Notes

1

GENERAL BIOCHEMISTRY

1.1 INTRODUCTION

Solutions of chemical reagents are a big part of biochemistry, biological and chemical based work. For a beginner of experimental procedure making solutions can also be the most frustrating part. Preparation and handling solutions are essential part of experimental biochemistry. Thus any of new science graduates should be clear in preparing reagents, buffers, and accuracy in pipeting.

The concentration of a dissolved salt in water refers to the amount of salt (solute) that is dissolved in water (solvent). Solutes are the substance of interest to be dissolved and the term solvent denotes the material in which the solute is dissolved.

Solution is a mixture that contains solute and a solvent. Solute can be denoted as the component of a solution present in the lesser amount and the solvent is the component of a solution present in the greater amount. Concentration can be written as the amount of a solute present in a solution per amount of solvent.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the importance of solution preparation in biochemistry
- explain different concentration units
- describe different terms of percent solutions

Notes

1.2 UNITS OF CONCENTRATION

- There are many ways to express concentrations. Concentration may be expressed several different ways and some of the more common concentration units are:
  1. Equivalent weight
  2. Molarity
  3. Molality
  4. Normality
  5. Percent solution (weight/weight)
  6. Percent solution (weight/volume)
  7. Percent solution (volume/volume)

1.2.1 Equivalent Weight

The equivalent weight is determined by dividing the atomic or molecular weight by the valence. A major use of the concept of equivalents is that one equivalent of an ion or molecule is chemically equivalent to one equivalent of a different ion or molecule. The mass of a substance especially in grams is chemically equivalent to eight grams of oxygen or one gram of hydrogen **:** the atomic or molecular weight divided by the valence

Valance could be determined as

1. The absolute value of ion charge
2. The number of H^+ or OH^- that a species can react with
3. The absolute value of change in charge on a species when undergoing a chemical reaction.

1.2.1.1 Preparation of NaOH

Solutions of NaOH can be prepared by either dissolving solid NaOH pellets in water or by diluting a concentrated solution of NaOH. However, the exact concentration of the solution prepared by these methods cannot be calculated from the weighed mass or using the dilution equation for two reasons:

1. Solid sodium hydroxide is hygroscopic ("water-loving"). Pellets of NaOH exposed to air will increase in mass as they become hydrated so the actual mass of pure NaOH is not accurately known.
2. Sodium hydroxide in solution reacts with carbonic acid and its concentration decreases over time. The acid is formed when small amounts of CO_2 gas (which is always present in air) dissolves in solution.

$$H_2CO_3(aq) + NaOH(aq) \rightleftharpoons H_2O + Na^+(aq) + HCO_3^-(aq)$$

The water used to make the NaOH solution can be boiled to expel the dissolved CO_2 gas but this time-consuming procedure is often not possible in a short laboratory period. A stock solution of NaOH can be made in advance with boiled water but will re-absorb CO_2 over a period of time unless stored in airtight containers. Therefore, if we want to know the exact concentration of a freshly made NaOH solution, we need to "standardize" it. That is, determine its exact concentration by titrating it with a known mass of a primary standard acid.

A "primary standard" is a substance that is used to determine the concentration of a solution. A primary standard should have the following properties: It should be available in very pure form at reasonable cost and should have a high equivalent weight to minimize weighing errors. It should be stable at room temperature, easy to dry, and should not easily absorb water when exposed to air (hygrophobic).

Potassium hydrogen phthalate ("KHP") is the primary standard reagent commonly used to standardize NaOH. It is a monoprotic acid whose formula is $KHC_8H_4O_4$ and molecular weight is 204.22 g/mol.

$$KHC_8H_4O_4(aq) + NaOH(aq) \longrightarrow H_2O + Na^+(aq) + K^+(aq) + C_8H_4O_4(aq)$$

The white powdery acid is normally heated at 110°C for one hour to remove any loosely bound waters of hydration and then cooled in a desiccator before use. The exact mass (and number of moles of acid) is determined by weighing the dried acid on an analytical balance. The acid is then dissolved in water and NaOH is added until an endpoint (the point at which an indicator changes color) is reached. The phenophthalein indicator used in this experiment is colorless in acid and pink in base. Therefore, the solution containing KHP will remain colorless as long as some KHP is still present. Once the last of the KHP has reacted, the solution will turn pink with one excess drop of base.

The exact concentration of NaOH is calculated by using the stoichiometry from reaction to convert the number of moles of KHP used to moles of NaOH and then dividing by the volume of NaOH used to reach the endpoint of the reaction.

1.2.1.2 Equivalent Weight of an Acid

In an acid base titration, the equivalence point is the volume of added base where the moles of –OH added (from the base) equal the moles of H+ initially present (from the acid). (i.e) moles of H^+ initially present = moles –OH added *(at equivalence point)*

To approximate the equivalence point, an indicator with an endpoint close to the equivalence point is added to the analyte solution. A balanced equation can be written describing the chemical reaction occurring between the titrant (the base in this experiment) and analyte (the acid in this experiment) if the identity of both is known.

Notes

For example, the titration of hydrochloric acid with potassium hydroxide can be written:

$$HCl(aq) + KOH(aq) \longrightarrow H_2O + KCl(aq)$$

In the case if the identity of the acid is unknown, but the number of acidic hydrogens (H^+) carried by the acid is known, a balanced equation can still be written.

For example, the titration of a triprotic acid (an acid with 3 H+) with sodium hydroxide can be written:

$$H_3X(aq) + 3KOH(aq) \longrightarrow 3H_2O(l) + K_3X(aq)$$

(X : the unknown anion of the acid)

The formula weight of this unknown acid can be calculated by using dimensional analysis. First, the base's concentration is used to convert the base's volume at the endpoint to moles. Then, multiplying by the mole ratio between acid and base from the balanced chemical equation allows for the calculation of the moles of the acid. Now the mass of acid titrated must be divided by the moles of acid calculated giving a result with the units of g/mol.

1.2.1.3 Equivalent Weight of an Oxidizing Agent

The concept of equivalents and equivalent mass is not restricted to acid-base reactions alone. Unlike acid-base reactions in redox reactions, the electrons are the active units (the equivalents) and the equivalent weights are the masses of oxidizing or reducing agent that deliver or accept 1 mole of electrons. But in case of acid and base the hydrogen or hydroxide ions plays key role in determination of equivalent weight.

INTEXT QUESTIONS 1.1

1 The equivalent weight is determined by dividing the atomic or molecular weight by the .....

2 The mass of a substance especially in grams is chemically equivalent to ..... grams of oxygen

(a) 2 (b) 5 (c) 8 (d) 10

3 ..... is the primary standard reagent commonly used to standardize NaOH

1.2.2 Molarity

Molarity is based on the volume of solution containing the solute. Since density is a temperature dependent property a solution's volume, and thus its molar

concentration, changes with temperature. By using the solvent's mass in place of the solution's volume, the resulting concentration becomes independent of temperature.

Molarity is the common way of referring to concentrations of solutions. The goal of most basic molarity problems shall be to get the moles from grams by dividing the molecular weight and then dividing by the total number of liters or by given the molarity find the number of grams of the solution by multiplying the volume then the molecular weight. Molarity might give you the density of the solution, from which you can obtain the mass by multiplying the density by the volume.

Although there are several ways in which the concentration of a solution can be quantified, molarity is one of the most basic and widely used. Molarity (M) is defined as the number of moles of solute dissolved in one liter of solution. The higher the molarity, the more concentrated or strong the solution is. For example, a 12 M (which is said "twelve molar") solution of HCl (ie. hydrochloric acid) is much more concentrated than a 0.10 M solution! The basic formula for calculating molarity is:

Molarity (M) = moles of solute (mol) per liters of solution (L)

To solve for moles of solvent, we can use algebra to manipulate the above equation producing the following derived formulas:

moles of solute (mol) = Molarity (M) × liters of solution (L)

In simple terms, the following formula could be used for preparation of molar solutions for lab solutions preparation

For preparation of molar solution

$$\frac{\text{Molecular weight of the compound (A)}}{1000} \times \text{Required morality (B)} \times \text{Required volume of solution (C)} = \text{D gram}$$

In the above equation for preparation of solution of 'B' molarity, 'D' grams of the solute could be dissolved in 'C' ml of solvent.

INTEXT QUESTIONS 1.2

1. ..... is based on the volume of solution containing the solute
   (a) Normality (b) Molarity (c) Molality (d) Percent
2. Molarity is the common way of referring to ..... of solutions

3. ..... is defined as the number of moles of solute dissolved in one liter of solution
4. The higher the ....., the more concentration of the solution

1.2.3 Molality

The molal unit is not used nearly as frequently as the molar unit and is used in thermodynamic calculations where a temperature independent unit of concentration is needed. A molality is the number of moles of solute dissolved in one kilogram of solvent. The term molality and molarity should not be confused. While expressing the Molality it is represented by a small "m," whereas molarity is represented by an upper case "M."

In case of preparation of molar solution except water all other solvent must be weighed. The water is exempted from weighing because; one liter of water has a specific gravity of 1.0 and weighs one kilogram. So one can measure out one liter of water and the solute could be directly added to it. But other solvents might have a specific gravity greater than or less than one. Therefore, one liter of any solvent other than water is not likely to occupy a liter of space.

For example to make a one molal aqueous (water) solution of sodium chloride (NaCl), measure out one kilogram of water and add one mole of the solute, NaCl to it. The atomic weight of sodium is 23 and the atomic weight of chlorine is 35. Therefore the formula weight for NaCl is 58. So 58 grams of NaCl could be dissolved in 1kg water for preparation of 1 molal solution of NaCl.

INTEXT QUESTIONS 1.3

1. ..... is the unit used in thermodynamic calculations where a temperature independent unit of concentration is needed.
2. A molality is the number of moles of solute dissolved in ..... kilogram of solvent

   (a) 3 (b) 2 (c) 1 (d) 6
3. Molality it is represented by a .....

   (a) Small 'm' (b) Capital 'M' (c) Small 'n' (d) Capital 'N'

1.2.4 Normality

The concentration of a solution could also be expressed in terms of Normality. It is based on an alternate chemical unit of mass called the equivalent weight. The normality of a solution is the concentration expressed as the number of

equivalent weights (equivalents) of solute per liter of solution. In a chemical mixture 1 normal (1 N) solution contains 1 equivalent weight of solute per liter of solution. Since normality simplifies the calculations required for chemical concentration, it is widely used in analytical chemistry.

Every substance may be assigned an equivalent weight. The equivalent weight may be equal to the formula weight (molecular weight, mole weight) of the substance or equal to an integral fraction of the formula weight (i.e., molecular weight divided by 2, 3, 4, and so on).

The above phenomenon could be better explained with the following example to gain an understanding of the meaning of equivalent weight:

$HCl(aq)$	+	$NaOH(aq)$	$\longrightarrow$	$NaCl(aq) + H_2O$
1 mole (36.5 grams)		1 mole (40.0 grams)		
$H_2SO_4(aq)$	+	$2NaOH(aq)$	$\longrightarrow$	$Na_2SO_4(aq) + 2H_2O$
1 mole (98.1 grams)		1 mole (80.0 grams)		

In the above chemical reaction 1 mole of hydrochloric acid (HCl) reacts with 1 mole of sodium hydroxide (NaOH) and 1 mole of sulfuric acid (H_2SO_4) reacts with 2 moles of NaOH. If you made 1 molar solutions of these substances, 1 liter of 1 M HCl will react with 1 liter of 1 M NaOH and 1 liter of 1 M H_2SO_4 will react with 2 liters of 1 M NaOH. Therefore, H_2SO_4 has twice the chemical capacity of HCl when reacting with NaOH. The equivalent weight of HCl is equal to its molecular weight, but that of H_2SO_4 is ½ its molecular weight.

Expressions for normality are shown below. Notice the similarity to molar solution definition.

$$\text{Normality (N)} = \frac{\text{Number of equivalents of solute}}{\text{1 liter of solution}} = \frac{\text{Equivalents}}{\text{liter}}$$

where $$\text{Number of equivalents of solute} = \frac{\text{grams of solute}}{\text{equivalent weight of solute}}$$

$$\text{finally N} = \frac{\text{grams of solute}}{\text{eq wt solute} \times \text{L solution}} = \frac{\text{grams}}{\text{eq wt} \times \text{L}}$$

So, 1 liter of solution containing 36.5 grams of HCl would be 1 N, and 1 liter of solution containing 49.0 grams of H_2SO_4 would also be 1 N. A solution containing 98.1 grams of H_2SO_4 (1 mole) per liter would be 2 N.

Notes

INTEXT QUESTIONS 1.4

1. ..... based on an alternate chemical unit of mass called the equivalent weight

   (a) Normality (b) Molarity (c) Percent (d) Molality

2. One mole of ..... reacts with 1 mole of sodium hydroxide (NaOH)
3. One mole of sulfuric acid (H_2SO_4) reacts with ..... moles of NaOH
4. The equivalent weight of HCl is equal to its .....

1.2.5 Percentage Solutions

The percentage solution could be expressed in terms of weight percent (% w/w), volume percent (% v/v) and weight-to-volume percent (% w/v) units of solute present in 100 units of solution. For example a solution of 1.5% w/v NH_4NO_3, contains 1.5 gram of NH_4NO_3 in 100 mL of solution.

1.2.5.1 Percent by weight (% w/w)

In case of preparing a solution based on percentage by weight, one would simply determine what percentage was required (for example, a 20% by weight aqueous solution of sodium chloride) and the total quantity to be prepared.

If the total quantity needed is 1 kg, then it would simply be a matter of calculating 20% of 1 kg which, of course is:

20 /100 * 1000 g/kg = 200 g NaCl/kg.

Thus finally to bring the total quantity to 1 kg, it would be necessary to add 800g water.

1.2.5.2 Percent by volume (% w/v)

Preparation of solutions based on percent by volume it requires the calculation same as for percent by weight, except that calculations are based on volume. In simple terms one should plan that what percentage was desired (for example, a 20% by volume aqueous solution of sodium chloride) and the total quantity to be prepared in terms of volume.

For example if 20 % is to be prepared for a total quantity of 1 liter, then it would simply be a matter of calculating 20% of NaCl in 1 liter, the formula can be written as:

20/100 * 1000 ml/l = 200 g NaCl/l.

1.2.5.3 Percent by volume (% v/v)

Volume percent or volume/volume percent most often is used when preparing solutions of liquids. This is typically only used for mixtures of liquids. Volume percent is relative to volume of solution, not volume of solvent. The advantage of volume/volume units is that gaseous concentrations reported in these units do not change as a gas is compressed or expanded.

For example, 70% v/v rubbing alcohol may be prepared by taking 700 ml of isopropyl alcohol and adding sufficient water to obtain 1000 ml of solution (which will not be 300 ml).

Table 1.1 Common Units for Reporting Concentration

Sl.No	Name	Units	Symbol
1.	Molarity	moles solute liters solution	M
2.	Normality	equivalents solute liters solution	N
3.	Molality	moles solute kilograms solvent	m
4.	Weight percent	grams solute 100 grams solution	% w/w
5.	Volume percent	mL solute 100 mL solution	% v/v
6.	weight-to-volume percent	grams solute 100 mL solution	% w/v

INTEXT QUESTIONS 1.5

1. Percent by weight could be expressed as .....
2. For preparation of 20 % NaCl by (w/v) ..... grams of NaCl is to be dissolved in 1 L of water.

   (a) 200 (b) 100 (c) 400 (d) 50
3. volume/volume percent most often is used when preparing solutions of .....
4. Match the following:

   (i) Molarity (a) N

   (ii) Normality (b) m

   (iii) Molality (c) M

WHAT HAVE YOU LEARNT

- Preparation solutions of chemical reagents are very important task for the beginners of biochemistry.
- Concentration of a biological solution could be expressed in terms of equivalent weight, normality, molarity, molality, percent solution (weight/ weight, weight/ volume, volume /volume).
- The equivalent weight is determined by dividing the atomic or molecular weight by the valence.
- Molarity could be simply termed as "M" and are units of moles of solute per liter of solution.
- Molality is units of moles of solute per kilogram of solution and is termed as "m". While the normality is termed as "N" and are units of equivalent of solute per liter of solution.
- The percentage solution could be expressed in terms of weight percent (% w/w), volume percent (% v/v) and weight-to-volume percent (% w/v) units of solute present in 100 units of solution.

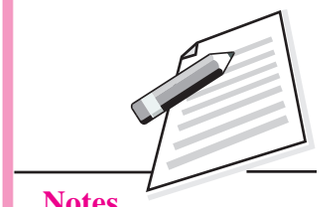
TERMINAL QUESTIONS

1. Write a brief note about the importance of solution preparation in biochemistry.
2. Write a note on Equivalent weight.
3. Describe about molarity and molality.
4. Write a short note on normality.
5. Write about different terms of percent solutions.
6. Tabulate the units and symbols of different modes of concentrations.

ANSWERS TO INTEXT QUESTIONS

1.1

1. Valence
2. 8
3. Potassium hydrogen phthalate



1.2

1. Molarity
2. Concentrations
3. Molarity
4. Molarity

1.3

1. Molality
2. 1
3. Small ‘n’

1.4

1. Normality
2. Hydrochloric acid
3. Two
4. Molecular weight

1.5

1. (% w/w)
2. 200
3. Liquids
4. Match the following:
   (i) (b)
   (ii) (a)
   (iii) (c)

Notes

2

CARBOHYDRATES

2.1 INTRODUCTION

A carbohydrate is a large biological molecule, or macromolecule, consisting only of carbon (C), hydrogen (H), and oxygen (O), usually with a hydrogen:oxygen atom ratio of 2:1. Carbohydrates are technically hydrates of carbon, structurally it is more accurate to view them as polyhydroxy aldehydes and ketones.

OBJECTIVES

After reading this lesson, you will be able to:

- define carbohydrates
- classify Common Carbohydrates
- describe the sources and composition of carbohydrates
- explain the Digestion and absorption of carbohydrates.

2.2 DEFINITION

Carbohydrates are polyhydroxy aldehydes or ketones, or compounds that can be hydrolyzed to them. Carbohydrate is an organic compound comprising only carbon, hydrogen, and oxygen, usually with a hydrogen: oxygen atom ratio of 2:1 (as in water). Carbohydrates are technically hydrates of carbon. The empirical formula is $C_n(H_2O)_n$.

2.3 SOURCES OF CARBOHYDRATES

Baked goods commonly contain dietary starch and added sugar. Most dietary carbohydrates come from plants. Sugars and starches are nutritive carbohydrates, meaning they are broken down and utilized by the body, primarily to generate energy. Although dietary fiber is also a carbohydrate, it contributes no calories because it is not digested or absorbed.

2.3.1 Grain Products

Grain products are the leading source of carbohydrates in the diet. Grains naturally contain high concentrations of starch, which our gastrointestinal system breaks down into sugars. Common grains in diet include wheat, oats, rice, barley and cornmeal. Any food that includes grain or grain flour as a primary ingredient contains carbohydrates, such as bread and other baked goods, pasta, cereal, crackers. Choosing whole-grain products instead of those made from refined grains boosts your dietary fiber intake, which supports your heart and digestive health.

2.3.2 Starchy Vegetables and Beans

Beans and starchy vegetables, such as potatoes, yams, green peas, and corn, contain high levels of complex carbohydrates that our body digests into sugars. In addition, starchy vegetables and beans contribute vitamins, minerals and fiber to our diet. Dry beans also serve as a good source of lean dietary protein.

2.3.3 Fruits

All fruit and fruit juices contain carbohydrates in the form of natural sugars, such as glucose and fructose. Fruit sugars contribute nearly all of the calories contained in these foods. With persistently low consumption rates, fruit contributes less than 8 percent of the average daily calories in the diet. Fresh fruit is a healthier option than fruit juice because it provides more dietary fiber and less carbohydrate by volume.

2.3.4 Beverages

Dairy milk is the only significant source of dietary carbohydrates not derived from plants. A cup of unflavored milk contains about 11 to 12 grams of carbohydrate in the form of milk sugar, or lactose. Chocolate milk contains more than twice the amount of carbohydrate per cup compared to plain milk because sugar is added to sweeten the flavor. Sugar-sweetened soda, fruit drinks and sports and energy drinks substantially contribute to dietary carbohydrate intake. Wine, beer and liqueurs also contain carbohydrates. Dessert wines typically contain roughly four times the amount of carbohydrates found in table wines.

Notes

2.3.5 Sweets and added Sugars

Eating candy and desserts markedly boosts the number of carbohydrates in our diet. Indulging in a 1.6-ounce milk chocolate bar adds more than 26 grams of carbohydrates to our daily intake; a slice of cherry pie adds approximately 47 to 69 grams. Sugar added to processed foods that you may not consider sweet can be an unrecognized source of carbohydrates in our diet. Commercial pasta sauces, salad dressings, sandwich bread, energy and nutrition bars, cereals, heat-and-eat meals and other convenience foods commonly contain high-fructose corn syrup or another form of sugar for added flavor. Opting for whole, fresh foods rather than processed foods helps us avoid hidden carbohydrates.

2.4 CLASSIFICATION

Carbohydrates are classified into following classes depending upon whether these undergo hydrolysis and if so on the number of products form:

Monosaccharides, Disaccharides, Trisaccharides, Oligosaccharides, Polysaccharides

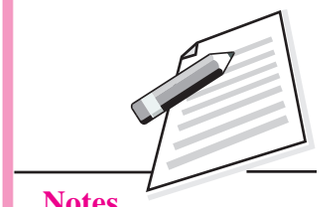
2.4.1 Monosaccharides

Molecules having only one actual or potential sugar group are called monosaccharides. They are simple carbohydrates that cannot be hydrolyzed further into polyhydroxy aldehydes or ketone unit

Sugars having aldehyde group are called aldoses and sugars with keto group are called ketoses. Depending on the number of carbon atoms monosaccharides are named as triose (C_3), tetrose (C_4), pentose (C_5), hexose (C_6), heptose (C_7) and so on.

Monosaccharide classifications based on the number of carbons

Number of Carbons	Category Name	Examples
4	Tetrose	Erythrose, Threose
5	Pentose	Arabinose, Ribose, Ribulose, Xylose, Xylulose, Lyxose
6	Hexose	Allose, Altrose, Fructose, Galactose, Glucose, Gulose, Idose, Mannose, Sorbose, Talose, Tagatose
7	Heptose	Sedoheptulose, Mannoheptulose



INTEXT QUESTIONS 2.1

1. Carbohydrate consists of ....., ... & ..... molecule
2. Hydrogen Oxygen atom ratio in Carbohydrate is .....
3. Molecules having only one actual sugar group is called as .....
4. Sugars having aldehyde group is called as .....
5. Sugars with keto group is called as .....
6. Match the following

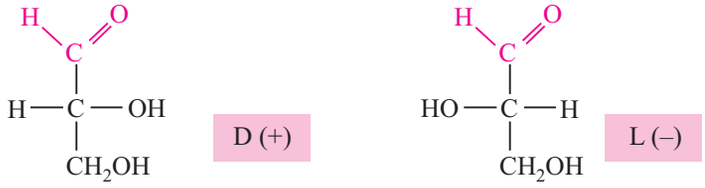
1. Monosaccharides with six carbons	(a) Tetrose
2. Monosaccharides with four carbons	(b) Heptose
3. Monosaccharides with five carbons	(c) Hexose
4. Monosaccharides with seven carbons	(d) Pentose

7. Match the following

1. Hexose	(a) Erythrose
2. Heptose	(b) Ribose
3. Tetrose	(c) Mannoheptulose
4. Pentose	(d) Fructose

2.4.2 Sterioisomers

Compounds having same structural formula but differing in spatial configuration are known as sterioisomers. While writing the molecular formula of monosaccharides, the spatial arrangements of H and OH groups are important, since they contain asymmetric carbon atoms. Asymmetric carbon means four different groups are attached to the same carbon. The reference molecule is glyceraldehyde which has a single asymmetric carbon.



glyceraldehyde

The number of possible sterioisomers depends on the number of asymmetric carbon atoms by the formula 2^n where n is the number of asymmetric carbon atoms

Reference carbon atom of sugars

The configuration of H and OH at the second carbon of glyceraldehyde will form two mirror images, they are denoted as D and L varieties. All monosaccharides are molecules derived from glyceraldehyde by successive addition of carbon atoms. Therefore the penultimate carbon atom is the reference carbon atom for naming the mirror images. This is also referred to as absolute configuration.

Optical activity

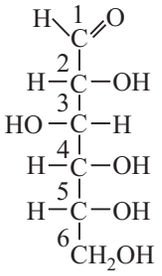
The presence of asymmetrical carbon atom causes optical activity. When a beam of plane polarized light is passed through a solution of carbohydrates, it will rotate the light either to right or to left. Depending on the rotation, molecules are called dextrorotatory (+) (d) or levorotatory (–).

Racemic mixture

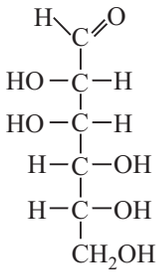
Equimolecular mixture of optical isomers has no net rotation and hence it is called as racemic mixture.

Epimers

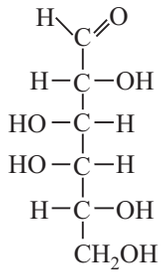
When sugars are different from one another, only in configuration with regard to the single carbon atom, other than the reference carbon atom, they are called Epimers. Example Glucose and Mannose are an epimeric pair which differ only with respect to C_2. Similarly galactose is the fourth epimer of glucose



D-Glucose



D-Mannose

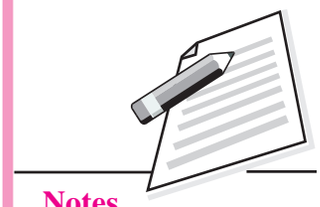


D-Galactose

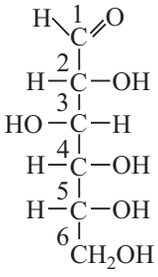
Anomers

When D glucose is crystallized at room temperature and a fresh solution is prepared, its specific rotation of polarized light is +112°; but after 12-18 hours it changes to +52.5°. If initial crystallization is taking place at 98° C and then solubilized, the specific rotation is found to be +19°, which also changes to+52.5° within few hours. This change in rotation with time is called **mutarotation**.

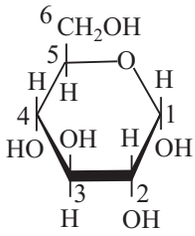
This is explained by the fact that D-glucose has two anomers, alpha and beta varieties. These anomers are produced by the spatial configuration with reference to the first carbon atom in aldoses and second carbon in ketoses. Hence



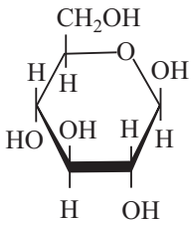
these carbons are called anomeric carbon atoms. Thus α-D-glucose has specific rotation of +112° and β=D-glucose has specific rotation of +19°. Both undergo mutarotation and at equilibrium one-third molecules are alpha type and two-third are beta variety to get the specific rotation +52.5°



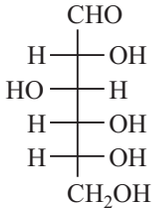
D-Glucose



α-D-Glucose



β-D-Glucose

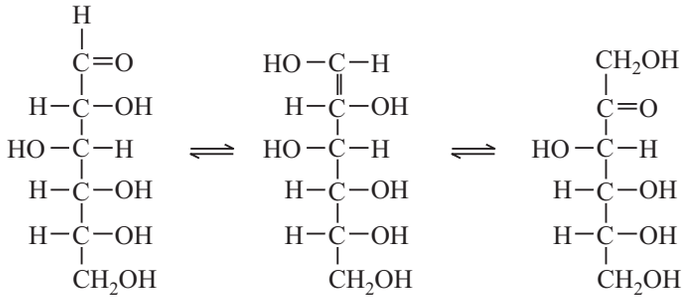


Cyclation of Glucose

2.5 REACTIONS OF MONOSACCHARIDES

2.5.1 Enediol formation

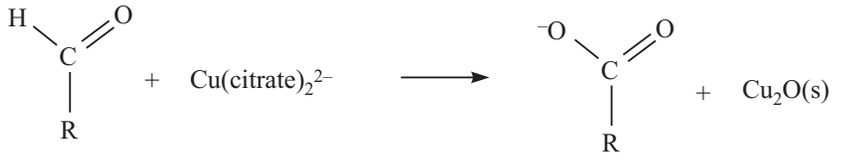
In mild alkaline solutions, carbohydrates containing free sugar group (aldehyde or keto group) will tautomerise to form enediols, where two hydroxyl groups are attached to the double-bonded carbon, in mild alkaline conditions, glucose is converted into fructose and mannose.



aldose D-Glucose — enediol — ketose D-fructose

Benedict's Reaction

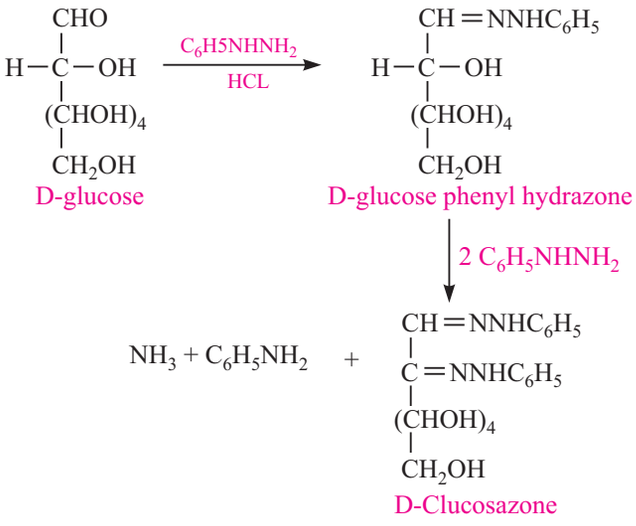
Benedict's reagent contains sodium carbonate, copper sulphate and sodium citrate. In alkaline medium, sugars form enediols which will reduce cupric ions and correspondingly the sugar is oxidized. Any sugar with free aldehyde or keto group will reduce benedict's reagent and called as reducing sugars.



An aldose + Benedict's reagent (blue solution) → Carboxylate anion + Brick-red precipitate

Osazone Formation

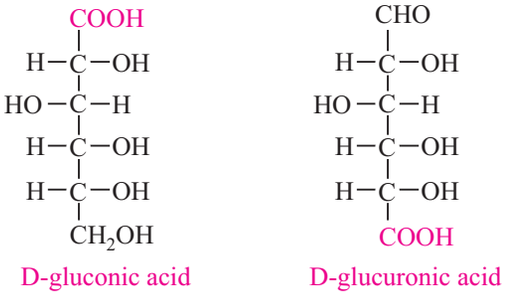
All reducing sugars will form osazone with excess of phenylhydrazine when kept at boiling temperature. Osazones are insoluable. Each sugar will have characteristic crystal form of osazone



Oxidation of Sugars

Under mild oxidation conditions the aldehyde group is oxidized to carboxyl group to produce aldonic acid. Ex: Glucose to Gluconic acid

When aldehyde group is protected and the molecule is oxidized the last carbon becomes COOH group to produce uronic acid. Ex: Glucose to Glucuronic acid.



Under strong oxidation conditions the first and last carbon atoms are simultaneously oxidized to form dicarboxyllic acids, known as saccharic acids. Ex: Glucose to Glucosaccharic acid.

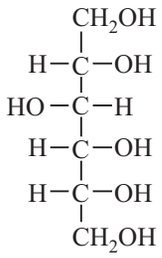
Furfural Derivatives

Monosaccharides when treated with concentrated sulphuric acid undergo dehydration with removal of 3 molecules of water. Therefore hexoses give

hydroxymethyl furfural and pentoses give furfural. These furfural derivatives can condense with phenolic compounds to give coloured products. This forms the basis of Molisch test, a general test for carbohydrates.

Reduction to form Alcohols

When treated with reducing agents such as sodium amalgam, hydrogen can reduce sugars. Aldose yields corresponding alcohols. But ketose forms two alcohols, because of appearance of a new asymmetric carbon atom. Ex: Glucose forms sorbitol and fructose forms sorbitol and mannitol.



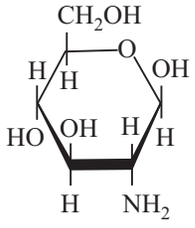
Glucitol or Sorbitol
(a sugar alcohol)

Glycosides: When the hemi-acatal group of a monosaccharide is condensed with an alcohol or phenol group, it is called glycoside.

Formation of esters: Hydroxyl groups of sugars can be esterified to form acetates, propionates, benzoates, phosphates, etc

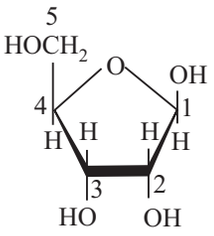
Amino sugars

Amino groups may be substituted for hydroxyl groups of sugars to give rise to amino sugars. Ex: Glucose to Glucosamine. Generally the amino group is added to the second carbon atom of hexoses. The amino group may be further acetylated to form N-acetylated sugars such N-acetyl-glucosamine.

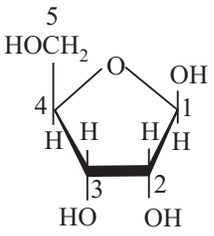


Glucosamine
(an amino sugar)

Deoxy sugars: Oxygen of the hydroxyl group may be removed to form deoxy sugars.



Ribose

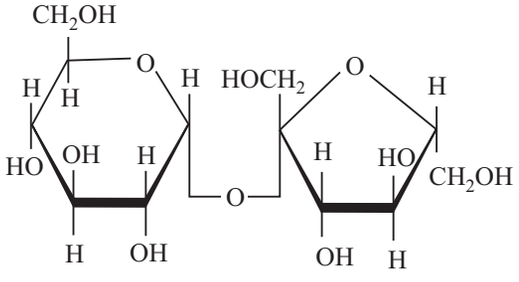


Deoxyribose

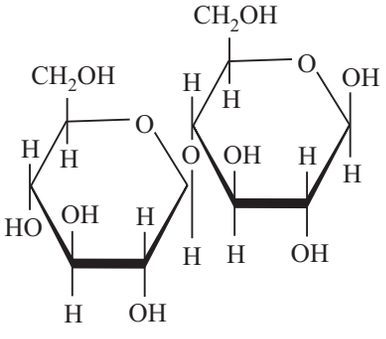
Disaccharides

When two monosaccharides are combined together with elimination of a water molecule it is called disaccharide. Monosaccharides are combined by glycosidic bond.

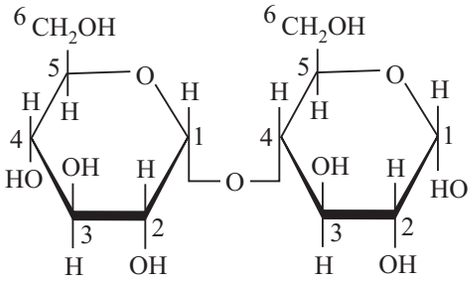
Disaccharide	Description	Component monosaccharides
Sucrose	common table sugar	glucose α1→2 fructose
Maltose	product of starch hydrolysis	glucose α1→4 glucose
Trehalose	found in fungi	glucose α1→1 glucose
Lactose	main sugar in milk	galactose β1→4 glucose
Melibiose	found in legumes	galactose β1→6 glucose



Sucrose



Lactose



Maltose

Sucrose

Sucrose also called saccharose, is ordinary table sugar refined from sugar cane or sugar beets. Sucrose is not a reducing sugar. This is because the glycosidic linkage inolves first carbon of glucose and second carbon of fructose, and hence there is no free reducing groups. When sucrose is hydrolyzed the resulting products have reducing property. Hydrolysis of sucrose (optical rotation +66.5°) will produce one molecule of glucose (+52.5°) and one molecule of fructose (-92°). Therefore the products will change the dextrorotation to levorotation, or the plane of rotation is inverted. Equimolecular mixture of glucose and fructose thus formed is called invert sugar.

Lactose

Lactose is the sugar present in milk. It is reducing disaccharide.

Maltose

Maltose consists of two α-D-glucose molecules. It is a reducing disaccharide

Polysaccharides

Polysaccharides are polymerized products of many monosaccharide units. They may be homo or hetero polysaccharides. Many polysaccharides, unlike sugars, are insoluble in water. Dietary fiber includes polysaccharides and oligosaccharides that are resistant to digestion and absorption in the human small intestine but which are completely or partially fermented by microorganisms in the large intestine.

Homopolysaccharides

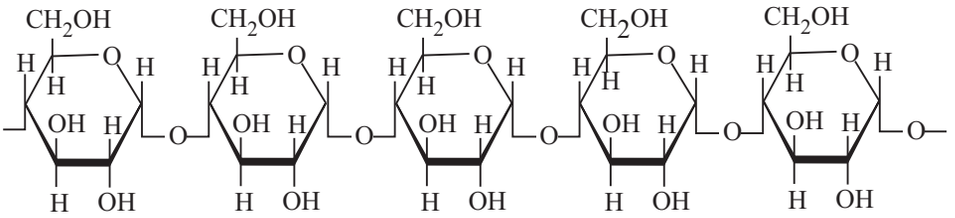
They have only one type of monosaccharide units.

Starch

Starch is the major form of stored carbohydrate in plants. Starch is composed of a mixture of two substances:amylose, an essentially linear polysaccharide,

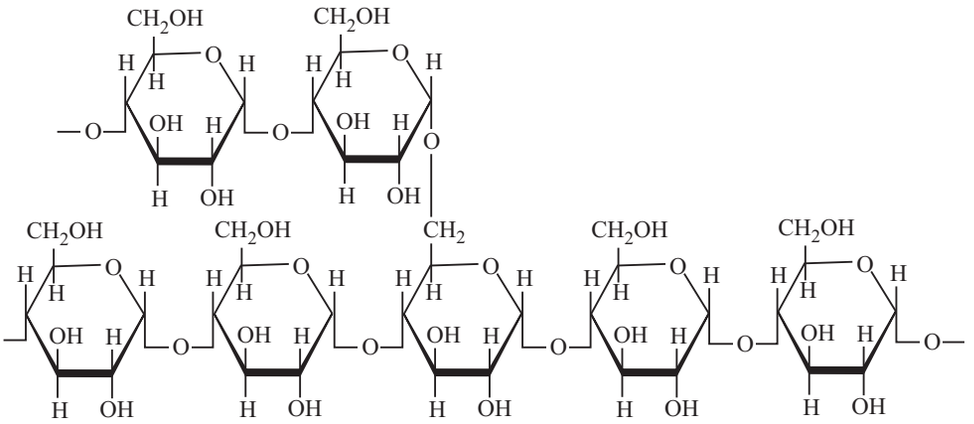
and amylopectin, a highly branched polysaccharide. Both forms of starch are polymers of α-D-Glucose. Natural starches contain 10-20% amylose and 80-90% amylopectin. Amylose forms a colloidal dispersion in hot water (which helps to thicken gravies) whereas amylopectin is completely insoluble.

- Amylose molecules consist typically of 200 to 20,000 glucose units which form a helix as a result of the bond angles between the glucose units.



Amylose

- Amylopectin differs from amylose in being highly branched. Short side chains of about 30 glucose units are attached with 1α→6 linkages approximately every twenty to thirty glucose units along the chain. Amylopectin molecules may contain up to two million glucose units.

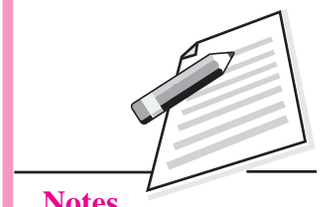


Amylopectin

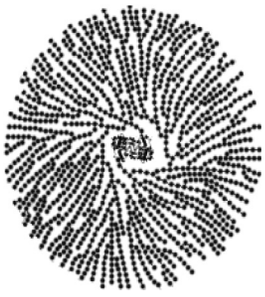
The side branching chains are clustered together within the amylopectin molecule

Glycogen

Glucose is stored as glycogen in animal tissues by the process of glycogenesis. When glucose cannot be stored as glycogen or used immediately for energy, it is converted to fat. Glycogen is a polymer of α-D-Glucose identical to amylopectin, but the branches in glycogen tend to be shorter (about 13 glucose units) and more frequent. The glucose chains are organized globularly like

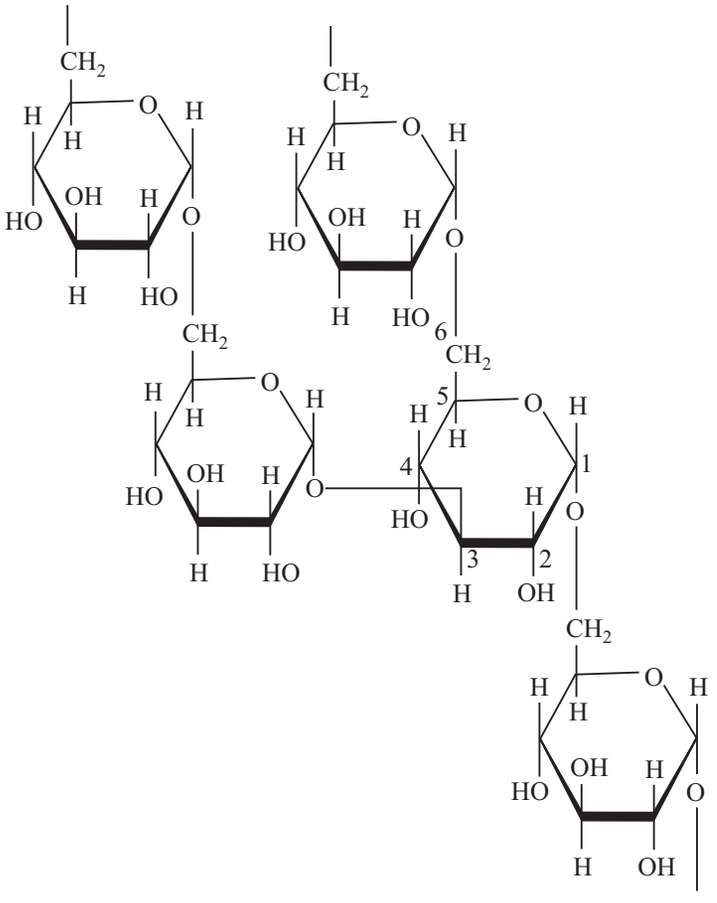


branches of a tree originating from a pair of molecules of glycogenin, a protein with a molecular weight of 38,000 that acts as a primer at the core of the structure. Glycogen is easily converted back to glucose to provide energy.



