be about 800 mg per day. The best sources are cheese and milk. Other sources are eggs, green vegetables, oranges, nuts, buttermilk, beans, and carrots. Meat, fish and fruits are poor sources of calcium. Hard water is a source of calcium.

Phosphorus:

This is taken in organic and inorganic form. Children should intake 1 g per day, adults 1.3 g and a pregnant or lactating woman 1.9 g. The best sources of phsophorus are animal foods such as meat, fish, milk, cheese and eggs. Considerable proportions of the total phosphorus are provided by the vegetable foods such as beans, rye, oatmeal and lentils. Cereals provide phosphorus in the form of phytic acid which interferes with the absorption of calcium. Hence, animal sources of phosphorus are better.

Iron:

It is an impotant constituent of the diet and its deficiency leads to anaemia. The more concentration of iron is from bread, meat and potatoes. Good sources are liver, kidney, egg yolk, green peas, cabbage, carrots and cereals. Only less than 10% of iron is absorbed in iron-deficient subjects about 20% may be absorbed. Milk is very poor in iron. The infant is born with a store of iron which maintains for six months. After this period, iron is given along with food human milk contains 1-2 mg iron per litre, cow's milk less. Children up to 12 years require 15 to 20 mg daily, adults require 40 mg daily and the daily requirements during pregnancy and lactation are 50 mg.

Iodine:

The daily requirement of iodine is 0.05 mg and iodine is obtained from water, vegetable and fish. In certain localities, where the soil and water lack this element, simple goitre results due to dietary deficiency. Fish, cod liver oil and vegetables can supplement iodine deficient diet.

Magnesium:

The average daily consumption of about 0.2 g is sufficient. Good sources are meat, green vegetables and bread.

Copper:

The daily adult requirement is 2 mg. Good sources are liver, oysters, cocoa, nuts etc. Human and cow's milk only contain about 0.6 mg per litre.

Sodium Chloride:

Ordinarily 20 g of the salt is consumed per day. The intake of the salt is only required if abnormal quantities are lost in sweat during severe muscular exercise at high temperature. Heat stroke is prevented by ingestion of salt solution instead of water.

Q. List the importance of vitamins in daily diet.

Ans. Normal persons get sufficient vitamins in their diets and hence supplementation with vitamin is not required. Only in disease state in which digestion and absorption are impaired, the supplementation with vitamin is most essential. Many of the vitamin are destroyed by cooking and storage. Hence, fresh fruits and vegetables should be taken daily. Cereals, when refined, lose B-vitamins. Therefore, food is improved by the addition of vitamins. Deficiency symptoms of water-soluble vitamins consist of dermatitis, anaemia, digestive difficulties, and neurologic disorders. Most of the water-soluble vitamins act as coenzymes in metabolism of substrates. Vitamin B_{12} is synthesized by microorganisms. It is present only in meat and dairy foods. Therefore, strict vegetarians may be at risk for vitamin B_{12} deficiency. The fat-soluble vitamins are mainly present in fatty meats, liver, dairy fats, egg-yolks, vegetable seed oil and leafy green vegetables. Fat-soluble hypervitaminosis produces symptom of toxicity. Deficiencies of fat-soluble vitamins occur primarily in young children who lack adequate body stores. Deficiencies are rare in adults unless there is malabsorption, biliary obstruction, or other conditions that affect fat metabolism.

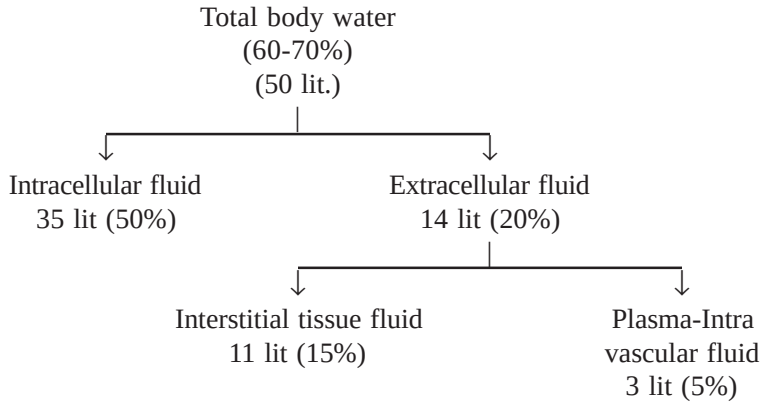
Q. Differentiate between intracellular fluids and extra cellular fluids.

Ans. In all living cells, all chemical reactions occur in aqueous environment. Approximately 75% of the mass of the living cell is water. The body water can be visualized to be distributed mainly in two components,

(1) Intra cellular fluids
(2) Extra cellular fluids

(1) **Intracellular fluids** — The fluid present in the cells which is approximately 35 litres.
(2) **Extra cellular fluids** — The fluid present outside the cells which constitutes approximately 14 litres. The extracellular fluid (ECF) is considered to be present in two compartments as follows—
   (*a*) Plasma — The fluid present in heart and blood. It is approximately 3 litre.
   (*b*) Interstitial tissue fluid — It is present in between the two cells and is approximately 11 litres.