Dextran

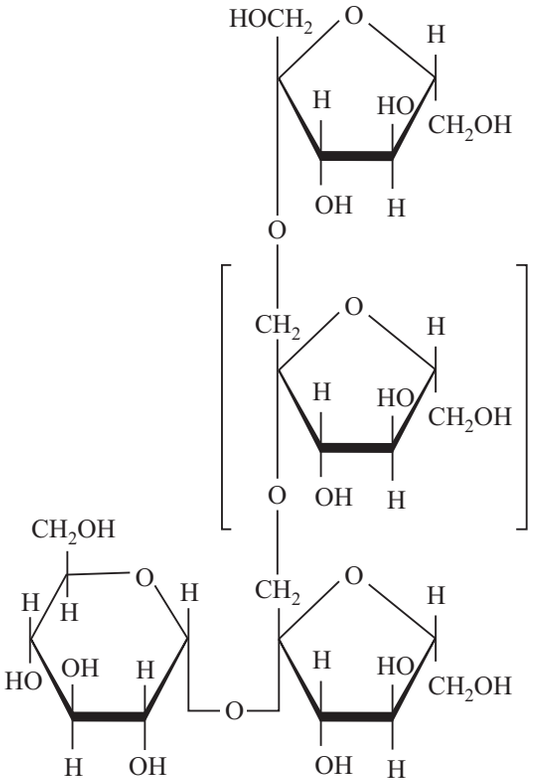
Dextran is a polysaccharide similar to amylopectin, but the main chains are formed by 1α→6 glycosidic linkages and the side branches are attached by 1α→3 or 1α→4 linkages. Dextran is an oral bacterial product that adheres to the teeth, creating a film called plaque. It is also used commercially in confections, in lacquers, as food additives, and as plasma volume expanders.



Dextran

Notes

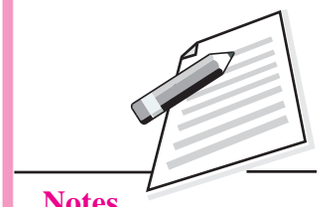
Inulin: Inulins, also called fructans, are polymers consisting of fructose units that typically have a terminal glucose. Oligofructose has the same structure as inulin, but the chains consist of 10 or fewer fructose units. Oligofructose has approximately 30 to 50 percent of the sweetness of table sugar. Inulin is less soluble than oligofructose and has a smooth creamy texture that provides a fat-like mouthfeel. Inulin and oligofructose are nondigestible by human intestinal enzymes, but they are totally fermented by colonic microflora. The short-chain fatty acids and lactate produced by fermentation contribute 1.5 kcal per gram of inulin or oligofructose. Inulin and oligofructose are used to replace fat or sugar and reduce the calories of foods like ice cream, dairy products, confections and baked goods.



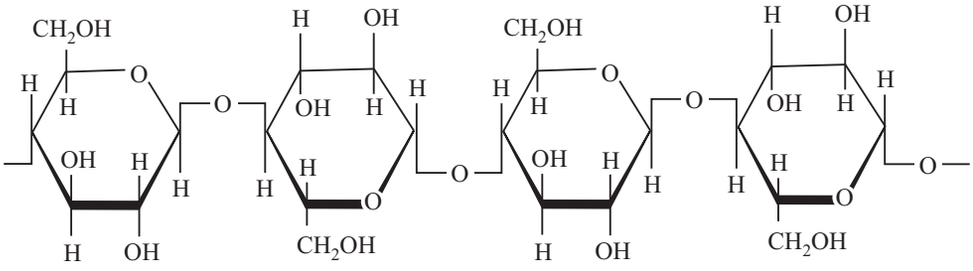
Inulin n = approx. 35

Cellulose

Cellulose is a polymer of β-D-Glucose, which in contrast to starch, is oriented with $-CH_2OH$ groups alternating above and below the plane of the cellulose molecule thus producing long, unbranched chains. The absence of side chains allows cellulose molecules to lie close together and form rigid structures. Cellulose is the major structural material of plants. Wood is largely cellulose, and cotton is almost pure cellulose. Cellulose can be hydrolyzed to its constituent glucose units by microorganisms that inhabit the digestive tract of termites and ruminants. Cellulose may be modified in the laboratory by treating it with nitric



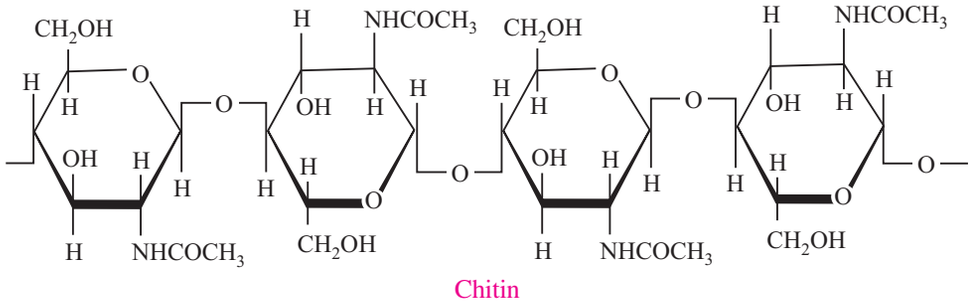
acid (HNO_3) to replace all the hydroxyl groups with nitrate groups ($-ONO_2$) to produce cellulose nitrate (nitrocellulose or guncotton) which is an explosive component of smokeless powder. Partially nitrated cellulose, known as pyroxylin, is used in the manufacture of collodion, plastics, lacquers, and nail polish.



Cellulose

Chitin

Chitin is an unbranched polymer of N-Acetyl-D-glucosamine. It is found in fungi and is the principal component of arthropod and lower animal exoskeletons, e.g., insect, crab, and shrimp shells. It may be regarded as a derivative of cellulose, in which the hydroxyl groups of the second carbon of each glucose unit have been replaced with acetamido ($–NH(C=O)CH_3$) groups.



Chitin

2.6 HETEROPOLYSACCHARIDES

Heteropolysaccarides contain two or more different kind of monosaccharides. Usually they provide extracellular support for organisms of all kingdoms: the bacteria cell envelope, or the matrix that holds individual cells together in animal tissues, and provides protection, shape and support to cells, tissues and organs.

Heteropolysaccharides provide extracellular support to very different organisms, from bacteria to humans; together with fibrous proteins, like collagen, elastin, fibronectin, laminin and others, heteropolysaccharides are the most important components of the extracellular matrix. Hyaluronic acid, condroitin sulfates and dermatan sulfates are important heteropolysaccharides in the extracellular matrix. These heteropolysaccharides usually are formed by the repetition of a disaccharide unit of an aminosugar and an acid sugar.

Notes

Other common constituents are sulfate groups linked to certain monosaccharides. Usually heteropolysaccharides are associated with proteins forming proteoglycans, glycosaminoglycans or mucopolysaccharides (since they are abundant in mucous secretions). As a group, they perform diverse functions: structural, water metabolism regulation (as a reservoir of water), cellular cement, biological sieve, biological lubricant, docking sites for growth factors, among other functions.

Established specific functions of some glycosaminoglycans are:

Hyaluronic Acid (Hyaluronate): It is a lubricant in the synovial fluid of joints, give consistency to vitreous humor, contributes to tensile strength and elasticity of cartilages and tendons (Answer to C-O6)

Chondroitin Sulfates: contributes to tensile strength and elasticity of cartilages, tendons, ligaments and walls of aorta.

Dermatan sulfate (former chondroitin sulfate B) is found mainly in skin, but also is in vessels, heart, lungs. It may be related to coagulation and vascular diseases and other conditions.

Keratan sulfate: Present in cornea, cartilage bone and a variety of other structures as nails and hair.

Digestion and absorption

All carbohydrates absorbed in the small intestine must be hydrolyzed to monosaccharides prior to absorption. The digestion of starch begins with the action of salivary alpha-amylase/ptyalin, although its activity is slight in comparison with that of pancreatic amylase in the small intestine. Amylase hydrolyzes starch to alpha-dextrin, which are then digested by gluco-amylase (alpha-dextrinases) to maltose and maltotriose. The products of digestion of alpha-amylase and alpha-dextrinase, along with dietary disaccharides are hydrolyzed to their corresponding monosaccharides by enzymes (maltase, isomaltase, sucrase and lactase) present in the brush border of small intestine. In the typical Western diet, digestion and absorption of carbohydrates is fast and takes place usually in the upper small intestine. However, when the diet contains carbohydrates not easily digestible, digestion and absorption take place mainly in the ileal portion of the intestine.

Digestion of food continues while simplest elements are absorbed. The absorption of most digested food occurs in the small intestine through the brush border of the epithelium covering the villi(small hair-like structure). It is not a simple diffusion of substances, but is active and requires energy use by the epithelial cells.

During the phase of carbohydrate absorption, fructose is transported into the intestinal cell's cytosol, glucose and galactose competes with other Na + transporter required for operation. From the cytosol, monosaccharides pass into the capillaries by simple or facilitated diffusion.

Carbohydrates that are not digested in the small intestine, including resistant starch foods such as potatoes, beans, oats, wheat flour, as well as several non-polysaccharides oligosaccharides and starch, are digested in a variable when they reach the large intestine. The bacterial flora metabolize these compounds in the absence of oxygen. This produces gases (hydrogen, carbon dioxide and methane) and short-chain fatty acids (acetate, propionate, butyrate). The gases are absorbed and excreted by breathing or through the anus. Fatty acids are rapidly metabolized. Thus butyrate, used mainly in the colonic, is an important nutritional source for these cells and regulates their growth, acetate into the blood and taken up by the liver, muscle and other tissues, and propionate, which is an important precursor of glucose in animals, it is not so in humans.

Are polymers made up of two to ten monosaccharide units joined together by glycosidic linkages. Oligosaccharides can be classified as di-, tri-, tetra- depending upon the number of monosaccharides present. Among these the most abundant are the disaccharides, with two monosaccharide units.

INTEXT QUESTIONS 2.2

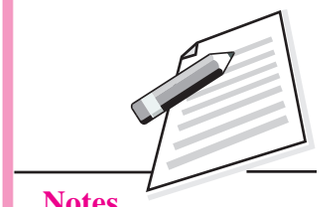
1. Compounds having same structural formula but differing in spatial configuration are known as .....
2. This change in rotation with time is called .....
3. Equimolecular mixture of glucose and fructose thus formed is called .....
4. Types of Polysaccharides are ..... & .....

WHAT HAVE YOU LEARNT

- Carbohydrates are polyhydroxy aldehydes or ketones, or compounds that can be hydrolyzed to Monosacchrides.
- Carbohydrate is an organic compound comprising only carbon, hydrogen, and oxygen, usually with a hydrogen: oxygen atom ratio of 2:1.

Notes

- Baked goods commonly contain dietary starch and added sugar. Most dietary carbohydrates come from plants
- Carbohydrates are classified depending on hydrolysis as Monosaccharides, Disaccharides, Trisaccharides, Oligosaccharides, Polysaccharides
- Molecules having only one actual or potential sugar group are called monosaccharides which cannot be hydrolyzed further into polyhydroxy aldehydes or ketone unit
- Sugars having aldehyde group are called aldoses and sugars with keto group are called ketoses. Depending on the number of carbon atoms monosaccharides are named as triose (C_3), tetrose (C_4), pentose (C_5), hexose (C_6), heptose (C_7) and so on.
- Compounds having same structural formula but differing in spatial configuration are known as sterioisomers
- The number of possible sterioisomers depends on the number of asymmetric carbon atoms by the formula 2^n where n is the number of asymmetric carbon atoms
- The presence of asymmetrical carbon atom causes optical activity. Depending on the rotation molecules are called dextrorotatory (+) (d) or levorotatory(-)
- When sugars are different from one another, only in configuration with regard to the single carbon atom are called Epimers
- When two monosaccharides are combined together with elimination of a water molecule it is called disaccharide. Monosaccharides are combined by glycosidic bond
- Sucrose, Maltose, Trehalose, Lactose and Melibose are disaccharide
- Polysaccharides are polymerized products of many monosaccharide units. They may be homo or hetero polysaccharides.
- Homopolysaccharides have only one type of monosaccharide units.
- Homopolysaccharides are starch, Glycogen, Dextran, Inulin, Cellulose, chitin
- Heteropolysaccarides contain two or more different kind of monosaccharides
- Carbohydrates absorbed in the small intestine and are hydrolyzed to monosaccharides prior to absorption.
- The digestion of starch begins with the action of salivary alpha-amylase/ ptyalin, although its activity is slight in comparison with that of pancreatic amylase in the small intestine.



TERMINAL QUESTIONS

1. Classify Monosaccharides with examples
2. Classify Polysaccharides with examples
3. Write short note on absorption of Carbohydrates

ANSWERS TO INTEXT QUESTIONS

2.1

1. Carbon, Hydrogen & Oxygen
2. 2 : 1
3. Monosaccharides
4. Aldoses
5. Ketoses
6. 1. (c)
   2. (a)
   3. (d)
   4. (b)
7. 1. (d)
   2. (c)
   3. (a)
   4. (b)

2.2

1. Sterioisomers
2. Mutarotation
3. Invert sugar
4. Homopolysaccharide & Heteropolysaccharide

Notes

3

CARBOHYDRATE METABOLISM

3.1 INTRODUCTION

All living cells require energy to carry out various cellular activities. This energy is stored in the chemical bonds of organic molecules (e.g. carbohydrates, fats, proteins) that we eat as food. These organic molecules are broken down by enzymatic reactions in cells to generate energy in the form of adenosine triphosphate (ATP). The ATP generated by these pathways in cells is used to drive fundamental cellular processes. The food we consume is mainly comprised of proteins, polysaccharides (carbohydrates) and fats. These are first broken down into smaller units: proteins into amino acids, polysaccharides into sugars, and fats into fatty acids and glycerol. This process of digestion occurs outside the cell. The amino acids, simple sugars and fatty acids then enter the cell and undergo oxidation by glycolysis (in the cytosol) and the citric acid cycle (in the mitochondria) to generate ATP (from ADP and P_i).

OBJECTIVES

After reading this lesson you will be able to

- describe glycolysis, Citric Acid Cycle
- explain Glycogenesis and Glycogenolysis
- describe the Hormonal regulation of blood sugar level

3.2 GLYCOLYSIS (EMBDEN-MEYERHOF PATHWAY)

Definition

In glycolysis pathway glucose is converted to pyruvate (aerobic condition) or lactate (anaerobic condition), along with production of a small quantity of energy.

Site of reaction: All the reaction steps take place in the cytoplasm.

Importance of the glycolysis pathway:

- It is the only pathway that is taking place in all the cells of the body.
- Glycolysis is the only source of energy in erythrocytes.
- In strenuous exercise, when muscle tissue lacks enough oxygen, anaerobic glycolysis forms the major source of energy for muscles.
- The glycolytic pathway may be considered as the preliminary step before complete oxidation.
- The glycolytic pathway provides carbon skeletons for synthesis of non-essential amino acids as well as glycerol part of fat.
- Most of the reactions are reversible.

Steps of glycolytic pathway

1. Glucose is phosphorylated to glucose -6-phosphate. The enzyme is hexokinase, which splits ATP into ADP and the Pi is added on to the glucose. The energy released by hydrolysis of ATP is utilised for the forward reaction. Hexokinase is the key glycolytic enzyme and the reaction is irreversible.
2. Glucose-6-phosphate is isomerised to fructose-6-phosphate by phosphohexose isomerase.
3. Fructose-6-phosphate is further phosphorylated to fructose-1,6-bisphosphate. The enzyme is phosphofructokinase, it is an important key enzyme and the reaction is irreversible.
4. Fructose-1, 6-bisphosphate is cleaved into two 3 carbon atoms; one glyceraldehyde-3-phosphate and another molecule of dihydroxyacetone phosphate. The enzyme is aldolase. Dihydroxyacetone phosphate is isomerised to glyceraldehyde-3-phosphate by the enzyme phophotriose isomerase.
5. Glyceraldehyde-3-phosphate is dehydrogenated and simultaneously phosphorylated to 1,3-bis-phosphoglycerate with the help of NAD^+. The enzyme is glyceraldehyde-3-phosphate dehydrogenase.
6. 1, 3-bis-phosphoglycerate is converted to 3-phosphoglycerate by the enzyme 1, 3-bis-phosphoglycerate kinase. Here one molecule of ATP is formed and this reaction is an example for Substrate level phosphorylation.
7. 3-phosphoglycerate is isomerised to 2-phosphoglycerate by shifting the phosphate group from 3^{rd} to 2^{nd} carbon atom. The enzyme is phosphoglucomutase.
8. 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme enolase. One water molecule is removed. A high energy phosphate bond is produced. This enzyme requires $Mg^{++}$and inhibited by fluoride.

9. Phosphoenol pyruvate is dephosphorylated to pyruvate, by pyruvate kinase. One molecule of ATP is generated. This step is irreversible.

10. In anaerobic condition pyruvate is reduced to lactate by lactate dehydrogenase.In aerobic conditions pyruvate enters citric acid cycle for complete oxidation. The lactate from anaerobic cycle enters cori's cycle.

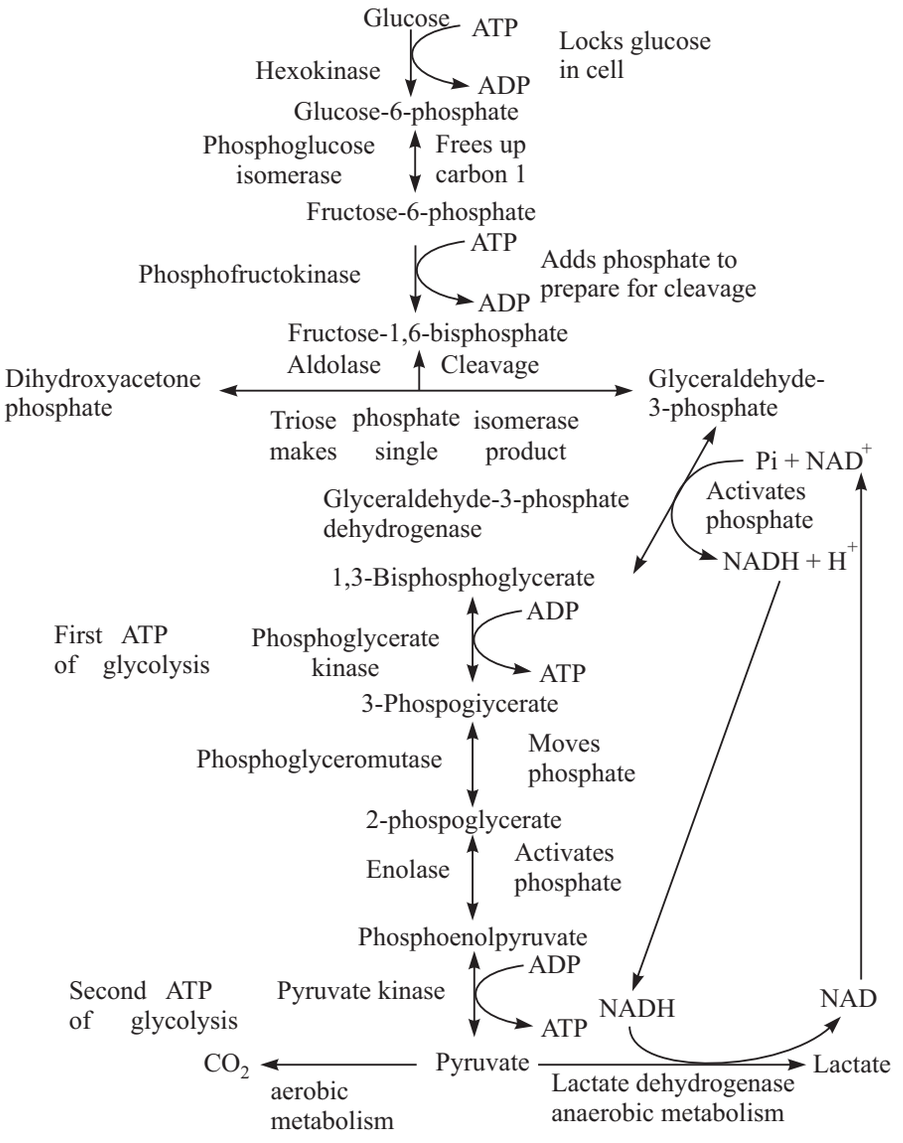


Fig. 3.1

Energy yield from glycolysis

Aerobic conditions

Number of ATPs gained per glucose molecule is 8 ATPs

Anaerobic conditions

Number of ATPs gained per glucose molecule is 2 ATPs

3.3 CITRIC ACID CYCLE: (KREB'S CYCLE)

Under aerobic conditions the end product of glycolysis is pyruvic acid. The next step is the formation of acetyl coenzyme A (acetyl CoA) - this step is technically not a part of the citric acid cycle, but is shown on the diagram on the top left.

Acetyl CoA, whether from glycolysis or the fatty acid spiral, is the initiator of the citric acid cycle. In carbohydrate metabolism, acetyl CoA is the link between glycolysis and the citric acid cycle. The initiating step of the citric acid cycle occurs when a four carbon compound (oxaloacetic acid) condenses with acetyl CoA (2 carbons) to form citric acid (6 carbons).

The whole purpose of a "turn" of the citric acid cycle is to produce two carbon dioxide molecules. This general oxidation reaction is accompanied by the loss of hydrogen and electrons at four specific places. These oxidations are connected to the electron transport chain where many ATP are produced.

Step 1

The acetic acid subunit of acetyl CoA is combined with oxaloacetate to form a molecule of *citrate*. The acetyl coenzyme A acts only as a transporter of acetic acid from one enzyme to another. After Step 1, the coenzyme is released by hydrolysis so that it may combine with another acetic acid molecule to begin the Krebs cycle again.

Step 2

The citric acid molecule undergoes an isomerization. A hydroxyl group and a hydrogen molecule are removed from the citrate structure in the form of water. The two carbons form a double bond until the water molecule is added back. Only now, the hydroxyl group and hydrogen molecule are reversed with respect to the original structure of the citrate molecule. Thus, *isocitrate* is formed.

Step 3

In this step, the isocitrate molecule is oxidized by a NAD molecule. The NAD molecule is reduced by the hydrogen atom and the hydroxyl group. The NAD binds with a hydrogen atom and carries off the other hydrogen atom leaving a carbonyl group. This structure is very unstable, so a molecule of CO_2 is released creating *alpha-ketoglutarate*.

Step 4

In this step, coenzyme A, returns to oxidize the alpha-ketoglutarate molecule. A molecule of NAD is reduced again to form NADH and leaves with another hydrogen. This instability causes a carbonyl group to be released as carbon

Notes

dioxide and a thioester bond is formed in its place between the former alpha-ketoglutarate and coenzyme A to create a molecule of *succinyl-coenzyme A* complex.

Step 5

A water molecule sheds its hydrogen atoms to coenzyme A. Then, a free-floating phosphate group displaces coenzyme A and forms a bond with the succinyl complex. The phosphate is then transferred to a molecule of GDP to produce an energy molecule of GTP. It leaves behind a molecule of *succinate*.

Step 6

In this step, succinate is oxidized by a molecule of FAD (Flavin adenine dinucleotide). The FAD removes two hydrogen atoms from the succinate and forces a double bond to form between the two carbon atoms, thus creating *fumarate*.

Step 7

An enzyme adds water to the fumarate molecule to form *malate*. The malate is created by adding one hydrogen atom to a carbon atom and then adding a hydroxyl group to a carbon next to a terminal carbonyl group.

Step 8

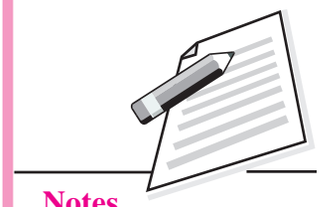
In this final step, the malate molecule is oxidized by a NAD molecule. The carbon that carried the hydroxyl group is now converted into a carbonyl group. The end product is *oxaloacetate* which can then combine with acetyl-coenzyme A and begin the Krebs cycle all over again.

Summary of Krebs Cycle

In summary, three major events occur during the Krebs cycle. One GTP (guanosine triphosphate) is produced which eventually donates a phosphate group to ADP to form one ATP; three molecules of NAD are reduced; and one molecule of FAD is reduced. Although one molecule of GTP leads to the production of one ATP, the production of the reduced NAD and FAD are far more significant in the cell's energy-generating process. This is because NADH and $FADH_2$ donate their electrons to an electron transport system that generates large amounts of energy by forming many molecules of ATP.

Yield of ATP

At this point the yield of ATP is 4 moles per mole of Glucose as it passes through the Krebs cycle.



- This is not much more than the 2 moles which would have been produced from glycolysis.
- However, NADH and FADH2 are energy rich molecules
- Their oxidation is highly exergonic and is coupled with the production of ATP from ADP
- Oxidation of 1 mole NADH produces 3 moles ATP
- Oxidation of 1 mole FADH2 produces 2 moles ATP
- Thus total ATP yield = (10 × 3) + (2 × 2) + 4 = 38 moles ATP per mole Glucose

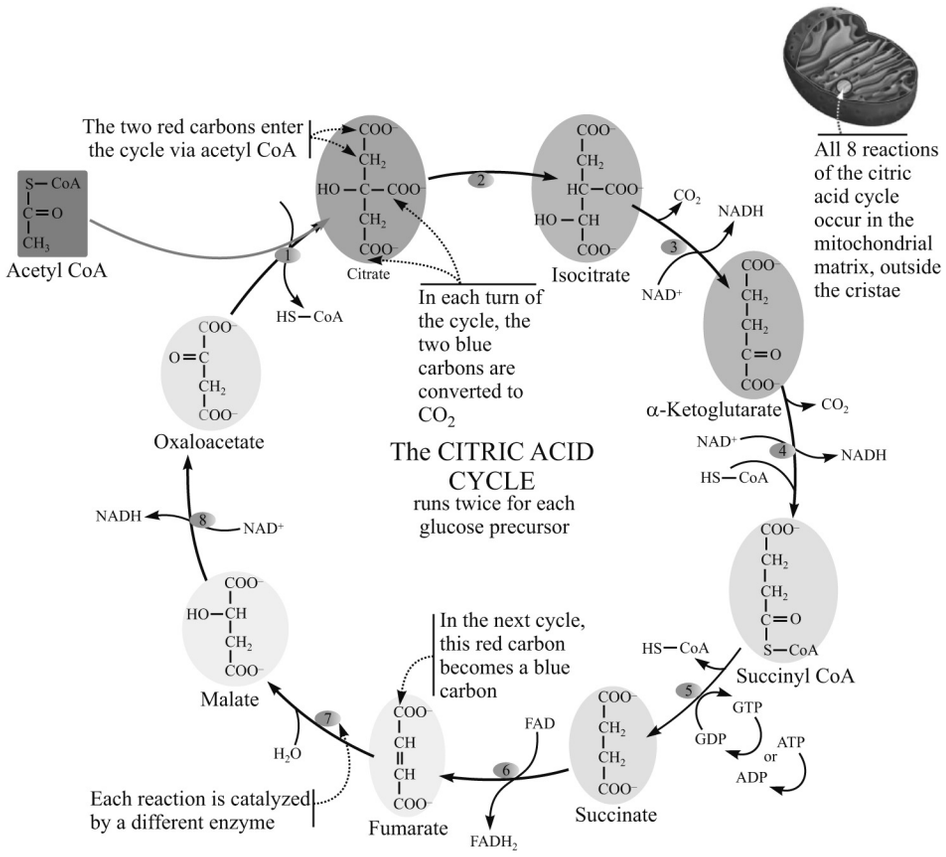


Fig. 3.2: Glycogenesis and Glycogenolysis

Biosynthesis of Glycogen

The goal of glycolysis, glycogenolysis, and the citric acid cycle is to conserve energy as ATP from the catabolism of carbohydrates. If the cells have sufficient supplies of ATP, then these pathways and cycles are inhibited. Under these conditions of excess ATP, the liver will attempt to convert a variety of excess molecules into glucose and/or glycogen.

Glycogenesis

Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy). If both are present in relatively high amounts, then the excess of insulin promotes the glucose conversion into glycogen for storage in liver and muscle cells.

In the synthesis of glycogen, one ATP is required per glucose incorporated into the polymeric branched structure of glycogen. actually, glucose-6-phosphate is the cross-roads compound. Glucose-6-phosphate is synthesized directly from glucose or as the end product of gluconeogenesis.

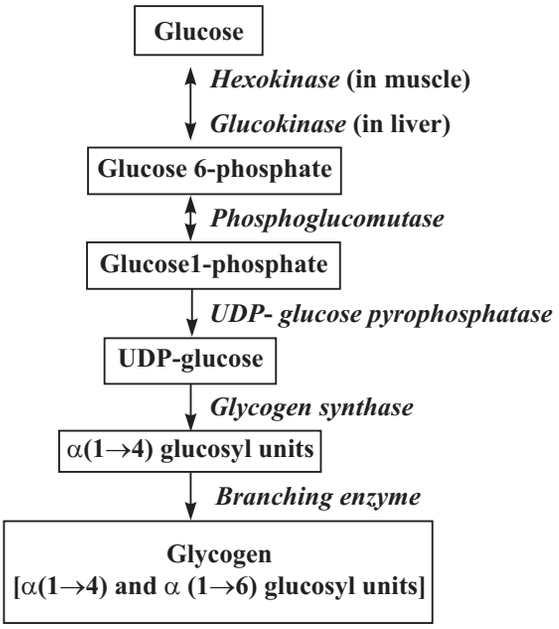


Fig. 3.3: Steps of glycogensis

Glycogenolysis

In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1- phosphate and then into glucose-6-phosphate. Two hormones which control glycogenolysis are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands.

Glucagon is released from the pancreas in response to low blood glucose and epinephrine is released in response to a threat or stress. Both hormones act upon enzymes to stimulate glycogen phosphorylase to begin glycogenolysis and inhibit glycogen synthetase (to stop glycogenesis).

Glycogen is a highly branched polymeric structure containing glucose as the basic monomer. First individual glucose molecules are hydrolyzed from the

chain, followed by the addition of a phosphate group at C-1. In the next step the phosphate is moved to the C-6 position to give glucose 6-phosphate, a cross road compound.

Glucose-6-phosphate is the first step of the glycolysis pathway if glycogen is the carbohydrate source and further energy is needed. If energy is not immediately needed, the glucose-6-phosphate is converted to glucose for distribution in the blood to various cells such as brain cells.

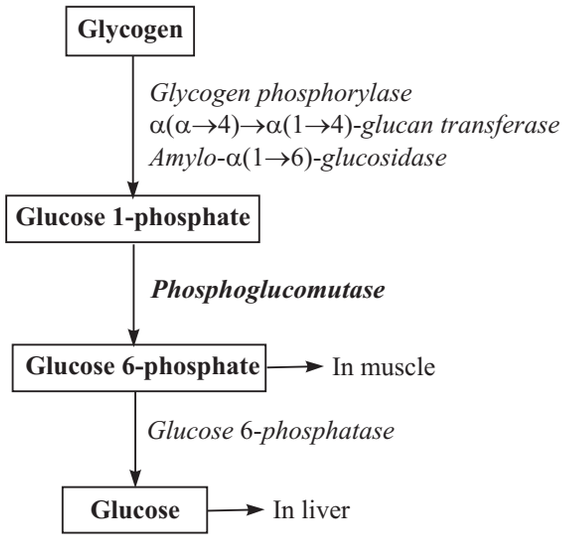


Fig. 3.4: Steps of glycogenolysis

Gluconeogenesis

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

The vast majority of gluconeogenesis takes place in the liver and, to a smaller extent, in the cortex of kidneys. This process occurs during periods of fasting, starvation, or intense exercise and is highly endergonic. Gluconeogenesis is often associated with ketosis.

Entering the pathway

Several non-carbohydrate carbon substrates can enter the gluconeogenesis pathway. One common substrate is lactic acid, formed during anaerobic respiration in skeletal muscle. Lactate is transported back to the liver where it is converted into pyruvate by the Cori cycle using the enzyme lactate

dehydrogenase. Pyruvate, the first designated substrate of the gluconeogenic pathway, can then be used to generate glucose. All citric acid cycle intermediates, through conversion to oxaloacetate, amino acids other than lysine or leucine, and glycerol can also function as substrates for gluconeogenesis. Amino acids must have their amino group removed by transamination or deamination before entering the cycle directly (as pyruvate or oxaloacetate), or indirectly via the citric acid cycle.

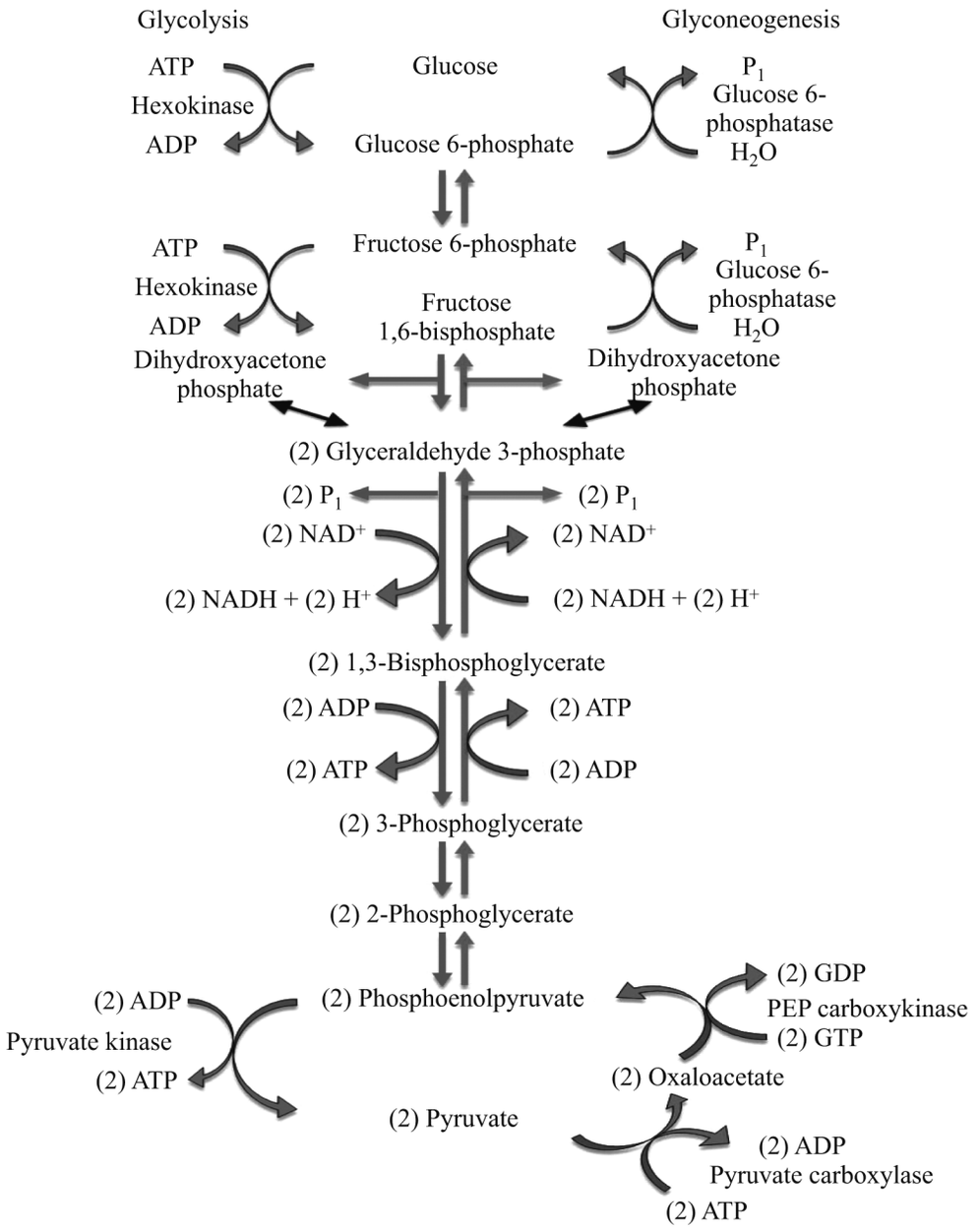


Fig. 3.5

Fatty acids cannot be converted into glucose in animals, the exception being odd-chain fatty acids which yield propionyl CoA, a precursor forsuccinyl CoA. In plants, specifically in seedlings, the glyoxylate cycle can be used to convert fatty acids (acetate) into the primary carbon source of the organism. The glyoxylate cycle produces four-carbon dicarboxylic acids that can enter gluconeogenesis. Glycerol, which is a part of alltriacylglycerols, can also be used in gluconeogenesis. In organisms in which glycerol is derived from glucose (e.g., humans and other mammals), glycerol is sometimes not considered a true gluconeogenic substrate, as it cannot be used to generate new glucose.

Gluconeogenesis is a pathway consisting of eleven enzyme-catalyzed reactions. The pathway can begin in the mitochondria or cytoplasm, depending on the substrate being used. Many of the reactions are reversible steps found in glycolysis.

Gluconeogenesis begins in the mitochondria with the formation of oxaloacetate through carboxylation of pyruvate at the expense of one molecule of ATP. This reaction is catalyzed by pyruvate carboxylase, which is stimulated by high levels of acetyl-CoA(when fatty acid oxidation is high in the liver) and inhibited by high levels of ADP.

Oxaloacetate must then be reduced into malate using NADH in order to be transported out of the mitochondria.

In the cytoplasm, malate is oxidized to oxaloacetate using NAD+, where the remaining steps of gluconeogenesis occur. Oxaloacetate is then decarboxylated and phosphorylated to produce phosphoenolpyruvate by phosphoenolpyruvate carboxykinase. One molecule of GTP is hydrolyzed to GDP in the course of this reaction.

The next steps in the reaction are the same as reversed glycolysis. However, fructose-1,6-bisphosphatase converts fructose-1,6-bisphosphate to fructose-6-phosphate. The purpose of this reaction is to overcome the large negative ΔG.

Glucose-6-phosphate is formed from fructose-6-phosphate by phospho gluco-isomerase. Glucose-6-phosphate can then be used for glucose generation or in other metabolic pathways. Free glucose is not generated automatically because glucose, unlike glucose-6-phosphate, tends to freely diffuse out of the cell.

The final reaction of gluconeogenesis, the formation of glucose, is carried out in the lumen of the endoplasmic reticulum. Glucose-6-phosphate is hydrolyzed by glucose-6-phosphatase to produce glucose. Glucose is then shuttled into the cytosol by glucose transporters located in the membrane of the endoplasmic reticulum.

Notes

Regulation

While most steps in gluconeogenesis are the reverse of those found inglycolysis, three regulated and strongly exergonic reactions are replaced with more kinetically favorable reactions. Hexokinase/glucokinase, phosphofructokinase, and pyruvate kinase enzymes of glycolysis are replaced with glucose-6-phosphatase, fructose-1,6-bisphosphatase, and PEP carboxykinase. This system of reciprocal control allow glycolysis and gluconeogenesis to inhibit each other and prevent the formation of afutile cycle.

The majority of the enzymes responsible for gluconeogenesis are found in the cytoplasm; the exceptions are mitochondrial pyruvate carboxylase, and, in animals, phosphoenol-pyruvate carboxykinase. The latter exists as an isozyme located in both the mitochondrion and the cytosol. As there is no known mechanism to transport phosphoenolpyruvate from the mitochondrion into the cytosol, the cytosolic enzyme is believed to be the isozyme important for gluconeogenesis. The rate of gluconeogenesis is ultimately controlled by the action of a key enzyme, fructose-1,6-bisphosphatase, which is also regulated through signal ransduction bycAMP and its phosphorylation.

Most factors that regulate the activity of the gluconeogenesis pathway do so by inhibiting the activity or expression of key enzymes. However, both acetyl CoA and citrate activate gluconeogenesis enzymes (pyruvate carboxylase and fructose-1,6-bisphosphatase, respectively). Due to the reciprocal control of the cycle, acetyl-CoA and citrate also have inhibitory roles in the activity of pyruvate kinase.

Insulin and Glucagon: Control of Blood Glucose

One of the most important and tightly regulated responses in the human body is the concentration of blood glucose (blood sugar). Glucose is the major breakdown product of cellular metabolism. As such, it is required both as an energy source and as a source of carbon for making organic molecules. Blood glucose concentrations are regulated by negative feedback pathways that are modulated by two separate hormones: insulin and glucagon. Both of these hormones are produced in special cells called islet cells, or islets of Langerhans – are found in clusters throughout the pancreas. Islet cells make up a very small percentage of the pancreas (about 1-2%); the remainder of the organ is an exocrine gland producing digestive enzymes and bicarbonate ion. This tiny number of endocrine cells is exceedingly important. Each islet contains two kinds of cells: alpha cells, which produce glucagon, and beta cells, which produce insulin.

Insulin vs. Glucagon

Normally, blood glucose concentrations in human blood should range between 70-110 milligrams (mg/ml). Insulin and glucagon operate in an antagonistic (opposing) manner. The result is a precise control of blood glucose levels within this range.

The insulin pathway is activated when blood glucose levels are too high. High blood glucose levels (e.g., occurring after the stomach has digested a food high in sugar) stimulate beta cells in the pancreas to release insulin. Insulin causes an increased uptake of glucose from the blood; promotes conversion of glucose into triglycerides in the liver, fat and muscle cells; and increases the cellular rate of glycolysis – breaking glucose into smaller components that can be used for synthesis of other compounds.

The glucagon pathway is activated when blood glucose levels are too low. Low blood glucose levels (e.g., due to exercise combined with not eating for several hours) stimulate the alpha cells in the pancreas to produce glucagon. Glucagon causes the liver to convert stored glycogen into glucose, then release the glucose into the blood (a process called glycogenolysis). The two hormones, insulin and glucagon, each regulate the other. A decrease in insulin (as well as low glucose levels) stimulates the secretion of glucagon, while an increase in insulin (as well as an increase in blood glucose) suppresses glucagon secretion. This results in a continuous cycle, with insulin and glucagon constantly monitoring blood glucose levels and regulating their secretion to maintain these levels as nearly constant as possible.

The main function of insulin is removal of excess blood glucose. Because all cells use glucose as an energy source and as a raw material for making other organic compounds, all cells except brain cells are targets for insulin. Since the function of glucagon is opposite that of insulin, it stimulates the addition of glucose to the bloodstream. Thus, it targets cells with high concentrations of energy stored as glycogen, including the liver and skeletal muscles. It also stimulates glucose production from fats, so adipose tissue cells are another target of glucagon.

Lactose intolerance

Lactose intolerance is a common digestive problem where the body is unable to digest lactose, a type of sugar mainly found in milk and dairy products. The body digests lactose by using an enzyme called lactase to break down lactose into two simpler sugars called glucose and galactose, which can then be easily absorbed into the bloodstream. Enzymes are proteins that cause chemical reactions to occur.

Notes

In cases of lactose intolerance, the body does not produce enough of the lactase enzyme so lactose stays in the digestive system, where it is fermented by bacteria (in the same way that yeast is fermented to produce beer). It's this fermentation process that causes the symptoms associated with lactose intolerance.

Levels of lactase often fall as people grow older and some health conditions can also reduce the production of lactase.

Symptoms of lactose intolerance include

- a bloated stomach
- flatulence (wind)
- diarrhoea

Treating lactose intolerance

Limiting intake of food and drink containing lactose is the main treatment for lactose intolerance.

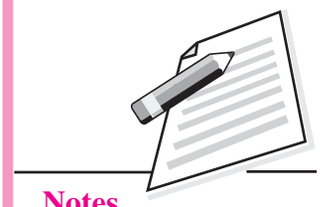
Depending on a person's levels of intolerance, they may also require additional calcium and vitamin D supplements to keep the bones strong and healthy.

Advice from a dietitian may sometimes be helpful in determining the best diet for a person.