The overall pattern of distribution of water is shown in Fig



Metabolic Functions of Water

It is difficult to sum up the functions of water. The great utility of water as a biologic medium, both intracellularly and extracellularly can be attributed to its ability to form hydrogen bonds and its dipole moment.

Under normal conditions the relative volumes of water in ECF and ICF are kept constant. The water can pass freely through the membrane which divide plasma from tissue fluid and tissue fluid from extracellular fluid. But the overall distribution of water is controlled by the osmotic pressure exerted by substances present in each compartment i.e. electrolytes and protein molecules. Membranes separating ICF from tissue fluid is semipermeable and allows only passage of water but not protein molecules and electrolytes.

Normally, there is osmotic equilibrium between these two compartments, but if it is distributed, water is drawn from the compartment with lower osmotic pressure into that with higher osmotic pressure until equilibrium is restored. The osmotic unbalance between these two compartments results, in water being either sucked out of the cells producing **cellular dehydration** or water is drawn into the cells producing **cellular oedema** to restore balance.

Q. What are Electrolytes? Discuss their movement in the body.

Ans. The body fluids consists of water and dissolved substances. Some substances such as glucose, urea and creatinine do not dissociate in solution. while substances like NaCl, KCl, in solution dissociate into Sodium (Na^+), Potassium (K^+) and Chloride (Cl^-) ions. These are called **electrolytes.** The water molecules completely surrounds these dissociated ions and prevent union of +vely charged particles with –vely charge ones. The +vely charged ions are called **cations** while –vely charged ions are called **anions.**

The solutes in the body fluid are mainly of two categories.

(1) **Organic** — There are organic compounds i.e. glucose, urea, uric acid etc. They are non-electrolytes, and do not dissociate or ionize in solution. Since these substances diffuse relatively freely across cell membrane and do not play an important role in the distribution of water. While some other organic compounds mainly proteins are of large molecular weight. The effect of protein is mainly on the transfer of fluid from one compartment to another and not on the total body water.

(2) **Inorganic** — These are inorganic compounds and are called **electrolytes.** They are present in large quantities and play most important role in distribution and retention of body water.

Electrolytes Composition of ECF

The plasma and tissue fluid are to be considered as single compartment.

Table Major cations and anions in ECF

		Cations mEq/L		*Anions mEq/L*	
I	Plasma	Na^+	143	Cl^-	103
		K^+	5	HCO_3^-	27
		Ca^{++}	5	HPO_4^-	2
		Mg^{++}	2	SO_4^-	1
				Proteins	16
		Total	125	Organic acids	6
				Total	155
II	Tissue fluid	Na^+	145	Cl^-	116
		K^+	5	HCO_3^-	27
		Ca^{++}	3	HPO_4^{-2}	3
		Mg^{++}	2	SO_4^{-2}	2
				Proteins	1
		Total	155	Organic acids	6
				Total	155

Electrolytes Composition of ICF

In intracellular fluid mainly K^+ and Mg^{++} are present. ICF contains 195 mEq of cations and anions.

Table Major cations and anions in ICF

Cations mEq/L	
K^+	150
Mg^{++}	40
Na^+	5
Total	195

Anions mEq/L	
HPO_4^-	110
Protein	50
SO_4^{-2}	20
HCO_3^-	5
Total	195

Movement of Electrolytes

The much higher concentration of Na^+ and Cl^- in interstitial fluid and K^+ in intracellular fluid are accompanied by a difference in electrical potential. The resting skeletal muscle cells are at about 90 mv –ve to the interstitial fluid. It is believed that the lipid-protein membrane plays an important role in determining and maintaining these differences in concentration and potential. K^+ ions tend to diffuse out of and the Cl^- ions into the cells because of their concentration gradients, but this is almost

counter balanced by a tendency to diffuse in the opposite direction due to the difference in electrical potential i.e. the relative negativity on the inside of the cells tend to keep Cl^- out and K^+ in.

In the case of Na^+, however diffusion into the cells is favoured by both the concentration gradient and electrical potential. Cells do not allow accumulation of Na^+, hence under normal healthy conditions, there must be some mechanism for removing Na^+ from the cell, virtually as rapidly it enters. Since, this has to be accomplished in opposition to forces of concentration and electrical potential. It involves expenditure of energy, derived from cellular metabolism. The process of active transport of Na^+ out of cells is done by **Sodium Pump,** which effectively extrudes Na^+ from the intracellular fluid. This extrusion of Na^+ from the cells is associated with spitting of ATP by $Na^+ – K^+$ ATPase located at the inner surface of the cell membrane. The enzyme is activated by Mg^{++}, and has a molecular weignt of 250,000 to 300,000. It consists of two large subunits, α and β, of which α subunit is catalytically active. The energy of hydrolysis of ATP is used by the transport mechanism for the coupled-exchange of Na^+ and K^+ ions between the intracellular and tissue fluids.