Lactase substitutes are also available. These are drops that you can add to your meals or drinks to improve your digestion of lactose.

Diabetes Mellitus

Diabetes mellitus (often referred to simply as *diabetes*) is a group of metabolic diseases characterized by high blood glucose levels. The term comes from two Greek words: "diabetes" comes from a verb that means "to pass through" and refers to the frequent, copious urination that is a characteristic of the disease; the word "meli" is Greek for "honey" so the term "mellitus" refers to the presence of high levels of glucose (sugar) in the blood. In addition to urination, other classic symptoms of diabetes are increased thirst and hunger. The diabetic's blood contains more glucose than can be taken up by the cells so this excess glusose is therefore released in the urine (a diagnostic characteristic of diabetes is sugar in the urine). The presence of sugar results in more water being drawn into the urine to balance the osmotic pressure, leading to copious urination.



INTEXT QUESTIONS 3.1

1. In glycolysis pathway glucose is aerobically converted to ..... and anaerobically .....
2. All the reaction steps take place in .....
3. Glycolysis is the only source of energy in ..... cells
4. Number of ATPs gained per glucose molecule in Aerobic conditions of glycolysis is .....
5. Number of ATPs gained per glucose molecule in Anaerobic conditions of glycolysis is .....
6. Krebs Cycle yield ..... ATP per mole Glucose

WHAT HAVE YOU LEARNT

- In glycolysis pathway glucose is converted to pyruvate in aerobic condition or lactate in anaerobic condition. All the reaction steps take place in the cytoplasm.
- Glycolysis is the only pathway that is taking place in all the cells of the body and is the only source of energy in erythrocytes.
- In Glycolyis, Aerobic conditions yields 8 ATPs and in Anaerobic conditions yields 2 ATPs per glucose molecule
- Citric acid cycle produces two carbon dioxide molecules. And oxidations are connected to the electron transport chain where many ATP are produced.
- A total of 38 moles ATP per mole Glucose is yielded in Krebs Cycle
- Glycogenesis is the formation of glycogen from glucose. Glycogen is synthesized depending on the demand for glucose and ATP (energy).
- In glycogenolysis, glycogen stored in the liver and muscles, is converted first to glucose-1- phosphate and then into glucose-6-phosphate.
- Two hormones which control glycogenolysis are a peptide, glucagon from the pancreas and epinephrine from the adrenal glands.
- Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.
- Blood glucose concentrations in human blood should range between 70-110 milligrams per milliliter (mg/mL). Insulin and glucagon operate in an antagonistic (opposing) manner.

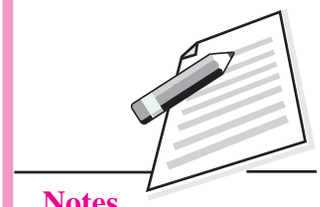
TERMINAL QUESTIONS

1. Explain glycolysis
2. Explain krebs cycle
3. Explain glycogenesis
4. What is the hormone control of blood sugar

ANSWERS TO INTEXT QUESTIONS

3.1

1. Pyruvate & Lactate
2. Cytoplasm
3. Erythrocytes
4. 8 ATPs
5. 2 ATPs
6. 38 moles



4

PROTEINS

4.1 INTRODUCTION

Proteins are the most abundant biological macromolecules, occurring in all cells and all parts of cells. Amino acids are the building blocks of proteins. All proteins, whether from the most ancient lines of bacteria or from the most complex forms of life, are constructed from the same set of 20 amino acids. What is most remarkable is that cells can produce proteins with strikingly different properties and activities by joining the same 20 amino acids in many different combinations and sequences. From these building blocks different organisms can make such widely diverse products as enzymes, hormones, antibodies, transporters, muscle fibers, the lens protein of the eye, feathers, spider webs, rhinoceros horn, milk proteins, antibiotics, and mushroom poisons and other substances having distinct biological activities. While proteins contain only L-α-amino acids, microorganisms elaborate peptides that contain both D- and L-α-amino acids.

OBJECTIVES

After reading this lesson, you will be able to

- describe amino acids
- explain the structure of amino acids
- classify amino acids
- describe proteins
- describe the structure of protein
- explain the function of proteins
- explain the digestion and absorption of proteins
- describe products of amino acids
- explain transamination, Deamination, Urea Cycle

Notes

4.2 AMINO ACIDS

Proteins are the essential agents of biological function, and amino acids are the building blocks of proteins. The diversity of the thousands of proteins found in nature arises from the commonly occurring 20 amino acids. Proteins are polymers of amino acids, with each amino acid residue joined to its neighbor by a specific type of covalent bond. Proteins can be broken down (hydrolyzed) to their constituent amino acids the free amino acids derived from them. Of the over 300 naturally occurring amino acids, 20 constitute the monomer units of proteins. All 20 amino acids (Table 4.1) are biologically essential. Humans can synthesize 12 (nutritionally nonessential) of the 20 common amino acids from the amphibolic intermediates of glycolysis and of the citric acid cycle. Of the 12 nutritionally nonessential amino acids, nine are formed from amphibolic intermediates and three (cysteine, tyrosine and hydroxylysine) from nutritionally essential amino acids.

Table 4.1 List of essential and nonessential amino acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Aspartic acid
Lysine	Cysteine
Methionine	Glutamic acid
Phenylalanine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
	Tyrosine
	Asparagine
	Selenocysteine
	Pyrrolysine

Essential amino acids are "essential" not because they are more important to life than the others, but because the body does not synthesize them. They must be present in the diet or they will not be present in the body. In addition, the amino acids arginine, cysteine, glycine, glutamine, histidine, proline, serine and tyrosine are considered conditionally essential, meaning they are not normally required in the diet, but must be supplied exogenously to specific populations that do not synthesize them in adequate amounts.

Selenocysteine, while not normally considered an amino acid present in proteins, selenocysteine occurs at the active sites of several enzymes. Examples include the human enzymes thioredoxin reductase, glutathione peroxidase, and the deiodinase that converts thyroxine to triiodothyronine. Pyrrolysine sometimes considered "the 22nd amino acid", is not listed here as it is not used by humans.

4.2.1 Amino Acids are Chiral Molecules

An α-amino acid consists of a central carbon atom, called the α carbon, linked to an amino group, a carboxylic acid group, a hydrogen atom, and a distinctive R group. For all the common amino acids except glycine, the α carbon is bonded to four different groups: a carboxyl group, an amino group, an R group, and a hydrogen atom (Fig. 4.1; in glycine, the R group is another hydrogen atom). The α-carbon atom is thus a **chiral center**. Because of the tetrahedral arrangement of the bonding orbitals around the α-carbon atom, the four different groups can occupy two unique spatial arrangements, and thus amino acids have two possible stereoisomers. Since they are nonsuperimposable mirror images of each other (Fig. 4.2), the two forms represent a class of stereoisomers called **enantiomers** (Fig. 4.3). The R group is often referred to as the side chain. Enantiomeric molecules display a special property called **optical activity** – the ability to rotate the plane of polarization of plane-polarized light. Clockwise rotation of incident light is referred to as **dextrorotatory** (D) behavior, and counterclockwise rotation is called **levorotatory** (L) behavior. Only L amino acids are constituents of proteins. The magnitude and direction of the optical rotation depend on the nature of the amino acid side chain.

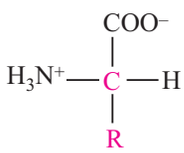


Fig. 4.1: General structure of an amino acid. This structure is common to all but one of the α-amino acids. (Proline, a cyclic amino acid, is the exception.) The R group or side chain (red) attached to the α carbon (blue) is different in each amino acid.

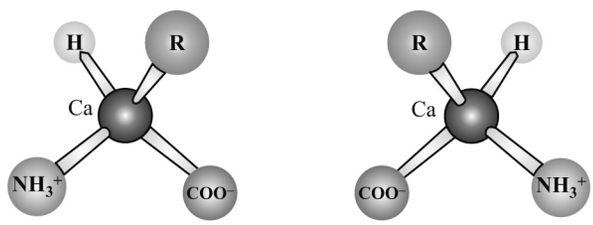


Fig. 4.2: The L and D Isomers of Amino Acids. R refers to the side chain. The L and D isomers are mirror images of each other.

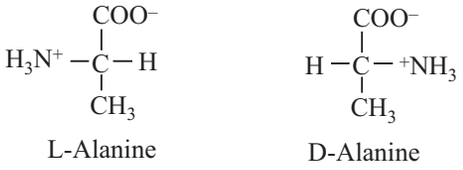


Fig. 4.3: Stereoisomerism in a-amino acids. The two stereoisomers of alanine, L- and D-alanine, are nonsuperimposable mirror images of each other (enantiomers).

4.2.2 Structure of a Typical Amino Acid

Amino acids in solution at neutral pH exist predominantly as dipolar ions (also called zwitterions). Amino acids can exist as zwitterions - substances containing equal numbers of positive and negative charge due to their carboxyl and amine groups, which can be negatively and positively charged, respectively. In the dipolar form, the amino group is protonated (NH_3^+) and the carboxyl group is deprotonated (COO^-). The ionization state of an amino acid varies with pH (Figure 4.4). They differ from each other in their side chains, or R groups, which vary in structure, size, and electric charge, and which influence the solubility of the amino acids in water.

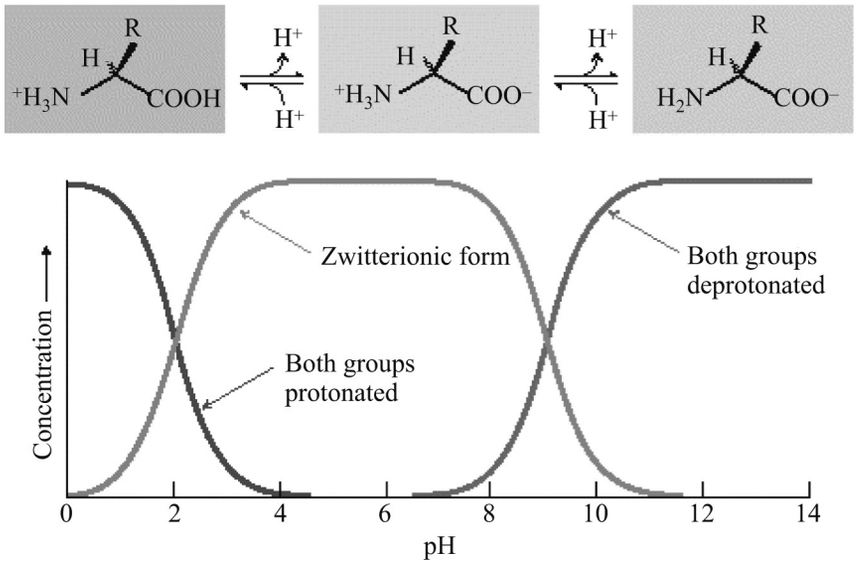


Fig. 4.4: Ionization State as a Function of pH. The ionization state of amino acids is altered by a change in pH. The zwitterionic form predominates near physiological pH.

4.2.3 Amino Acids can join via Peptide Bonds

The crucial feature of amino acids that allows them to polymerize to form peptides and proteins is the existence of their two identifying chemical groups:

the amino (NH_3^+) and carboxyl (COO^-) groups. The amino and carboxyl groups of amino acids can react in a head-to-tail fashion, eliminating a water molecule and forming a covalent amide linkage, which, in the case of peptides and proteins, is typically referred to as a peptide bond.

4.3 CLASSIFICATION

The structures and abbreviations for the 20 amino acids commonly found in proteins are shown in Figure 4.5. All the amino acids except proline have both free amino and free carboxyl groups. The classifications of amino acids is based on the polarity of the side chains. Thus, the structures shown in Figure 4.5 are grouped into the following categories: (1) nonpolar or hydrophobic amino acids, (2) neutral (uncharged) but polar amino acids, (3) acidic amino acids (which have a net negative charge at pH 7.0), and (4) basic amino acids (which have a net positive charge at neutral pH).

4.3.1 Nonpolar Amino Acids

The nonpolar amino acids include all those with alkyl chain R groups (alanine, valine, leucine, and isoleucine), as well as proline (with its unusual cyclic structure), methionine (one of the two sulfur-containing amino acids), and two aromatic amino acids, phenylalanine and tryptophan. Tryptophan is sometimes considered a borderline member of this group because it can interact favorably with water via the N–H moiety of the indole ring. Proline, strictly speaking, is not an amino acid but rather an α-imino acid.

4.3.2 Polar, Uncharged Amino Acids

The polar, uncharged amino acids except for glycine contain R groups that can form hydrogen bonds with water. Thus, these amino acids are usually more soluble in water than the nonpolar amino acids. Tyrosine displays the lowest solubility in water of the 20 common amino acids. Glycine, the simplest amino acid, has only a single hydrogen for an R group, and this hydrogen is not a good hydrogen bond former. Glycine's solubility properties are mainly influenced by its polar amino and carboxyl groups, and thus glycine is best considered a member of the polar, uncharged group. It should be noted that tyrosine has significant nonpolar characteristics due to its aromatic ring and could arguably be placed in the nonpolar group.

4.3.3 Acidic Amino Acids

There are two acidic amino acids – aspartic acid and glutamic acid – whose R groups contain a carboxyl group. Aspartic acid and glutamic acid thus have a net negative charge at pH 7. Many proteins that bind metal ions for structural

or functional purposes possess metal binding sites containing one or more aspartate and glutamate side chains.

4.3.4 Basic Amino Acids

Three of the common amino acids have side chains with net positive charges at neutral pH: histidine, arginine, and lysine. The ionized group of histidine is an imidazolium, that of arginine is a guanidinium, and lysine contains a protonated alkyl amino group. Arginine and lysine side chains, which are protonated under physiological conditions, participate in electrostatic interactions in proteins.

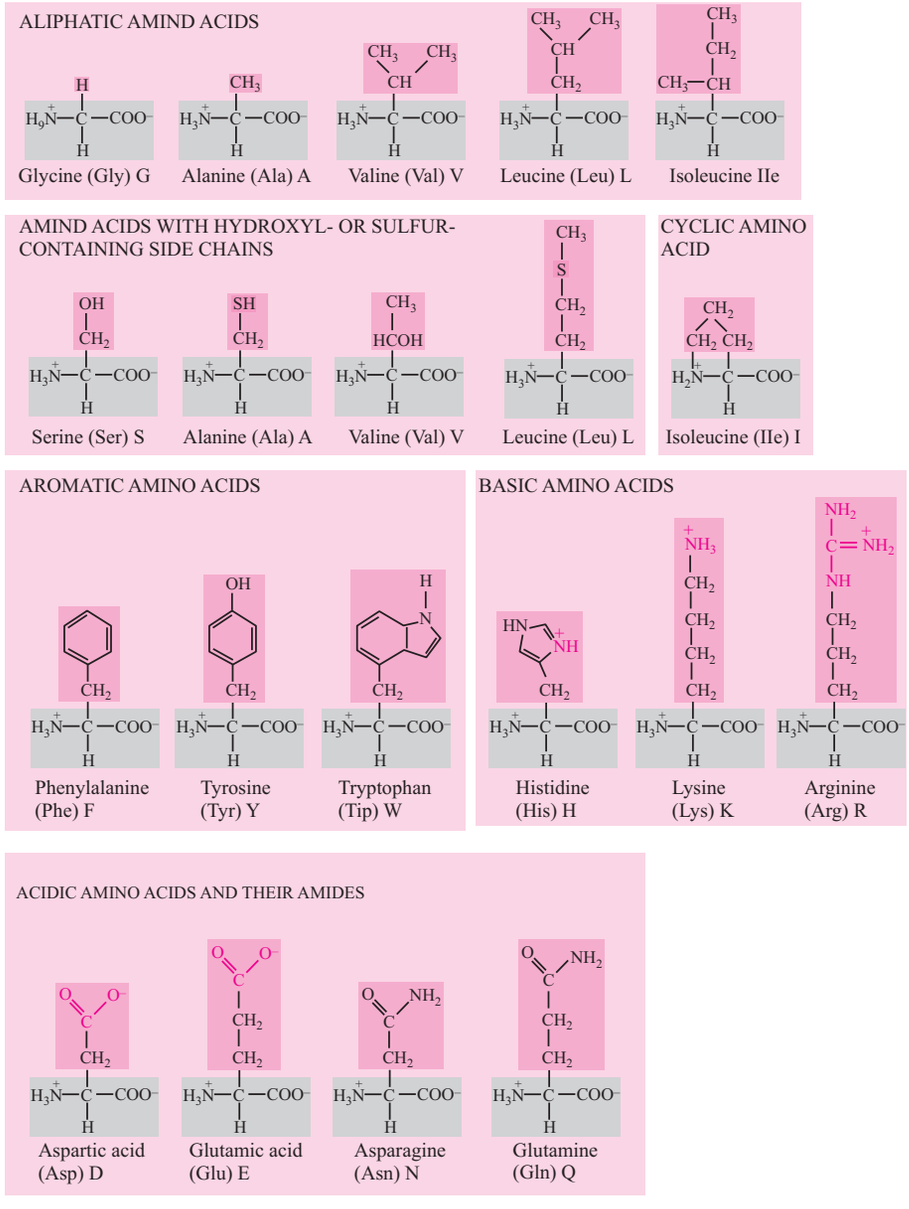


Fig. 4.5: Classification of amino acids.

4.4 PROTEIN

Proteins are a diverse and abundant class of biomolecules, constituting more than 50% of the dry weight of cells. This diversity and abundance reflect the central role of proteins in virtually all aspects of cell structure and function. Biologically occurring polypeptides range in size from small to very large, consisting of two or three to thousands of linked amino acid residues. Peptides are chains of amino acids, two amino acid molecules can be covalently joined through a substituted amide linkage, termed a peptide bond (Figure 4.6), to yield a dipeptide. Such a linkage is formed by removal of the elements of water (dehydration) from the α-carboxyl group of one amino acid and the α-amino group of another. Peptide bond formation is an example of a condensation reaction, a common class of reactions in living cells. Three amino acids can be joined by two peptide bonds to form a tripeptide; similarly, amino acids can be linked to form tetrapeptides, pentapeptides, and so forth. When a few amino acids are joined in this fashion, the structure is called an oligopeptide. When many amino acids are joined, the product is called a polypeptide. Proteins may have thousands of amino acid residues. Although the terms "protein" and "polypeptide" are sometimes used interchangeably, molecules referred to as polypeptides generally have molecular weights below 10,000, and those called proteins have higher molecular weights. Proteins can be assigned to one of three global classes on the basis of shape and solubility: fibrous, globular, or membrane.

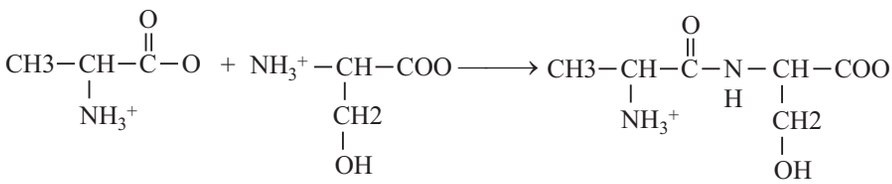


Fig. 4.6: Peptide bond formation between two amino acids Alanine and Serine.

Fibrous proteins tend to have relatively simple, regular linear structures. These proteins often serve structural roles in cells. Typically, they are insoluble in water or in dilute salt solutions. In contrast, **globular proteins** are roughly spherical in shape. The polypeptide chain is compactly folded so that hydrophobic amino acid side chains are in the interior of the molecule and the hydrophilic side chains are on the outside exposed to the solvent, water. **Membrane proteins** are found in association with the various membrane systems of cells. For interaction with the nonpolar phase within membranes, membrane proteins have hydrophobic amino acid side chains oriented outward. As such, membrane proteins are insoluble in aqueous solutions but can be solubilized in solutions of detergents.

Notes

4.5 THE LEVELS OF PROTEIN STRUCTURE

The various levels of protein structural organization are defined as follows.

4.5.1 Primary Structure

The amino acid sequence is the primary (1°) structure of a protein, such as that shown in Figure 4.7.

4.5.2 Secondary Structure

Through hydrogen bonding interactions between adjacent amino acid residues the polypeptide chain can arrange itself into characteristic helical or pleated segments. These segments constitute structural conformities, so-called regular structures that extend along one dimension, like the coils of a spring. Such architectural features of a protein are designated secondary (2°) structures (Figure 4.7). Secondary structures are just one of the higher levels of structure that represent the three-dimensional arrangement of the polypeptide in space.

4.5.3 Tertiary Structure

When the polypeptide chains of protein molecules bend and fold in order to assume a more compact three-dimensional shape, a tertiary (3°) level of structure is generated (Figure 4.7). It is by virtue of their tertiary structure that proteins adopt a globular shape. A globular conformation gives the lowest surface to-volume ratio, minimizing interaction of the protein with the surrounding environment.

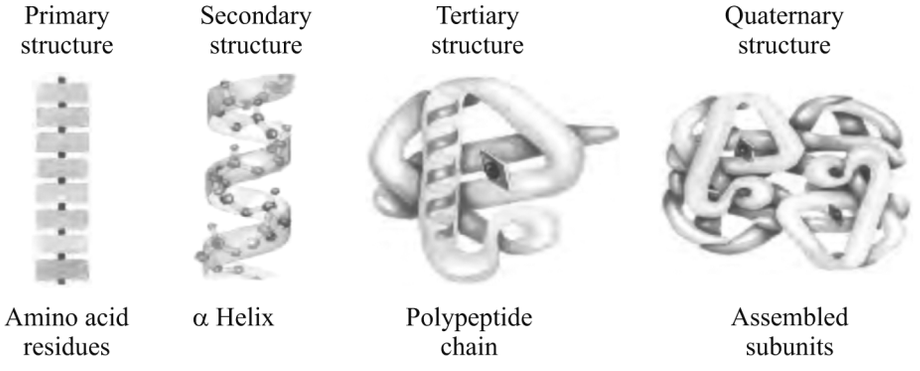


Fig. 4.7: Structures of protein.

4.5.4 Quaternary Structure

Many proteins consist of two or more interacting polypeptide chains of characteristic tertiary structure, each of which is commonly referred to as a subunit of the protein. Subunit organization constitutes another level in the

hierarchy of protein structure, defined as the protein's quaternary (4°) structure (Figure 4.7). Whereas the primary structure of a protein is determined by the covalently linked amino acid residues in the polypeptide backbone, secondary and higher orders of structure are determined principally by noncovalent forces such as hydrogen bonds and ionic, van der Waals, and hydrophobic interactions.

4.6 FUNCTIONS OF PROTEINS

Proteins are the agents of biological function. Virtually every cellular activity is dependent on one or more particular proteins. Thus, a convenient way to classify the enormous number of proteins is by the biological roles they fill. The various functions of proteins are as follows.

4.6.1 Enzymes

By far the largest class of proteins is enzymes. More than 3000 different enzymes are listed in *Enzyme Nomenclature*, the standard reference volume on enzyme classification. **Enzymes** are catalysts that accelerate the rates of biological reactions. Each enzyme is very specific in its function and acts only in a particular metabolic reaction. Virtually every step in metabolism is catalyzed by an enzyme. Enzymes are systematically classified according to the nature of the reaction that they catalyze, such as the transfer of a phosphate group (phosphotransferase) or an oxidation–reduction (oxidoreductase). The formal names of enzymes come from the particular reaction within the class that they catalyze, as in ATP: D-fructose-6-phosphate 1-phosphotransferase. Often, enzymes have common names in addition to their formal names. ATP: D-fructose-6-phosphate 1-phosphotransferase is more commonly known as phosphofructokinase (kinase is a common name given to ATP-dependent phosphotransferases).

4.6.2 Regulatory Proteins

A number of proteins do not perform any obvious chemical transformation but nevertheless can regulate the ability of other proteins to carry out their physiological functions. Such proteins are referred to as regulatory proteins. A well-known example is insulin, the hormone regulating glucose metabolism in animals. Insulin is a relatively small protein and consists of two polypeptide chains held together by disulfide cross-bridges. Other hormones that are also proteins include pituitary somatotropin and thyrotropin, which stimulates the thyroid gland.

4.6.3 Transport Proteins

A third class of proteins is the transport proteins. These proteins function to transport specific substances from one place to another. One type of transport

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is exemplified by the transport of oxygen from the lungs to the tissues by haemoglobin or by the transport of fatty acids from adipose tissue to various organs by the blood protein serum albumin.Membrane transport proteins take up metabolite molecules on one side of a membrane, transport them across the membrane, and release them on the other side. Examples include the transport proteins responsible for the uptake of essential nutrients into the cell, such as glucose or amino acids.

4.6.4 Storage Proteins

Proteins whose biological function is to provide a reservoir of an essential nutrient are called storage proteins. Because proteins are amino acid polymers and because nitrogen is commonly a limiting nutrient for growth, organisms have exploited proteins as a means to provide sufficient nitrogen in times of need. For example, ovalbumin, the protein of egg white, provides the developing bird embryo with a source of nitrogen during its isolation within the egg. Casein is the most abundant protein of milk and thus the major nitrogen source for mammalian infants. The seeds of higher plants often contain as much as 60% storage protein to make the germinating seed nitrogen-sufficient during this crucial period of plant development. In corn (Zea mays or maize), a family of low molecular weight proteins in the kernel called zeins serve this purpose. Ferritin is a protein found in animal tissues that binds iron, retaining this essential metal so that it is available for the synthesis of important iron-containing proteins such as hemoglobin.

4.6.5 Contractile and Motile Proteins

Certain proteins endow cells with unique capabilities for movement. Cell division, muscle contraction, and cell motility represent some of the ways in which cells execute motion. Examples include actin and myosin, the filamentous proteins forming the contractile systems of cells, and tubulin, the major component of microtubules.

4.6.6 Structural Proteins

An apparently passive but very important role of proteins is their function in creating and maintaining biological structures. Structural proteins provide strength and protection to cells and tissues. Monomeric units of structural proteins typically polymerize to generate long fibers (as in hair). α-*Keratins* are insoluble fibrous proteins making up hair, horns, and fingernails. Collagen, another insoluble fibrous protein, is found in bone, connective tissue, tendons, and cartilage, where it forms inelastic fibrils of great strength. One-third of the total protein in a vertebrate animal is collagen. A structural protein having elastic properties is, appropriately, elastin, an important component of ligaments.

Certain insects make a structurally useful protein, fibroin (a a-keratin), the major constituent of cocoons (silk) and spider webs.

4.6.7 Scaffold Proteins (Adapter Proteins)

Some proteins play a recently discovered role in the complex pathways of cellular response to hormones and growth factors. These proteins, the scaffold or adapter proteins, have a modular organization in which specific parts (modules) of the protein's structure recognize and bind certain structural elements in other proteins through protein–protein interactions.

4.6.8 Protective and Exploitive Proteins

In contrast to the passive protective nature of some structural proteins, another group can be more aptly classified as protective or exploitive proteins because of their biologically active role in cell defense, protection, or exploitation. Prominent among the protective proteins are the immunoglobulins or antibodies produced by the lymphocytes of vertebrates. Antibodies have the remarkable ability to specifically recognize and neutralize "foreign" molecules resulting from the invasion of the organism by bacteria, viruses, or other infectious agents. Another group of protective proteins is the blood-clotting proteins, thrombin and fibrinogen, which prevent the loss of blood when the circulatory system is damaged. Arctic and Antarctic fishes have antifreeze proteins to protect their blood against freezing in the below-zero temperatures of high-latitude seas. Another class of exploitive proteins includes the toxins produced by bacteria, such as diphtheria toxin and cholera toxin. It is worth repeating that the great diversity of function in proteins, as reflected is attained using just 20 amino acids.

4.7 DIGESTION AND ABSORPTION OF PROTEINS

Several groups of enzymes catalyze the digestion of proteins. There are two main classes of proteolytic digestive enzymes **(proteases),** with different specificities for the amino acids forming the peptide bond to be hydrolyzed. **Endopeptidases** hydrolyze peptide bonds between specific amino acids throughout the molecule. They are the first enzymes to act, yielding a larger number of smaller fragments, eg, **pepsin** in the gastric juice and **trypsin, chymotrypsin,** and **elastase** secreted into the small intestine by the pancreas. **Exopeptidases** catalyze the hydrolysis of peptide bonds, one at a time, from the ends of polypeptides. **Carboxypeptidases,** secreted in the pancreatic juice, release amino acids from the free carboxyl terminal, and **aminopeptidases,** secreted by the intestinal mucosal cells, release amino acids from the amino terminal. Dipeptides, which are not substrates for exopeptidases, are hydrolyzed in the brush border of intestinal mucosal cells by **dipeptidases.** The proteases are secreted as inactive

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zymogens; the active site of the enzyme is masked by a small region of its peptide chain, which is removed by hydrolysis of a specific peptide bond. **Pepsinogen** is activated to pepsin by gastric acid and by activated pepsin (autocatalysis). In the small intestine, **trypsinogen,** the precursor of trypsin, is activated by **enteropeptidase,** which is secreted by the duodenal epithelial cells; trypsin can then activate **chymotrypsinogen** to chymotrypsin, **proelastase** to elastase, **procarboxypeptidase** to carboxypeptidase, and **proaminopeptidase** to aminopeptidase.

4.7.1 Free amino acids and small peptides are absorbed by different mechanisms

The end product of the action of endopeptidases and exopeptidases is a mixture of free amino acids, di- and tripeptides, and oligopeptides, all of which are absorbed. Free amino acids are absorbed across the intestinal mucosa by sodium-dependent active transport. There are several different amino acid transporters, with specificity for the nature of the amino acid side chain (large or small; neutral, acidic, or basic). Dipeptides and tripeptides enter the brush border of the intestinal mucosal cells, where they are hydrolyzed to free amino acids, which are then transported into the hepatic portal vein.

4.8 SPECIALIZED PRODUCTS OF AMINO ACIDS PHENYLALANINE, TYROSINE

4.8.1 Phenylalanine

Phenylalanine is first converted to tyrosine. **Hyperphenylalaninemias** arise from defects in phenylalanine hydroxylase itself (**type I, classic phenylketonuria** or **PKU**), in dihydrobiopterin reductase (**types II and III**), or in dihydrobiopterin biosynthesis (**types IV and V**). DNA probes facilitate prenatal diagnosis of defects in phenylalanine hydroxylase or dihydrobiopterin reductase. A diet low in phenylalanine can prevent the mental retardation of PKU (frequency 1:10,000 births). Elevated blood phenylalanine may be detectable by a less reliable screening test that employs FeCl3 to detect urinary phenylpyruvate. FeCl3 screening for PKU of the urine of newborn infants is compulsory in the United States and many other countries.

4.8.2 Tyrosine

The probable metabolic defect in **type I tyrosinemia (tyrosinosis)** is at **fumarylacetoacetate hydrolase**. Therapy employs a diet low in tyrosine and phenylalanine. Untreated acute and chronic tyrosinosis leads to death from liver failure. Alternate metabolites of tyrosine are also excreted in **type II tyrosinemia**

(Richner-Hanhart syndrome), a defect in **tyrosine aminotransferase**, and in **neonatal tyrosinemia,** due to lowered *p*-hydroxyphenylpyruvate hydroxylase activity. Therapy employs a diet low in protein.

4.9 TRANSAMINATION (IMPORTANCE OF TRANSAMINASES)

The first step in the catabolism of most L-amino acids, once they have reached the liver, is removal of the α-amino groups, promoted by enzymes called **aminotransferases** or **transaminases.** The effect of transamination reactions is to collect the amino groups from many different amino acids in the form of L-glutamate. The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products. Cells contain different types of aminotransferases. All aminotransferases have the same prosthetic group and the same reaction mechanism. The prosthetic group is **pyridoxal phosphate (PLP),** the coenzyme form of pyridoxine, or vitamin B6. Its primary role in cells is in the metabolism of molecules with amino groups. Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases.

4.10 DEAMINATION

While ammonia, derived mainly from the α-amino nitrogen of amino acids, is highly toxic, tissues convert ammonia to the amide nitrogen of nontoxic glutamine. Subsequent deamination of glutamine in the liver releases ammonia, which is then converted to nontoxic urea. The deamination of amino acids leaves α-keto acid carbon skeletons. Several of these α-keto acids are citric acid cycle intermediates. Amino acids are used to synthesize liver and plasma proteins, or their carbon skeletons are converted to glucose and glycogen by gluconeogenesis; the ammonia formed by deamination is converted to urea. The α-amino acids collected in the liver in the form of the amino group of L-glutamate molecules must be removed from glutamate to prepare them for excretion. In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase**. In mammals, this enzyme is present in the mitochondrial matrix.

4.11 UREA CYCLE

Urea is the major end product of nitrogen catabolism in humans. Urea synthesis is a cyclic process. Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the α-amino nitrogen of aspartate. Five enzymes

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catalyze the numbered reactions of Figure 3.8. Of the six participating amino acids, N-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea. The major metabolic role of **ornithine, citrulline**, and **argininosuccinate** in mammals is urea synthesis. Since the ornithine consumed in reaction 2 is regenerated in reaction 5, there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine. Ammonium ion, CO_2, ATP, and aspartate are, however, consumed. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol.

4.11.1 Carbamoyl phosphate synthase I initiates Urea biosynthesis

Condensation of CO_2, ammonia, and ATP to form **carbamoyl phosphate** is catalyzed by mitochondrial **carbamoyl phosphate synthase I**. Carbamoyl phosphate synthase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of its allosteric activator ***N*-acetylglutamate,** which enhances the affinity of the synthase for ATP.

4.11.2 Carbamoyl phosphate plus Ornithine forms Citrulline

L-Ornithine transcarbamoylase catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate. While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.

4.11.3 Citrulline plus Aspartate forms Argininosuccinate

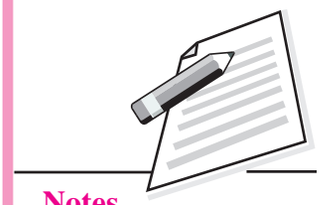
Argininosuccinate synthase links aspartate and citrulline via the amino group of aspartate and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms citrulline.

4.11.4 Cleavage of Argininosuccinate forms Arginine and Fumarate

Cleavage of argininosuccinate, catalyzed by **argininosuccinase**, proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate.

4.11.5 Cleavage of Arginine releases Urea and re-forms Ornithine

Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver arginase, releases urea. The other product, ornithine, reenters liver mitochondria for additional rounds of urea synthesis.



4.11.6 Carbamoyl phosphate synthase I is the pacemaker enzyme of the Urea cycle

The activity of carbamoyl phosphate synthase I is determined by N-acetylglutamate, whose steady-state level is dictated by its rate of synthesis from acetyl-CoA and glutamate and its rate of hydrolysis to acetate and glutamate. These reactions are catalyzed by N-acetylglutamate synthase and N-acetylglutamate hydrolase, respectively. Major changes in diet can increase the concentrations of individual urea cycle enzymes 10-fold to 20-fold. Starvation, for example, elevates enzyme levels, presumably to cope with the increased production of ammonia that accompanies enhanced protein degradation.

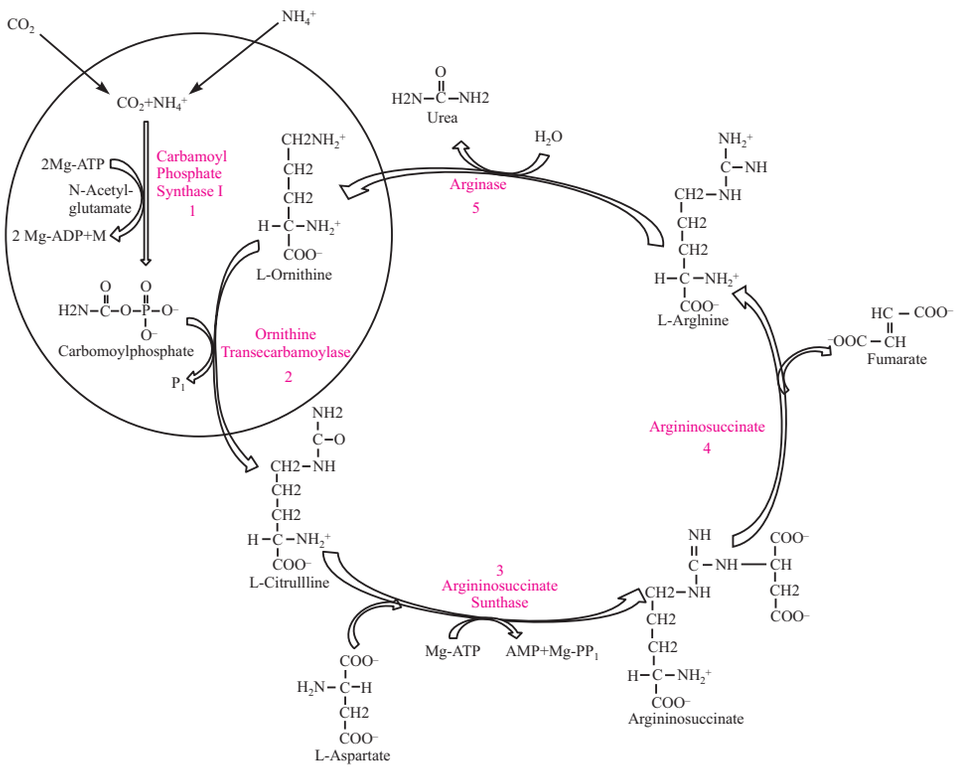


Fig. 4.8: Reactions and intermediates of urea biosynthesis. Reactions 1 and 2 occur in the matrix of liver mitochondria and reactions 3, 4, and 5 in liver cytosol. CO_2 (as bicarbonate), ammonium ion, ornithine, and citrulline enter the mitochondrial matrix via specific carriers present in the inner membrane of liver mitochondria.

INTEXT QUESTIONS 4.1

I. Choose the best answer

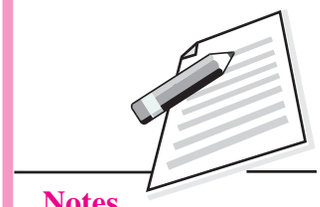
1. The simplest amino acid having only a single hydrogen for an R group is
   (a) Valine (b) Glycine
   (c) Histidine (d) Alanine
2. Peptide bond formation is an example of the reaction
   (a) Condensation (b) Hydrolysis
   (c) Deamination (d) Transformation
3. The characteristic helical or pleated segments formed through hydrogen bonding interactions between adjacent amino acid residues in the polypeptide chain of protein is seen in
   (a) Primary structure (b) Tertiary structure
   (c) Secondary structure (d) Quaternary structure
4. For the synthesis of hemoglobin, the following protein binds iron
   (a) Ferritin (b) Myosin
   (c) Keratin (d) Tubulin
5. The blood clotting proteins thrombin and fibrinogen comes under
   (a) Adapter proteins (b) Structural proteins
   (c) Protective proteins (d) Transport proteins

II. Fill in the blanks

6. Trypsinogen, the precursor of trypsin, is activated by ..... in the small intestine
7. Pyridoxal phosphate (PLP), the coenzyme form of .....
8. The oxidative deamination of glutamate catalysed by L-glutamate dehydrogenase takes place in ..... of hepatocytes
9. The rate-limiting enzyme of the urea cycle is .....
10. Some reactions of urea synthesis occur in the matrix of mitochondria, other reactions in the .....

III. Match the following

11. Essential amino acid (a) Ovalbumin
12. Non-essential amino acid (b) Leucine



13. Storage protein (c) L-glutamate dehydrogenase
14. Oxidative deamination (d) proteases
15. Proteolytic enzymes (e) Alanine

WHAT HAVE YOU LEARNT

- Both D-amino acids and non-α-amino acids occur in nature, but only L-α-amino acids are present in proteins.
- The 20 amino acids commonly found as residues in proteins contain an α-carboxyl group, an a-amino group, and a distinctive R group substituted on the a-carbon atom. The a-carbon atom of all amino acids except glycine is asymmetric, and thus amino acids can exist in at least two stereoisomeric forms.
- Amino acids can be joined covalently through peptide bonds to form peptides and proteins.
- Cells generally contain thousands of different proteins, each with a different biological activity.
- Differences in protein function result from differences in amino acid composition and sequence.
- Every protein has a three-dimensional structure that reflects its function. Tertiary structure is the complete three dimensional structure of a polypeptide chain. There are two general classes of proteins based on tertiary structure: fibrous and globular.
- Proteins are the agents of biological functions, virtually occurring in every cellular activity in the form of enzymes, regulatory proteins, transport proteins, storage proteins, contractile and motile proteins, structural proteins, adapter proteins, and protective and exploitive proteins.
- Proteins are digested at the intestinal region by several groups of proteolytic digestive enzymes (proteases), with different specificities for the amino acids forming the peptide bond to be hydrolyzed.
- Phenylalanine is first converted to tyrosine. The defects in phenylalanine hydroxylase leads to Hyperphenylalaninemias (type I, classic phenylketonuria or PKU). The metabolic defect in fumarylacetoacetate hydrolase leads to type I tyrosinemia (tyrosinosis). Alternate metabolites of tyrosine are also excreted in type II tyrosinemia (Richner-Hanhart syndrome), a defect in tyrosine aminotransferase.

Notes

- The catabolism of most L-amino acids in the liver is promoted by transamination and deaminations reactions. Glutamate functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the elimination of nitrogenous waste products.
- Urea is the major end product of nitrogen catabolism in humans. Urea synthesis is a cyclic process. Some reactions of urea synthesis occur in the matrix of the mitochondrion, other reactions in the cytosol.

ANSWERS TO INTEXT QUESTIONS

4.1

I. 1. (b) 2. (a) 3. (c) 4. (a) 5. (c)

II. 6. Enteropeptidase

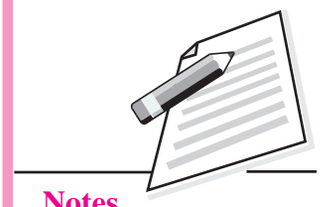
7. Pyridoxine

8. Mitochondrial matrix

9. Carbamoyl phosphate synthase I

10. Cytosol

III. 11. (b) 12. (e) 13. (a) 14. (c) 15. (d)



5

LIPIDS

5.1 INTRODUCTION

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, which are related more by their physical than by their chemical properties. Lipids are a class of compounds distinguished by their insolubility in water and solubility in nonpolar solvents. Lipids are important in biological systems because they form the cell membrane, a mechanical barrier that divides a cell from the external environment. Lipids also provide energy for life and several essential vitamins are lipids. Lipids can be divided in two major classes, nonsaponifiable lipids and saponifiable lipids. A nonsaponifiable lipid cannot be broken up into smaller molecules by hydrolysis, which includes triglycerides, waxes, phospholipids, and sphingolipids. A saponifiable lipid contains one or more ester groups allowing it to undergo hydrolysis in the presence of an acid, base, or enzyme. Nonsaponifiable lipids include steroids, prostaglandins, and terpenes. Within these two major classes of lipids, there are several specific types of lipids important to life, including fatty acids, triglycerides, glycerophospholipids, sphingolipids, and steroids. Each of these categories can be further broken down. Nonpolar lipids, such as triglycerides, are used for energy storage and fuel. Polar lipids, which can form a barrier with an external water environment, are used in membranes. Polar lipids include glycerophospholipids and sphingolipids. Fatty acids are important components of all of these lipids.

OBJECTIVES

After reading this lesson, you will be able to:

- classify lipids
- describe fatty acids and classify them
- enlist functions of lipids
- describe cholesterol and its importance

Notes

5.2 BIOLOGICAL ROLES OF LIPID

Lipids have the common property of being relatively insoluble in water and soluble in nonpolar solvents such as ether and chloroform. They are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods. Fat is stored in adipose tissue, where it also serves as a thermal insulator in the subcutaneous tissues and around certain organs. Nonpolar lipids act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves. Combinations of lipid and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria, and serving also as the means of transporting lipids in the blood. Knowledge of lipid biochemistry is necessary in understanding many important biomedical areas, e.g., obesity, diabetes mellitus, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

5.3 CLASSIFICATION OF LIPIDS

Lipids are classified as follows:

1. Simple lipids: Esters of fatty acids with various alcohols.
   (a) Fats: Esters of fatty acids with glycerol. Oils are fats in the liquid state.
   (b) Waxes: Esters of fatty acids with higher molecular weight monohydric alcohols.
2. Complex lipids: Esters of fatty acids containing groups in addition to an alcohol and a fatty acid.
   (a) Phospholipids: Lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, eg, in glycerophospholipids the alcohol is glycerol and in sphingophospholipids the alcohol is sphingosine.
   (b) Glycolipids (glycosphingolipids): Lipids containing a fatty acid, sphingosine, and carbohydrate.
   (c) Other complex lipids: Lipids such as sulfolipids and aminolipids. Lipoproteins may also be placed in this category.
3. Precursor and derived lipids: These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, and ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones. Because they are uncharged, acylglycerols (glycerides), cholesterol, and cholesteryl esters are termed neutral lipids.

5.3.1. Fatty Acids

A fatty acid is a molecule characterized by the presence of a carboxyl group attached to a long hydrocarbon chain. Therefore these are molecules with a formula R–COOH where R is a hydrocarbon chain. Fatty acids can be said to be carboxylic acids, and come in two major varieties.

- Saturated fatty acids do not have any double bonds. A fatty acid is saturated when every carbon atom in the hydrocarbon chain is bonded to as many hydrogen atoms as possible (the carbon atoms are saturated with hydrogen). Saturated fatty acids are solids at room temperature. Animal fats are a source of saturated fatty acids. In addition, fatty acids pack easily and form rigid structures (e.g., fatty acids are found in membranes).
- Unsaturated fatty acids can have one or more double bonds along its hydrocarbon chain. A fatty acid with one double bond is called monounsaturated. If it contains two or more double bonds, we say that the fatty acid is polyunsaturated. The melting point of a fatty acid is influenced by the number of double bonds that the molecule contains and by the length of the hydrocarbon tail. The more double bonds it contains, the lower the melting point. As the length of the tail increases, the melting point increases. Plants are the source of unsaturated fatty acids (Figure 5.1).

–CH = CH – CH = CH –

Unsaturated fatty acid chain

–CH – CH – CH –

Saturated fatty acid chain

Fig. 5.1: Aliphatic chain showing structure of unsaturated fatty acid chain with double bonds and saturated fatty acid chain with single bonds.

5.4 CLASSIFICATION OF FATTY ACIDS

Naturally occurring fatty acids have cis bonds. Trans-fatty acids are created artificially using a process called hydrogenation. A trans-fatty acid has a trans configuration rather than cis configuration at each double bond. This causes the molecule to straighten. These two stereoisomers can be distinguished in the following way:

- In a cis stereoisomer, two similar groups attached to the carbon double bond are found on the same side.
- In a trans stereoisomer, two similar groups attached to the carbon double bond are found on opposite sides.

Notes

5.5 ESSENTIAL AND NONESSENTIAL FATTY ACIDS

If a fatty acid can only be obtained from the diet (for humans) then the fatty acid is an essential fatty acid. Two fatty acids cannot be synthesized in the human body and are therefore essential. These are linoleic and linolenic fatty acids, which are both unsaturated. Nonessential fatty acids can be made by the human body and so do not need to be obtained from diet alone. These are made from carbohydrates and proteins or from other fatty acids. Fatty acids are an important source of energy. While carbohydrates or proteins only provide 4 kcal/g of energy, fatty acids provide more than twice the energy per unit weight of 9 kcal/g. This is one reason why a high-fat diet can lead to obesity.

5.5.1 Fatty acids are aliphatic carboxylic acids

Fatty acids occur mainly as esters in natural fats and oils but do occur in the unesterified form as free fatty acids, a transport form found in the plasma. Fatty acids that occur in natural fats are usually straight-chain derivatives containing an even number of carbon atoms. The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds).

5.5.2 Most naturally occurring unsaturated fatty acids have cis double bonds

The carbon chains of saturated fatty acids form a zigzag pattern when extended, as at low temperatures. At higher temperatures, some bonds rotate, causing chain shortening, which explains why biomembranes become thinner with increases in temperature. A type of geometric isomerism occurs in unsaturated fatty acids, depending on the orientation of atoms or groups around the axes of double bonds, which do not allow rotation. If the acyl chains are on the same side of the bond, it is cis-, as in oleic acid; if on opposite sides, it is trans-, as in elaidic acid, the trans isomer of oleic acid.

Naturally occurring unsaturated long-chain fatty acids are nearly all of cis configuration. Thus, oleic acid has an L shape, whereas elaidic acid remains "straight." Increase in the number of cis double bonds in a fatty acid leads to a variety of possible spatial configurations of the molecule – e.g., arachidonic acid, with four cis double bonds, has "kinks" or a U shape. This has profound significance on molecular packing in membranes and on the positions occupied by fatty acids in more complex molecules such as phospholipids. Trans double bonds alter these spatial relationships. Trans fatty acids are present in certain foods, arising as a by-product of the saturation of fatty acids during hydrogenation, or "hardening," of natural oils in the manufacture of margarine. An additional small contribution comes from the ingestion of ruminant fat that contains trans fatty acids arising from the action of microorganisms in the rumen.

5.6 COMMON LIPIDS AND THEIR FUNCTIONS

5.6.1 Triglycerides

A triglyceride (often called triacylgycerol) is a fatty acid trimester of glycerol. Triglycerides are important for human health in that they provide most of the lipids in our diet. Glycerol has three hydroxyl groups. Fatty acids can be attached at these three sites forming a triglyceride. One important characteristic of a tryglycerol is its state at room temperature. The degree of saturation and the length of their chains attached to the glycerol backbone both determine their state at room temperature.

- Short-chain unsaturated triglycerides are liquid at room temperature.
- Long-chain saturated triglycerides are solid at room temperature.

Animal fats (lard) contain a high amount of saturated triglycerides while plant oils (vegetable oil) contain a high amount of unsaturated triglycerides. While neither is healthy when consumed in excess, vegetable oils are far healthier than lard.

5.6.2 Triglycerides are the main storage forms of fatty acids

The triglycerides are esters of the trihydric alcohol glycerol and fatty acids. Mono- and diacylglycerols wherein one or two fatty acids are esterified with glycerol are also found in the tissues. These are of particular significance in the synthesis and hydrolysis of triacylglycerols.

5.6.3 The role of triglycerides in health

While fat may seem bad, triglycerides play many important roles in the body. For example, triglycerides can be used for energy storage in animals. This food reserve can be called upon during periods of starvation, with the high-calorie content of the fatty acids adding to the value of storing fat and providing much needed energy. In addition, triglycerides can provide insulation for animals in the form of body fat, which allows them to survive in colder temperatures. These two roles played by fat in the body, which arose over eons of evolution, are now deemed undesirable in modern industrialized society where humans no longer face starvation or have to deal directly with cold weather.

5.6.4 Phospholipids are the main lipid constituents of membranes

Phospholipids may be regarded as derivatives of phosphatidic acid, in which the phosphate is esterified with the OH of a suitable alcohol. Phosphatidic acid is important as an intermediate in the synthesis of triacylglycerols as well as phosphoglycerols but is not found in any great quantity in tissues. Amphipathic lipids self-orient at oil:water interfaces. They form Membranes, Micelles,

Liposomes, & Emulsions (Figure 5.2). In general, lipids are insoluble in water since they contain a predominance of nonpolar (hydrocarbon) groups. However, fatty acids, phospholipids, sphingolipids, bile salts, and, to a lesser extent, cholesterol contain polar groups. Therefore, part of the molecule is hydrophobic, or water-insoluble; and part is hydrophilic, or water-soluble. Such molecules are described as amphipathic. They become oriented at oil:water interfaces with the polar group in the water phase and the nonpolar group in the oil phase.

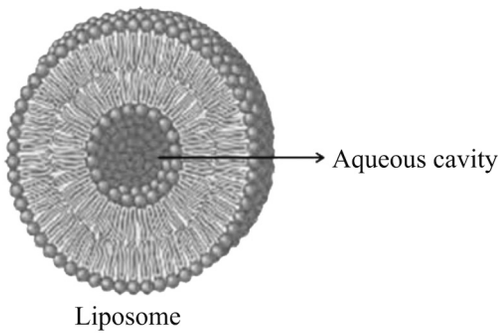
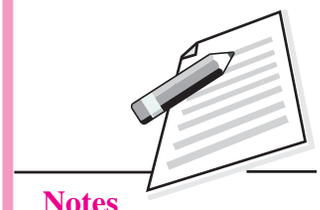


Fig. 5.2: Structure of liposome showing aqueous cavity at the centre of fatty acid bilayer.

A bilayer of such amphipathic lipids has been regarded as a basic structure in biologic membranes. When a critical concentration of these lipids is present in an aqueous medium, they form micelles. Aggregations of bile salts into micelles and liposomes and the formation of mixed micelles with the products of fat digestion are important in facilitating absorption of lipids from the intestine. Liposomes may be formed by sonicating an amphipathic lipid in an aqueous medium. They consist of spheres of lipid bilayers that enclose part of the aqueous medium. They are of potential clinical use – particularly when combined with tissue specific antibodies – as carriers of drugs in the circulation, targeted to specific organs, eg, in cancer therapy. In addition, they are being used for gene transfer into vascular cells and as carriers for topical and transdermal.

5.7 CHOLESTEROL AND ITS IMPORTANCE

Cholesterol is an important lipid found in the cell membrane. It is a sterol, which means that cholesterol is a combination of a steroid and an alcohol (Figure 5.3). It is an important component of cell membranes and is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D. In the cell membrane, the steroid ring structure of cholesterol provides a rigid hydrophobic structure that helps boost the rigidity of the cell membrane. Without cholesterol the cell membrane would be too fluid. In the human body, cholesterol is synthesized in the liver.



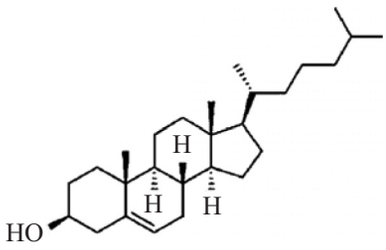


Fig. 5.3: Structure of cholesterol.

Cholesterol is insoluble in the blood, so when it is released into the blood stream it forms complexes with lipoproteins. Cholesterol can bind to two types of lipoprotein, called high-density lipoprotein (HDL) and low-density lipoprotein (LDL). A lipoprotein is a spherical molecule with water soluble proteins on the exterior. Therefore, when cholesterol is bound to a lipoprotein, it becomes blood soluble and can be transported throughout the body. HDL cholesterol is transported back to the liver. If HDL levels are low, then the blood level of cholesterol will increase.

High levels of blood cholesterol are associated with plaque formation in the arteries, which can lead to heart disease and stroke. While most cholesterol in the body is synthesized in the liver, dietary cholesterol also adds to the total blood levels. Cholesterol intake from the diet enters the bloodstream in the LDL form. This helps explain why consumption of foods with high-cholesterol content can lead to increased blood levels of cholesterol which is bad for health. So reducing the cholesterol in the diet can lower the blood level of cholesterol. This can reduce the amount of plaque formation. Aerobic exercise also contributes to health by increasing HDL levels in the blood. Hence more cholesterol is returned to the liver leading to a lower blood level of cholesterol, and reduced plaque formation.

5.8 DIGESTION AND ABSORPTION OF LIPIDS

The major lipids in the diet are triacylglycerols and, to a lesser extent, phospholipids. These are hydrophobic molecules and must be hydrolyzed and emulsified to very small droplets (micelles) before they can be absorbed. The fat-soluble vitamins – A, D, E, and K – and a variety of other lipids (including cholesterol) are absorbed dissolved in the lipid micelles. Absorption of the fat-soluble vitamins is impaired on a very low fat diet. Hydrolysis of triacylglycerols is initiated by lingual and gastric lipases that attack the sn-3 ester bond, forming 1,2-diacylglycerols and free fatty acids, aiding emulsification. Pancreatic lipase is secreted into the small intestine and requires a further pancreatic protein, colipase, for activity. It is specific for the primary ester links – ie, positions 1 and 3 in triacylglycerols – resulting in 2-monoacylglycerols and free fatty acids as the major end-products of luminal triacylglycerol digestion. Monoacylglycerols

are hydrolyzed with difficulty to glycerol and free fatty acids, so that less than 25% of ingested triacylglycerol is completely hydrolyzed to glycerol and fatty acids.

Bile salts, formed in the liver and secreted in the bile, enable emulsification of the products of lipid digestion into micelles and liposomes together with phospholipids and cholesterol from the bile. Because the micelles are soluble, they allow the products of digestion, including the fat soluble vitamins, to be transported through the aqueous environment of the intestinal lumen and permit close contact with the brush border of the mucosal cells, allowing uptake into the epithelium, mainly of the jejunum. The bile salts pass on to the ileum, where most are absorbed into the enterohepatic circulation. All long-chain fatty acids absorbed are converted to triacylglycerol in the mucosal cells and, together with the other products of lipid digestion, secreted as chylomicrons into the lymphatics, entering the blood stream via the thoracic duct. Free fatty acids are removed from the blood very rapidly and oxidized (fulfilling 25–50% of energy requirements in starvation) or esterified to form triacylglycerol in the tissues. In starvation, esterified lipids from the circulation or in the tissues are oxidized as well, particularly in heart and skeletal muscle cells, where considerable stores of lipid are to be found.

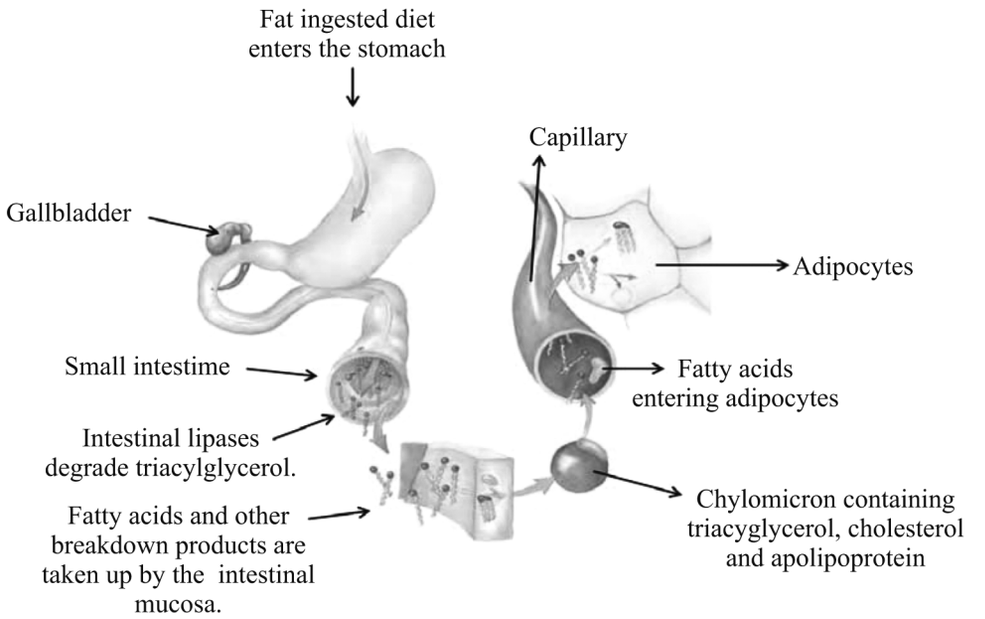


Fig. 5.4: Schematic representation of fat digestion at the intestinal region and absorption and transport of lipids in the form of triacylglycerol, fatty acids, cholesterol by chylomicrons. Fatty acids enter the blood circulation and stored in the adipose tissue.

The free fatty acid uptake by tissues is related directly to the plasma free fatty acid concentration, which in turn is determined by the rate of lipolysis in adipose tissue. After dissociation of the fatty acid-albumin complex at the plasma membrane, fatty acids bind to a membrane fatty acid transport protein that acts

as a transmembrane cotransporter with Na+. On entering the cytosol, free fatty acids are bound by intracellular fatty acid-binding proteins. The role of these proteins in intracellular transport is thought to be similar to that of serum albumin in extracellular transport of long chain fatty acids. Triacylglycerol is transported from the intestines in chylomicrons and from the liver in very low density lipoproteins. By definition, chylomicrons are found in chyle formed only by the lymphatic system draining the intestine. They are responsible for the transport of all dietary lipids into the circulation. Small quantities of VLDL are also to be found in chyle; however, most of the plasma VLDL is of hepatic origin. They are the vehicles of transport of triacylglycerol from the liver to the extrahepatic tissues (Figure 5.4).

5.9 CLASSIFICATION AND FUNCTIONS OF LIPOPROTEINS

Cholesterol and other lipids are carried on plasma lipoproteins. Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are essentially insoluble in water, yet must be moved from the tissue of origin to the tissues in which they will be stored or consumed. They are carried in the blood plasma as plasma lipoproteins, macromolecular complexes of specific carrier proteins, apolipoproteins, with various combinations of phospholipids, cholesterol, cholesteryl esters, and triacylglycerols. Apolipoproteins ("apo" designates the protein in its lipid-free form) combine with lipids to form several classes of lipoprotein particles, spherical complexes with hydrophobic lipids in the core and hydrophilic amino acid side chains at the surface.

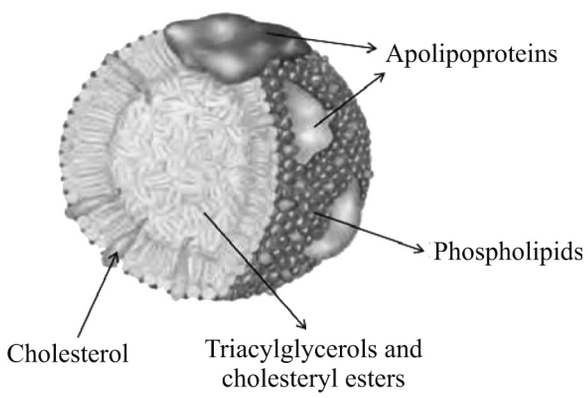
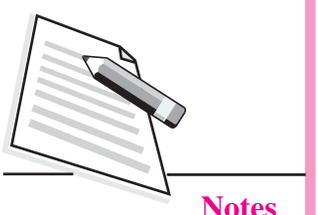


Fig. 5.5: Structure of chylomicron.

Different combinations of lipids and proteins produce particles of different densities, ranging from chylomicrons (Figure 5.5) to high-density lipoproteins. Each class of lipoprotein has a specific function, determined by its point of synthesis, lipid composition, and apolipoprotein content. At least nine different



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apolipoproteins are found in the lipoproteins of human plasma (Table), distinguishable by their size, their reactions with specific antibodies, and their characteristic distribution in the lipoprotein classes. These protein components act as signals, targeting lipoproteins to specific tissues or activating enzymes that act on the lipoproteins. Chylomicrons with the movement of dietary triacylglycerols from the intestine to other tissues are the largest of the lipoproteins and the least dense, containing a high proportion of triacylglycerols. Chylomicrons are synthesized in the ER of epithelial cells that line the small intestine, then move through the lymphatic system and enter the bloodstream via the left subclavian vein.

The apolipoproteins of chylomicrons include apoB-48 (unique to this class of lipoproteins), apoE, and apoC-II. ApoC-II activates lipoprotein lipase in the capillaries of adipose, heart, skeletal muscle, and lactating mammary tissues, allowing the release of free fatty acids to these tissues. Chylomicrons thus carry dietary fatty acids to tissues where they will be consumed or stored as fuel. The remnants of chylomicrons (depleted of most of their triacylglycerols but still containing cholesterol, apoE, and apoB-48) move through the bloodstream to the liver. Receptors in the liver bind to the apoE in the chylomicron remnants and mediate their uptake by endocytosis. In the liver, the remnants release their cholesterol and are degraded in lysosomes. When the diet contains more fatty acids than are needed immediately as fuel, they are converted to triacylglycerols in the liver and packaged with specific apolipoproteins into very-low-density lipoprotein (VLDL). Excess carbohydrate in the diet can also be converted to triacylglycerols in the liver and exported as VLDLs. In addition to triacylglycerols, VLDLs contain some cholesterol and cholesteryl esters, as well as apoB-100, apoC-I, apoC-II, apoC-III, and apo-E. These lipoproteins are transported in the blood from the liver to muscle and adipose tissue, where activation of lipoprotein lipase by apoC-II causes the release of free fatty acids from the VLDL triacylglycerols.

Adipocytes take up these fatty acids, reconvert them to triacylglycerols, and store the products in intracellular lipid droplets; myocytes, in contrast, primarily oxidize the fatty acids to supply energy. Most VLDL remnants are removed from the circulation by hepatocytes. The uptake, like that for chylomicrons, is receptor-mediated and depends on the presence of apoE in the VLDL remnants. The loss of triacylglycerol converts some VLDL to VLDL remnants (also called intermediate density lipoprotein, IDL); further removal of triacylglycerol from VLDL produces low-density lipoprotein (LDL). Very rich in cholesterol and cholesteryl esters and containing apoB-100 as their major apolipoprotein, LDLs carry cholesterol to extrahepatic tissues that have specific plasma membrane receptors that recognize apoB-100 (Table 5.1).

The fourth major lipoprotein type, high-density lipoprotein (HDL), originates in the liver and small intestine as small, protein-rich particles that contain relatively little cholesterol and no cholesteryl esters. HDLs contain apoA-I, apoC-I, apoC-II, and other apolipoproteins, as well as the enzyme lecithin-cholesterol acyl transferase (LCAT), which catalyzes the formation of cholesteryl esters from lecithin (phosphatidylcholine) and cholesterol. LCAT on the surface of nascent (newly forming) HDL particles converts the cholesterol and phosphatidylcholine of chylomicron and VLDL remnants to cholesteryl esters, which begin to form a core, transforming the disk-shaped nascent HDL to a mature, spherical HDL particle. This cholesterol-rich lipoprotein then returns to the liver, where the cholesterol is unloaded; some of this cholesterol is converted to bile salts.

Table 5.1. Lipoproteins source and components and apolipoproteins.

Lipoprotein	Source	Main lipid components	Apolipoproteins
Chylomicrons	Intestine	Triacyglycerol	A-I, A-II, A-IV, B 48, C-I, C-II, C-III,E
Chylomicron remnants	Chylomicrons	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons	Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D, E

5.10 KETONE BODY TYPES AND IMPORTANCE

Ketone bodies formed in liver to free up CoA for b-oxidation metabolized in other tissues, including brain, as an energy source. The formation of ketone bodies is a consequence of prolonged metabolism of fat. Their formation in the liver actually enables liver to metabolize even more fat by freeing up CoA that would otherwise be tied up as acetyl-CoA waiting to get into the TCA cycle. The liver exports the ketone bodies; and other tissues, particularly the brain, can adapt to use them. With increasing metabolism of fat through b-oxidation, much of the mitochondrial CoA pool may become tied up as acyl- or acetyl-CoA. In such cases, the supply of free CoA can be diminished, and this may limit the

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rate of b-oxidation. Upon prolonged fasting and heavy reliance on fat for energy, the liver induces the enzymes required for the formation of ketone bodies and brain induces enzymes required for their metabolism.

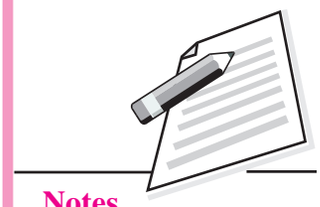
Ketone bodies are formed in the liver mitochondria by the condensation of three acetyl-CoA units. Two acetyl-CoAs are condensed to form acetoacetyl-CoA. The acetoacetyl-CoA is condensed with another acetyl-CoA to give hydroxymethylglutaryl-CoA (HMG-CoA). This is then split by HMG-CoA lyase to acetyl-CoA and acetoacetate. The hydroxybutyrate arises from acetoacetate by reduction. The overall sum of ketone body formation is the generation of acetoacetate (or hydroxybutyrate) and the freeing-up of the 2 CoAs that were trapped as acetyl-CoA. Ketone bodies are utilized in other tissues (but not the liver) by converting the acetoacetate to acetoacetyl-CoA and then converting the acetoacetyl-CoA to 2 acetyl-CoA, which are burned in the muscle mitochondria. Increased fatty acid oxidation is a characteristic of starvation and of diabetes mellitus, leading to ketone body production by the liver (ketosis). Ketone bodies are acidic and when produced in excess over long periods, as in diabetes, cause ketoacidosis, which is ultimately fatal. Because gluconeogenesis is dependent upon fatty acid oxidation, any impairment in fatty acid oxidation leads to hypoglycemia. This occurs in various states of carnitine deficiency or deficiency of essential enzymes in fatty acid oxidation, eg, carnitine palmitoyltransferase, or inhibition of fatty acid oxidation by poisons, eg, hypoglycin.

5.10.1 Ketogenesis occurs when there is a high rate of fatty acid oxidation in the liver

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of acetoacetate and β-hydroxybutyrate. Acetoacetate continually undergoes spontaneous decarboxylation to yield acetone. These three substances are collectively known as the ketone bodies. Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme D(β)-3-hydroxybutyrate dehydrogenase; the equilibrium is controlled by the mitochondrial [NAD+]/ [NADH] ratio, ie, the redox state. The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L. Extrahepatic tissues utilize them as respiratory substrates. The net flow of ketone bodies from the liver to the extrahepatic tissues results from active hepatic synthesis coupled with very low utilization. The reverse situation occurs in extrahepatic tissues.

5.11 ATHEROSCLEROSIS

Unregulated cholesterol production can lead to serious human disease. When the sum of cholesterol synthesized and cholesterol obtained in the diet exceeds the amount required for the synthesis of membranes, bile salts, and steroids,



pathological accumulations of cholesterol in blood vessels (atherosclerotic plaques) can develop, resulting in obstruction of blood vessels (atherosclerosis). Atherosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL-bound cholesterol; there is a negative correlation between HDL levels and arterial disease. In familial hypercholesterolemia, a human genetic disorder, blood levels of cholesterol are extremely high and severe atherosclerosis develops in childhood.

The metabolism of LDL and HDL intersects in the production and control of fatty streaks and potential plaques in blood vessels. Damage to the endothelium may be related to many factors, including normal turbulence of the blood, elevated LDL, especially modified or oxidized LDL, free radicals from cigarette smoking, homocystinemia, diabetes (glycation of LDL), and hypertension. The atherosclerotic lesion represents an inflammatory response sharing several characteristics with granuloma formation, and not simple deposition of cholesterol in the blood vessel.

Local inflammation recruits monocytes and macrophages with subsequent production of reactive oxygen species. LDL can become oxidized and then taken up, along with other inflammatory debris, by macrophages, which can become laden with cholesterol (foam cells). Initially the subendothelial accumulation of cholesterol-laden macrophages produces fatty streaks. As the fatty streak enlarges over time, necrotic tissue and free lipid accumulates, surrounded by epithelioid cells and eventually smooth muscle cells, an advanced plaque with a fibrous cap. The plaque eventually begins to occlude the blood vessel, causing ischemia and infarction in the heart, brain, or extremities. Eventually the fibrous cap may thin, and the plaque becomes unstable, leading to rupture and thrombosis.

HDL may be protective by picking up accumulating cholesterol before the advanced lesion forms. Apo-l activates LCAT, which in turn adds a fatty acid to cholesterol to produce a cholesterol ester that dissolves in the core of the HDL. The HDL may subsequently be picked up by the liver through the apoE receptor or deliver cholesterol through the scavenger receptor SR-Bl (reverse cholesterol transport from the periphery to the liver). The HDL may also transfer the cholesterol to an IDL reforming a normal, unoxidized LDL particle.

INTEXT QUESTIONS 5.1

I. Choose the best answer

1. Lipid stored in the form of energy in the body adipose tissue is

   (a) Steroid (b) Fatty acid

   (c) Sphingolipid (d) Phospholipid

Notes

2. Combination of lipid and protein serving as the means of transporting lipids in the blood is

   (a) Glycoprotein (b) Phosphoprotein

   (c) Lipoprotein (d) None

3. The enzyme which catalyzes the formation of cholesteryl esters from lecithin and cholesterol.

   (a) Lecithin-cholesterol acyl transferase

   (b) D(b)-3-hydroxybutyrate dehydrogenase

   (c) Lipoprotein lipase

   (d) Carnitine palmitoyltransferase

4. A human genetic disorder in which the extremely high levels of blood cholesterol develops atherosclerosis is

   (a) Familial hypercholesterolemia

   (b) Obesity

   (c) Apolipoproteinemia

   (d) None

5. The molecule acting as both hydrophobic and hydrophilic is termed as

   (a) Amphipathic (b) Aliphatic

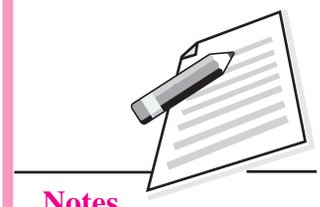
   (c) Polar (d) Nonpolar

II. Fill in the blanks

1. ..... act as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves.
2. Vegetable oil contains high amount of ..... triglycerides.
3. Aggregation of bile salts into ..... facilitates the absorption of lipids from the intestine.
4. A lipoprotein is a spherical molecule with water soluble ..... on the exterior.
5. Triglyceride is hydrolysed to ..... and .....

III. Match the following

1.	Cholestrol	(a)	Phospholipid
2.	Liposome	(b)	Diabetes mellitus
3.	Linoleic	(c)	Steroid
4.	Glycerophospholipid	(d)	Unsaturated fatty acid
5.	Ketoacidosis	(e)	Amphipathic molecule



WHAT HAVE YOU LEARNT

- Lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds and can be divided in two major classes as nonsaponifiable and saponifiable lipids.
- Saturated fatty acids do not have any double bonds. Animal fats are a source of saturated fatty acids. Unsaturated fatty acids can have one or more double bonds along its hydrocarbon chain. Plants are the source of unsaturated fatty acids.
- Fatty acid that cannot be synthesized by the body is an essential fatty acid. Linoleic and linolenic are the two essential fatty acids (both are unsaturated). Nonessential fatty acids can be made by the human body and so do not need to be obtained from diet alone.
- Animal fats (lard) contain a high amount of saturated triglycerides while plant oils (vegetable oil) contain a high amount of unsaturated triglycerides. While neither is healthy when consumed in excess, vegetable oils are far healthier than lard.
- Phospholipids are amphipathic lipids seen as a bilayer and form the structure in biologic membranes.
- Cholesterol is a sterol and an important component of cell membranes. It is also the basis for the synthesis of other steroids, including the sex hormones estradiol and testosterone, as well as other steroids such as cortisone and vitamin D.
- Cholesterol is insoluble in the blood, so when it is released into the blood stream it forms complexes with lipoproteins. Cholesterol can bind to two types of lipoprotein, called high-density lipoprotein (HDL) and low-density lipoprotein (LDL).
- High levels of blood cholesterol are associated with plaque formation in the arteries, which can lead to heart disease and stroke.
- The major lipids in the diet are triacylglycerols and, to a lesser extent, phospholipids. These are hydrophobic molecules and must be hydrolyzed and emulsified to very small droplets (micelles) before they can be absorbed.
- The fat-soluble vitamins – A, D, E, and K – and a variety of other lipids (including cholesterol) are absorbed dissolved in the lipid micelles.
- Bile salts, formed in the liver and secreted in the bile, enable emulsification of the products of lipid digestion into micelles and liposomes together with phospholipids and cholesterol from the bile. Because the micelles are soluble, they allow the products of digestion absorbed at the intestinal region.

Notes

- Low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), and intermediate density lipoprotein (IDL) are the types of lipoprotein.
- Ketogenesis occurs when there is a high rate of fatty acid oxidation in the liver. Acetoacetate, β-hydroxybutyrate and acetone are the products of fatty acid oxidation.
- Atherosclerosis is linked to high levels of cholesterol in the blood, and particularly to high levels of LDL-bound cholesterol; there is a negative correlation between HDL levels and arterial disease. LDL-cholesterol is called as bad cholesterol and HDL-cholesterol is called as good cholesterol.

TERMINAL QUESTIONS

1. Classify lipids with suitable examples.
2. What are essential and nonessential fatty acids.
3. Write short on importance of cholesterol.
4. Write short note on digestion and absorption of lipids.

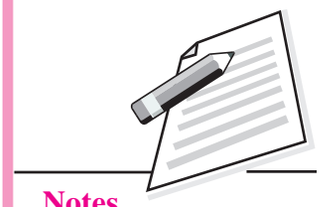
ANSWERS TO INTEXT QUESTIONS

5.1

I. 1. (b) 2. (c) 3. (a) 4. (a) 5. (a)

II. 1. Nonpolar lipids
2. Unsaturated
3. Micelles
4. Proteins
5. Glycerol and free fatty acid

III. 11. (c) 12. (e) 13. (d) 14. (a) 15. (b)



6

NUCLEOTIDES

6.1 INTRODUCTION

Nucleotides play major important roles in cellular metabolism. They are very essential for chemical links in the response of cells to hormones and other extracellular stimuli. They also act as the structural components of an array of enzyme cofactors and metabolic intermediates. Most importantly they are the constituents of nucleicacids: deoxyribonucleic acid (DNA) and ribonucleicacid (RNA). Nucleic acids are the molecular repositories of genetic information. The structure of every protein, and ultimately of every biomolecule and cellular component are programmed in form of nucleotide sequence of a cell's nucleic acids. The ability of nucleic acids to store and transmit genetic information from one generation to the next is a fundamental condition for life.

This chapter provides an overview of the structure of nucleic acids, chemistry of nucleotides, nucleotide metabolism, function of nucleic acid and diseases of error in nucleotide metabolism with special reference to gout.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the chemistry of nucleotides
- describe the structure of nucleic acids
- explain the characteristics of nucleic acids
- describe the nucleotide metabolism
- enlist the functions of nucleic acid

6.2 STRUCTURE AND CHEMISTRY OF NUCLEIC ACIDS

In all living organisms the amino acid sequence of every protein and the nucleotide sequence of every RNA, is specified by a nucleotide sequence in the cell's DNA. Segment of DNA molecule that encodes a protein or RNA, is referred to as a gene.

6.2.1 Chemistry of nucleic acid

Nucleotides are organic compounds that are monomeric units of nucleic acids,they are of different types and structures and have variety of physiological functions. Nucleic acids are responsible for storage and transmission of genetic information. Nucleic acids are chemically composed of polymers of nucleotides joined together by phosphodiester linkages (bonds). Nucleic acids are broadly divided into two major types; Ribonucleic acid (RNA) which is single stranded containing Adenine (A), Uracil (U), Cytosine (C) and Guanine (G) ribonucleotides and deoxyribonucleic acid which is double stranded containing Adenine, Thymine (T), Cytosine and Guanine deoxyribonucleotides.

6.2.1.1 Nucleosides

The nitrogenous bases and pentose sugars associated structure gives a compound called nucleoside. Based on the type of nitrogenous base and the types of sugar it is liked to, different types of nucleotides are formed each having its own characteristic and structure.

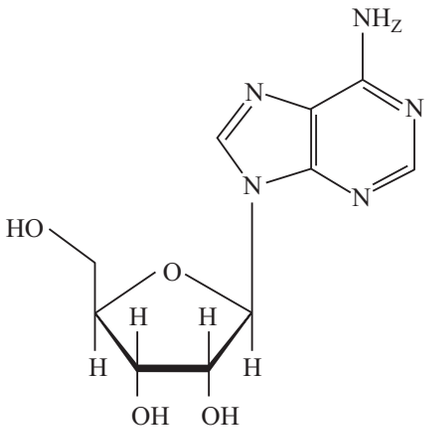


Fig. 6.1

Purines and pyrimidines are the components of nitrogenous bases. The purine bases contains the purine ring (double ringsystem) while the pyrimidine base contain pyrimidine ring (single ring structure). The purine bases include Adenine

and Guanine. The unusual forms of purines are hypoxathine, 1 methylguanine, 1 methylhypoxanthine etc. While the pyrimidine includes cytosine, thymine anduracil and its unusual forms are 5-methylcytosine, Thiouracil etc.

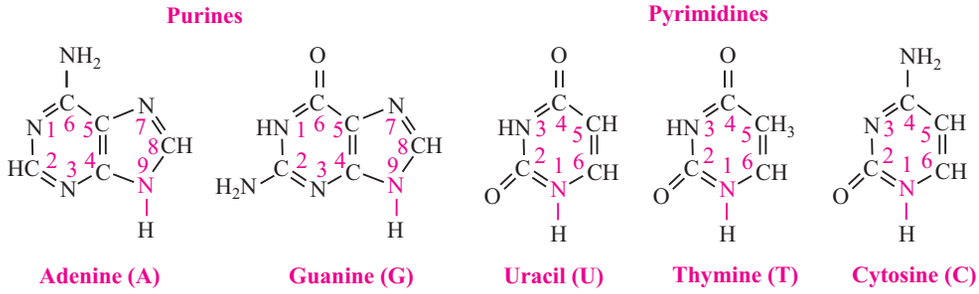


Fig. 6.2

In nucleosides, nitrogenous bases are joined to pentose sugar through the hemiacetal hydroxyl group on the C-1 (first carbon atom of the sugar). The purines are attached to the sugar through the N-9 nitrogen atom while pyrimidine are attached through the N-1 nitrogen atom.

6.2.1.2 Nucleotides

Nucleotides are phosphoric acid esters of nucleosides. Nucleotides contain nitrogenous bases, sugars and phosphoric acids in ester linkage. The nitrogenous base, presentin nucleotides are purines: Adenine and Guanine; pyrimidines: Cytosine,Thymine and Uracil. The uracil can only be found in ribonucleotides while thymine base can only be found in deoxyribonucleotides.

The sugar in nucleotides is the pentose sugar which could be ribose and deoxyribose. Sugar are esterified to a phosphoric acid residue at positions (2, 3 or 5) in ribose and (3 or 5) in the deoxyribose where the ester bonds could be formed. In addition, the nucleotides could be in form of mono,di and triphosphates.

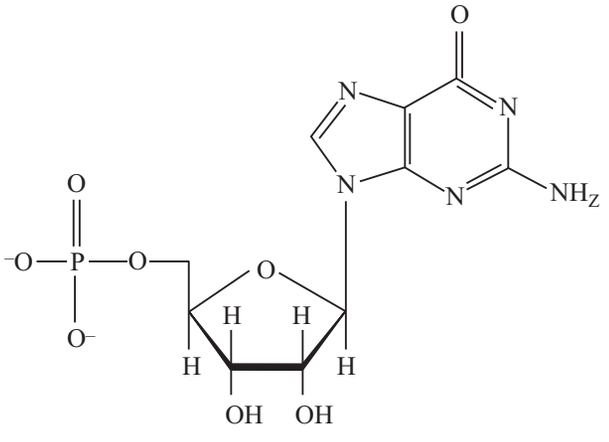
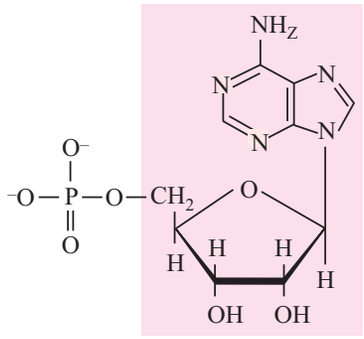


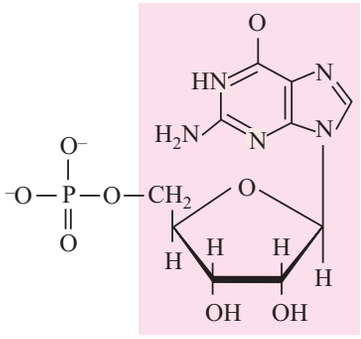
Fig. 6.3



Nucleotide: Deoxyadenylate (deoxyadenosine 5′-monophosphate)

Symbols: A. dA. dAMP

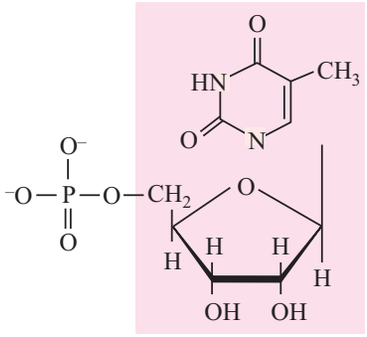
Nucleoside: Deoxyadenosine



Deoxyguanylate (deoxyguanosine 5′-monophosphate)

G. dG. dGMP

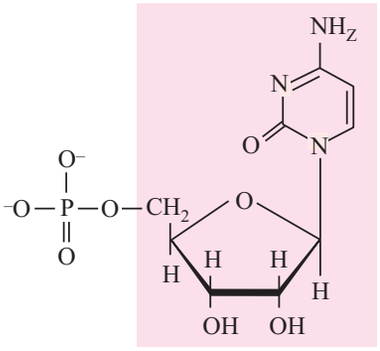
Deoxyadenosine



Deoxythymidylate (deoxythymidine 5′-monophosphate)

T. dT. dTMP

Deoxythymidine

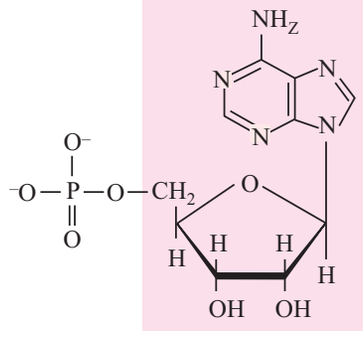


Deoxycytidylate (deoxycytidine 5′-monophosphate)

G. dG. dGMP

Deoxycytidine

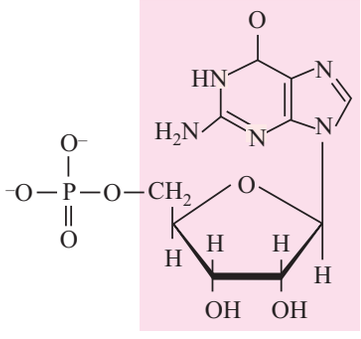
(a) Deoxyribonucleotides



Nucleotide: Adenylate (adenosine) 5′-monophosphate

Symbols: A. AMP

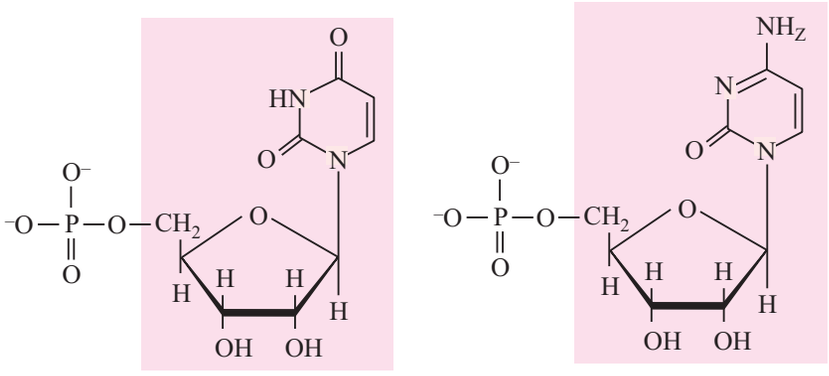
Nucleoside: Adenosine



Guanylate (guanosine 5′-monophosphate)

G. dGMP

Deoxyadenosine



Uridylate (uridine 5′-monophosphate)
U. UMP
Uridine

Cytidylate (cytidine 5′-monophosphate)
C. CMP
Cytidine

(b) Ribonucleotides

Fig. 6.4

Compound that have their structures derived from nucleotide structures are called as nucleotide derivatives. They share close structural features of nucleotides. Nicotinamide Adenine dinucleotide (NAD), Nicotinamide Adenine dinucleotide phosphate (NADP), flavine adeninedinucleotide (FAD) are some of the examples for nucleotide derivatives.