Normal Electrolyte balance

The human systems consume fluids and foods which vary markedly both in quantity and quality, electrolyte levels in subjects from any two widely located regions of the world are within narrow normal ranges. The organs which are constantly regulating the electrolyte levels are intestine, kidneys, gastrointestinal tract and kidneys.

In the kidneys, the principal ECF ions enter the lumen of GI tract and renal tubules and their near complete reabsorption regulate electrolyte levels. About 3 litres of fluid of different electrolytes enter GI tract, everyday and is reabsorbed almost completely with fluid loss. Approximately 100 to 150 ml, and electrolyte loss of Na^+ approximately 10-30 mEq and of K^+ approximately 10 mEq. Internal circulation of salts is continuously occuring in kidneys, and is at a much faster rate than that observed in GI tract. In kidneys, a volume of plasma equal to ECF(12 to 15 lit) is filtered and reabsorbed every 2 hours and about 25,000 mEq of Na^+ are filtered and reabsorbed everyday. Na^+ is reabsorbed from the renal tubules in exchange with H^+ and NH_4^+ in proximal and distal tubules respectively.

Q. Write a note on following electrolytes present in the body.

Chloride.
Sodium
Phosphorus
Calcium
Potassium

Chloride/Chlorine

Occurrence and Distribution

The chlorine is taken in the form of chloride, i.e. sodium chloride. Many vegetables and meats have small proportions of chloride. It is also available in the chlorinated water, which is normally supplied as a process of purification of water for drinking purposes. Approximately 250 mg/dl of chloride is present in body, 375 mg/dl is present in plasma, 440 mg/dl in CSF, 40 mg/dl in muscles and 190 mg/dl in cells.

Functions

It is important in the function of HCl in gastric juice. It is important in **chloride shift.** The CO_2 that is derived as an end product from cellular metabolism, diffuses from tissues through plasma and

into RBC where the CO_2 conc. is relatively low. Within the erythrocyte the CO_2 is combined with water to form H_2CO_3 by carbonic anhydrase. The acid then dissociates into a bicarbonate and H^+. The H^+ is buffered by the Hb and the HCO_3^- diffuses from the red cells into the plasma in exchange of Cl^-. The reverse reaction occur when the erythrocyte reaches the lung where its CO_2 content exceeds the alveoli. Thus, arterial and venous plasma will differ slightly in their plasma chloride concentration.

Cl^- is the chief anion in the body, particularly in extracellular fluids. Its excretion in the urine is slightly higher than that of Na^+, however, the kidney is capable of secreting urine atmost free of Na^+ and Cl^-.

Clinical determination and Significance

To find normal or abnormal level of chloride in body during dehydration, routine urine analysis is performed. The excess level of chloride in urine states the depletion of Cl^- in body.

Sodium

Occurrence and distribution

Sodium is an electrolyte which is found in large concentration in extracellular fluid compartment. The total concentration of sodium in body is about 3150 mmol/litre. The sodium is mainly associated with chloride and bicarbonate as NaCl and $NaHCO_3$ respectively.

Functions

It is almost found in all body fluids. The adult human body contains about 100 g of Na^+. It is lost from the body through urine and sweat, which is passed out in urine and is regulated by the kidney, but that which is passed out in sweat is not regulated by the kidneys. The requirement of NaCl depends upon climate, occupation and physical activity. People engaged in hard work will need more sodium.

The sodium maintains crystalloids osmotic pressure of extracellular fluids and help in retaining water in ECF. Sodium is water distributing tissue salt and it enters into the composition of every fluid and solid of the body. Because of its powerful affinity for water, it controls the inflow and outflow of the body fluids. Its prime functions is to maintain a proper degree of moisture throughout the system. Along with the other cations Na+ is also involved in neuromuscular irritability.

Phosphorus

Occurrence and distribution

Phosphorus in an essential mineral required for the formation of bones and teeth, it plays an important part in all metabolism.

Total phosphate in body is about 700 g. More than 60% (600g) is found in bones, 15% in soft tissues and 1% is found in ECF. About 5g is in brain and 2g in blood.

Functions

About 1.5g of phosphate is required to be taken in the daily diet. 90% of the daily dietary phosphate is absorbed. The absorption is stimulated by both parathyroid hormone and Vit D_3. The Ca:P ratio in the diet affects the absorption and excretion of phosphorus. If one is in excess in diet, the excretion of other in increased.