INTEXT QUESTIONS 6.1

1. Segment of DNA molecule that encodes a protein is called as .....
   (a) RNA (b) gene (c) interon (d) operon
2. Nucleotides are organic compounds that are monomeric units of .....
3. The nitrogenous bases and ..... associated structure gives a compound called nucleoside
4. ..... are phosphoric acid esters of nucleosides

6.2.1.3 Structure of different types of DNA

The primary structure of a nucleicacid is based on nucleotide sequence. Any regular, stable structure taken up by some or all of the nucleotides in a nucleic acid can be referred to as secondary structure. The complex folding of large chromosomes within eukaryotic chromatin and bacterial nucleoids is generally considered tertiary structure. The structure of DNA was worked out by bringing together a number of observations from various sources such as

(a) That DNA from different sources have remarkable similarity in their Xray diffraction patterns; suggesting that DNA molecules have uniform molecular pattern and consists polynucleotide chains arranged in helical structure.

(b) The ratio of the bases (A: T and C: G) is very close to one. In base pairing event A and T can be paired with a maximum of two hydrogen bonds between them while C and G will have a maximum of three bonds.

(c) The long polynucleotide chains were held together through bonds between these residues.

Using these observations, Watson and Crick constructed a model of DNA structures in 1953.

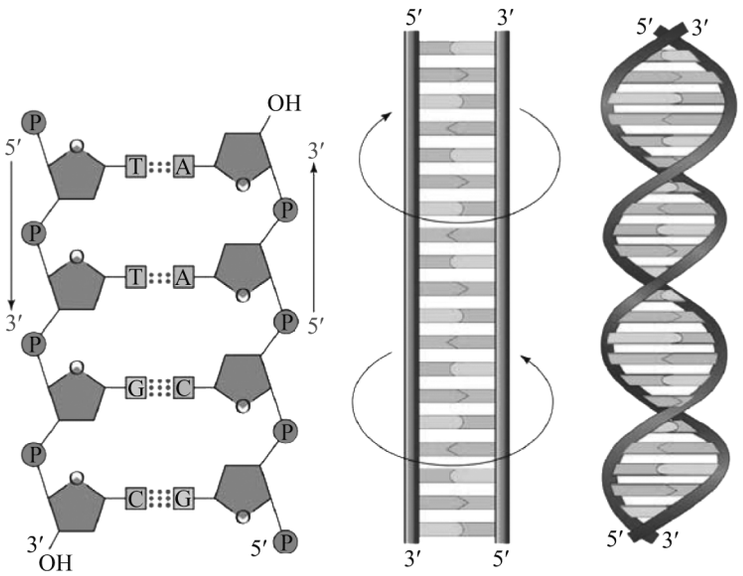
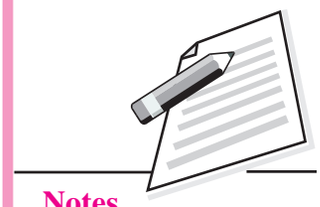


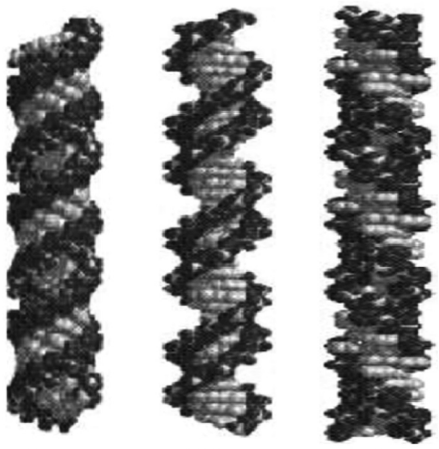
Fig. 6.5

The Watson-Crick Model consists of sugar molecule joined together by phosphate diesters. Bases were observed to be projecting perpendicularly from the chains into the central axis. For each adenine projecting inwards, there is a corresponding thymine from the other chain and for each cytosine, there is a guanine. A-T and C-G are held together by two and 3 hydrogen bonds respectively. The two chains are however not identical because of base pairings. The chains donot run in the same direction with respect to linkage between the nucleotides, rather they are anti-parallel.

Structurally DNA exists as three different forms namely: A, B and Z form. The A form of DNA posses' right handed helix with a diameter of 26Å. The A form of DNA contains 11 base pair per helix turn and base turns rise of helix is 2.6Å. The B form of the DNA is considered as Watson-Crick DNA structure. In B form, the DNA helix is arranged as left handed. The B form of DNA contains 0.34 nm



between bp, 3.4 nm per turn, about 10 bp per turn1.9 nm (about 2.0 nm or 20 Angstroms) in diameter. The Z form of DNA is a more radical departure from B-DNA with left handed helical rotation. Ion Z form of DNA each helical turn consist of 12 base pairs. Structures appear to be more slender and elongated.



A Form B Form Z Form

Fig. 6.6

Comparison between different forms of DNA

Character	A form	B form	Z form
helix sense	Right handed	Right handed	Left handed
bp per turn	10	11	12
vertical rise per bp	3.4 Å	2.56 Å	3.7Å
rotation per bp	+36°	+33°	+30°
helical diameter	19 Å	23 Å	18 Å

INTEXT QUESTIONS 6.2

1. The primary structure of a nucleic acid is based on ..... nucleotide sequence
2. In base pairing event A and T can be paired with a maximum of two hydrogen bonds

   (a) 1 (b) 2 (c) 3 (d) 4
3. Watson and Crick constructed a model of DNA structures in the year 1953
4. Structurally DNA exists as three different forms namely .....

Notes

6.2.2 Structure of different types of RNA

RNA in solution is dynamic molecules in solution. They undergo changes in conformation during synthesis, processing and functioning. RNA (Ribo nucleic acid) molecule exist as three different forms namely,

(a) Primary structure

(b) Secondary structure

(c) Tertiary structure

Unlike DNA, RNA molecules in primary structure are single stranded linear polymer containing nucleotides joined by phosphodiester linkage. The linkage of the ribonucleotides in RNA is 3'5' phosphodiester link involve 3'-OH group of ribose and 5'-phosphate group of another ribonucleotide. In eukaryotes the length of RNA molecule ranges from 65 nucleotides to 6000 nucleotides.

In secondary structure the single stranded RNA molecule may have double helical regions formed by hydrogen bonding between complimentary base sequences within the RNA molecule. In tertiary structure the association of RNA with proteins enables the RNA molecule to be stable and also fold into specific conformations. The "L shaped" conformation of the tRNA conformations held in position not only by the base pairing interactions but also other interactions.

RNA exist mainly as 3 major types namely:

(a) mRNA

(b) tRNA

(c) rRNA

6.2.2.1 Messenger RNA (mRNA)

The structure of messenger RNA (mRNA) especially the eukaryotic mRNA has some unique. The 5'terminus of mRNA is "capped" with a methylated base of Guanosine 5' triphosphate. The methylation is on the 2'-hydroxyl group of the ribose sugar. The methylated capping is followed by a non translated or "leader" sequence. The leader sequence is followed by an initiation codon, most often AUG. Coding region of mRNA are terminated by stop codon, usually UAA, UAG, UGA. The stop codon are followed by a second non translated sequence at the 3' end. A series of adenylic acids called poly A tail which makes up 3' terminus of the mRNA molecule.

6.2.2.2 Transfer RNA (tRNA)

The length of tRNAs ranges from 65-110 nucleotides with a corresponding molecular weight of 22, 000-37500 Daltons. The tRNA is a single stranded. As a result of intramolecular hydrogen bonding the 5' to 3' nucleotide stretch folded

into a conformation and forms different loops. The various loops are: the D-loop, anticodon loop, variable loop or arm, TøC loop and the acceptor system, each having its own function.

6.2.2.3 Ribosomal RNA (rRNA)

Ribosomal RNA are found associated with large number of proteins in an ordered complex. Ribosomal RNA has a helical structure resulting from the folding back of single stranded polymer and constitute about 74-80% of total RNA in a cell.

INTEXT QUESTIONS 6.3

1. RNA molecules in primary structure are single stranded linear polymer containing nucleotides joined by ..... phosphodiester linkage
2. The three types of RNA are .....
3. The 5'terminus of mRNA is "capped" with a methylated base of .....
   (a) Guanosine 5' triphosphate (b) Adenosine 5' triphosphate
   (c) Cytocine 5' triphosphate (d) Thyamine 5' triphosphate
4. The length of tRNAs ranges from ..... to ..... nucleotides

6.2.3 Characteristics of nucleic acids

(a) The components of nucleic acids are nucleotides joined by phosphodiester linkage to one another.

(b) Based on the type of sugar in nucleic acids, nucleic acids can either be deoxyribonucleic acids in which case they contain deoxyribose sugar or ribonucleic acids when the sugar is a ribose one.

(c) The double stranded forms of both DNA or RNA can be denatured.

(d) DNA is highly viscous at pH 7.0 and room temperature (25°C).

(e) At extreme pH or temperature above 80°C the viscosity of DNA decreases sharply, which is an indicator of physical change.

(f) The denaturation of double helical DNA associated with the disruption of hydrogen bonds between the paired bases is called melting of the.

(g) The temperature midpoint in the transition is called melting temperature (tm).

(h) For nucleic acids the marked absorption in the ultraviolet (UV) region of the lights pectrum with Absorption maxima is near 260nm.

INTEXT QUESTIONS 6.4

1. The components of nucleic acids are nucleotides joined by .....
2. ..... is highly viscous at pH 7.0
3. At temperature above ..... the viscosity of DNA decreases
4. The Absorption maxima of nucleic acid is near .....

6.2.4 Metabolism of Nucleotides

Nucleotide metabolism involves several interconnected pathways. Nucleotides can be synthesized de novo, or from components "salvaged" from the degradation products of nucleic acids. When in excess, nucleotides are degraded to products that can either be consumed by other pathways or excreted. Defects in the pathways for de novo synthesis, salvage, and degradation of nucleotides result in clinical disorders, and many drugs target these pathways.

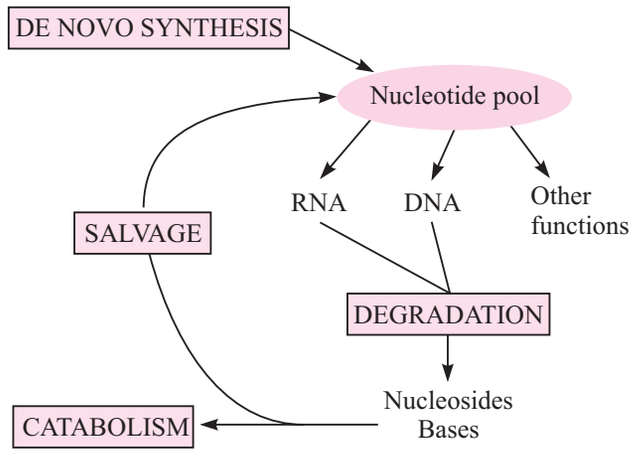
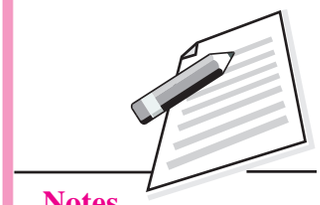


Fig. 6.7

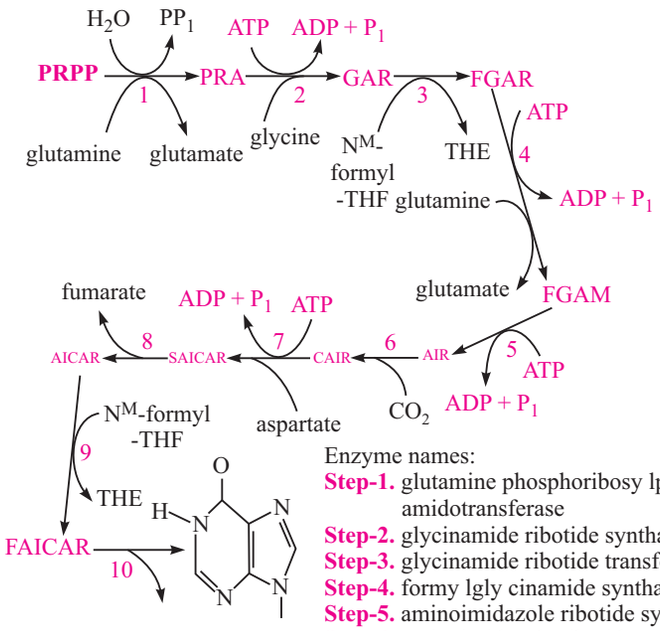
Nucleotide anabolism can be broadly characterized as purine and pyrimidine biosynthesis.

6.2.4.1 Biosynthesis of purine nucleotides

Purine nucleotides are synthesized in cytoplasm of most of the tissues. The major site for purine synthesis is liver. Since purine ring are synthesized from different



small components, they are makorly denoted by de novo synthesis. Different sources such as respiratory CO_2, amino group from aspartate, formyl group, amide group from glutamine and glycine etc., are required for formation of purine ring. As a primary requirement of purine synthesis, purine ring are first built upon a ribose-5-phosphate molecule. De novo synthesis of purine is a multi enzyme reaction composed of 10 steps as follow as



Enzyme names:

Step-1. glutamine phosphoribosy lpyrophosphate amidotransferase
Step-2. glycinamide ribotide synthase
Step-3. glycinamide ribotide transformylase
Step-4. formy lgly cinamide synthase
Step-5. aminoimidazole ribotide synthase
Step-6. aminoimidazole ribotide carboxy lase
Step-7. succiny laminoimidazolecarboxamideribotide synthase
Step-8. adenylosuccinate lyase
Step-9. aminoimidazole carboxamide ribotide transformy lase
Step-10. IMP cyclohy drolase

Fig. 6.8

In salvage pathway purines were recycled from degraded nucleotide. Both nucleosides and deoxy-nucleosides can be salvaged. Phospho ribosyl phyrophosphate (PRPP) is the starting material for salvage pathway. In salvage pathway purines are salvaged by adenine phosphor ribosyl transferase (APRTase) and hypoxanthine guanine phosphoribosyl transferase (HGPRTase). Salvage pathway are encountered in tissues such as RBC and brain, where there is absence of de novo pathway.

6.2.4.2 Disorders of purine metabolism

The most common abnormality of purine metabolism is elevation of uric acid level in blood. Elevated level of uric acid in blood is called as hyper-uricemia. In hyperuricemia the serum uric acid level exceeds the level of 7 mg/dl for male

Notes

and 6 mg/dl in female. This increased uric acid may or may not be excreated via urine and the condition is called as uricosuria.

Gout

In gout the urate crystals accumulates in synovial fluid. The above condition leads to inflammation mediated acute arthritis. The solubility of uric acid is lowered to 4.5 mg/dl at 30°C. Thus the uric acid is deposited in cooler zone such as tophi. Deposition of uric acid crystrals are noticed with increase in excretion of uric acid in urine. The deposition of uric acid crystals leads to calculi or stone formation. The gout may be either primary or secondary in nature

Primary gout

Primary gout may show familial incidence and are about 1:500 in total population. About 10 % of primary gout are idiopathic in nature. The main cause of primary gout is because of error in synthesis of enzymes such as

(a) 5-phosphoribosyl amido transferase

The error in 5-phospho ribosyl amido transferase leads to over production of purine nucleotides. Even though the abnormal nucleotide is active, they are not sensitive to feedback inhibition by inhibitory nucleotides.

(b) Phospho ribosyl phyrophosphate (PRPP) synthetase

The abnormal leads to increase and accumulation of PRPP and are X-linked recessive in inheritance.

(c) Glucose-6-phosphatase

Deficiency in glucose-6-phosphatase leads to a glycogen storage disease called as Gierke's disease. In this case more glucose is channeled to the pentose-phosphate shunt pathway, leading increased availability of ribose5-phosphate. As a result of this there will be a increase in PRPP formation.

(d) Glutathione reductase

Abnormality in glutathione reductase leads to increased production of ribose-5-phosphate and thereby increases in PRPP.

Secondary gout

Secondary gout is characterized by increase in uric acid production and reduced excretion rate. The increase in uric acid is due to increase in turnover rate of nucleic acids. The increase in nucleic acid turnover rate are seen in

(a) Rapidly growing malignant tissues

(b) Increase in tissue breakdown after treatment of large malignant tumors

(c) Increase in tissue damage due to trauma

(d) Increase in rate of catabolism as in starvation

Reduction in excretion rate as encountered in secondary gout during

(a) Renal failure

(b) Treatment with thiazide diuretics

(c) Interfrence of tubular secretion due to lactic acidosis and keto-acidosis.

6.2.4.3 Pyrimidine synthesis

Purines are synthesized by building the ring system on the ribose. In contrast, the pyrimidine ring is constructed first, followed by attachment of the pyrimidine base to ribose using a phosphoribosyltransferase similar to those used for purine salvage reactions. In both purine and pyrimidine synthesis, PRPP is used as the ribose donor, but the stage of the pathway is different.

The first step of the pyrimidine synthesis pathway is the condensation of bicarbonate with nitrogen derived from glutamine to form carbamoyl phosphate. The enzyme involved is **carbamoyl phosphate synthetase II** and is different from the enzyme catalyzing the equivalent step in the urea cycle.

Carbamoyl phosphate synthetase II has three **major differences**:

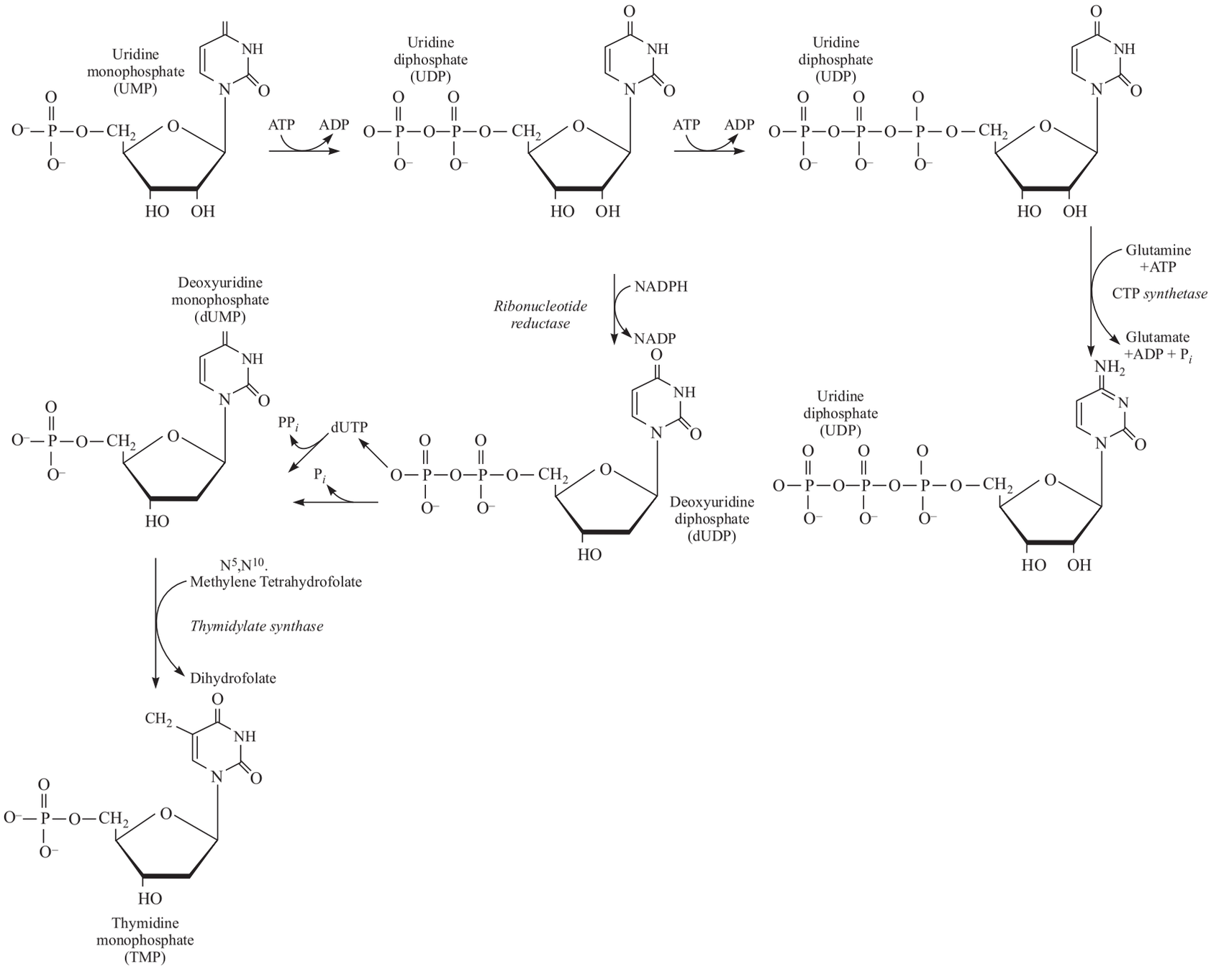
1. It uses nitrogen from glutamine rather than from ammonium
2. It is a cytosolic rather than a mitochondrial enzyme
3. Its regulation is completely different.

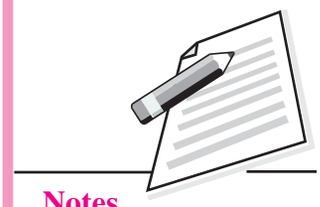
In animals two separate pools of carbamoyl phosphate were noticed, they are

1. Mitochondrial pool, used for the urea cycle
2. Cytosolic pool, used for pyrimidine synthesis.

While in bacteria a single pool of carbamoyl phosphate was used for both purposes, and therefore their pathways are regulated slightly differently. The pyrimidine ring skeleton comes from two molecules, the carbamoyl phosphate from the first step, and the aspartate added in the second step.

The ribose ring is not added until the synthesis of the pyrimidine orotic acid is complete. This orotic acid is then attached to PRPP with release of pyrophosphate. UMP is the first "completed" product. UMP can then be phosphorylated to produce UDP. UDP acts as a branch point; it can be converted to UTP and used as a nucleotide, or it can serve as a substrate for the synthesis of the two other major pyrimidine nucleotides. Both CTP and TTP were synthesized as described in the following reaction.


Uridine monophosphate (UMP)
ATP
ADP
Uridine diphosphate (UDP)
ATP
ADP
Uridine diphosphate (UDP)
Glutamine +ATP
CTP synthetase
Glutamate +ADP + Pi
Deoxyuridine monophosphate (dUMP)
Ribonucleotide reductase
NADPH
NADP
PPi
dUTP
Pi
Deoxyuridine diphosphate (dUDP)
Uridine diphosphate (UDP)
N5,N10. Methylene Tetrahydrofolate
Thymidylate synthase
Dihydrofolate
Thymidine monophosphate (TMP)



INTEXT QUESTIONS 6.5

1. Nucleotide anabolism can be broadly characterized as ..... biosynthesis
2. Purine nucleotides are synthesized in ..... of most of the tissues and major site is .....
3. In salvage pathway purines both ..... and ..... can be salvaged
4. The most common abnorbality of purine metabolism is ..... in blood
5. In gout the ..... accumulates in synovial fluid

6.2.5 Functions of nucleic acids

Both structurally and chemically, the nucleic acids differ from other macromolecule found in living systems and poses their roles and functions. Nucleic acid itself exist as two different forms namely DNA and RNA, both differ chemically and structurally.

6.2.5.1 Functions of DNA

(a) DNA acts as a genetic material.

(b) It is worthwhile emphasizing that the main role of the DNA is storage and transmission of genetic information from parents to offsprings or from one generation to another.

(c) DNA is capable of undergoing replication (synthesis of another copy of DNA) and being transcribed into RNA (transcription).

(d) These two processes enables the genetic information encoded in the DNA found in the nucleus to be transformed into a functional biological material e.g protein in the cytoplasm.

6.2.5.2 Functions of RNA

(a) RNA, like DNA has shown to be a general constituent of prokaryotic and eukaryotic cells.

(b) RNA molecules are not as stable as DNA, they also serve as genetic information carrier in some organisms e.g some viruses where RNA is their genetic material.

(c) The main functions of tRNA include: Transportation of specific amino acids to the ribosome's decoding the genetic information in the messenger RNA in terms of the proper amino acid to be inserted in the sequence of protein/polypeptide synthesised.

Notes

(d) tRNA carries one amino acid and also possess an anticodon by which it recognizes the message on the mRNA template during protein synthesis.

(e) Transfer RNAs have two primary active sites, the 3'hydroxyl terminus to which specific aminoacid are attached covalently and the anticodon triplet.

(f) Ribosomal RNAs (rRNA) serve as a structural framework for the ribosomes. The hinging mechanism between the two ribosomal subunits enables translocation and mRNA movement.

(g) Messenger RNA (mRNA) are direct carriers of genetic information from the nucleus to the cytoplasmic ribosomes.

(h) Eukaryotic mRNA contains information for only one polypeptide and is therefore monocistronic whereas prokaryotic mRNA can contain information for more than one polypeptide chain and therefore designated polycistronic.

INTEXT QUESTIONS 6.6

1. Nucleic acid itself exist as two different forms namely ..... and .....
2. DNA acts as a .....
3. ..... are not as stable as DNA
4. ..... are direct carriers of genetic information from the nucleus to the cytoplasmic ribosomes

   (a) mRNA (b) rRNA (c) tRNA (d) DNA
5. Prokaryotic mRNA can contain information for more than one polypeptide chain and therefore designated .....

WHAT HAVE YOU LEARNT

- There are different types of nucleic acids, each having its own characteristic function.
- DNA has a structure different from RNA. Structurally there are 3 forms of DNA. Structures of mRNA, rRNA and rRNA have been described. RNA has primary, secondary and tertiary level structural organization.
- Nucleic acids are polymers of nucleotides. There are two types of nucleic acids, DNA and RNA.
- The DNA is further subdivided into three (3) forms namely: A form, B form and Z form.

- DNA can be denatured and renatured. DNA is highly condensed in the chromosome and its size can be determined under centrifugal field.
- RNA has a primary, secondary and tertiary structures. DNA and RNA have differences and similarities. DNA serves in storage and transmission of genetic material from one generation to another or from parents to offsprings.
- Messenger RNA carries genetic information from nucleus to the cytoplasm.
- Transfer RNA carries amino acids to the site of protein synthesis. Ribosomal rRNA serves as the structural framework of the ribosome.

TERMINAL QUESTIONS

1. Describe the structure of DNA
2. Write short notes on the mRNA and tRNA
3. Elaborate in detail about nucleotide metabolism
4. Give details on Gout.

ANSWERS FOR INTEXT QUESTIONS

6.1

1. Gene
2. Nucleic acids
3. Pentose sugars
4. Nucleotides

6.2

1. Nucleotide sequence
2. 2
3. 1953
4. A, B and Z form

6.3

1. Phosphodiester linkage

Notes

2. mRNA, rRNA and tRNA
3. Guanosine 5' triphosphate
4. 65 to 110

6.4

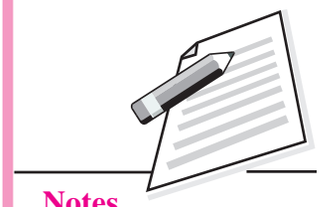
1. Phosphodiester linkage
2. DNA
3. 80°C
4. 260nm

6.5

1. Purine and pyrimidine
2. Cytoplasm and liver
3. Nucleosides and deoxy-nucleosides
4. Elevation of uric acid level
5. Urate crystals

6.6

1. DNA and RNA
2. Genetic material
3. RNA
4. Messenger RNA
5. Polycistronic



7

CLINICAL CHEMISTRY

7.1 INTRODUCTION

The clinical chemistry measures chemical changes in the body for diagnosis, therapy and prognosis of disease. Primarily testing is performed using body fluids such as serum, plasma, and urine to determine the chemical components.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the reglulation of blood sugar
- explain various blood glucose laboratory tests
- describe laboratory tests of Urea, Calcium and Phosphates
- describe Lipid Profile, Urine Creatinine

7.2 BLOOD SUGAR

The blood sugar concentration or blood glucose level is the amount of glucose (sugar) present in the blood of a human or animal. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis. Glucose is the primary source of energy for the body's cells. Glucose is transported from the intestines or liver to body cells via the bloodstream, and is made available for cell absorption via the hormone insulin, produced by the body primarily in the pancreas.

The mean normal blood glucose level in humans is about 100 mg/dL; however, this level fluctuates throughout the day. Glucose levels are usually lowest in the morning, before the first meal of the day (termed "the fasting level"), and rise after meals for an hour or two by a few milligram. The normal blood glucose level (tested while fasting) for non-diabetics, should be between 70 and 100

Notes

milligrams per deciliter (mg/dL). Blood sugar levels for those without diabetes and who are not fasting should be below 125 mg/dL. The blood glucose target range for diabetics, according to the American Diabetes Association, should be 70–130 (mg/dL) before meal, and less than 180 mg/dL after meals (as measured by a blood glucose monitor).

Blood sugar levels outside the normal range may be an indicator of a medical condition. A persistently high level is referred to as hyperglycemia; low levels are referred to as hypoglycemia. Diabetes mellitus is characterized by persistent hyperglycemia from any of several causes, and is the most prominent disease related to failure of blood sugar regulation. Intake of alcohol causes an initial surge in blood sugar, and later tends to cause levels to fall. Also, certain drugs can increase or decrease glucose levels.

7.2.1 Regulation

The body's homeostatic mechanism keeps blood glucose levels within a narrow range. It is composed of several interacting systems, of which hormone regulation is the most important.

There are two types of mutually antagonistic metabolic hormones affecting blood glucose levels:

- catabolic hormones (such as glucagon, cortisol and catecholamines) which increase blood glucose
- anabolic hormone (insulin), which decreases blood glucose.

7.2.2 Abnormality in blood sugar levels

7.2.3 High blood sugar

If blood sugar levels remain too high the body suppresses appetite over the short term. Long-term hyperglycemia causes many of the long-term health problems including heart disease, eye, kidney, and nerve damage. The most common cause of hyperglycemia is diabetes. When diabetes is the cause, physicians typically recommend an anti-diabetic medication as treatment. From the perspective the majority of patients, treatment with an old, well-understood diabetes drug such as metformin will be the safest, most effective, least expensive, most comfortable route to managing the condition. Diet changes and exercise implementation may also be part of a treatment plan for diabetes.

7.2.4 Low blood sugar

If blood sugar levels drop too low, a potentially fatal condition called hypoglycemia develops. Symptoms may include lethargy, impaired mental functioning; irritability; shaking, twitching, weakness in arm and leg muscles; pale complexion; sweating; paranoid or aggressive mentality and loss of consciousness.

7.2.5 Glucose measurement

7.2.5.1 Sample type

Glucose is measured in whole blood, plasma or serum. Historically, blood glucose values were given in terms of whole blood, but most laboratories now measure and report plasma or serum glucose levels. Because red blood cells (erythrocytes) have a higher concentration of protein (e.g., hemoglobin) than serum, serum has a higher water content and consequently more dissolved glucose than does whole blood. Collection of blood in clot tubes for serum chemistry analysis permits the metabolism of glucose in the sample by blood cells until separated by centrifugation. Red blood cells, for instance, do not require insulin to intake glucose from the blood. Higher than normal amounts of white or red blood cell counts can lead to excessive glycolysis in the sample, with substantial reduction of glucose level if the sample is not processed quickly. Ambient temperature at which the blood sample is kept prior to centrifuging and separation of plasma/serum also affects glucose levels. At refrigerator temperatures, glucose remains relatively stable for several hours in a blood sample.

Loss of glucose can be prevented by using Fluoride tubes since fluoride inhibits glycolysis. However, these should only be used when blood will be transported from one hospital laboratory to another for glucose measurement. Red-top serum separator tubes also preserve glucose in samples after being centrifuged isolating the serum from cells. Arterial, capillary and venous blood has comparable glucose levels in a fasting individual. Following meals, venous levels are somewhat lower than those in capillary or arterial blood; a common estimate is about 10%.

7.2.6 Measurement techniques

Two major methods have been used to measure glucose. The first, still in use in some places, is a chemical method exploiting the nonspecific reducing property of glucose in a reaction with an indicator substance that changes color when reduced. The more recent technique, using enzymes specific to glucose, is less susceptible to this kind of error. The two most common employed enzymes are glucose oxidase and hexokinase. In either case, the chemical system is commonly contained on a test strip which is inserted into a meter, and then has a blood sample applied. Test-strip shapes and their exact chemical composition vary between meter systems and cannot be interchanged. More precise blood glucose measurements are performed in a medical laboratory, using hexokinase, glucose oxidase or glucose dehydrogenase enzymes.

7.2.7 Blood glucose laboratory tests

1. fasting blood sugar (i.e., glucose) test (FBS)
2. two-hr postprandial blood sugar test (2-h PPBS)
3. oral glucose tolerance test (OGTT)

Notes

4. intravenous glucose tolerance test (IVGTT)
5. glycosylated hemoglobin (HbA_{1C})
6. self-monitoring of glucose level via patient testing
7. Random blood sugar (RBS)
8. Average blood glucose may be estimated by measuring glycated hemoglobin (HbA1c)

7.2.8 Clinical Correlation

The fasting blood glucose level, which is measured after a fast of 8 hours, is the most commonly used indication of overall glucose homeostasis, largely because disturbing events such as food intake are avoided. The metabolic response to a carbohydrate challenge is conveniently assessed by a postprandial glucose level drawn 2 hours after a meal or a glucose load. In addition, the glucose tolerance test, consisting of several timed measurements after a standardized amount of oral glucose intake, is used to aid in the diagnosis of diabetes.

Finally, there are several influences on blood glucose level aside from food intake. Infection, for instance, tends to change blood glucose levels, as does stress either physical or psychological. Exercise, especially if prolonged or long after the most recent meal, will have an effect as well.

7.3 UREA

7.3.1 Blood urea nitrogen (BUN)

The liver produces urea in the urea cycle as a waste product of the digestion of protein. Normal human adult blood should contain between 6 to 20 mg of urea nitrogen per 100 ml. Individual laboratories may have different reference ranges, and this is because the procedure may vary.

7.3.2 Interpretation

BUN is an indication of renal health. Normal ranges 8-20 mmol/L. If Glomerular Filtration Rate (GFR) and blood volume decrease (hypovolemia) then BUN will increase. Other factors responsible for its increment are fever, increased catabolism, high protein diet and gastrointestinal bleeding.

7.4 CALCIUM-PHOSPHATES

Calcium metabolism or calcium homeostasis is the mechanism by which the body maintains adequate calcium levels. Derangements of this mechanism lead to hypercalcemia or hypocalcemia, both of which can have important consequences for health.

7.4.1 Calcium location and quantity

Calcium is the most abundant mineral in the human body. The average adult body contains in total approximately 1 kg, 99% in the skeleton in the form of calcium phosphate salts. The extracellular fluid (ECF) contains approximately 22.5 mmol, of which about 9 mmol is in the serum. Approximately 500 mmol of calcium is exchanged between bone and the ECF over a period of twenty-four hours.

7.4.2 Biological functions

- Structural function: Supporting material in bones. Present as calcium phosphate.
- Signalling function: Intracellular calcium functions as a second messenger for some hormones.
- Enzymatic function: Calcium acts as a coenzyme for clotting factors.

Calcium also causes the release of Acetylcholine from Pre-synaptic terminal in the transmission of nerve impulse. Calcium causes the contraction of muscles.

7.4.3 Normal ranges

The serum level of calcium is closely regulated with normal total calcium of 2.2-2.6 mmol/L (9-10.5 mg/dL) and normal ionized calcium of 1.1-1.4 mmol/L (4.5-5.6 mg/dL). The amount of total calcium varies with the level of serum albumin, a protein to which calcium is bound. The biologic effect of calcium is determined by the amount of ionized calcium, rather than the total calcium. Ionized calcium does not vary with the albumin level, and therefore it is useful to measure the ionized calcium level when the serum albumin is not within normal ranges, or when a calcium disorder is suspected despite a normal total calcium level.

7.4.4 Effector organs

7.4.4.1 Absorption

Calcium enters the body in a normal diet and is absorbed across the intestinal brush border membrane. Calbindin is a vitamin D-dependent calcium-binding protein inside intestinal epithelial cells actively transports calcium into the body.

7.4.4.2 Excretion

The kidney excretes 250 mmol a day in pro-urine, and resorbs 245 mmol, leading to a net loss in the urine of 5 mmol/d. In addition to this, the kidney processes Vitamin D into calcitriol, the active form that is most effective in assisting intestinal absorption. Both processes are stimulated by parathyroid hormone.

Notes

7.4.4.3 The role of bone

Although calcium flow to and from the bone is neutral, about 5 mmol is turned over a day. Bone serves as an important storage point for calcium, as it contains 99% of the total body calcium. Calcium release from bone is regulated by parathyroid hormone. Calcitonin stimulates incorporation of calcium in bone, although this process is largely independent of calcitonin. Low calcium intake may also be a risk factor in the development of osteoporosis.

7.4.5 Interaction with other chemicals

7.4.5.1 Potential positive interactions

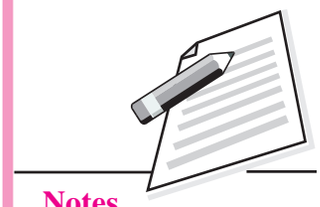
- Vitamin D is an important co-factor in the intestinal absorption of calcium, as it increases the number of calcium binding proteins, involved in calcium absorption through the apical membrane of enterocytes in small intestine. It also promotes re-absorption of calcium in the kidneys.
- Magnesium also plays an important role in calcium absorpsion by bones. Release of calcium from the sarcoplasmic reticulum is inhibited by magnesium. Thus hypomagnesemia results in an increased intracellular calcium level. This inhibits the release of parathyroid hormone, which can result in hypoparathyroidism and hypocalcemia. Furthermore, it makes skeletal and muscle receptors less sensitive to parathyroid hormone.

7.4.5.2 Potential negative interactions

- Unesterified long-chain saturated fatty acids, i.e. palmitic acid, have a melting point above body temperature and, with sufficient calcium in the intestinal lumen, form insoluble calcium soaps.
- Sodium binding to calcium
- Phytic acid binding to calcium
- Oxalic acid binding to calcium
- Cortisol binding to calcium
- Low pH food and proteins (the latter promotes gastric acid)

7.5 LIPID PROFILE

Lipid profile or lipid panel, is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases.



7.5.1 Components

The lipid profile typically includes:

- Low-density lipoprotein
- High-density lipoprotein
- Triglycerides
- Total cholesterol

Using these values, a laboratory may also calculate:

- Very low-density lipoprotein
- Cholesterol:HDL ratio

7.5.2 Procedure

Traditionally, most laboratories have required patients to fast for 9–12 hours before screening. However, recent studies have questioned the utility of fasting before lipid panels, and some diagnostic labs now routinely accept non-fasting samples.

7.5.3 Implications

This test is used to identify hyperlipidemia (various disturbances of cholesterol and triglyceride levels), many forms of which are recognized risk factors for cardiovascular disease and rarely pancreatitis. A total cholesterol reading can be used to assess an individual's risk for heart disease; however, it should not be relied upon as the only indicator. The individual components that make up total cholesterol reading LDL, HDL, and VLDL are also important in measuring risk. The lipid profile includes total cholesterol, HDL-cholesterol (often called good cholesterol), LDL-cholesterol (often called bad cholesterol), and triglycerides. Sometimes the report will include additional calculated values such as the Cholesterol/HDL ratio or a risk score based on lipid profile results, age, sex, and other risk factors. The lipid profile is used to guide providers in deciding how a person at risk should be treated. The results of the lipid profile are considered along with other known risk factors of heart disease to develop a plan of treatment and follow-up.

7.5.4 Normal range

LDL	:	60–130 mg/dL
HDL	:	> 40 mg/dL
Total cholesterol	:	< 200 mg/dL
Triglycerides	:	10–150 mg/dL
VLDL	:	2–38 mg/dL

Notes

7.6 URINE LEVELS OF SUGAR

7.6.1 Glycosuria

Glycosuria or glucosuria is the excretion of glucose into the urine. Ordinarily, urine contains no glucose because the kidneys are able to reclaim all of the filtered glucose back into the bloodstream. Glycosuria is nearly always caused by elevated blood glucose levels, most commonly due to untreated diabetes mellitus. Rarely, glycosuria is due to an intrinsic problem with glucose reabsorption within the kidneys themselves, a condition termed renal glycosuria. Glycosuria leads to excessive water loss into the urine with resultant dehydration, a process called osmotic diuresis.

7.6.2 Pathophysiology

Blood is filtered by millions of nephrons, the functional units that comprise the kidneys. In each nephron, blood flows from the arteriole into the glomerulus, a tuft of leaky capillaries. The Bowman's capsule surrounds each glomerulus, and collects the filtrate that the glomerulus forms. The filtrate contains waste products (e.g. urea), electrolytes (e.g. sodium, potassium, chloride), amino acids, and glucose. The filtrate passes into the renal tubules of the kidney. In the first part of the renal tubule, the proximal tubule, glucose is reabsorbed from the filtrate, across the tubular epithelium and into the bloodstream.

The proximal tubule can only reabsorb a limited amount of glucose. When the blood glucose level exceeds about 160 – 180 mg/dl, the proximal tubule becomes overwhelmed and begins to excrete glucose in the urine. This point is called the renal threshold of glucose (RTG). Some people, especially children and pregnant women, may have a low RTG (less than ~7 mmol/L glucose in blood to have glucosuria). If the RTG is so low that even normal blood glucose levels produce the condition, it is referred to as renal glycosuria. Glucose in urine can be identified by Benedict's qualitative test. A urine dipstick can show a false-positive glucosuria if someone is taking Pyridium standard, medications that relieve symptoms of urinary tract infection.