The regulation of Ca and P is under the similar control mechanisms by kidney with respect to PTH and Vit D. The phosphate uptake is sodium dependent, about 85% of filter phosphate is

reabsorbed by the proximal tubules. Phosphate reabsorption is increased where dietary intake is reduced by PTH dependent mechanism. The plasma inorganic phosphate is a major regulator of 25OH Vit D 1 hydroxylase activity.

Clinical determination and Significance

The phosphorus is a trace element in the body. The normal or abnormal level in body is estimated by performing serum analysis.

Calcium

Occurrence and Distribution

Calcium is a mineral element of the body. There is dynamic equilibrium between the calcium in the blood and that in skeleton. This equilibrium is maintained by the interaction of Vitamin D, parathyroid hormone (PTH) and probably calcitonin.

The total calcium of the body is 25-35 mols (100g-170g) about 99% of it is found in bones. The normal level of plasma calcium is 9-11 mg/dl. The calcium in plasma is of three types i.e. ionized calcium (diffusible), protein bound calcium and complexed calcium, it is probably complexed with organic acids. About 40% of total calcium is in ionized form. Albumin is the major protein with which calcium is bound. All three forms of calcium in plasma remain in equilibrium with each other. Ionized calcium is physiologically active form of calcium.

Functions

Calcium has a role in neuromuscular transmission. Calcium ions are needed for excitability of nerves. It also plays role in permeability of gap junctions. It plays role as secondary or tertiary messanger in hormone action. Calcium plays active role in blood coagulation by producing substances for thromboplastic activity of blood. The process of bone formation and teeth formation is continuous process, where it is the chief acting mineral.

Potassium

Potassium is the major intracellular cation. The adult human body contains about 250 g of potassium. It is readily distributed in the body fluids and tissues as follows-cells-440 mg/dl.

Whole blood	200 mg/dl
Nerves	530 mg/dl
Plasma	20 mg/dl
Muscles	250-400 mg/dl

Potassium is easily absorbed. As soon as it is absorbed, it enters the cells. It is excreted in the urine. The amount of potassium excretion increases, when there is an excessive dietary intake of sodium. Average normal human body contains 3 - 6 mols of potassium. The concentrations of intracellular K^+ is 150 mE/L which is roughly equal to the concentration of Na^+ outside the cell. The normal concentration of plasma potassium is 3.5 - 5 mEq/L. the $Na^+ – K^+$ ATPase or sodium pump maintains this concentration gradient. Potassium is also excreted in gastro-intestinal tract, saliva, gastric juice, bile, pancreatic juice and intestinal juices.

The most of the functions of potassium and sodium are carried out in coordination with each other and are common. They are body's main positively charged ions and are found in every cell of the body. Sodium is mainly found outside the cells and potassium inside the cells.

15

ORGAN FUNCTION TEST

Q. What are Renal Function Tests? List the Renal function tests? Describe in detail any one RFT.

Ans. Kidney plays an important role in the maintenance of acid base balance and volume of water in the body. It serves an important function of excretion of products of metabolism and other harmful substances. The assessment of renal functions involves urine analysis, blood chemistry, urography and some special renal function tests.

Each kidney contains about 1 million nephrons. A nephron has a glomerulus and a long tubule that has three parts

(1) The proximal convoluted tubule (PCT)

(2) The thin loop of Henle (LH)

(3) The distal convoluted tubule (DCT)

The glomeruli are the ultra filter and the filtrate produced is like plasma except that it has almost no protein, 180 litres of this filtrate is produced in 24 hrs, of which 178 litres of water and most of the organic and inorganic solutes are reabsorbed. Normally, some components of the filtered solutes are actively absorbed; glucose, phosphate, amino acids, sodium etc. for some solutes, the maximum reabsorptive capacity of the tubule is limited and filtered material in excess of this limit is passed on in the urine. Normal renal threshold for glucose is 180 mg m%; if excess is presented to the nephrons, it would result in glycosuria. Other solutes are not reabsorbed or are only passively and partially reabsorbed or are actively secreted by the tubule. Inulin, a carbohydrate used for renal function studies is not at all reabsorbed by the tubules. Some urea is passively reabsorbed but most of the filtered urea escapes reabsorption. Exogenous creatinine, H^+, K^+, phenol red (PSP), para aminohippurate and penicillin are actively secreted by the tubule cells, thus increasing excretion over the amount filtered.

Classification of Renal function Tests

On the basis of different functions of kidney the renal function test can be classified as follows

I. Test based on glomerular filtration
Urea Clearance Test
Endogenous Creatinine Clearance Test
Inulin Clearance Test
Cr^{51} EDTA Test

II. Test based on tubular function
Concentration and Dilution Test

PSP excretion test
Measurement of tubular secretary mass

III. Tests to measure Renal Plasma Flow (RPF)
Para amino hippurate test (PAH)
Filtration fraction
Maximal Tubular Capacity (Tm)

I. Tests based on Glomerular Filtration

Clearance Tests

Urea clearance, creatinine clearance and inulin clearance test are used to examine for impairment of glomerular filtration. Clearance is means of expressing quantitatively the rate of excretion of a given substance by the kidney. This is defined as a volume of blood or plasma, which contains the amount of substance, which is excreted in the urine in one minute or alternatively, the clearance of a substance, may be defined as that volume of blood or plasma cleared of the amount of substance found in one minute excretion of urine.

Urea Clearance Test

The normal blood urea level is in the range of 20 – 40 mg/dl. Uraemia donates a high level of blood urea and is often due to retention caused by kidney damage. It can also rise in dehydration and it catabolic states. Urea clearance is defined as the volume of blood or plasma cleared of urea per minute. If the urine volume exceeds 2 ml/min, the rate of urea elimination is at a maximum and is directly proportional to the concentration of urea in the blood.

Volume of blood-cleared urea per minute can be calculated from the formula

$$\frac{U \times V}{B}$$

Where U = concentration of urea in urine (mg).
V = volume of urine in ml/min.
B = the concentration of urea in blood (mg).

Substituting average values, the number of ml of blood cleared of urea per minute

$$= \frac{100 \times 2.1}{2.8} = 75$$

It means that the amount of urea excreted in the urine in one minute is equal to the amount found in 75 ml of blood.

The clearance which occurs when the urinary volume exceeds 2 ml/min is termed as **Maximum Urea Clearance (Cm)** and averages normal value is 75.

Cm = 75 ml (normal range 75 ± 10)

Standard Clearance

When the urinary volume is less than 2 ml/min, the rate of urea elimination is reduced, because relatively more urea is reabsorbed in the tubules, and is proportional to the square root of the urinary volume. Such clearance is termed as **Standard Clearance of Urea (Cs),** and average normal value is 54.

$$Cs = \frac{U \times \sqrt{V}}{B} = 54 \text{ (normal range } 54 \pm 10)$$

If a large volume than normal is cleared/mt, renal function is satisfactory. If a smaller volume is cleared, renal function is impaired.

Expression as %

Sometimes, the result of a urea clearance test is expressed as a % of the normal maximum or of the normal standard urea clearance depending on whether the urinary output is greater or lesser than 2 ml/min.

Expressed as % of normal

$$Cm = \frac{U \times V}{B} \times \frac{100}{75}$$

$$\% = 1.33$$

$$Cs = \frac{U \times \sqrt{V}}{B} \times \frac{100}{54}$$

$$\% = 1.85$$

Endogenous Creatinine Clearance Test

At normal levels of creatinine, this metabolite is filtered at the glomerulus, but neither secreted nor reabsorbed by the tubules. Hence its clearance gives glomerular filtration rate (GFR). This is a convenient method for estimation of GFR since

(1) It is a normal metabolite in the body.

(2) It does not require the intravenous administration of any test material.

(3) Estimation of creatinine is simple.

Measurement of 24 hour excretion of endogenous creatinine is convenient. This longer collection period minimizes the timing error.

Inulin Clearance Test

Inulin, a homopolysaccharide, is a polymer of fructose and is ideal substance, as it is not metabolized in the body. It is excreted entirely through glomerular filtration, being neither excreted nor reabsorbed by renal tubules. Hence, the number of ml of plasma, which is, cleared of Inulin in one minute is equivalent to the volume of glomerular filtrate formed in one minute.

Cr^{51} EDTA Clearance Test

This is simplified single injection method for determination of Cr^{51} EDTA plasma clearance. This test is used for routine assessment of Glomerular Filtration Rate (GFR) in adults as well as children. It is particularly convenient in children where it is not easy to collect 24 hour urine sample. It has been used for children younger than 1 year old.

Q. What are Liver function Tests. Describe any one test in detail.

Ans. Liver, is a versatile organ, which is involved in metabolism and independently involved in many other biochemical functions. The regenerating power of liver cells is tremendous. There are different functions, which are actively performed by liver cells.

Functions of liver

Metabolic functions

Liver is the key organ and the principle site where the metabolism of carbohydrates, lipids and proteins take place.

(1) It is the principle organ where cholesterol is synthesized, and catabolized to form bile acids and bile salts. Esterification of cholesterol solely occurs here.
(2) In this organ the monosaccharides other than glucose are converted to glucose i.e. galactose is converted to glucose, fructose is converted to glucose.
(3) Liver besides other organs can bring about metabolism of nucleic acids, vitamins and minerals.

Secretory function

Liver is responsible for the formation and secretion of bile in the intestine. Bile pigments-bilirubin formed from heme catabolism, is conjugated in liver cells and secreted in the bile.

Excretory function

Certain exogenous dyes like BSP (Bromosulphthalein) and Rose Bengal dye are exclusively excreted through liver cells.