7.6.3 Diagnosis

A doctor normally can diagnose renal glycosuria when a routine urine test (Urinalysis) detects glucose in the urine, while a blood test indicates that the blood glucose level is normal. In most affected individuals, the condition causes no apparent symptoms (asymptomatic) or serious effects. When renal glycosuria occurs as an isolated finding with otherwise normal kidney function, the condition is thought to be inherited as an autosomal recessive trait.

7.7 URINE LEVELS OF CREATININE

Creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body (depending on muscle mass). Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism that is excreted unchanged by the kidneys. Creatinine itself is produced via a biological system involving creatine, phosphocreatine (also known as creatine phosphate), and adenosine triphosphate (ATP, the body's immediate energy supply). Creatine is synthesized primarily in the liver from the methylation of glycocyamine by S-adenosyl methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high-energy compound phosphocreatine. During the reaction, creatine and phosphocreatine are catalyzed by creatine kinase, and a spontaneous conversion to creatinine may occur.

7.7.1 Creatinine clearance

Creatinine is removed from the blood chiefly by the kidneys, primarily by glomerular filtration but also via proximal tubular secretion. There is little or no tubular reabsorption of creatinine. If the filtration in the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which correlates with the glomerular filtration rate (GFR). Blood creatinine levels may also be used alone to calulate the estimated GFR (eGFR).

The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will overestimate the GFR because hypersecretion of creatinine by the proximal tubules will account for a larger fraction of the total creatinine cleared. Ketoacids, cimetidine, and trimethoprim reduce creatinine tubular secretion and, therefore, increase the accuracy of the GFR estimate, in particular in severe renal dysfunction. An alternate estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. BUN-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a pre-renal problem such as volume depletion. Each day, 1-2% of muscle creatine is converted to creatinine. Men tend to have higher levels of creatinine than women because, in general, they have a greater mass of skeletal muscle. Increased dietary intake of creatine or eating a lot of meat can increase daily creatinine excretion.

7.7.2 Diagnostic use

7.7.2.1 Serum creatinine

Measuring serum creatinine is a simple test, and it is the most commonly used indicator of renal function. A rise in blood creatinine level is observed only with

Notes

marked damage to functioning nephrons. Therefore, this test is unsuitable for detecting early-stage kidney disease. A better estimation of kidney function is given by calculating the estimated glomerular filtration rate (eGFR). eGFR can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race.

7.7.2.2 Urine creatinine

Creatinine concentration is also checked during standard urine drug tests. Normal creatinine levels indicate the test sample is undiluted, whereas low amounts of creatinine in the urine indicate either a manipulated test or low individual baseline creatinine levels. Random urine creatinine levels have no standard reference ranges. They are usually used with other tests to reference levels of other substances measured in the urine. Diuretics, such as coffee and tea, cause more frequent urination, thus potently decreasing creatinine levels. A decrease in muscle mass will also cause a lower reading of creatinine, as will pregnancy.

7.7.3 Interpretation

The typical human reference ranges for serum creatinine are 0.5 to 1.0 mg/dl (about 45-90 µmol/l) for women and 0.7 to 1.2 mg/dl (60-110 µmol/L) for men. While a baseline serum creatinine of 2.0 mg/dl (150 µmol/l) may indicate normal kidney function in a male body builder, a serum creatinine of 1.2 mg/dl (110 µmol/l) can indicate significant renal disease in an elderly female. Creatinine levels may increase when ACE inhibitors (ACEI) or angiotensin II receptor antagonists (or angiotensin receptor blockers, ARBs) are taken. Using both ACEI and ARB concomitantly will increase creatinine levels to a greater degree than either of the two drugs would individually. An increase of <30% is to be expected with ACEI or ARB use.

7.7.4 Normal Results

Urine creatinine (24-hour sample) values can range from 500 to 2000 mg/day. Results depend greatly on your age and amount of lean body mass.

Another way of expressing the normal range for these test results are:

- 14 to 26 mg per kg of body mass per day for men
- 11 to 20 mg per kg of body mass per day for women

7.7.5 Abnormal Results Mean

Abnormal results of urine creatinine are nonspecific, but may be due to any of the following conditions such as, glomerulonephritis, high meat diet, kidney infection (pyelonephritis), kidney failure, muscular dystrophy (late stage), myasthenia gravis, prerenal azotemia, reduced kidney blood flow (as in shock or congestive heart failure), urinary tract obstruction.

7.8 URINE LEVELS OF PROTEINS

7.8.1 Proteinuria

Proteinuria means the presence of an excess of serum proteins in the urine. The excess protein in the urine often causes the urine to become foamy, although foamy urine may also be caused by bilirubin in the urine (bilirubinuria), or drugs such as pyridium. Up to 150 mg a day of protein may be excreted by a normal person. Proteinuria can also be caused by certain biological agents, such as Avastin used in cancer treatment, or by excessive fluid intake (drinking in excess of 4 litres of water per day).

7.8.2 Causes

There are three main mechanisms to cause proteinuria:

- Due to disease in glomerulus
- Because of increased quantity of proteins in serum (overflow proteinuria)
- Due to low reabsorption at proximal tubule (Fanconi syndrome)

7.8.3 Measurement

Conventionally, proteinuria is diagnosed by a simple dipstick test, although it is possible for the test to give a false negative reading, if the protein in the urine is composed mainly of globulins or Bence-Jones proteins. Alternatively the concentration of protein in the urine may be compared to the creatinine level in a spot urine sample. This is termed the protein/creatinine ratio (PCR). Proteinuria is defined as a protein/creatinine ratio greater than 45 mg/mmol (which is equivalent to albumin/creatinine ratio of greater than 30 mg/mmol or approximately 300 mg/g) with very high levels of proteinuria being for a PCR greater than 100 mg/mmol.

7.8.4 Associated conditions

Proteinuria may be a sign of renal (kidney) damage. Since serum proteins are readily reabsorbed from urine, the presence of excess protein indicates either an insufficiency of absorption or impaired filtration. Diabetics may suffer from damaged nephrons and develop proteinuria.

7.8.5 Conditions with proteinuria as a sign

Proteinuria may be a feature of the following conditions such as, nephrotic syndromes (i.e. intrinsic renal failure), toxic lesions of kidneys, amyloidosis, collagen vascular diseases (e.g. systemic lupus erythematosus), dehydration, glomerular diseases, strenuous exercise, stress, IgA nephropathy, IgM nephropathy, diabetes mellitus (diabetic nephropathy), drugs (e.g. NSAIDs, nicotine, penicillamine, lithium carbonate, antibiotics, or opiates (especially heroin),

infections (e.g. HIV, syphilis, hepatitis, poststreptococcal infection), aminoaciduria, hypertensive nephrosclerosis, sickle cell disease, hemoglobinuria, multiple myeloma, myoglobinuria, organ rejection (Kidney transplant patients may have gamma-globulins in their urine if the kidneys start to reject), systemic lupus erythematosus, rheumatoid arthritis, glycogen storage disease type 1, and urinary tract infection which has spread to the kidney(s). Conditions with proteinuria consisting mainly of Bence-Jones proteins as a sign are waldenstrom's macroglobulinemia, chronic lymphocytic leukemia, amyloidosis, malignancies (e.g., lymphoma, other cancers), multiple myeloma.

7.8.6 Treatment

Treating proteinuria mainly needs proper diagnosis of the cause. The most common cause is diabetic nephropathy; in this case, proper glycemic control may slow the progression. Medical management consists of angiotensin converting enzyme (ACE) inhibitors, which are typically first-line therapy for proteinuria. In patients whose proteinuria is not controlled with ACE inhibitors, the addition of an aldosterone antagonist or angiotensin receptor blocker may further reduce protein loss. Caution must be used if these agents are added to ACE inhibitor therapy due to the risk of hyperkalemia. Proteinuria secondary to autoimmune disease should be treated with steroids or steroid-sparing agent plus the use of ACE inhibitors.

INTEXT QUESTIONS 7.1

I. Choose the best answer

1. Measuring glucose levels before the first meal of the day is termed as

(a) Post prandial blood glucose

(b) Fasting blood glucose

(c) Normal blood glucose

(d) None

2. The catabolic hormone which increases blood glucose level is

(a) Glucagon (b) Insulin

(c) Histamine (d) None

3. The coenzyme acts as blood clotting factor is

(a) Magnesium (b) Iron

(c) Calcium (d) Lead

4. The vitamin D-dependent calcium-binding protein that actively transports calcium into the body
   (a) Calbindin (b) Calmodulin
   (c) Transferrin (d) Globulin
5. Good cholesterol is termed for
   (a) LDL-cholesterol (b) VLDL-cholesterol
   (c) HDL-cholesterol (d) Triglycerides

II. Fill in the blanks

6. Excretion of glucose into the urine is called as .....
7. ..... is the by-product of muscle metabolism
8. The protein in the urine is composed mainly of globulins is termed medically as .....
9. The two most common employed enzymes in the blood glucose measurement are .....
10. ..... increases if glomerular filtration rate and blood volume decrease.

III. Match the following

11.	Bone formation	(a)	LDL
12.	Bad cholesterol	(b)	Calcium
13.	Glycosuria	(c)	Diabetes mellitus
14.	Bilirubin in the urine	(d)	Benedict's qualitative test
15.	Metformin	(e)	Bilirubinuria

WHAT HAVE YOU LEANRT

- Glucose is the primary source of energy for the body's cells. Its level should be between 70 and 100 mg/dL for normal person.
- Anabolic hormone (insulin) decreases blood glucose andcatabolic hormones (such as glucagon, cortisol and catecholamines) increase blood glucose.
- More precise blood glucose measurements are performed in a medical laboratory, using hexokinase, glucose oxidase or glucose dehydrogenase enzymes.
- The liver produces urea in the urea cycle as a waste product of the digestion of protein.
- Derangements of the calcium mechanism lead to hypercalcemia or hypocalcemia, both of which can have important consequences for health.

- Calbindin is a vitamin D-dependent calcium-binding protein inside intestinal epithelial cells actively transports calcium into the body.
- Hyperlipidemia is recognized as a risk factor which leads to cardiovascular disease.
- HDL-cholesterol is often called good cholesterol and LDL-cholesterol is often called as bad cholesterol.
- Glycosuria is nearly always caused by elevated blood glucose levels. Glucose in urine can be identified by Benedict's qualitative test.
- Serum creatinine is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism. Creatinine is removed from the blood chiefly by the kidneys.
- Proteinuria means the presence of an excess of serum proteins in the urine.
- The three main mechanisms that cause proteinuria are disease in glomerulus, increased quantity of proteins in serum, and low reabsorption at proximal tubule (Fanconi syndrome).

TERMINAL QUESTIONS

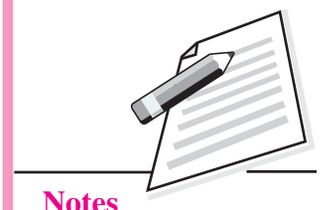
1. Write short note on Laboratory tests of Blood glucose
2. Write short note on Lipid profile
3. Write short notes on urine levels of creatinine

ANSWERS TO INTEXT QUESTIONS

I. 1. (b) 2. (a) 3. (c) 4. (a) 5. (c)

II. 6. Glycosuria,
7. Creatinine,
8. Bence-Jones proteins,
9. Glucose oxidase and Hexokinase,
10. Blood urea nitrogen,

III. 11. (b) 12. (a) 13 (d) 14. (e) 15. (c)



8

ENZYMES

8.1 INTRODUCTION

The global life depends on a series of chemical reactions. Most of the chemical reactions proceed too slowly on their own to sustain life. Hence catalysts are required to greatly accelerate the rates of these chemical reactions. In nature enzymes posses the catalytic power to facilitate life processes in essentially all life-forms from viruses to man. Most of the enzymes retain their catalytic potential even after extraction from the living organism. The above catalytic power of enzyme leads to commercial usage of enzymes.

In ancient days enzymes are used in manufacture of cheeses, breads, and alcoholic beverages, and for the tenderizing of meats. Today enzymes are also of fundamental interest in the health sciences. Human disease processes can be linked to the aberrant activities of one or a few enzymes. Because of their role in maintaining life processes, the assay and pharmacological regulation of enzymes have become key elements in clinical diagnosis and therapeutics.

The macromolecular components of almost all enzymes are composed of protein, except for a class of RNA modifying catalysts known as ribozymes. Ribozymes are molecules of ribonucleic acid that catalyze reactions on the phosphodiester bond of other RNAs. Enzymes are found in all tissues and fluids of the body. Intracellular enzymes catalyze the reactions of metabolic pathways. Plasma membrane enzymes regulate catalysis within cells in response to extracellular signals, and enzymes of the circulatory system are responsible for regulating the clotting of blood.

Almost every significant life process is dependent on enzyme activity. So the modern pharmaceutical research is based on the search for potent and specific inhibitors of these enzymes. This chapter briefs you about basic nature, classification and clinical significance of enzymes.

Notes

OBJECTIVES

After reading this lesson, you will be able to:

- define enzymes
- classify enzymes
- explain co-enzymes
- explain the factors affecting enzyme activity
- describe isoenzymes
- explain the Clinical significance of enzymes

8.2 DEFINITION AND CHARACTERISTICS OF ENZYMES

Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions. Enzymes are biological catalysts responsible for supporting almost all of the chemical reactions that maintain animal homeostasis. Enzyme reactions are always reversible. The substance, upon which an enzyme acts, is called as substrate. Enzymes are involved in conversion of substrate into product. Almost all enzymes are globular proteins consisting either of a single polypeptide or of two or more polypeptides held together (in quaternary structure) by non-covalent bonds. Enzymes do nothing but speed up the rates at which the equilibrium positions of reversible reactions are attained. In terms of thermodynamics, enzymes reduce the activation energies of reactions, enabling them to occur much more readily at low temperatures - essential for biological systems. The basic characteristics of enzymes includes

(i) Almost all the enzymes are proteins and they follow the physical and chemical reactions of proteins

(ii) Enzymes are sensitive and labile to heat

(iii) Enzymes are water soluble

(iv) Enzymes could be precipitated by protein precipitating agents such as ammonium sulfate and trichloroacetic acid.

INTEXT QUESTIONS 8.1

1. Enzymes are ..... biocatalyst produced by cells.
2. Enzymes follow physical and chemical properties of .....

3. Enzymes are ..... soluble.
   (a) Water (b) acid (c) organic acids
4. Precipitation of enzymes could be achieved by using ..... and .....

8.3 CLASSIFICATION OF ENZYMES

Since earlier days to still date, fanciful names such as pepsin, chymotrypsin, etc were used to name enzymes. Later the suffix "ase" to the substrate was used to name enzymes. For example the enzymes lactase acts upon the lactate and produces glucose and galactose. The above method is known as "trivial naming" of enzymes. Currently enzymes are grouped into six functional classes by the International Union of Biochemists and Molecular Biology (IUBMB). As per the IUBMB system, each enzyme name starts with EC (enzyme class) followed by 4 digits.

The first digit represents the class, the second digit strands for the subclass, the third digit represents the sub-subclass or subgroup and the fourth digit provides the particular enzyme (Table 8.1)

Table 8.1 Classification of enzymes

Sl.No.	Classification	Biochemical Properties
1.	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. e.g. Lactate dehydrogenase
2.	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. e.g. Aminotransferase.
3.	Hydrolases	Add water across a bond, hydrolyzing it. E.g. Acetyl choline esterase
4.	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. e.g. Aldolase.
5.	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. e.g. Triose phosphate isomerase
6.	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. e.g. Acetyl CoA carboxylase

Notes

These rules give each enzyme a unique number and specifies a textual name for each enzyme. Enzymes are also classified on the basis of their composition. Enzymes composed wholly of protein are known as simple enzymes in contrast to complex enzymes, which are composed of protein plus a relatively small organic molecule. Complex enzymes are also known as holo-enzymes. The non-protein component of an enzyme may be as simple as a metal ion or as complex as a small non-protein organic molecule. Enzymes that require a metal in their composition are known as metalloenzymes. Metalloenzymes bind and retain their metal atom(s) under all conditions with very high affinity. Enzymes with lower affinity for metal ion, but still require the metal ion for activity, are known as metal-activated enzymes. Based on requirement of ATP, enzymes are further classified into two types namely synthetases and synthase. Synthetases are ATP-dependent enzymes catalyzing biosynthetic reactions. Synthetases are enzyme belong to the class 6 (Ligases). Enzymes such as Carbamoyl phosphate synthetase, Arginino succinate synthetase and Glutamine synthetase are examples of the synthetases group of enzymes. The enzyme class other than ligases includes synthases. Synthases group of enzymes involves in catalyzing biosynthetic reactions that do not require ATP directly. Enzymes such as glycogen synthase and Alanine synthase are examples of synthase group.

INTEXT QUESTIONS 8.2

1. There are ..... main groups of enzymes.
2. Lactate dehydrogenase is example for ..... group of enzyme.
3. Enzymes that require a metal in their composition are known as .....
4. ..... are ATP dependent enzymes.
5. ..... and ..... are examples for synthase group of enzymes.

8.4 COENZYMES

Enzymes may be simple proteins, or complex enzymes. A complex enzyme contains a non-protein part, called as prosthetic group (co-enzymes). Co-enzymes are heat stable low molecular weight organic compound. The combined form of protein and the co-enzyme are called as holo-enzyme. The heat labile or unstable part of the holo-enzyme is called as apo-enzyme. The apo-enzyme gives necessary three dimensional structures required for the enzymatic chemical reaction. Co-enzymes are very essential for the biological activities of the enzyme. Co-enzymes combine loosely with apo-enzyme and are released easily by dialysis. Most of the co-enzymes are derivatives of vitamin B complex

group of substance. One molecule of the co-enzyme with its enzyme is sufficient to convert a large group of substrate.

Co-enzymes are further divided into two groups. The first groups of co-enzymes are a part of reaction catalyzed by oxidoreductase by donating or accepting hydrogen atoms or electrons. The first group of co-enzymes are also called as co-substrates or secondary substrates. Because they are involved in counter-balance in change occurring in the substrate. The second group of co-enzymes involves in reactions transferring groups other than hydrogen.

8.4.1 Role of Coenzymes

The functional role of coenzymes is to act as transporters of chemical groups from one reactant to another. The chemical groups carried can be as simple as the hydride ion ($H^+ + 2e^-$) carried by NAD or the mole of hydrogen carried by FAD; or they can be even more complex than the amine ($-NH_2$) carried by pyridoxal phosphate. Since coenzymes are chemically changed as a consequence of enzyme action, it is often useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different holoenzymes. In all cases, the coenzymes donate the carried chemical grouping to an acceptor molecule and are thus regenerated to their original form. This regeneration of coenzyme and holoenzyme fulfills the definition of an enzyme as a chemical catalyst, since (unlike the usual substrates, which are used up during the course of a reaction) coenzymes are generally regenerated.

INTEXT QUESTIONS 8.3

1. Non protein part of complex enzymes is called as .....
2. Combination of ..... and ..... are called as holo enzymes.
3. Co-enzymes are heat stable low molecular weight ..... compound.
4. There are ..... groups of co-enzymes.

8.5 FACTORS AFFECTING ENZYME ACTIVITY

Velocity or rate of enzymatic reaction is assessed by the rate of change in concentration of substrate or product at a given time duration. Various factors which affect the activity of enzymes include:

1. Substrate concentration
2. Enzyme concentration
3. Product concentration

4. Temperature
5. Hydrogen ion concentration (pH)
6. Presence of activators
7. Presence of inhibitor

8.5.1 Effect of substrate Concentration

Reaction velocity of an enzymatic process increases with constant enzyme concentration and increase in substrate concentration. The velocity (V) is expressed in micromoles of substrate converted per minute. As the concentration of substrate increases, the velocity of the reaction increases. Continued increase in substrate concentration may lead to a reduction in rate of the reaction and leads to flattened curve. The maximum velocity obtained from a enzymatic reaction is called as Vmax. Vmax represents the maximum reaction rate possible in the presence of excess substrate.

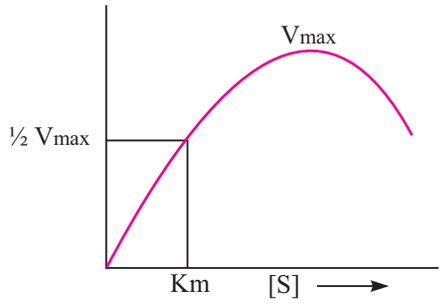


Fig. 8. 1: Effect of substrate concentration

8.5.2 Effect of enzyme Concentration

As there is optimal substrate concentration, rate of an enzymatic reaction or velocity (V) is directly proportional to the enzyme concentration. Presence of excess substrate and an increase in the enzyme concentration may result in some limitations. It is worthy of note that the enzymes are rarely saturated with substrates under physiological conditions.

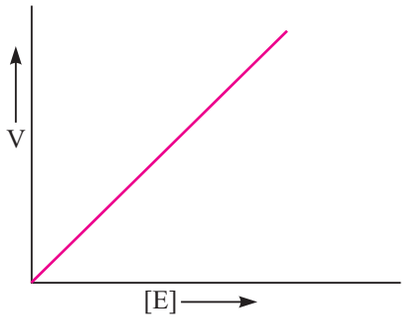


Fig. 8.2: Effect of enzyme concentration

8.5.3 Effect of product concentration

In case of a reversible reaction catalyzed by a enzyme, as per the law of mass action the rate of reaction is slowed down with equilibrium. So, rate of reaction is slowed, stopped or even reversed with increase in product concentration. This phenomena can be better explained by the equation

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D$$

In the above equation, in case of absence of the enzyme E3, the product C will accumulate. Enzymatic activity of E2 will be inhibited with accumulation of the product C. In such inborn error of one enzyme will block the whole pathway.

8.5.4 Effect of temperature

Velocity of enzymatic reaction increases with temperature of the medium which they are most efficient and the same is termed as optimum temperature. As temperatures increases it leads to denaturation; a molecular arrangement which causes a loss of the active sites of the enzyme surfaces and results in a loss of efficiency.

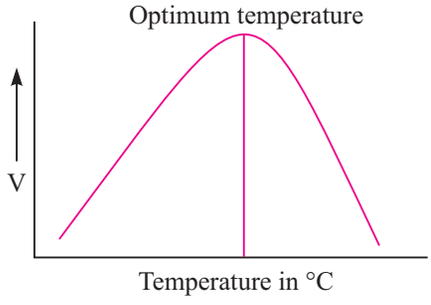


Fig. 8.3: Effect of temperature

8.5.5 Effect of pH

Like temperature, all enzymes have a optimum pH, at which the enzymatic activity will be at maximum. Many enzymes are most efficient in the region of pH 6-7, which is the pH of the cell. Outside this range, enzyme activity drops off very rapidly. Reduction in efficiency caused by changes in the pH is due to changes in the degree of ionization of the substrate and enzyme. Highly acidic or alkaline conditions bring about a denaturation and subsequent loss of enzymatic activity. Some exceptions such as pepsin (with optimum pH 1-2), alkaline phosphatase (with optimum pH 9-10) and acid phosphatase (with optimum pH 4-5) are even noticed.

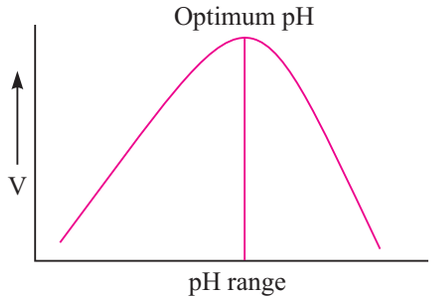


Fig. 8.4: Effect of pH

8.5.6 Presence of activators

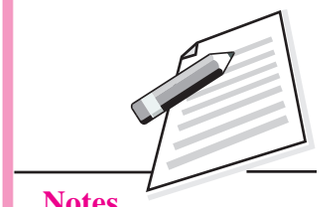
Presence of certain inorganic ions increases the activity of enzymes. The best examples are chloride ions activated salivary amylase and calcium activated lipases.

8.5.7 Effect of Inhibitors

The catalytic enzymatic reaction may be inhibited by substances which prevent the formation of a normal enzyme-substrate complex. The level of inhibition then depends entirely upon the relative concentrations of the true substrate and the inhibitor. Such inhibition, which depends on competition with the substrate for the active sites of the enzyme, is termed competitive inhibition. In other cases, the inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further reaction to give the usual product. This is termed uncompetitive inhibition. Non competitive inhibition involves combination of the inhibitor with the enzyme or the enzyme substrate complex, to give inactive complexes. In this case, the inhibitor binds to sites, on the enzyme other than enzyme sites, resulting in deformation of the enzyme molecule so that the formation of the enzyme-substrate complex is slower than normal. Some enzymes undergo irreversible inactivation; reaction of the inhibitor with a functional group of the enzyme, resulting in a loss of its catalytic activity. Enzyme inhibitor plays a vital role in clinical utility and is listed in table 8.2.

Table 8.2: Effect of Enzyme Inhibitors

Sl.No	Enzymatic inhibitor/drug	Enzyme inhibited	Clinical use
1.	Allopurinol	Xanthine oxidase	gout
2.	Dicoumarol	Vitamin-K-epoxide-reductase	Anti-coagulant
3.	Penicillin	Transpeptidase	Anti-bacterial
4.	Sulphonamide	Pteroid synthetase	Anti-bacterial
5.	Pyrimethamine	FH2-reductase	Anti-malarial
6.	5-fluorouracil	Thymidylate synthetase	Anti-cancer



INTEXT QUESTIONS 8.4

1. At optimal substrate concentration the rate of an enzymatic reaction or velocity (V) is directly proportional to the .....
2. Velocity of ..... increases with temperature of the medium.
3. The optimum pH of alkaline phosphatase are .....
4. Allopurinol are ..... inhibitor and used for treatment of .....

8.6 ISO-ENZYMES

Iso-enzymes are physically distinct forms of the same enzyme activity. Higher organisms have several physically distinct versions of a given enzyme, each of which catalyzes the same reaction. Isozymes arise through gene duplication and exhibit differences in properties such as sensitivity to particular regulatory factors or substrate affinity that adapts them to specific tissues or circumstances. Some isozymes enhance survival by providing a "backup" copy of an essential enzyme. Isozymes are expressed only in specific cell types. The expression of isozymes in specific cells occurs during certain periods of development, or in response to specific physiologic or pathophysiologic changes. Thus analysis of the presence and distribution of enzymes and isozymes are often useful in diagnosis. Isoforms of Lactate dehydrogenase is useful in diagnosis of myocardial infarction. While study of alkaline phosphatase isoforms are helpful in diagnosis of various bone disorder and obstructive liver diseases.

INTEXT QUESTIONS 8.5

1. Physically distinct forms of the same enzyme are called as .....
2. Isozymes arise through ..... duplication.
3. Isoforms of ..... is useful in diagnosis of myocardial infarction.
4. Alkaline phosphatase isoforms are helpful in diagnosis of various ..... disorder.

8.7 CLINICAL SIGNIFICANCE OF ENZYMES

The measurement of enzymes level in serum is applied in diagnostic application. Detection of certain enzymes in the serum indicates that tissue or cellular damage has occurred resulting in the release of intracellular components into the blood. Hence, when a physician indicates that he/she is going to assay for liver enzymes, the purpose is to ascertain the potential for liver cell damage.

Notes

Commonly assayed enzymes are the amino transferases: alanine transaminase, ALT (sometimes still referred to as serum glutamate-pyruvate aminotransferase, SGPT) and aspartate aminotransferase, AST (also referred to as serum glutamate-oxaloacetate aminotransferase, SGOT); lactate dehydrogenase, LDH; creatine kinase, CK (also called creatine phosphokinase, CPK); gamma-glutamyl transpeptidase, GGT. Other enzymes are assayed under a variety of different clinical situations but they will not be covered here.

8.7.1 Pancreatic Enzymes

Acute pancreatitis is an inflammatory process where auto digestion of gland was noticed with activation of the certain pancreatic enzymes. Enzymes which involves in pancreatic destruction includes α-amylase, lipase etc.,

8.7.1.1 α-amylase

α-amylase (AMYs) are calcium dependent hydrolyase class of metaloenzyme that catalyzes the hydrolysis of 1, 4- α-glycosidic linkages in polysaccharides. Molecular weights of AMYs are human plasma ranges from 54 to 62 kDa. Due to its smaller size they could easily pass the glomeruli of the kidneys and AMY is the only plasma enzyme physiologically found in urine. The normal values of amylase is in range of 28-100 U/L. Marked increase of 5 to 10 times the upper reference limit (URL) in AMYs activity indicates acute pancreatitis and severe glomerular impairment. Pancreatic pseudocyst occurs if the plasma level of amylase activity fails to fall after an attack of acute pancreatitis.

8.7.1.2 Lipase

Lipase is single chain glycoprotein of molecular weight 48 kDa. Bile salts and a cofactor called colipase are required for full catalytic activity of lipase. Colipase is secreted by pancreas. Lipase is small molecule filtered through the glomerulus and totally reabsorbed by the renal tubules. Lipase is not normally detected in urine samples. The normal value of lipase ranges from 40-200 U/L. Increase in plasma lipase activity indicates acute pancreatitis and carcinoma of the pancreas. So determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

8.7.2 Liver Enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various liver disorders. Liver enzymes acts as marker of hepatocellular damage, cholestasis and disturbances in the hepatocellular synthesis.

8.7.2.1 Markers of Hepatocellular Damage

In case of hepatocellular damage, the enzymes which are normally present inside the hepatocytes are released into the blood. Aminotransaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) are routinely used in diagnosis of hepatocellular damages. Transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid. The 2-oxoglutarate acts as amino group acceptor and the L-glutamate serves as donor in all amino-transfer reactions. The specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group.

8.7.2.1.1 Aspartate transaminase (AST)

Aspartate transaminase is present in high concentrations in cells of cardiac and skeletal muscle, liver, kidney and erythrocytes. Damage to any of these tissues may increase plasma AST levels. The normal value of AST for male is <35 U/L and for female it is <31 U/L. Marked increase of AST activity in the range of 10 to 100 times the upper adult reference limit indicates myocardial infarction or acute viral or toxic hepatitis.

8.7.2.1.2 Alanine transaminase (ALT)

Alanine transaminase is present at high concentrations in liver and to a lesser extent, in skeletal muscle, kidney and heart. Thus in case of liver damage increase in both AST and ALT were noticed. While in myocardial infarction AST is increased with little or no increase in ALT. The normal value of ALT is <45 U/L and <34 U/L for male and female respectively. In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST.

8.7.2.2 Markers of cholestasis

Enzymatic markers of cholestasis are membrane bound enzymes. Markers of cholestasis includes alkaline phosphatases, gamma-glutamyltransferase and glutamate dehydrogenase.

8.7.2.2.1 Alkaline phosphatases

Alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in osteoblasts of bone, the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta.

8.7.2.2.2 Gamma-glutamyl-transferase (GGT)

Gamma-glutamyl-transferase catalyzes the transfere of the γ–glutamyl group from peptides. The activity of GGT is higher in men than in women. In male

Notes

the normal value of GGT activity is <55 U/L and for female it is <38 U/L. Rise in plasma GGT activity is due to infectious hepatitis and induction of enzyme synthesis, without cell damage, by drugs or alcohol.

8.7.2.2.3 Glutamate dehydrogenase (GLD)

Glutamate dehydrogenase is a mitochondrial enzyme found in liver, heart muscle and kidneys. Small amounts of GLD are even observed in brain, skeletal muscle tissue and leukocytes. GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease. GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. The GLD upper reference limits are 6U/L (women) and 8U/L (men).

8.7.3 Muscle Enzymes

Clinically important muscle enzymes include creatine kinase and lactate dehydrogenase.

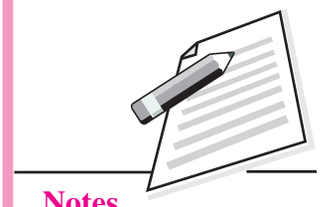
8.7.3.1 Creatine Kinase

Creatine kinase (CK) is most abundant in cells of brain, cardiac and skeletal. In addition to their abundance in above tissues, it also occurs in other tissues such as smooth muscle. In normal physiological condition the CK activity is 46-171 U/L (for male) and 34-145 U/L (for female). Serum CK level elevates in all types of muscular dystrophy. Quite high values of CK are noted in viral myositis, polymyositis and similar muscle disease. Under the circumstances of neurogenic muscle disease such as: myasthenia gravis, multiple sclerosis and Parkinsonism, the level of serum CK is normal. CK consist of two protein subunits, M (for muscle) and B (for brain) and exist as three different isoforms namely BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts.

8.7.3.2 Lactate Dehydrogenase

Lactate dehydrogenase (LD) catalyses the reversible interconversion of lactate and pyruvate. LD has a molecular weight of 134 kDa and is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes. Five isoforms (LD-1 to LD-5) of LD are existing. The normal physiological limit of LD is 180-360 U/L.

Other clinically important enzymes includes acid phosphatase, glucose -6-phosphate dehydrogenase, cystathionine α-synthase and sphingomyelinase.



INTEXT QUESTIONS 8.6

1. ..... and ..... are enzymes which involves in pancreatic destruction.
2. Molecular weights of amylases in human plasma ranges ..... to ..... kDa.
3. Plasma level of amylase activity fails to fall after an attack of .....
4. Alkaline phosphatases, gamma-glutamyltransferase and glutamate dehydrogenase are markers of .....
5. Clinically important muscle enzymes include ..... and .....

WHAT HAVE YOU LEARNT

- Enzymes are protein catalyst produced by a cell and responsible 'for the high rate' and specificity of one or more intracellular or extracellular biochemical reactions.
- Enzymes posses the catalytic power to facilitates life processes in essentially all life-forms from viruses to man.
- Enzymes are found in all tissues and fluids of the body.
- Intracellular enzymes catalyze the reactions of metabolic pathways.
- Plasma membrane enzymes regulate catalysis within cells in response to extracellular signals.
- Enzymes are broadly classified into 6 major groups namely Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases and Ligases.
- Enzymes may be simple proteins, or complex enzymes (presence of non-protein part, called as prosthetic group).
- Coenzymes functions as transporters of chemical groups from one reactant to another.
- Activity of a particular enzyme may be affected by many external factors such as concentration of substrate, product, enzyme, hydrogen ion, temperature, activators and inhibitors.
- Enzyme inhibitors plays a vital role in clinical utility and are useful as anti-bacterial, anti-malarial, anti-cancer molecule and treatment of metabolic disorders such as gout. Thus measurement of enzymes level in serum is applied in diagnostic application.

TERMINAL QUESTIONS

1. Define enzyme?
2. Write about classification of enzymes?
3. Give a brief discussion about various factors affecting enzyme activity?
4. What are metallo enzymes?
5. Define isozymes?
6. What are co-enzymes?
7. Differentiate synthase and synthatase?
8. Write a note on clinically important enzymes?
9. Describe in detail about the liver enzymes used in clinical diagnosis?
10. Give some examples of enzyme inhibitory drugs?

ANSWERS TO INTEXT QUESTIONS

8.1

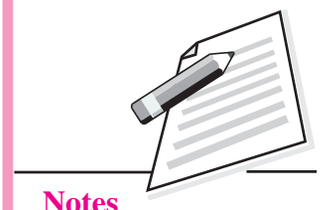
1. Protein
2. Proteins
3. Water
4. Ammonium sulfate and Trichloroacetic acid

8.2

1. Six
2. Oxidoreductases
3. Metallo enzymes
4. Synthetases
5. Glycogen synthase and Alanine synthase

8.3

1. Co-enzymes
2. Protein and co-enzyme
3. Organic
4. Two



8.4

1. Enzyme concentration
2. Enzyme reaction
3. 9-10
4. Xanthine oxidase and gout

8.5

1. Isozymes
2. Gene
3. Lactate dehydrogenase
4. Bone

8.6

1. α-amylase and lipase
2. 54 to 62 kDa
3. Acute pancreatitis
4. Cholestasis
5. Creatine kinase and lactate dehydrogenase

Notes

9

BIOLOGICAL OXIDATION, ELECTRON TRANSFER CHAIN AND OXIDATIVE PHOSPHORYLATION

9.1 INTRODUCTION

Chemically, oxidation is defined as the removal of electrons and reduction as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Many biologic oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations. The life of higher animals is absolutely dependent upon a supply of oxygen for respiration, the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as oxygenases; many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the cytochrome P450 system. Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

OBJECTIVES

After reading this lesson, you will be able to

- describe biological oxidation
- explain Electron transfer chain
- describe oxidation phosphorylation

9.2 BIOLOGICAL OXIDATION-REDUCTION

9.2.1 Redox potential – free energy changes

In reactions involving oxidation and reduction, the free energy change is proportionate to the tendency of reactants to donate or accept electrons. Free energy change expressed as oxidation-reduction or redox potential. The redox potential of a system is usually compared with the potential of the hydrogen electrode (0.0 volts at pH 0.0). However, for biologic systems, the redox potential is normally expressed at pH 7.0, at which pH the electrode potential of the hydrogen electrode is -0.42 volts. Enzymes involved in oxidation and reduction are called oxidoreductases and are classified into four groups: oxidases, dehydrogenases, hydroperoxidases, and oxygenases. Oxidases use oxygen as a hydrogen acceptor. Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor and form water or hydrogen peroxide as a reaction product.

9.2.2 Some oxidases contain copper

Cytochrome oxidase is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes. It is the terminal component of the chain of respiratory carriers found in mitochondria and transfers electrons resulting from the oxidation of substrate molecules by dehydrogenases to their final acceptor, oxygen. The enzyme is poisoned by carbon monoxide, cyanide, and hydrogen sulfide. It has also been termed cytochrome *a*3. It is now known that cytochromes *a* and *a*3 are combined in a single protein, and the complex is known as cytochrome *aa*3. It contains two molecules of heme, each having one Fe atom that oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Furthermore, two atoms of Cu are present, each associated with a heme unit.

9.2.3 Other oxidases are Flavoproteins

Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin. FMN and FAD are usually tightly – but not covalently – bound to their respective apoenzyme proteins. Metalloflavoproteins contain one or more metals as essential cofactors. Examples of flavoprotein enzymes include L-amino acid oxidase, an FMN-linked enzyme found in kidney with general specificity for the oxidative deamination of the naturally occurring L-amino acids.

9.2.4 Dehydrogenases cannot use oxygen as a hydrogen acceptor

There are a large number of enzymes in this class. They perform two main functions:

1. Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction. These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+ (Figure 9.1). Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell. This type of reaction, which enables one substrate to be oxidized at the expense of another, is particularly useful in enabling oxidative processes to occur in the absence of oxygen, such as during the anaerobic phase of glycolysis.

$$NAD^+ + AH_2 \longleftrightarrow NADH + H^+ + A$$

Fig. 9.1: NAD acting as a hydrogen carrier in the dehydrogenase reaction.

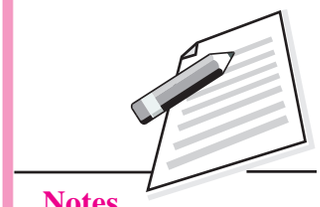
2. As components in the respiratory chain of electron transport from substrate to oxygen.

9.2.5 Many dehydrogenases depend on Nicotinamide Coenzymes

These dehydrogenases use nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+)—or both—and are formed in the body from the vitamin niacin. These coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor. They may freely and reversibly dissociate from their respective apoenzymes. Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in the oxidative pathways of metabolism, particularly in glycolysis, in the citric acid cycle, and in the respiratory chain of mitochondria. NADP-linked dehydrogenases are found characteristically in reductive syntheses, as in the extramitochondrial pathway of fatty acid synthesis and steroid synthesis— and also in the pentose phosphate pathway.

9.2.6 Other dehydrogenases depend on Riboflavin

The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases. They are generally more tightly bound to their apoenzymes than are the nicotinamide coenzymes. Most of the riboflavin-linked dehydrogenases are concerned with electron transport in (or to) the respiratory chain. NADH dehydrogenase acts as a carrier of electrons between NADH and the components of higher redox potential. Other dehydrogenases such as succinate dehydrogenase, acyl-CoA dehydrogenase, and mitochondrial glycerol-3-phosphate dehydrogenase transfer reducing equivalents directly from the substrate to the respiratory chain. Another role of the flavin-dependent dehydrogenases is in the dehydrogenation of reduced lipoate, an intermediate in the oxidative decarboxylation of pyruvate and α-ketoglutarate. The electron-



transferring flavoprotein is an intermediary carrier of electrons between acyl-CoA dehydrogenase and the respiratory chain.

9.2.7 Cytochromes may also be regarded as dehydrogenases

The cytochromes are iron-containing hemoproteins in which the iron atom oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Except for cytochrome oxidase, they are classified as dehydrogenases. In the respiratory chain, they are involved as carriers of electrons from flavoproteins on the one hand to cytochrome oxidase on the other. Several identifiable cytochromes occur in the respiratory chain, ie, cytochromes b, c1, c, a, and a3 (cytochrome oxidase). Cytochromes are also found in other locations, eg, the endoplasmic reticulum (cytochromes P450 and b5), and in plant cells, bacteria, and yeasts.

9.2.8 Hydroperoxidases use hydrogen peroxide or organic peroxide as substrate

Two type of enzymes found both in animals and plants fall into this category: peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides. Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and perhaps cause cancer and atherosclerosis.

9.2.9 Peroxidases reduce peroxides using various electron acceptors

Peroxidases are found in milk and in leukocytes, platelets, and other tissues involved in eicosanoid metabolism. The prosthetic group is protoheme. In the reaction catalyzed by peroxidase, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate, quinones, and cytochrome c. The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows: In erythrocytes and other tissues, the enzyme glutathione peroxidase, containing selenium as a prosthetic group, catalyzes the destruction of H2O2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids and hemoglobin against oxidation by peroxides.

9.2.10 Catalase uses hydrogen peroxide as electron donor and electron acceptor

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor (Figure 9.2). Under most conditions in vivo, the peroxidase activity of catalase seems to be favored. Catalase is found in blood, bone marrow, mucous membranes,

kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases. Peroxisomes are found in many tissues, including liver. They are rich in oxidases and in catalase, Thus, the enzymes that produce H_2O_2 are grouped with the enzyme that destroys it. However, mitochondrial and microsomal electron transport systems as well as xanthine oxidase must be considered as additional sources of H_2O_2.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Fig. 9.2: Catalase uses hydrogen peroxide as electron donor and electron acceptor.

9.2.11 Cytochromes P450 are monooxygenases important for the detoxification of many drugs

Cytochromes P450 are an important superfamily of heme-containing monooxgenases, and more than 1000 such enzymes are known. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes, which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the hydroxylase cycle (Figure 9.3). In liver microsomes, cytochromes P450 are found together with cytochrome *b*5 and have an important role in detoxification. Benzpyrene, aminopyrine, aniline, morphine, and benzphetamine are hydroxylated, increasing their solubility and aiding their excretion. Many drugs such as Phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochromes P450. Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol.

$$\text{Reduced cytochrome P450} \longrightarrow \text{Oxidized cytochrome P450}$$

$$RH + O_2 \longrightarrow R - OH + H_2O$$

Fig. 9.3: Reduction and oxidation of cytochrome.