Detoxication and Protective function

Kupffer cells of liver remove foreign bodies from blood by phagocytosis. Liver cells can detoxicate drugs, hormones and convert them into less toxic substances for excretion.

Storage function

Liver stores glucose in the form of glycogen. It also stores Vit B_{12}, Vit A etc.

Miscellaneous function

Liver cells are responsible for conversion of preprothrombin (inactive) to active prothrombin in the presence of Vit K. It also produces other clotting factors like factor V, VII and X. Albumin is solely synthesized in liver and to some extent α and β globulins also.

Classification of Liver function Tests

The tests used in the study of patients with liver and biliary tract diseases can be classified according to the specific functions of the liver involved.

I Tests based on abnormalities of pigment metabolism
Serum bilirubin and VD Bergh reaction
Icteric index
Urine bilirubin
Urine and faecal urobilinogen

II Tests based on liver's role in carbohydrate metabolism
Galactose tolerance test
Fructose tolerance test
Epinephrine tolerance test

III Tests based on changes in plasma proteins
Estimation of total plasma proteins(albumin and globulin).
Flocculation tests

IV Tests based on Abnormalities of lipids
Cholesterol – Cholesteryl Ester Ratio
V Tests based on the detoxicating function of the liver
Hippuric acid test
VI BSP Tests based on Excretory function of liver
Bromosulphthalein test (– reaction test)
I^{131} Rose Bengal Test
VII Formation of Prothrombin by liver
Determination of Prothrombin time
VIII Determination of Serum Enzyme activities.

I. Tests based on Abnormalities of Bile pigment metabolism

VD Bergh Reaction and Serum Bilirubin

The methods for detecting and estimating bilirubin in serum are based on the formation of a purple compound 'azo-bilirubin' where bilirubin in serum is allowed to react with a freshly prepared solution of VD Bergh's diazo reagent.

Serum bilirubin

It gives a measure of the intensity of jaundice, higher values are found in obstructive jaundice than in hemolytic jaundice.

Icterix Index

It measures the degree of jaundice by measuring the intensity of the yellow colour of the serum. The serum or plasma is diluted with physiological saline until it matches in colour a 1 in 10,000 solution of potassium dichromate (Standard solution). The dilution factor is termed the icteric index.

Interpretation

The normal range is from 4 to 6. In latent jaundice, the index is from 7 to 15.

Urine Bilirubin

Most of the tests used for detection of bile pigments depend upon the oxidation of bilirubin to differently coloured compounds such as biliverdin (green) and bilicyanin (blue).

Interpretation

Bilirubin is found in the urine in obstructive jaundice due to various causes and in 'cholestasis'. Conjugated bilirubin can pass through the glomerular filtrate. Bilirubin is not present in most cases of hemolytic jaundice, as unconjugated bilirubin is carried in plasma attached to albumin hence it cannot pass through the glomerular filtrate.

Bilirubin in faeces

Bilirubin is not normally present in faeces since bacteria in the intestine reduce it to urobilinogen. Some may be found if there is very rapid passage of materials among the intestine. It is regularly found in faces of patients who are being treated with gut sterilizing antibiotics such as neomycin.

Urinary and Faecal Urobilinogen

Urine urobilinogen

Normally there are mere traces of urobilinogen in the urine. Average is 0.64 mg, maximum normal is 4 mg/24 hours.

Faecal urobilinogen

Normal quantity of urobilinogen excreted in the faeces per day is from 50 – 250 mg. Since urobilinogen is formed in the intestine by the reduction of bilirubin the amount of faecal urobilinogen depends primarily on the amount of bilirubin entering the intestine.

Q. What are Thyroid function tests? Mention any one test in detail.

Ans. The principal hormones secreted by the follicular cells of thyroid are:
Thyroxine (T_4)
Tri-iodo thyronine (T_3)
'Reverse' T_3

The hormones T_4, T_3 and "reverse" T_3 are iodinated amino acid tyrosine. The iodine in thyroxine accounts for 80% of the organically bound iodine in thyroid venous blood. Small amounts of 'reverse' tri-iodo thyronine, Monoiodotyrosine (MIT) and other compound are also liberated.

Classification of Thyroid function tests

The assessment of hormones of thyroid gland can be performed by various procedures. But the most commonly and widely used tests are based on the primary functions of thyroid gland.