9.2.12 Superoxide dismutase protects aerobic organisms against oxygen toxicity

Transfer of a single electron to O_2 generates the potentially damaging superoxide anion free radical (O2"-) (Figure 9.4), the destructive effects of which are amplified by its giving rise to free radical chain reactions. The ease with which superoxide can be formed from oxygen in tissues and the occurrence of superoxide dismutase, the enzyme responsible for its removal in all aerobic organisms (although not in obligate anaerobes) indicate that the potential toxicity of oxygen is due to its conversion to superoxide. Superoxide is formed when reduced flavins – present, for example, in xanthine oxidase – are reoxidized univalently by molecular oxygen.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} H_2O + O_2$$

Fig. 9.4: Removal of superoxide anion free radical by superoxide dismutase enzyme.

9.3 THE RESPIRATORY CHAIN AND OXIDATIVE PHOSPHORYLATION

Aerobic organisms are able to capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms. Most of this takes place inside mitochondria, which have been termed the "powerhouses" of the cell. Respiration is coupled to the generation of the high-energy intermediate, ATP, by oxidative phosphorylation, and the chemiosmotic theory offers insight into how this is accomplished. A number of drugs (eg, amobarbital) and poisons (eg, cyanide, carbon monoxide) inhibit oxidative phosphorylation, usually with fatal consequences. Several inherited defects of mitochondria involving components of the respiratory chain and oxidative phosphorylation have been reported. Patients present with myopathy and encephalopathy and often have lactic acidosis.

9.3.1 Specific enzymes act as markers

Mitochondria have an outer membrane that is permeable to most metabolites, an inner membrane that is selectively permeable, and a matrix within. The outer membrane is characterized by the presence of various enzymes, including acyl-CoA synthetase and glycerolphosphate acyltransferase. Adenylyl kinase and creatine kinase are found in the intermembrane space. The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain.

9.3.2 The respiratory chain collects and oxidizes reducing equivalents

Most of the energy liberated during the oxidation of carbohydrate, fatty acids, and amino acids is made available within mitochondria as reducing equivalents (H+ or electrons). Mitochondria contain the respiratory chain, which collects and transports reducing equivalents directing them to their final reaction with oxygen to form water, the machinery for trapping the liberated free energy as high-energy phosphate, and the enzymes of β-oxidation and of the citric acid cycle that produce most of the reducing equivalents.

9.3.3 Components of the respiratory chain are arranged in order of increasing redox potential

The respiratory chain consists of a number of redox carriers that proceed from the NAD-linked dehydrogenase systems, through flavoproteins and cytochromes,

to molecular oxygen. Not all substrates are linked to the respiratory chain through NAD-specific dehydrogenases; some, because their redox potentials are more positive (eg, fumarate/succinate; are linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain.

9.3.4 Ubiquinone or Q (coenzyme Q)

Coenzyme Q links the flavoproteins to cytochrome b, the member of the cytochrome chain of lowest redox potential. Q exists in the oxidized quinone or reduced quinol form under aerobic or anaerobic conditions, respectively. The structure of Q is very similar to that of vitamin K and vitamin E and of plastoquinone, found in chloroplasts. Q acts as a mobile component of the respiratory chain that collects reducing equivalents from the more fixed flavoprotein complexes and passes them on to the cytochromes. An additional component is the iron-sulfur protein (FeS; nonheme iron). It is associated with the flavoproteins (metalloflavoproteins) and with cytochrome b. The sulfur and iron are thought to take part in the oxidoreduction mechanism between flavin and Q, which involves only a single e-change, the iron atom undergoing oxidoreduction between Fe^{2+} and Fe^{3+}.

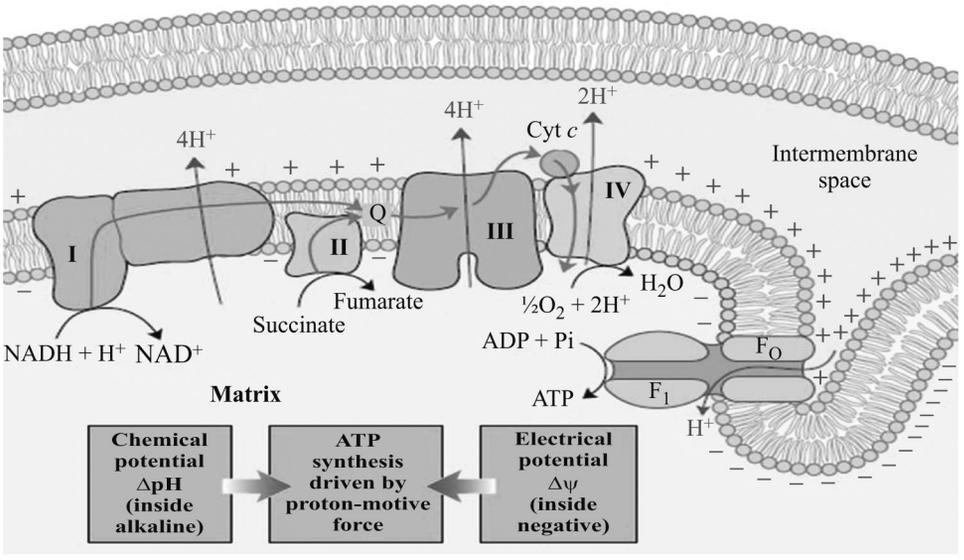
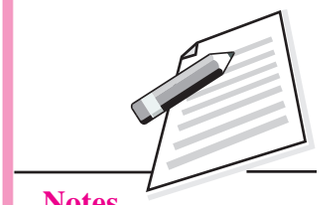


Fig. 9.5: In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient (Δψ). The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (Fo). The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F1 complex associated with Fo.



Pyruvate and α-ketoglutarate dehydrogenase have complex systems involving lipoate and FAD prior to the passage of electrons to NAD, while electron trans fers from other dehydrogenases, e.g., L(+)-3-hydroxyacyl- CoA dehydrogenase, couple directly with NAD. The reduced NADH of the respiratory chain is in turn oxidized by a metalloflavoprotein enzyme – NADH dehydrogenase. This enzyme contains FeS and FMN, is tightly bound to the respiratory chain, and passes reducing equivalents on to Q. Electrons flow from Q through the series of cytochromes in order of increasing redox potential to molecular oxygen. The terminal cytochrome aa3 (cytochrome oxidase), responsible for the final combination of reducing equivalents with molecular oxygen, has a very high affinity for oxygen, allowing the respiratory chain to function at maximum rate until the tissue has become depleted of O_2. Since this is an irreversible reaction (the only one in the chain), it gives direction to the movement of reducing equivalents and to the production of ATP, to which it is coupled. Functionally and structurally, the components of the respiratory chain are present in the inner mitochondrial membrane as four protein-lipid respiratory chain complexes that span the membrane. Cytochrome c is the only soluble cytochrome and, together with Q, seems to be a more mobile component of the respiratory chain connecting the fixed complexes. The overall reaction is given in the figure 9.5.

9.3.5 The respiratory chain provides most of the energy captured during catabolism

ADP captures, in the form of high-energy phosphate, a significant proportion of the free energy released by catabolic processes. The resulting ATP has been called the energy "currency" of the cell because it passes on this free energy to drive those processes requiring energy. There is a net direct capture of two high-energy phosphate groups in the glycolytic reactions, equivalent to approximately 103.2 kJ/mol of glucose. (In vivo, ΔG for the synthesis of ATP from ADP has been calculated as approximately 51.6 kJ/mol. (It is greater than ΔG0' for the hydrolysis of ATP, which is obtained under standard concentrations of 1.0 mol/ L.) Since 1 mol of glucose yields approximately 2870 kJ on complete combustion, the energy captured by phosphorylation in glycolysis is small. Two more high-energy phosphates per mole of glucose are captured in the citric acid cycle during the conversion of succinyl CoA to succinate.

All of these phosphorylations occur at the substrate level. When substrates are oxidized via an NAD-linked dehydrogenase and the respiratory chain, approximately 3 mol of inorganic phosphate are incorporated into 3 mol of ADP to form 3 mol of ATP per half mol of O_2 consumed; ie, the P:O ratio = 3. On the other hand, when a substrate is oxidized via a flavoprotein- linked dehydrogenase, only 2 mol of ATP are formed; ie, P:O = 2. These reactions are

known as oxidative phosphorylation at the respiratory chain level. Such dehydrogenations plus phosphorylations at the substrate level can now account for 68% of the free energy resulting from the combustion of glucose, captured in the form of high-energy phosphate. It is evident that the respiratory chain is responsible for a large proportion of total ATP formation.

9.3.6 Respiratory control ensures a constant supply of ATP

The rate of respiration of mitochondria can be controlled by the availability of ADP. This is because oxidation and phosphorylation are tightly coupled; ie, oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP. When work is performed, ATP is converted to ADP, allowing more respiration to occur, which in turn replenishes the store of ATP. Under certain conditions, the concentration of inorganic phosphate can also affect the rate of functioning of the respiratory chain. There is also the possibility that the ADP/ATP transporter, which facilitates entry of cytosolic ADP into and ATP out of the mitochondrion, becomes rate limiting. Thus, the manner in which biologic oxidative processes allow the free energy resulting from the oxidation of foodstuffs to become available and to be captured is stepwise, efficient (approximately 68%), and controlled – rather than explosive, inefficient, and uncontrolled, as in many nonbiologic processes. The remaining free energy that is not captured as high-energy phosphate is liberated as heat. This need not be considered "wasted," since it ensures that the respiratory system as a whole is sufficiently exergonic to be removed from equilibrium, allowing continuous unidirectional flow and constant provision of ATP. It also contributes to maintenance of body temperature.

9.3.7 Many poisons inhibit the respiratory chain

Much information about the respiratory chain has been obtained by the use of inhibitors, and, conversely, this has provided knowledge about the mechanism of action of several poisons (Figure 9.6). They may be classified as inhibitors of the respiratory chain, inhibitors of oxidative phosphorylation, and uncouplers of oxidative phosphorylation. Barbiturates such as amobarbital inhibit NAD linked dehydrogenases by blocking the transfer from FeS to Q. At sufficient dosage, they are fatal in vivo. Antimycin A and dimercaprol inhibit the respiratory chain between cytochrome b and cytochrome c. The classic poisons H2S, carbon monoxide, and cyanide inhibit cytochrome oxidase and can therefore totally arrest respiration. Malonate is a competitive inhibitor of succinate dehydrogenase. Atractyloside inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion. The

action of uncouplers is to dissociate oxidation in the respiratory chain from phosphorylation. These compounds are toxic in vivo, causing respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or Pi. The uncoupler that has been used most frequently is 2,4-dinitrophenol, but other compounds act in a similar manner. The antibiotic oligomycin completely blocks oxidation and phosphorylation by acting on a step in phosphorylation.

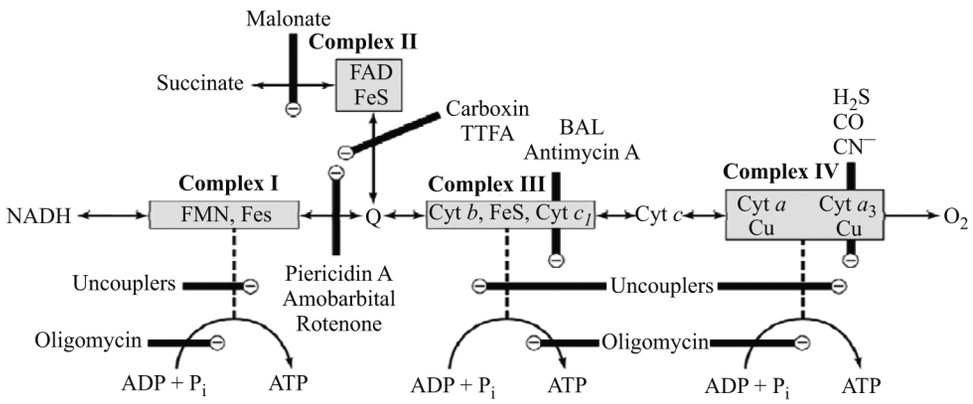


Fig. 9.6: Proposed sites of inhibition (--) of the respiratory chain by specific drugs, chemicals, and antibiotics. The sites that appear to support phosphorylation are indicated. BAL, dimercaprol. TTFA, an Fe-chelating agent. Complex I, NADH:ubiquinone oxidoreductase; complex II, succinate:ubiquinone oxidoreductase; complex III, ubiquinol:ferricytochrome c oxidoreductase; complex IV, ferrocytochrome c:oxygen oxidoreductase.

9.3.8 The chemiosmotic theory explains the mechanism of oxidative phosphorylation

Mitchell's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions (protons, H+) from the inside to the outside of the inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP (Figure 9.7).

9.3.9. The respiratory chain is a proton pump

Each of the respiratory chain complexes I, III, and IV act as a proton pump. The inner membrane is impermeable to ions in general but particularly to protons, which accumulate outside the membrane, creating an electrochemical potential difference across the membrane. This consists of a chemical potential (difference in pH) and an electrical potential.

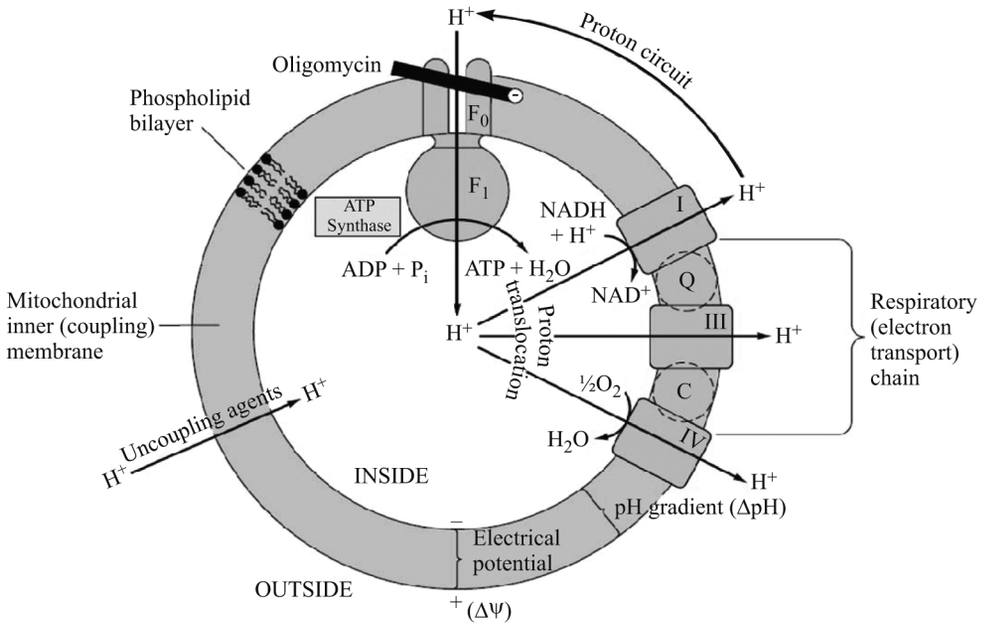
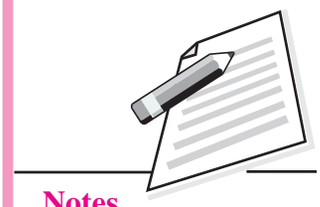


Fig. 9.7: Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the inside to the outside of the membrane, driven by the respiratory chain complexes I, III, and IV, each of which acts as a proton pump. Q, ubiquinone; C, cytochrome c; F1, F0, protein subunits which utilize energy from the proton gradient to promote phosphorylation. Uncoupling agents such as dinitrophenol allow leakage of H+ across the membrane, thus collapsing the electrochemical proton gradient. Oligomycin specifically blocks conduction of H^+ through F0.

9.3.10 A membrane-located ATP synthase functions as a rotary motor to form ATP

The electrochemical potential difference is used to drive a membrane-located ATP synthase which in the presence of Pi + ADP forms ATP. Scattered over the surface of the inner membrane are the phosphorylating complexes, ATP synthase, responsible for the production of ATP. These consist of several protein subunits, collectively known as F1, which project into the matrix and which contain the phosphorylation mechanism. These subunits are attached to a membrane protein complex known as F0, which also consists of several protein subunits. F0 spans the membrane and forms the proton channel. The flow of protons through F0 causes it to rotate, driving the production of ATP in the F1 complex. Estimates suggest that for each NADH oxidized, complex I translocates four protons and complexes III and IV translocate 6 between them. As four protons are taken into the mitochondrion for each ATP exported, the P:O ratio would not necessarily be a complete integer, ie, 3, but possibly 2.5. However, for simplicity, a value of 3 for the oxidation of NADH + H+ and 2 for the oxidation of FADH2 will continue to be used throughout this text.



9.3.11 The chemiosmotic theory can account for respiratory control and the action of uncouplers

The electrochemical potential difference across the membrane, once established as a result of proton translocation, inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back translocation of protons across the membrane through the vectorial ATP synthase. This in turn depends on availability of ADP and Pi. Uncouplers (eg, dinitrophenol) are amphipathic and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.

9.3.12 Impermeability of the inner mitochondrial membrane

The relative impermeability of the inner mitochondrial membrane necessitates exchange transporters. Exchange diffusion systems are present in the membrane for exchange of anions against OH- ions and cations against H+ ions. Such systems are necessary for uptake and output of ionized metabolites while preserving electrical and osmotic equilibrium. The inner bilipoid mitochondrial membrane is freely permeable to uncharged small molecules, such as oxygen, water, CO2, and NH3, and to monocarboxylic acids, such as 3-hydroxybutyric, acetoacetic, and acetic. Long-chain fatty acids are transported into mitochondria via the carnitine system, and there is also a special carrier for pyruvate involving a symport that utilizes the H+ gradient from outside to inside the mitochondrion. However, dicarboxylate and tri- carboxylate anions and amino acids require specific transporter or carrier systems to facilitate their passage across the membrane. Monocarboxylic acids penetrate more readily in their undissociated and more lipid-soluble form. The transport of di- and tricarboxylate anions is closely linked to that of inorganic phosphate, which penetrates readily as the $H_2PO_4^-$ ion in exchange for OH^-.

9.3.13. Ionophores permit specific cations to penetrate membranes

Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes, eg, valinomycin (K^+). The classic uncouplers such as dinitrophenol are, in fact, proton ionophores.

9.3.14 A proton-translocating transhydrogenase is a source of intramitochon-drial NADPH

Energy-linked transhydrogenase, a protein in the inner mitochondrial membrane, couples the passage of protons down the electrochemical gradient from outside

Notes

to inside the mitochondrion with the transfer of H from intramitochondrial NADH to NADPH for intramitochondrial enzymes such as glutamate dehydrogenase and hydroxylases involved in steroid synthesis.

9.3.15 Oxidation of extramitochondrial NADH is mediated by substrate shuttles

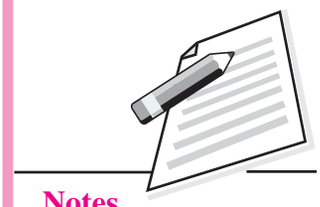
NADH cannot penetrate the mitochondrial membrane, but it is produced continuously in the cytosol by 3-phosphoglyceraldehyde dehydrogenase, an enzyme in the glycolysis sequence. However, under aerobic conditions, extramitochondrial NADH does not accumulate and is presumed to be oxidized by the respiratory chain in mitochondria. The transfer of reducing equivalents through the mitochondrial membrane requires substrate pairs, linked by suitable dehydrogenases on each side of the mitochondrial membrane. The mechanism of transfer uses glycerophosphate shuttle. Since the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein rather than NAD, only 2 mol rather than 3 mol of ATP are formed per atom of oxygen consumed. Although this shuttle is present in some tissues (eg, brain, white muscle), in others (eg, heart muscle) it is deficient. It is therefore believed that the malate shuttle system is of more universal utility. The complexity of this system is due to the impermeability of the mitochondrial membrane to oxaloacetate, which must react with glutamate and transaminate to aspartate and á-ketoglutarate before transport through the mitochondrial membrane and reconstitution to oxaloacetate in the cytosol.

9.3.16. Ion transport in mitochondria is energy-linked

Mitochondria maintain or accumulate cations such as K+, Na+, Ca2+, and Mg2+, and Pi. It is assumed that a primary proton pump drives cation exchange.

9.3.17 The creatine phosphate shuttle facilitates transport of high-energy phosphate from mitochondria

The creatine phosphate shuttle augments the functions of creatine phosphate as an energy buffer by acting as a dynamic system for transfer of high-energy phosphate from mitochondria in active tissues such as heart and skeletal muscle. An isoenzyme of creatine kinase is found in the mitochondrial intermembrane space, catalyzing the transfer of high-energy phosphate to creatine from ATP emerging from the adenine nucleotide transporter. In turn, the creatine phosphate is transported into the cytosol via protein pores in the outer mitochondrial membrane, becoming available for generation of extramitochondrial ATP.



INTEXT QUESTIONS 9.1

I. Choose the best answer

1. Chemically, the removal and the gain of electrons is defined respectively as

(a) Oxidation and reduction

(b) Reduction and oxidation

(c) Oxidation and dehydrogenase

(d) Reduction and dehydrogenase

2. Chemical carcinogens (xenobiotics) are metabolized by the enzymes system known as

(a) Cytochrome P450 (b) Xanthine oxidase

(c) Succinate dehydrogenase (d) Hydroperoxides

3. Oxidative phosphorylation is inhibited usually with fatal consequences by

(a) Quinalones (b) Cyanide

(c) Anacin (d) Amoxycillin

4. The action of uncouplers is to dissociate oxidation in the respiratory chain from

(a) Gluconeogenesis (b) Glycolysis

(c) TCA cycle (d) Phosphorylation

5. This antibiotic completely blocks oxidation and phosphorylation by acting on a step in phosphorylation.

(a) Dinitrophenol (b) Benzpyrene

(c) Oligomycin (d) Morphine

II. Fill in the blanks

6. Flavoprotein enzymes contain ..... or ..... as prosthetic groups.

7. Generally, NAD-linked dehydrogenases catalyze ..... reactions in the oxidative pathways of metabolism.

8. ..... protect the body against harmful peroxides.

9. The function of ..... is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases.

10. Cytochromes P450 are an important superfamily of heme-containing .....

III. Match the following

11.	Mitochondria	(a)	Energy currency of the cell
12.	Cardiolipin	(b)	Uncouplers
13.	ATP	(c)	Powerhouses of the cell
14.	Valinomycin	(d)	Phospholipid
15.	Dinitrophenol	(e)	Ionophores

WHAT HAVE YOU LEARNT

- Oxidation is defined as the removal of electrons and reduction as the gain of electrons.
- Many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized oxygenases known as cytochrome P450 system.
- Enzymes involved in oxidation and reduction are called oxidoreductases and are classified into four groups: oxidases, dehydrogenases, hydroperoxidases, and oxygenases.
- Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin.
- Oxidation-reduction reactions carried out by dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+.
- The cytochromes are iron-containing hemoproteins in which the iron atom oscillates between Fe3+ and Fe2+ during oxidation and reduction.
- Cytochromes are also found in the endoplasmic reticulum (cytochromes P450 and b5), and in plant cells, bacteria, and yeasts.
- Two type of enzymes found both in animals and plants are peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides.
- Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and perhaps cause cancer and atherosclerosis.
- Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol.
- The potential toxicity of oxygen is due to its conversion to superoxide in tissues and the enzyme superoxide dismutase is responsible for its removal.
- Mitochondria are termed as the “powerhouses” of the cell. Respiration is coupled to the generation of the high-energy intermediate, ATP, by oxidative

phosphorylation, and the chemiosmotic theory offers insight into how this is accomplished.

- A number of drugs (eg, amobarbital) and poisons (eg, cyanide, carbon monoxide) inhibit oxidative phosphorylation, usually with fatal consequences.
- Mitochondria contain the respiratory chain, which collects and transports reducing equivalents directing them to their final reaction with oxygen to form water, the machinery for trapping the liberated free energy as high-energy phosphate, and the enzymes of β-oxidation and of the citric acid cycle that produce most of the reducing equivalents.
- Mitchell's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions (protons, H+) from the inside to the outside of the inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP.
- Uncouplers (eg, dinitrophenol) are amphipathic and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.
- Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes, eg, valinomycin (K+).

TERMINAL QUESTIONS

1. Write short note on electron transfer chain.
2. Write short note on oxidative phosphorylation.

ANSWERS TO INTEXT QUESTIONS

I. 1. (a) 2. (a) 3. (b) 4. (d) 5. (c)

II. 6. Flavin mononucleotide (FMN) or Flavin adenine dinucleotide (FAD)
7. Oxidoreduction
8. Hydroperoxidases
9. Catalase
10. Monooxgenases

III. 11. (c) 12. (d) 13. (a) 14. (e) 15. (b)

Notes

10

VITAMINS

10.1 INTRODUCTION

The term "vitamin" is used to describe certain organic compounds that are needed by the body but that cannot be manufactured by the body. They mainly serve as catalysts for certain reactions in the body. The amounts of vitamins required are very small, perhaps hundredths of grams. Vitamins are mainly obtained from our foods.

OBJECTIVES

After reading this lesson, you will be able to

- classify vitamins
- describe Water soluble vitamins
- describe Fat soluble vitamins

10.2 CLASSIFICATION OF VITAMINS

Based on solubility Vitamins are classified as either fat-soluble (lipid soluble) or water-soluble. Vitamins A, D, E and K are fat-soluble Vitamin C and B is water soluble.

WATER-SOLUBLE VITAMINS

10.3 B-COMPLEX VITAMINS

Eight of the water-soluble vitamins are known as the vitamin B-complex group: thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6 (pyridoxine), folate (folic acid), vitamin B12, biotin and pantothenic acid. The

B vitamins are widely distributed in foods and their influence is felt in many parts of the body. They function as coenzymes that help the body obtain energy from food. The B vitamins are also important for normal appetite, good vision, and healthy skin, nervous system, and red blood cell formation.

10.3.1 Thiamin: Vitamin B1

Thiamin, or vitamin B1, helps to release energy from foods, promotes normal appetite, and is important in maintaining proper nervous system function.

Food Sources for Thiamin

Sources include peas, pork, liver, and legumes. Most commonly, thiamin is found in whole grains and fortified grain products such as cereal, and enriched products like bread, pasta, rice, and tortillas. The process of enrichment adds back nutrients that are lost when grains are processed. Among the nutrients added during the enrichment process are thiamin (B1), niacin (B3), riboflavin (B2), folate and iron.

RDA (Required Daily allowance)

Males: 1.2 mg/day; **Females**: 1.1 mg/day

Thiamin Deficiency

Under-consumption of thiamin is rare due to wide availability of enriched grain products. However, low calorie diets as well as diets high in refined and processed carbohydrates may place one at risk for thiamin deficiency. Alcoholics are especially prone to thiamin deficiency because excess alcohol consumption often replaces food or meals. Symptoms of thiamin deficiency include: mental confusion, muscle weakness, wasting, water retention (edema), impaired growth, and the disease known as beriberi. Thiamin deficiency is currently not a problem in the United States.

Thiamin toxicity

No problem with overconsumption are known for thiamin.

10.3.2 Riboflavin: Vitamin B2

Riboflavin, or vitamin B2, helps to release energy from foods, promotes good vision, and healthy skin. It also helps to convert the amino acid tryptophan (which makes up protein) into niacin.

Food Sources

Sources include liver, eggs, dark green vegetables, legumes, whole and enriched grain products, and milk. Ultraviolet light is known to destroy riboflavin, which is why most milk is packaged in opaque containers instead of clear.

Notes

RDA

Males: 1.3 mg/day; **Females:** 1.1 mg/day

Deficiency

Under consumption of riboflavin is rare. However, it has been known to occur with alcoholism, malignancy, hyperthyroidism, and in the elderly. Symptoms of deficiency include cracks at the corners of the mouth, dermatitis on nose and lips, light sensitivity, cataracts, and a sore, red tongue.

Riboflavin toxicity

No problems with overconsumption are known for riboflavin.

10.3.3 Niacin: Vitamin B3, Nicotinamide, Nicotinic Acid

Niacin, or vitamin B3, is involved in energy production, normal enzyme function, digestion, promoting normal appetite, healthy skin, and nerves.

Food Sources for Niacin

Sources include liver, fish, poultry, meat, peanuts, whole and enriched grain products.

RDA

Males: 16 mg/day; **Females:** 14 mg/day

Niacin Deficiency

Niacin deficiency is known to occur with alcoholism, protein malnourishment, low calorie diets, and diets high in refined carbohydrates. Pellagra is the disease state that occurs as a result of severe niacin deficiency. Symptoms include cramps, nausea, mental confusion, and skin problems.

Niacin toxicity

Consuming large doses of niacin supplements may cause flushed skin, rashes, or liver damage. Over consumption of niacin is not a problem if it is obtained through food.

10.3.4 Vitamin B6: Pyridoxine, Pyridoxal, Pyridoxamine

Vitamin B6, otherwise known as pyridoxine, pyridoxal or pyridoxamine, aids in protein metabolism and red blood cell formation. It is also involved in the body's production of chemicals such as insulin and hemoglobin.

Food Sources for Vitamin B6

Sources include pork, meats, whole grains and cereals, legumes, and green, leafy vegetables. The RDA for vitamin B6 is 1.3 mg/day for adult males and females through age fifty.

Vitamin B6 Deficiency

Deficiency symptoms include skin disorders, dermatitis, cracks at corners of mouth, anemia, kidney stones, and nausea. A vitamin B6 deficiency in infants can cause mental confusion.

Too much Vitamin B6

Over consumption is rare, but excess doses of vitamin B6 over time have been known to result in nerve damage.

10.3.5 Folate: Folic Acid, Folacin

Folate, also known as folic acid or folacin, aids in protein metabolism, promoting red blood cell formation, and lowering the risk for neural tube birth defects. Folate may also play a role in controlling homocysteine levels, thus reducing the risk for coronary heart disease.

Food Sources for Folate

Sources of folate include liver, kidney, dark green leafy vegetables, meats, fish, whole grains, fortified grains and cereals, legumes, and citrus fruits. Not all whole grain products are fortified with folate..

RDA

The RDA for folate is 400 mcg/day for adult males and females. Pregnancy will increase the RDA for folate to 600 mcg/day.

Folate Deficiency

Folate deficiency affects cell growth and protein production, which can lead to overall impaired growth. Deficiency symptoms also include anemia and diarrhea. A folate deficiency in women who are pregnant or of child bearing age may result in the delivery of a baby with neural tube defects such as spina bifida.

Folate toxicity

Over consumption of folate offers no known benefits, and may mask B12 deficiency as well as interfere with some medications.

10.3.6 Vitamin B12: Cobalamin

Vitamin B12, also known as cobalamin, aids in the building of genetic material, production of normal red blood cells, and maintenance of the nervous system.

Notes

Food Sources for Vitamin B12

Vitamin B12 can only be found only in foods of animal origin such as meats, liver, kidney, fish, eggs, milk and milk products, oysters, shellfish. Some fortified foods may contain vitamin B12.

RDA

The Recommended Dietary Allowance (RDA) for vitamin B12 is 2.4 mcg/day for adult males and females

Vitamin B12 Deficiency

Vitamin B12 deficiency most commonly affects strict vegetarians (those who eat no animal products), infants of vegan mothers, and the elderly. Symptoms of deficiency include anemia, fatigue, neurological disorders, and degeneration of nerves resulting in numbness and tingling. In order to prevent vitamin B12 deficiency, a dietary supplement should be taken. Some people develop a B12 deficiency because they cannot absorb the vitamin through their stomach lining. This can be treated through vitamin B12 injections.

Vitamin B12 toxicity

No problems with overconsumption of vitamin B12 are known.

10.3.7 Biotin

Biotin helps release energy from carbohydrates and aids in the metabolism of fats, proteins and carbohydrates from food.

Food Sources for Biotin

Sources of Biotin include liver, kidney, egg yolk, milk, most fresh vegetables, yeast breads and cereals. Biotin is also made by intestinal bacteria.

RDA

The Adequate Intake (AI) for Biotin is 30 mcg/day for adult males and females

Biotin Deficiency

Biotin deficiency is uncommon under normal circumstances, but symptoms include fatigue, loss of appetite, nausea, vomiting, depression, muscle pains, heart abnormalities and anemia.

Biotin toxicity

No problems with overconsumption are known for Biotin.

10.3.8 Pantothenic Acid

Pantothenic Acid is involved in energy production, and aids in the formation of hormones and the metabolism of fats, proteins, and carbohydrates from food.

Food Sources for Pantothenic Acid

Sources include liver, kidney, meats, egg yolk, whole grains, and legumes. Pantothenic Acid is also made by intestinal bacteria.

RDA

The Adequate Intake (AI) for Pantothenic Acid is 5 mg/day for both adult males and females.

Pantothenic Acid Deficiency

Pantothenic Acid deficiency is uncommon due to its wide availability in most foods.

Pantothenic Acid toxicity

No problems with overconsumption are known for Pantothenic Acid. Rarely, diarrhea and water retention will occur with excessive amounts.

10.4 VITAMIN C: ASCORBIC ACID, ASCORBATE

The body needs vitamin C, also known as ascorbic acid or ascorbate. Vitamin C benefits the body by holding cells together through collagen synthesis; collagen is a connective tissue that holds muscles, bones, and other tissues together. Vitamin C also aids in wound healing, bone and tooth formation, strengthening blood vessel walls, improving immune system function, increasing absorption and utilization of iron, and acting as an antioxidant.

Since our bodies cannot produce or store vitamin C, an adequate daily intake of this nutrient is essential for optimum health. Vitamin C works with vitamin E as an antioxidant, and plays a crucial role in neutralizing free radicals throughout the body. An antioxidant can be a vitamin, mineral, or a carotenoid, present in foods, that slows the oxidation process and acts to repair damage to cells of the body. Studies suggest that vitamin C may reduce the risk of certain cancers, heart disease, and cataracts. Research continues to document the degree of these effects.

Food Sources for Vitamin C

Consuming vitamin C-rich foods is the best method to ensure an adequate intake of this vitamin. While many common plant foods contain vitamin C, the best sources are citrus fruits (orange, kiwi fruit, grape etc,)

Notes

RDA

The Recommended Dietary Allowance (RDA) for Vitamin C is 90 mg/day for adult males and 75 mg/day for adult females For those who smoke cigarettes, the RDA for vitamin C increases by 35 mg/day, in order to counteract the oxidative effects of nicotine.

Vitamin C Deficiency

Severe vitamin C deficiency result in the disease known as scurvy, causing a loss of collagen strength throughout the body. Loss of collagen results in loose teeth, bleeding and swollen gums, and improper wound healing. More commonly, vitamin C deficiency presents as a secondary deficiency in alcoholics, the elderly, and in smokers.

Vitamin C toxicity

Despite being a water-soluble vitamin that the body excretes when in excess, vitamin C overdoses have been shown to cause kidney stones, gout, diarrhea, and rebound scurvy.

FAT-SOLUBLE VITAMINS

The fat-soluble vitamins, A, D, E, and K, are stored in the body for long periods of time and generally pose a greater risk for toxicity when consumed in excess than water-soluble vitamins. Eating a normal, well-balanced diet will not lead to toxicity in otherwise healthy individuals. However, taking vitamin supplements that contain megadoses of vitamins A, D, E and K may lead to toxicity. The body only needs small amounts of any vitamin.

While diseases caused by a lack of fat soluble vitamins are rare symptoms of mild deficiency can develop without adequate amounts of vitamins in the diet. Additionally, some health problems may decrease the absorption of fat, and in turn, decrease the absorption of vitamins A, D, E and K. Consult a medical professional about any potential health problems that may interfere with vitamin absorption.

10.5 VITAMIN A: RETINOL

Vitamin A, also called retinol, has many functions in the body. In addition to helping the eyes adjust to light changes, vitamin A plays an important role in bone growth, tooth development, reproduction, cell division, gene expression, and regulation of the immune system. The skin, eyes, and mucous membranes of the mouth, nose, throat and lungs depend on vitamin A to remain moist.

Vitamin A is also an important antioxidant that may play a role in the prevention of certain cancers.

Food Sources for Vitamin A

Eating a wide variety of foods is the best way to ensure that the body gets enough vitamin A. The retinol, retinal, and retinoic acid forms of vitamin A are supplied primarily by foods of animal origin such as dairy products, fish and liver. Some foods of plant origin contain the antioxidant, betacarotene, which the body converts to vitamin A. Beta-carotene, comes from fruits and vegetables, especially those that are orange or dark green in color. Vitamin A sources also include carrots, pumpkin, winter squash, dark green leafy vegetables and apricots, all of which are rich in beta-carotene.

How much Vitamin A

The recommendation for vitamin A intake is expressed as micrograms (mcg) of retinol activity equivalents (RAE). Retinol activity equivalents account for the fact that the body converts only a portion of betacarotene to retinol. One RAE equals 1 mcg of retinol or 12 mcg of beta-carotene. The Recommended Dietary Allowance (RDA) for vitamin A is 900 mcg/ day for adult males and 700 mcg/ day for adult females.

Compared to vitamin A, it takes twice the amount of carotene rich foods to meet the body's vitamin A requirements, so one may need to increase consumption of carotene containing plant foods. Recent studies indicate that vitamin A requirements may be increased due to hyperthyroidism, fever, infection, cold, and exposure to excessive amounts of sunlight. Those that consume excess alcohol or have renal disease should also increase intake of vitamin A.

Vitamin A Deficiency

Vitamin A deficiency is rare, but the disease that results is known as xerophthalmia. It most commonly occurs in developing nations usually due to malnutrition. Since vitamin A is stored in the liver, it may take up to 2 years for signs of deficiency to appear. Night blindness and very dry, rough skin may indicate a lack of vitamin A. Other signs of possible vitamin A deficiency include decreased resistance to infections, faulty tooth development, and slower bone growth.

Vitamin A toxicity

The Tolerable Upper Intake Level (UL) for adults is 3,000 mcg RAE. It would be difficult to reach this level consuming food alone, but some multivitamin

Notes

supplements contain high doses of vitamin A. If you take a multivitamin, check the label to be sure the majority of vitamin A provided is in the form of beta-carotene, which appears to be safe. Symptoms of vitamin A toxicity include dry, itchy skin, headache, nausea, and loss of appetite. Signs of severe overuse over a short period of time include dizziness, blurred vision and slowed growth. Vitamin A toxicity also can cause severe birth defects and may increase the risk for hip fractures.

10.6 VITAMIN D

Vitamin D plays a critical role in the body's use of calcium and phosphorous. It works by increasing the amount of calcium absorbed from the small intestine, helping to form and maintain bones. Vitamin D benefits the body by playing a role in immunity and controlling cell growth. Children especially need adequate amounts of vitamin D to develop strong bones and healthy teeth.

Food Sources for Vitamin D

The primary food sources of vitamin D are milk and other dairy products fortified with vitamin D. Vitamin D is also found in oily fish (e.g., herring, salmon and sardines) as well as in cod liver oil. In addition to the vitamin D provided by food, we obtain vitamin D through our skin which produces vitamin D in response to sunlight.

RDA

The Recommended Dietary Allowance (RDA) for vitamin D appears as micrograms (mcg) of cholecalciferol (vitamin D3). From 12 months to age fifty, the RDA is set at 15 mcg. Twenty mcg of cholecalciferol equals 800 International Units (IU), which is the recommendation for maintenance of healthy bone for adults over fifty.

Exposure to ultraviolet light is necessary for the body to produce the active form of vitamin D. Ten to fifteen minutes of sunlight without sunscreen on the hands, arms and face, twice a week is sufficient to receive enough vitamin D. This can easily be obtained in the time spent riding a bike to work or taking a short walk. In order to reduce the risk for skin cancer one should apply sunscreen with an SPF of 15 or more, if time in the sun exceeds 10 to 15 minutes.

Vitamin D Deficiency

Symptoms of vitamin D deficiency in growing children include rickets (long, soft bowed legs) and flattening of the back of the skull. Vitamin D deficiency in adults may result in osteomalacia (muscle and bone weakness), and osteoporosis (loss of bone mass).

Recently published data introduces a concern that some adults and children may be more prone to developing vitamin D deficiency due to an increase in sunscreen use. In addition, those that live in inner cities, wear clothing that covers most of the skin, or live in northern climates where little sun is seen in the winter are also prone to vitamin D deficiency. Since most foods have very low vitamin D levels (unless they are enriched) a deficiency may be more likely to develop without adequate exposure to sunlight. Adding fortified foods to the diet such as milk, and for adults including a supplement, are effective at ensuring adequate vitamin D intake and preventing low vitamin D levels.

Vitamin D deficiency has been associated with increased risk of common cancers, autoimmune diseases, hypertension, and infectious disease. In the absence of adequate sun exposure, at least 800 to 1,000 IU of vitamin D3 may be needed to reach the circulating level required to maximize vitamin D's benefits.

Vitamin D toxicity

The Tolerable Upper Intake Level (UL) for vitamin D is set at 100 mcg for people 9 years of age and older. High doses of vitamin D supplements coupled with large amounts of fortified foods may cause accumulations in the liver and produce signs of poisoning. Signs of vitamin D toxicity include excess calcium in the blood, slowed mental and physical growth, decreased appetite, nausea and vomiting.

It is especially important that infants and young children do not consume excess amounts of vitamin D regularly, due to their small body size.

10.7 VITAMIN E: TOCOPHEROL

Vitamin E benefits the body by acting as an antioxidant, and protecting vitamins A and C, red blood cells, and essential fatty acids from destruction. Research from decades ago suggested that taking antioxidant supplements, vitamin E in particular, might help prevent heart disease and cancer. However, newer findings indicate that people who take antioxidant and vitamin E supplements are not better protected against heart disease and cancer than non-supplement users. Many studies show a link between regularly eating an antioxidant rich diet full of fruits and vegetables, and a lower risk for heart disease, cancer, and several other diseases. Essentially, recent research indicates that to receive the full benefits of antioxidants and phytonutrients in the diet, one should consume these compounds in the form of fruits and vegetables, not as supplements.

Food Sources for Vitamin E

About 60 percent of vitamin E in the diet comes from vegetable oil (soybean, corn, cottonseed, and safflower). This also includes products made with

Notes

vegetable oil (margarine and salad dressing). Vitamin E sources also include fruits and vegetables, grains, nuts (almonds and hazelnuts), seeds (sunflower) and fortified cereals.

RDA

The Recommended Dietary Allowance (RDA) for vitamin E is based on the most active and usable form called alpha-tocopherol. Food and supplement labels list alpha-tocopherol as the unit International units (IU) not in milligrams (mg). One milligram of alpha-tocopherol equals to 1.5 International Units (IU). RDA guidelines state that males and females over the age of 14 should receive 15 mcg of alpha-tocopherol per day. Consuming vitamin E in excess of the RDA does not result in any added benefits.

Vitamin E Deficiency

Vitamin E deficiency is rare. Cases of vitamin E deficiency usually only occur in premature infants and in those unable to absorb fats. Since vegetable oils are good sources of vitamin E, people who excessively reduce their total dietary fat may not get enough vitamin E.