Tests based on Primary function of thyroid, viz. Substrate input and hormone synthesis:

Radio-iodine "uptake" studies and 'turn-over' (RAI or RIU) studies
PBI^{131} in serum
T_3-suppression test
TSH-stimulation test
TRH-stimulation test

Tests measuring blood levels of thyroid hormones

Serum PBI and BEI levels
Serum T_4 levels
Effective Thyroxine ratio(ETR)
Serum T_3 levels
Serum TSH level
Erythrocyte uptake of 131 Triodothyronine
Plasma Tyrosine level

Tests based on metabolic effects of thyroid hormones

Serum cholesterol
Serum creatinine
Serum uric acid
Serum CK
BMR

"Scanning" of thyroid gland

Immunological tests to detect auto-immune diseases of thyroid gland

Determination of anti thyroid auto antibodies
Determination of antimicrosomal antibodies

Tests based on primary function of thyroid

Radioactive "Uptake" Studies

Iodine plays a key role in the metabolism of the thyroid gland. I^{131} "tracer" is most commonly used for thyroid function studies because of low cost, easy availability, and convenient shelf life. Short lived isotopes of Iodine like I^{132} and I^{123} are preferred for use in pediatric practice and in pregnant and lactating women. Recently, Tc^{99m} has also been used as it behaves like iodine and has advantage of lower radiation dose to the patient.

Urinary excretion of I^{131} and "T" Index:

Renal excretion of I^{161} is an indirect evidence of thyroid function. Proportion of the administered dose excreted is inversely proportional to thyroid uptake. If uptake is "more", less of I^{131} will be excreted and vice versa. 24 hours urine is collected accurately and radioactivity is measured.

"T"-index

Activity is measured in urine sample, 0 to 8 hours after, 0 to 24 hours and 0 to 48 hours. "T"– index is calculated as follows:

$$T = \frac{\text{0–8 hrs excretion expressed as \%} \times 100}{(\text{0–24 hrs excretion expressed as \%}) \times (\text{0–48 hrs excretion expressed as \%})}$$

Interpretations:

A 'T'– index > 17 indicates hyper functioning of the gland.

A "T"– index < 2.5 indicates hypothyroidism.

Thyroid "Clearance" Rate

The amount of I^{131} that is accumulated in thyroid over a fixed interval, in relation to the mean plasma concentration of I^{131} mid-way in that time period provides the index of rate at which the thyroid gland is handling I^{131}.

$$\text{Hence, Thyroid Clearance rate} = \frac{\text{Thyroid } I^{131} \text{ accumulation rate}}{\text{Plasma } I^{131} \text{ concentration (Midway between the time period)}}$$

The above gives a direct index of thyroid activity with regard to I_2 accumulation.

Normal value: 60 ml/mt.

Serum PBI^{131}

The administered I^{131} accumulates in the thyroid gland and appears as "labeled" hormone bound to proteins. Normally it is a slow process, but in hyperthyroidism, level of Protein-bound radioactivity increases in plasma, which can be measured accurately by Scintillation counter. The result is conveniently expressed as "conversion ratio", which indicates the proportion of the total plasma radioactivity at 24 hrs.

- Normal value: 35%

T-Suppression Test:

After a 24 hrs RIU studies and obtaining the basal value and serum T_4 values, 20 µg of T_3 four times daily is given for 7 to 10 days (or alternatively 25 µg three times a day for 7 days). RIU is repeated after T_3 administration and serum T_4 values are also determined.

TSH-Stimulation Test:

Following completion of 24 hrs RIU studies, 3 injections of TSH, each 5 USP units are given at 24 hrs intervals.24-hour thyroidal RIU is measured after 42 hours after the final TSH dose.

TRH-Stimulation Test

With the availability of synthetic TRH, which is a tripeptide, suitable for human use, it is now possible to assess the functional integrity of thyrotropic cells or the factors that influence the secretory response.

Q. Mention the important functions of pancreas and list the important Pancreatic function tests.

Ans. The pancreas is a large gland situated between the duodenum and stomach. The pancreatic secretions are drained through the pancreatic duct, which join the bile duct coming from the liver. The two form the common bile duct which open in the duodenum.

Function of pancreas

The pancreas, due to dual nature functions endocrinally as well as exocrinally.

Exocrine function

The pancreatic juice produced from pancreas is a colourless, viscous fluid, alkaline in reaction, due to $NaHCO_3$ present in it. The juice contains different enzymes, which perform different functions during metabolism.

Digestive enzymes

The enzymes present in pancreatic juice are mainly proteolytic, but nucleases and amylolytic enzymes are also produced.

(1) Proteolytic enzymes

a. Trypsin—Trypsin is present in inactive form i.e., trypsinogen, which is activated by enterokinase.

$$\text{Trypsinogen} \xrightarrow{\text{Enterokinase}} \text{Trypsin}$$

Trypsin hydrolyses proteins at peptide bonds and is much more active than pepsin.

b. Chymotrypsin—It exists in two inactive forms A and B. these two forms are activated by trypsin.

$$\text{Chymotrypsinogen (A and B)} \xrightarrow{\text{Trypsin}} \text{Chymotrypsin}$$

Its action is similar to that of trypsin.

c. Collagenase—It digests collagen and initates tissue destruction in necrotizing pancreatitis.

d. Elastases—It digests elastin, which is the most resistant of all body proteins to lytic agents.

(2) Peptidases

a. Carboxypeptidase—This enzyme removes amino acids one by one from the carboxyl ends of the peptide chains.

b. Aminopeptidase—This enzyme amino acids one by one from the ends of the peptide chains bearing the free amino groups.

(3) Nucleases
   (*a*) Ribonuclease—This enzyme exists in more than one form, and hydrolyze ribonucleic acid.
   (*b*) Deoxyribonuclease—This also exists in more than one form, and hydrolyze deoxyribonucleic acid.

(4) Amylolytic enzymes
   (*a*) α - amylases—This enzyme attacks the α 1 - 4 glucosidic bonds of starches breaking them down to the disaccharide maltose.

(5) Lipolytic enzyme
   (*a*) Lipases—This hydrolyses neutral fats, splitting off one fatty acid at a time, and forms diglycerides, monoglycerides and liberate free fatty acids. It is active when the substrate is emulsified. The emulsifying action of bile salts and bile acids are helpful for its activity.
   (*b*) Lecithinase/Phospholipase—It exists in two forms A and B. They both act in succession, both of these remove fatty acids, and ultimately forms glyceryl phosphoryl choline, glyceryl, phosphoryl, ethanolamine and glyceryl phosphoryl serine.

Endocrine function

The α and β cells of the islets of Langerhans are responsible for the endocrine secretions of pancreas. They secrete glucagon and insulin respectively.

The pancreatic function tests depend on an estimation of one or more of the three digestive enzymes. The pancreatic juice obtained by duodenal incubation may be examined, but an analysis of the blood is more widely used.

Classification of Pancreatic Function tests

The pancreatic function tests can be broadly classified as

I. Determination of exocrine functions

(A) Determination of enzymes — (a) Lipase (b) Amylase

Determination of Exocrine functions

(A) Determination of enzymes (Serum)

Amylase (Serum)

Amylase attacks the α 1-4 glycosidic bonds of starches and break down to maltose.

Procedure—Two procedures are widely employed for serum amylase activity.

(1) **Sacchrometric method**—Serum is incubated with starch solution and the amount of reducing substances present is determined before and after the incubation. The difference gives a measure of amylolytic activity of the serum.

(2) **Somogyi's iodine method**—In this procedure, the time required for complete digestion of a certain amount of starch is determined by periodic testing with iodine.

Interpretations—Serum amylase estimation has been widely used in the diagnosis of acute pancreatitis. Serum amylase activity rises within hours following an episode. Values over 5 times the upper limit of normal are suggestive of the diagnosis. Values may return to normal within 5 days following a mild edematous attack. Persisting elevated values longer than this suggests continuous necrosis or possible pseudocyst formation. Its activity may be raised to 1000 Somogyi units in intestinal obstruction, strangulation, upper abdominal surgery, mumps etc. Values over 5000 units suggests a diagnosis of acute pancreatic necrosis.

Chronic pancreatitis—The determination of serum amylase activity is helpful in diagnosizing for variable degree of fibrosis and atrophy in pancreatic parenchyma.

Carcinoma of pancreas—Serum amylase activity is elevated, but is of little diagnostic importance.

II. Determination of endocrine functions

The test for determining the endocrine functions of pancreas are related chiefly to the integrity of the beta cells of the islets of Langerhans. Examination of urine for glucose followed by quantitation of blood sugar level is the routine procedure for assessing the endocrine function of pancreas.

Glucose tolerance test (GTT)

Procedure—The conventional glucose tolerance test is done after an oral ingestion of glucose. GTT is done in the morning after a twelve hour overnight fast. The blood in the fasting state is drawn and the sugar is estimated. Urine is collected in the some state and tested for glucose with Benedicts reagent. The patient is now given, orally 50 g of glucose dissolved in a glass of water. The time of ingestion is noted and blood is drawn at 30 minutes intervals with simultaneous collection of urine samples, over a period of 150 minutes. The blood samples are preserved with fluoride. Totally there will be six blood samples and six urine samples. Sugar in these samples are estimated. The results are expressed in the form of a graph with time intervals on the abscissa and the blood sugar levels on the ordinate.

Interpretations—A normal response shows a fasting level of 70 - 90 mg/dl of true sugar (Nelson – Somogyi), the maximum is reached in 60 minutes and the level reaches normal almost in 120 minutes. The Benedicts test will be negative with all the urine samples.

In diabetes, the glucose tolerance is diminished the graph will indicate elevated blood sugar levels not returning to normal at 120 minutes. There will be also sugar in urine of different concentrations corresponding to the blood sugar level, showing that the hyperglycemia of diabetes is associated with glycosuria. Glucose tolerance is lowered not only in the diabetic state but also in hyperadrenalism on the other hand, insulin sensitivity is increased in hyperinsulinism, hypopituitarism, hypoadrenalism and hypothyroidism.