Vitamin E toxicity

Vitamin E obtained from food usually does not pose a risk for toxicity. Supplemental vitamin E is not recommended due to lack of evidence supporting any added health benefits. Megadoses of supplemental vitamin E may pose a hazard to people taking blood-thinning medications such as Coumadin (also known as warfarin) and those on statin drugs.

10.8 VITAMIN K

Vitamin K is naturally produced by the bacteria in the intestines, and plays an essential role in normal blood clotting, promoting bone health, and helping to produce proteins for blood, bones, and kidneys.

Food Sources for Vitamin K

Good food sources of vitamin K are green, leafy-vegetables such as turnip greens, spinach, cauliflower, cabbage and broccoli, and certain vegetables oils including soybean oil, cottonseed oil, canola oil and olive oil. Animal foods, in general, contain limited amounts of vitamin K.

RDA

Males and females age 14 - 18: 75 mcg/day; Males and females age 19 and older: 90 mcg/day

Vitamin K Deficiency

Hemorrhage can occur due to sufficient amounts of vitamin K. Vitamin K deficiency may appear in infants or in people who take anticoagulants, such as Coumadin (warfarin), or antibiotic drugs. Newborn babies lack the intestinal bacteria to produce vitamin K and need a supplement for the first week. Those on anticoagulant drugs (blood thinners) may become vitamin K deficient, but should not change their vitamin K intake without consulting a physician. People taking antibiotics may lack vitamin K temporarily because intestinal bacteria are sometimes killed as a result of long-term use of antibiotics. Also, people with chronic diarrhea may have problems absorbing sufficient amounts of vitamin K through the intestine and should consult their physician to determine if supplementation is necessary.

Vitamin K toxicity

Although no Tolerable Upper Intake Level (UL) has been established for vitamin K, excessive amounts can cause the breakdown of red blood cells and liver damage. People taking blood-thinning drugs or anticoagulants should moderate their intake of foods with vitamin K, because excess vitamin K can alter blood clotting times. Large doses of vitamin K are not advised.

INTEXT QUESTIONS 10.1

1. Fill in the balnks:

1. Vitamins are classified into ..... and .....
2. Vitamin B complex comprises ..... vitamins in total.
3. Pellagra and Scurvy are caused by ..... and ..... deficiency.
4. Water soluble vitamins are excreted through .....
5. ..... can be synthesized by human body.

2. Match the following

1. Vitamin A deficiency	(a) Vitamin D
2. Vitamin K	(b) Vitamin E
3. Bone formation	(c) Vitamin B12
4. Cobalamin	(d) Night blindness
5. Tocopherol	(e) Coagulation

Notes

3. True or false:

1. Vitamins are required in large amounts.
2. Vitamin B2 is otherwise known as Riboflavin.
3. All vitamins are synthesized in our body. (false)
4. Fat malabsorption leads to deficiency of fat soluble vitamins.
5. Water soluble vitamins are stored in our body.

WHAT HAVE YOU LEARNT

- The term vitamin is used to describe certain orgain compounds that are needed by the body but they cannot be manufactured by the body
- Vitamins serve as catalysts for certain reactions in the body
- Based on solubility vitamins are classified as either fat soluble or vitamin soluble
- Vitamins A, D, E and K are fat soluble and vitamins C and B is water soluble

TERMINAL QUESTIONS

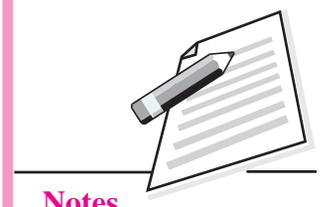
1. Name the B complex vitamins.
2. Classification of vitamins.
3. Give the RDA of thiamine, folate, niacin, vitamin C.
4. Name the fat soluble vitamins and where they are stored?
5. What are the symptoms of Pellagra and Scurvy?

ANSWERS TO INTEXT QUESTIONS

1.
1. fat soluble and water soluble
2. Eight
3. Niacin and vitamin C
4. urine
5. vitamin D

2. 1. (d) 2. (e) 3. (a) 4. (c) 5. (b)

3. 1. false 2. true 3. false 4. true 5. false



11

MINERALS

11.1 INTRODUCTION

Minerals are indispensable part of a complete diet of farm animals. In this unit, dietary essential minerals functions, factors affecting the requirements and sources will be discussed. Minerals are essential for the normal growth and maintance of the body. If the daily requirement is more than 100mg/day they are called major elements and if the daily requirements is less than 100mg/day they are called minor elements. The roles of minerals and vitamins in the maintenance of homeostatic balance and mediation of metabolic reactions in the skeleton, tissues, body fluids, digestive juices, etc.

OBJECTIVES

After reading this lesson, you will be able to:

- classify minerals
- describe the functions, Daily requirements of minerals

11.2 CALCIUM

Total calcium in the human body is 1 to 1.5kg, out of which 99% is seen in bone and 1% in extracellular fluid. The main source of calcium is milk. But in India cereals is major source of calcium. The daily requirement of calcium for child is 1200mg/day and for adult it is 500mg/day. During pregnancy /lactation the calcium requirement is 1500mg/day.

The absorption of calcium takes place in 1^{st} and 2^{nd} part of deuodenum. Calcium absorption requires carrier protein, helped by Ca^{2+} - dependent ATpase.

Factors responsible for increase in calcium absorption include Vitamin D, Parathyroid hormone, acidity and amino acids. Factors such as phytic acid,oxalates,

Notes

malabsorption syndromes and Phosphates decreases calcium absorption. The normal calcium level in blood is 9-11mg/dl.

11.2.1 Function of Calcium

The major functions of calcium are

(a) Excitation and contraction of muscle fibres needs calcium. The active transport system utilizing calcium binding protein is called Calsequestrin. Calcium decreases neuromuscular irritability.

(b) Calcium is necessary for transmission of nerve impulse from presynaptic to postsynaptic region.

(c) Calcium is used as second messenger in system involving protein and inositol triphosphate.

(d) Secretion of insulin, parathyroid hormone, calcium etc, from the cells requires calcium.

(e) Calcium decrease the passage of serum through capillaries thus, calcium is clinically used to reduce allergic exudates.

(f) Calcium is also required for coagulation factors such as prothrombin.

(g) Calcium prolongs systole.

(h) Bone and teeth contains bulk quantity of calcium.

11.2.2 Factors regulating blood calcium level

The factors regulating the blood calcium level includes

(i) Vitamin D

(a) Vitamin D and absorption of calcium:

Active form of calcium is calcitriol. Calcitriol enters intestinal wall and binds to cytoplasmic receptor and then binds with DNA causes depression and consequent transcription of gene code for calbindin. Due to increased availability of calbindin, absorption of calcium increases leading to increased blood calcium level.

(b) Vitamin D and Bone:

Vitamin D activates osteoblast, bone forming cells & also stimulates secretion of alkaline phosphatase. Due to this enzyme, calcium and phosphorus increase.

(c) Vitamin D and Kidney:

Calcitriol increase reabsorption of calcium and phosphorus by renal tubules.

(ii) Parathyroid hormone (PTH)

Normal PTH level in serum is 10-60ng/l.

(a) PTH and bones:

In bone, PTH causes demineralization. It also causes recreation of collagenase from osteoclast leads to loss of matrix and bone resorption. As a result, mucopolysacharides and hydroxyproline are excreted in urine.

(b) PTH and Kidney:

In kidney, PTH causes increased reabsorption of calcium but decreases reabsorption of phosphorus from kidney tubules.

(iii) Calcitonin

Calcitonin decreases serum calcium level. It inhibits resorption of bone. It decreases the activity of osteoclasts and increases osteoblasts.

11.2.3 Hyper Calcemia

When plasma Ca^{2+} level is more than 11mg/dl is called Hypercalcemia. It is due to parathyroid adenoma or ectopic PTH secreting tumor. In this condition, calcium excreted in urine decreases excretion of chloride causing hyperchloremic acidosis. The main symptoms of hyperchloremic acidosis are:

(a) Anorexia, nausea,vomiting

(b) polyuria, polydypsia

(c) Confusion, depression, psychosis

(d) renal stones

(e) osteoporosis

11.2.4 Hypocalcemia

Plasma calcium level less than 8mg/dl is called hypocalcemia. Tetany due to accidental surgical removal of parathyroid glands or by autoimmune disease. In tetany, neuromuscular irritability is increased. Increased Q-7 internal in ECG is seen. Main manifestation is carpopedal spasm. Laryngismus and stridor are also observed. Laryngeal spasm may lead is death. The causes of laryngeal spasm includes

(a) Deficiency of vitamin D

(b) Deficiency of parathyroid

(c) Increased calcitonin

(d) Deficiency of calcium intake

INTEXT QUESTION 11.1

1. The total calcium range in human is .....
2. Calcium absorption requires ..... dependent ATpase.
   (a) Ca (b) Mn (c) Mg (d) fe
3. Factors responsible for increase in calcium absorption include .....
4. When plasma Ca^{2+} level is more than 11mg/dl is called .....
   (a) Hypercalcemia (b) Anemia (c) Fluorosis (d) Selenosis

11.3 PHOSPHORUS

Total body content of phosphorus is 1 kg. Bone posses 80 % of total phosphorus and muscle contains 10 % of total phosphorus. Human body requires 500 mg of phosphorus per day. Milk is the good source of phosphorus and it contains about 100 mg/dl of phosphrous. Apart from milk, cereals, nuts and meat are moderate sources of phosprous.

Serum level of phosphate is 3-4 mg/dl for adults and 5-6 mg/dl in children. Consumption of calcitriol increases phosphate absorption.

11.3.1 Functions of phosphorus

(a) Plays key role in formation of tooth and bone
(b) Production of high energy phosphate compounds such as ATP, CTP, GTP etc.,
(c) Synthesis of nucleotide co-enzymes such as NAD and NADP
(d) Formation of phosphodiester backbone structure for DNA and RNA synthesis

Hypophosphatemia is the condition which leads to decrease in absorption of phosphorus. Further it leads to hypercalcamia, chronic alcoholism and increased excretion of urinary phosphate. In case of hyperphosphatemia, increase in absorption of phosphate was noticed. Hyperphosphatemia leads to cell lysis, hypocalcemia and thyrotoxicosis.

INTEXT QUESTIONS 11.2

1. Bone posses ..... % of total phosphorus.
2. Serum level of phosphate is ..... mg/dl for adults.
   (a) 4-8 (b) 3-4 (c) 8-9 (d) 1-2
3. ..... plays key role in formation of tooth and bone

11.4 MAGNESIUM

Optimal level of Magnesium for human consumption ranges 300-400 mg/day. The main source of Magnesium includes cereals, beans, leafy vegetables and fish. The normal serum level of Magnesium is 1.8 to 2.2. mg/dl.

11.4.1 Functions of Magnesium

(a) Irritability of neuromuscular tissues is lowered by Magnesium

(b) Magnesium deficiency leads to decrease in Insulin dependent uptake of glucose

(c) Magnesium supplementation improves glucose tolerance

Decrease in Magnesium content leads to the condition called as hypomagnesemia. It causes increase in urinary loss. Causes such as liver cirrhosis, protein calorie malnutrition and hypo para thyroidism leads to hypomagnesemia. Increase in the level of Magnesium is called as hypermagnesemia. The main causes of hypermagnesemia includes renal failure, hyper para thyroidism, rickets, oxalate poisoning and multiple myeloma.

INTEXT QUESTIONS 11.3

1. Optimal level of ..... for human consumption ranges 300-400 mg/day.
2. Irritability of neuromuscular tissues is lowered by .....
3. Magnesium supplementation improves ..... tolerance

   (a) lactose (b) glucose (c) toxin (d) microbial

11.5 IRON

Total body content of iron is 3 to 5 gm out of which 75 % is recorded in blood and rest of them are recorded from liver, spleen, bone marrow and muscle. The normal limit for iron consumption is 20 mg/day for adults, 20-30 mg/day for children and 40 mg/day for pregnant women. The main source of iron is jaggery. Other source of iron includes leafy vegetables and meat etc., Milk is considered as a poor source of iron.

11.5.1 Factors influencing absorption of iron

Iron is absorbed by upper part of duodenum and is affected by various factors

(a) Only reduced form of iron (ferrous) is absorbed and ferric form are not absorbed

Notes

(b) Ascorbic acid (Vitamin C) increases the absorption of iron

(c) The interfering substances such as phytic acid and oxalic acid decreases absorption of iron

11.5.2 Regulation of absorption of Iron

Absorption of iron is regulated by three main mechanisms, which includes

(a) Mucosal Regulation

(b) Storer regulation

(c) Erythropoietic regulation

In mucosal regulation absorption of iron requires DM-1 and ferroportin. Both the proteins are down regulated by hepcidin secreted by liver. The above regulation occurs when the body irons reserves are adequate. When the body iron content gets felled, storer regulation takes place. In storer regulation the mucosal is signaled for increase in iron absorption. The erythropoietic regulation occurs in response to anemia. Here the erythroid cells will signal the mucosa to increase the iron absorption.

11.5.3 Iron transport in blood

The transport form of iron in blood is transferin. Transferin are glycoprotein secreted by liver. In blood, the ceruloplasmin is the ferroxidase which oxidizes ferrous to ferric state.

$$\text{Apo-Transferin} + 2Fe^{2+} \longrightarrow \text{Transferin combined with } 2Fe^{3+}$$

Storage form of iron is ferritin. Almost no iron is excreted through urine. Feces contains iron as well as iron trapped in the intestine cells.

11.5.4 Anemia

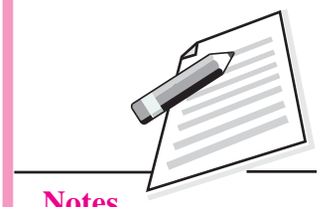
Anemia is the most common nutritional deficiency disease. The microscopic appearance of anemia is characterized by microcytic hypochromic anemia. Iron toxicity or excess of iron is called as hemosiderosis. Iron toxicity occurs due to repeated blood transfusion. The abnormal gene responsible for hemosiderosis is located on the short arm of chromosome No.6. The main causes of iron deficiency or anemia are

(a) Nutritional deficiency of iron

(b) Lack of iron absorption

(c) Hook worm infection

(d) Repeated pregnancy



(e) Chronic blood loss

(f) Nephrosis

(g) Lead poisoning

INTEXT QUESTION 11.4

1. Match the following

Adults	20 - 30 mg/day
Children	40 mg/day
Pregnant women	20 mg/day

2. ..... is the most common nutritional deficiency disease
3. The transport form of iron in blood is .....

11.6 COPPER

Total human body contains about 100 mg of copper and are encountered in muscle, liver, bone marrow, brain, kidney, heart and in hairs. Enzymes such as cytochrome oxidase, tyrosinase, lysyl oxidase, allanine synthase, monoamine oxidase, superoxide dismutase and phenol oxidase contains copper. The normal copper limit for human are 1.5 to 3 mg/day. The main sources of copper are cereals, meat, liver, nuts and green leafy vegetables. From the total dietary copper only 10% are absorbed. Copper are mainly excreated through bile. The normal serum level of copper is 25 to 50 mg/dl.

11.6.1 Functions of copper

(a) Copper is necessary for iron absorption and incorporation of iron into hemoglobin.

(b) It is very essential for tyrosinase activity

(c) It is the co-factor for vitamin C requiring hydroxylation

(d) Copper increases the level of high density lipo protein and protects the heart

11.6.2 Abnormal metabolism of copper

The abnormal metabolism of copper leads to Wilson's disease and Menke's kidney hair syndrome.

(a) Wilson's disease

In case of Wilson's disease ceruloplasmin level in blood is drastically reduced. The incidence of Wilson's disease is noticed for 1 in 50,000 populations. The

Notes

defect in a gene encoding copper binding ATPase of liver cells leads to Wilson's disease. Wilson's disease leads to

(i) Accumulation of copper in liver leads to hepatocellular degeneration and cirrhosis

(ii) Deposition of copper in brain basal ganglia leads to leticular degeneration

(iii) Copper deposits as green pigmented ring around cornea and the condition is called as Kayser-Kleischer ring

Over accumulation of copper can be treated by consumption of diet containg low copper and injection of D-penicillamine, which excretes copper through urine.

(b) Menke's kidney hair syndrome

It is X-linked defect. In this condition copper is absorbed by GI tract, but cannot be transported to blood. The defect in transport of copper to blood is due to absence of an intracellular copper binding ATPase, which is due to mutation in gene encoding them.

INTEXT QUESTIONS 11.5

1. Total human body conatins about ..... mg of copper

   (a) 100 (b) 200 (c) 300 (d) 400

2. Menke's kidney hair syndrome is ..... linked defect.

3. Deposition of copper in ..... leads to leticular degeneration

11.7 ZINC

The daily requirement of Zinc for human consumption ranges 10mg /day. The major sources of Zinc includes grains, beans, nuts cheese, meat and shellfish. The normal serum level of Zinc in human is 100mg/day. In the human total body the content of Zinc is 2gm, out of which 60 % is encountered in skeletal muscle and 30% in bones highest concentration is seen in hippocampus area of brain and prostatic secretion.

More than 300 enzymes in human body are zinc-dependent; some of them are carboxypeptidase, carbonic anhydrase, alkaline phosphatase, lactate dehydrogenase, alcohol dehydragenase. The enzyme RNA polymerase, which is required for transcription, contains zinc and it is essential for protein bio synthesis.

Deficiency in Zinc leads to poor wound healing, lesions of skin impaired spermatogenesis, hyperkeratosis, dermatitis and alopecia. Consumption of more than 1000 mg /day leads to zinc toxicity. Zinc toxicity leads to gastric ulcer, pancreatitis, anemia, nausea and vomiting.

INTEXT QUESTIONS 11.6

1. ..... and ..... are major source of Zinc

2. ..... number of enzymes are zinc dependent

   (a) 100 (b) 300 (c) 500 (d) 50

3. The normal serum level of Zinc in human is ..... mg/day

11.8 FLUORIDE

Fluoride is well known for their protective effect on caries. The safe limit of fluorine is about 1PPM in water. But excess of fluoride causes Flourosis. Flourosis is more dangerous than caries. When Fluoride content is more than 2 PPM, it will cause chronic intestinal upset, gastroenteritis, loss of weight, osteosclerosis, stratification and discoloration of teeth. In India Flourosis is widespread in Punjab, Rajasthan, Delhi and Tamilnadu. Fluoride rich source includes sea fish, cheese, tea and jowar.

Flourosis could be prevented by providing and consumption of fluoride free water, Further supplementation of vitamin c and usage of toothpaste containing regulated level of Fluoride could prevent Fluorosis.

INTEXT QUESTIONS 11.7

1. ..... is well known for caries protection.

   (a) calcium (b) fluoride (c) iron (d) Magnesium

2. Fluoride rich source includes .....

11.9 SELENIUM

The normal limit of Selenium for human consumption is 50-100 mg/day and the normal serum level is 50-100 mg/day. Selenium dependent enzymes include glutathione Peroxidase and 5-de-iodinase. Selenium concentration in testis is the

Notes

highest in adult. It is very necessary for normal development and maturation of sperm.

Selenium toxicity could be termed as selenosis. The toxicity symptoms include hair loss, falling of nails, diarrhea, weight loss and garlicky odor in breath.

INTEXT QUESTIONS 11.8

1. The normal limit of Selenium for human consumption is ..... mg/day
2. Glutathione peroxides is a ..... dependent enzyme

11.10 MANGANESE

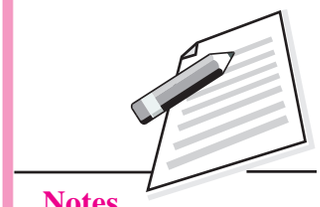
The normal limit of Manganese for human is 5mg/day. The major source of Manganese are nuts. In metabolism, its absorption is inhibited by iron. In blood, it binds with transmanganin and excreted through bile.

INTEXT QUESTIONS 11.9

1. The major source of Manganese is .....
2. Manganese are excreted through .....

WHAT HAVE YOU LEARNT

- In this lesson you have learnt about the importance and types of clinically significant mineral elements.
- Mineral elements are solid crystalline, chemical elements that cannot be decomposed and synthesized by ordinary chemical reactions.
- They are present in both plants and animals to execute specific functions and the amount to be required is largely determined by certain inherent factors.
- Minerals although nutritionally required in smaller amounts are essential for maintenance and production purposes. However the major and trace minerals are needed by the body in large and small amount respectively.
- Generally, deficiencies of most minerals are shown by a reduced appetite and production, slow growth and occasionally death.



TERMINAL QUESTIONS

1. Write a short note on minerals?
2. What are the elements of major and minor minerals?
3. Write a note on calcium and its finctions?
4. What are the functions of phosphorous?
5. Narrate the importance of iron?

ANSWERS TO INTEXT QUESTIONS

11.1

1. 1 to 1.5 kg
2. Ca
3. Vitamin D
4. Hypercalcemia

11.2

1. 80 %
2. 3-4
3. Phosphorus

11.3

1. 300 to 400
2. Magnesium
3. Glucose

11.4

1. Adults 20 mg/day
   Children 20 – 30 mg/day
   Pregnant women 40 mg/day
2. Anemia
3. Transferin

Notes

11.5

1. 100
2. X-linked
3. Brain basal ganglia

11.6

1. Beans and nuts
2. 300
3. 10

11.7

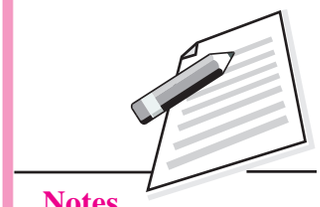
1. Fluoride
2. Fish

11.8

1. 50-100
2. Selenium

11.9

1. Nuts
2. Bile



Notes

12

HORMONES

12.1 INTRODUCTION

A hormone is a chemical that acts as a messenger transmitting a signal from one cell to another. When it binds to another cell which is the target of the message, the hormone can alter several aspects of cell function, including cell growth, metabolism, or other function. Hormones can be classified according to chemical composition, solubility properties, location of receptors, and the nature of the signal used to mediate hormonal action within the cell. Hormones that bind to the surfaces of cells communicate with intracellular metabolic processes through intermediary molecules called second messengers (the hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction. The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP. This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones. To date, only one hormone, atrial natriuretic factor (ANF), uses cGMP as its second messenger.

OBJECTIVES

After reading this lesson, you will be able to

- explain the characterizing hormone
- describe functional importance of hormones

12.2 CHARACTERIZING HORMONE

The first way of characterizing a hormone is by looking at the distance over which the hormone acts. Hormones can be classified on three primary ways as following:

Notes

- Autocrine: An autocrine hormone is one that acts on the same cell that released it.
- Paracrine: A paracrine hormone is one that acts on cells which are nearby relative to the cell which released it. An example of paracrine hormones includes growth factors, which are proteins that stimulate cellular proliferation and differentiation. Specifically, consider the binding of white blood cells to T cells. When the white blood cell binds to a T cell, it releases a protein growth factor called interleukin-1. This causes the T cell to proliferate and differentiate.
- Endocrine: An endocrine hormone is one that is released into the bloodstream by endocrine glands. The receptor cells are distant from the source. An example of an endocrine hormone is insulin, which is released by the pancreas into the bloodstream where it regulates glucose uptake by liver and muscle cells.

There are three major classifications one should be aware of:

- Steroids: Steroid hormones are for the most part derivatives of cholesterol.
- Amino acid derivatives: Several hormones (and neurotransmitters) are derived from amino acids.
- Polypeptides: Many hormones are chains of amino acids.

12.3 FUNCTIONAL IMPORTANCE OF HORMONES

12.3.1 Insulin

Insulin is a polypeptide hormone synthesized in the pancreas by β-cells, which construct a single chain molecule called proinsulin. Enzymes excise a portion of the proinsulin molecule called the C peptide, producing the actual insulin molecule. When in demand, the β-cells will release insulin together with the c peptide into the blood stream via exocytosis. The role of insulin in the body is well known, with its primary role being to control the uptake of glucose by liver and muscle cells and also the storage of glucose in the form of glycogen.

Diabetes results from a lack of insulin secretion by the pancreas. Without insulin, cells take up glucose very slowly. The lack of insulin results in an inability to use blood glucose for fuel. Insulin is a signal for high blood glucose levels and increases glucose transport into cells. It stimulates synthesis of glycogen, fat, and protein. It inhibits breakdown of glycogen, fat, and protein. Insulin, secreted by the β-cells of the pancreas in response to rising blood glucose levels, is a signal that glucose is abundant. Insulin binds to a specific receptor on the cell surface and exerts its metabolic effect by a signaling pathway that involves a receptor tyrosine kinase phosphorylation cascade. Note that insulin stimulates storage processes and at the same time inhibits degradative pathways.

12.3.1.1 The pancreas secretes insulin or glucagon in response to changes in blood glucose

When glucose enters the bloodstream from the intestine after a carbohydrate-rich meal, the resulting increase in blood glucose causes increased secretion of insulin (and decreased secretion of glucagon). Insulin release by the pancreas is largely regulated by the level of glucose in the blood supplied to the pancreas. The peptide hormones insulin, glucagon, and somatostatin are produced by clusters of specialized pancreatic cells, the islets of Langerhans. Each cell type of the islets produces a single hormone: α-cells produce glucagon; β-cells, insulin; and δ-cells, somatostatin.

12.3.1.2 Insulin secretion

When blood glucose rises, GLUT2 transporters carry glucose into the b-cells, where it is immediately converted to glucose 6-phosphate by hexokinase IV (glucokinase) and enters glycolysis. The increased rate of glucose catabolism raises [ATP], causing the closing of ATP-gated K^+ channels in the plasma membrane. Reduced efflux of K^+ depolarizes the membrane, thereby opening voltage-sensitive Ca^{2+} channels in the plasma membrane. The resulting influx of Ca^{2+} triggers the release of insulin by exocytosis. Stimuli from the parasympathetic and sympathetic nervous systems also stimulate and inhibit insulin release, respectively. Insulin lowers blood glucose by stimulating glucose uptake by the tissues; the reduced blood glucose is detected by the β-cell as a diminished flux through the hexokinase reaction; this slows or stops the release of insulin. This feedback regulation holds blood glucose concentration nearly constant despite large fluctuations in dietary intake.

12.3.1.3 Insulin counters high blood glucose

Insulin stimulates glucose uptake by muscle and adipose tissue, where the glucose is converted to glucose 6-phosphate. In the liver, insulin also activates glycogen synthase and inactivates glycogen phosphorylase, so that much of the glucose 6-phosphate is channelled into glycogen. Insulin also stimulates the storage of excess fuel as fat. In the liver, insulin activates both the oxidation of glucose 6-phosphate to pyruvate via glycolysis and the oxidation of pyruvate to acetyl-CoA. If not oxidized further for energy production, this acetyl- CoA is used for fatty acid synthesis in the liver, and the fatty acids are exported as the triglyerides to the adipose tissue. These fatty acids are ultimately derived from the excess glucose taken up from the blood by the liver. In summary, the effect of insulin is to favor the conversion of excess blood glucose to two storage forms: glycogen (in the liver and muscle) and triacylglycerols (in adipose tissue).

Notes

12.3.1.4 Diabetes Mellitus arises from defects in insulin production or action

Diabetes mellitus, caused by a deficiency in the secretion or action of insulin, is a relatively common disease. There are two major clinical classes of diabetes mellitus: type I diabetes, or insulin-dependent diabetes mellitus (IDDM), and type II diabetes, or non-insulin-dependent diabetes mellitus (NIDDM), also called insulin-resistant diabetes. In type I diabetes, the disease begins early in life and quickly becomes severe. This disease responds to insulin injection, because the metabolic defect stems from the pancreatic β-cells and a consequent inability to produce sufficient insulin. IDDM requires insulin therapy and careful, lifelong control of the balance between dietary intake and insulin dose. Characteristic symptoms of type I (and type II) diabetes are excessive thirst and frequent urination (polyuria), leading to the intake of large volumes of water (polydipsia) ("diabetes mellitus" means "excessive excretion of sweet urine"). These symptoms are due to the excretion of large amounts of glucose in the urine, a condition known as glucosuria. Type II diabetes is slow to develop (typically in older, obese individuals), and the symptoms are milder and often go unrecognized at first. This is really a group of diseases in which the regulatory activity of insulin is defective: insulin is produced, but some feature of the insulin-response system is defective. These individuals are insulin-resistant. Individuals with either type of diabetes are unable to take up glucose efficiently from the blood; recall that insulin triggers the movement of GLUT4 glucose transporters to the plasma membrane of muscle and adipose tissue. Another characteristic metabolic change in diabetes is excessive but incomplete oxidation of fatty acids in the liver. Insulin secretion reflects both the size of fat reserves (adiposity) and the current energy balance (blood glucose level). Insulin acts on insulin receptors in the hypothalamus to inhibit eating. Insulin also signals muscle, liver, and adipose tissues to increase catabolic reactions, including fat oxidation, which results in weight loss.

12.3.2 Glucagon

Glucagon, a peptide hormone synthesized and secreted from the α-cells of the islets of Langerhans of pancreas, raises blood glucose levels. Its effect is opposite that of insulin, which lowers blood glucose levels. The pancreas releases glucagon when blood sugar (glucose) levels fall too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues. Thus, glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level.

12.3.2.1 Regulation and function

Secretion of glucagon is stimulated by hypoglycemia, epinephrine, arginine, alanine, acetylcholine, and cholecystokinin. Secretion of glucagon is inhibited by somatostatin, insulin, increased free fatty acids and keto acids into the blood, and increased urea production. Glucose is stored in the liver in the form of glycogen, which is a starch-like polymer chain made up of glucose molecules. Liver cells (hepatocytes) have glucagon receptors. When glucagon binds to the glucagon receptors, the liver cells convert the glycogen polymer into individual glucose molecules, and release them into the bloodstream, in a process known as glycogenolysis. As these stores become depleted, glucagon then encourages the liver and kidney to synthesize additional glucose by gluconeogenesis. Glucagon turns off glycolysis in the liver, causing glycolytic intermediates to be shuttled to gluconeogenesis.

Glucagon is a signal for low blood glucose levels. It stimulates breakdown of glycogen, fat, and protein. It inhibits synthesis of glycogen, fat, and protein. Several hours after the intake of dietary carbohydrate, blood glucose levels fall slightly because of the ongoing oxidation of glucose by the brain and other tissues. Although its primary target is the liver, glucagon (like epinephrine) also affects adipose tissue, activating TAG breakdown by activating triacylglycerol lipase and liberates free fatty acids, which are exported to the liver and other tissues as fuel, sparing glucose for the brain. The net effect of glucagon is therefore to stimulate glucose synthesis and release by the liver and to mobilize fatty acids from adipose tissue, to be used instead of glucose as fuel for tissues other than the brain.

12.3.2.2 During fasting and starvation, metabolism shifts to provide fuel for the brain

The fuel reserves of a healthy adult human are of three types: glycogen stored in the liver and, in relatively small quantities, in muscles; large quantities of triacylglycerols in adipose tissues; and tissue proteins, which can be degraded when necessary to provide fuel. In the first few hours after a meal, the blood glucose level is diminished slightly, and tissues receive glucose released from liver glycogen. There is little or no synthesis of lipids. By 24 hours after a meal, blood glucose has fallen further, insulin secretion has slowed, and glucagon secretion has increased.

12.3.3 Thyroid Hormones

Thyroid hormones (T_4 and T_3) are tyrosine-based hormones produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland, are primarily responsible for

Notes

regulation of metabolism. Iodine is necessary for the production of T_3 (triiodothyronine) and T_4 (thyroxine). A deficiency of iodine leads to decreased production of T_3 and T_4, enlarges the thyroid tissue and will cause the disease known as goitre. The thyronines act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline). The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyroid hormone leads to heat generation in humans. However, the thyronamines function via some unknown mechanism to inhibit neuronal activity; this plays an important role in the hibernation cycles of mammals and the moulting behaviour of birds.

12.3.3.1 Iodine is essential for thyroid hormone synthesis

Iodide is actively absorbed from the bloodstream by a process called iodide trapping. In this process, sodium is cotransported with iodide from the basolateral side of the membrane into the cell and then concentrated in the thyroid follicles to about thirty times its concentration in the blood. Via a reaction with the enzyme thyroperoxidase, iodine is bound to tyrosine residues in the thyroglobulin molecules, forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). Linking two moieties of DIT produces thyroxine. Combining one particle of MIT and one particle of DIT produces triiodothyronine. If there is a deficiency of dietary iodine, the thyroid will not be able to make thyroid hormone. The lack of thyroid hormone will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid-stimulating hormone, which causes the thyroid to enlarge (the resulting medical condition is called endemic colloid goiter).

12.3.3.2 Thyroid hormones are transported by Thyroid-Binding Globulin

Thyroxinebinding globulin (TBG), a glycoprotein binds T4 and T3 and has the capacity to bind 20 µg/dL of plasma. Under normal circumstances, TBG binds – noncovalently – nearly all of the T4 and T3 in plasma, and it binds T4 with greater affinity than T3. The small, unbound (free) fraction is responsible for the biologic activity. Thus, in spite of the great difference in total amount, the free fraction of T3 approximates that of T4, and given that T3 is intrinsically more active than T4, most biologic activity is attributed to T3. TBG does not bind any other hormones.

12.3.3.3 Circulation and transport

Most of the thyroid hormone circulating in the blood is bound to transport proteins. Only a very small fraction of the circulating hormone is free (unbound) and biologically active, hence measuring concentrations of free thyroid hormones is of great diagnostic value.

When thyroid hormone is bound, it is not active, so the amount of free T_3/T_4 is what is important. For this reason, measuring total thyroxine in the blood can be misleading.

12.3.3.4 Related diseases

Both excess and deficiency of thyroxine can cause disorders.

- Hyperthyroidism (an example is Graves Disease) is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. It is a common disorder that affects approximately 2% of women and 0.2% of men.
- Hypothyroidism (an example is Hashimoto's thyroiditis) is the case where there is a deficiency of thyroxine, triiodiothyronine, or both.

12.3.4 Parathyroid Hormone

Parathyroid hormone (PTH), parathormone or parathyrin, is secreted by the chief cells of the parathyroid glands. It acts to increase the concentration of calcium (Ca^{2+}) in the blood, whereas calcitonin (a hormone produced by the parafollicular cells of the thyroid gland) acts to decrease calcium concentration. PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone 1 receptor (high levels in bone and kidney) and the parathyroid hormone 2 receptor (high levels in the central nervous system, pancreas, testis, and placenta). PTH was one of the first hormones to be shown to use the G-protein, adenylyl cyclase second messenger system. Parathyroid hormone regulates serum calcium through its effects (Table 12.1).

Table 12.1: Effect of parathyroid hormone in regulation of serum calcium.

Region	Effect
Bone	PTH enhances the release of calcium from the large reservoir contained in the bones. Bone resorption is the normal destruction of bone by osteoclasts, which are indirectly stimulated by PTH forming new osteoclasts, which ultimately enhances bone resorption.

Notes

Kidney	PTH enhances active reabsorption of calcium and magnesium from distal tubules of kidney. As bone is degraded, both calcium and phosphate are released. It also decreases the reabsorption of phosphate, with a net loss in plasma phosphate concentration. When the calcium:phosphate ratio increases, more calcium is free in the circulation.
Intestine	PTH enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D. Vitamin D activation occurs in the kidney. PTH converts vitamin D to its active form (1,25-dihydroxy vitamin D). This activated form of vitamin D increases the absorption of calcium (as Ca^{2+} ions) by the intestine via calbindin.

12.3.4.1 Regulation of PTH secretion

Secretion of parathyroid hormone is controlled chiefly by serum [Ca^{2+}] through negative feedback. Calcium-sensing receptors located on parathyroid cells are activated when [Ca^{2+}] is low. Hypomagnesemia inhibits PTH secretion and also causes resistance to PTH, leading to a form of hypoparathyroidism that is reversible. Hypermagnesemia also results in inhibition of PTH secretion. Stimulators of PTH includes decreased serum [Ca^{2+}], mild decreases in serum [Mg^{2+}], and an increase in serum phosphate. Inhibitors include increased serum [Ca^{2+}], severe decreases in serum [Mg^{2+}], which also produces symptoms of hypoparathyroidism (such as hypocalcemia), and calcitriol.

12.3.4.2 Clinical significance

- Hyperparathyroidism, the presence in the blood of excessive amounts of parathyroid hormone, occurs in two very distinct sets of circumstances. Primary hyperparathyroidism is due to autonomous, abnormal hypersecretion of PTH in the parathyroid gland while secondary hyperparathyroidism is an appropriately high PTH level seen as a physiological response to hypocalcemia
- A low level of PTH in the blood is known as hypoparathyroidism and is most commonly due to damage to or removal of parathyroid glands during thyroid surgery.
- There are a number of rare but well-described genetic conditions affecting parathyroid hormone metabolism, including pseudohypoparathyroidism, familial hypocalciuric hypercalcaemia, and autosomal dominant hypercalciuric hypocalcaemia.

12.3.5 Growth hormone

Growth hormone (GH or HGH), also known as somatotropin or somatropin, is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans. It is a type of mitogen which is specific only to certain kinds of cells. Growth hormone is a single-chain polypeptide that is synthesized, stored, and secreted by somatotropic cells within the lateral wings of the anterior pituitary gland. The anterior pituitary gland secretes hormones that tend to elevate the blood glucose and therefore antagonize the action of insulin. Growth hormone secretion is stimulated by hypoglycemia; it decreases glucose uptake in muscle. Some of this effect may not be direct, since it stimulates mobilization of free fatty acids from adipose tissue, which themselves inhibit glucose utilization. Growth hormone increases amino acid transport in all cells, and estrogens do this in the uterus. A deficiency of this hormone produces dwarfism, and an excess leads to gigantism.

12.3.5.1 Regulation of growth hormone secretion

Secretion of growth hormone (GH) in the pituitary is regulated by the neurosecretory nuclei of the hypothalamus. These cells release the peptides Growth hormone-releasing hormone (GHRH or somatocrinin) and Growth hormone-inhibiting hormone (GHIH or somatostatin) into the hypophyseal portal venous blood surrounding the pituitary. GH release in the pituitary is primarily determined by the balance of these two peptides, which in turn is affected by many physiological stimulators (e.g., exercise, nutrition, sleep) and inhibitors (e.g., free fatty acids) of GH secretion. A number of factors are known to affect GH secretion, such as age, sex, diet, exercise, stress, and other hormones.

12.3.5.2 Regulation

Stimulators of growth hormone (GH) secretion include peptide hormones, ghrelin, sex hormones, hypoglycemia, deep sleep, niacin, fasting, and vigorous exercise. Inhibitors of GH secretion include somatostatin, circulating concentrations of GH and IGF-1 (negative feedback on the pituitary and hypothalamus), hyperglycemia, glucocorticoids, and dihydrotestosterone.

12.3.5.3 Effects of growth hormone

Increased height during childhood is the most widely known effect of GH. In addition to increasing height in children and adolescents, growth hormone has many other effects on the body such as:

Notes

- Increases calcium retention, and strengthens and increases the mineralization of bone
- Increases muscle mass through sarcomere hypertrophy
- Promotes lipolysis
- Increases protein synthesis
- Stimulates the growth of all internal organs excluding the brain
- Plays a role in homeostasis
- Reduces liver uptake of glucose
- Promotes gluconeogenesis in the liver
- Contributes to the maintenance and function of pancreatic islets
- Stimulates the immune system

12.3.5.4 Clinical significance

12.3.5.4.1 Excess

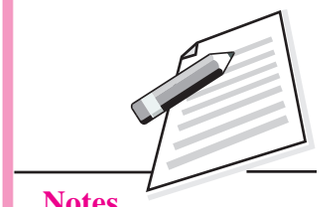
The most common disease of GH excess is a pituitary tumor composed of somatotroph cells of the anterior pituitary. These somatotroph adenomas are benign and grow slowly, gradually producing more and more GH. For years, the principal clinical problems are those of GH excess. Eventually, the adenoma may become large enough to cause headaches, impair vision by pressure on the optic nerves, or cause deficiency of other pituitary hormones by displacement.

Prolonged GH excess thickens the bones of the jaw, fingers and toes. Resulting heaviness of the jaw and increased size of digits is referred to as acromegaly. Accompanying problems can include sweating, pressure on nerves (e.g., carpal tunnel syndrome), muscle weakness, excess sex hormone-binding globulin (SHBG), insulin resistance or even a rare form of type 2 diabetes, and reduced sexual function.

GH-secreting tumors are typically recognized in the fifth decade of life. It is extremely rare for such a tumor to occur in childhood, but, when it does, the excessive GH can cause excessive growth, traditionally referred to as pituitary gigantism.

12.3.5.4.2 Deficiency

The effects of growth hormone deficiency vary depending on the age at which they occur. In children, growth failure and short stature are the major manifestations of GH deficiency, with common causes including genetic conditions and congenital malformations. It can also cause delayed sexual



maturity. In adults, deficiency is rare, with the most common cause a pituitary adenoma, and others including a continuation of a childhood problem, other structural lesions or trauma, and very rarely idiopathic GHD. Adults with GHD "tend to have a relative increase in fat mass and a relative decrease in muscle mass and, in many instances, decreased energy and quality of life".

INTEXT QUESTIONS 12.1

I. Choose the best answer

1. The hormone that is released into the bloodstream having the receptor cells at distant from the source is

   (a) Exocrine (b) Paracrine

   (c) Endocrine (d) None

2. This hormone is a chain of amino acids

   (a) Thyroxine (b) Insulin

   (c) Vitamin D_3 (d) Epinephrin

3. Glucose transporter to the plasma membrane of muscle and adipose tissue is

   (a) GLUT1 (b) GLUT2

   (c) GLUT3 (d) GLUT4

4. The storage form of lipid in adipose tissue is

   (a) Triacylglycerol (b) Free fatty acid

   (c) Low density lipoprotein (d) Phospholipid

5. Excess of somatotropin leads to

   (a) Dwarfism (b) Gigantism

   (c) Goitre (d) Diabetes Mellitus

II. Fill in the blanks

6. Hormones communicate with intracellular metabolic processes through intermediary molecules called .....
7. ..... transporters carry glucose into the b-cells of pancreas
8. ..... stimulates breakdown of glycogen, fat, and protein.
9. Thyroid hormones are transported by .....
10. Vitamin D3 increases the absorption of calcium (as Ca^{2+} ions) by the intestine via .....

Notes

III. Match the following

11. Frequent urination	(a) Insulin
12. Endocrine hormone	(b) Glucokinase
13. Second messenger	(c) Hypothyroidism
14. Hashimoto's thyroiditis	(d) cAMP
15. Hexokinase IV	(e) Polyuria

WHAT YOU HAVE LEARNT

- A hormone acts as a messenger by transmitting signal from one cell to another.
- Hormones are derived from steroids, amino acids and polypeptides.
- Insulin secreted from β-cells of pancreas controls the uptake of glucose by liver and muscle cells and also the storage of glucose in the form of glycogen.
- Diabetes Mellitus results from the lack of insulin secretion by the pancreas.
- Glucagon, a peptide hormone synthesized and secreted from the α-cells of pancreas, raises blood glucose levels.
- Secretion of glucagon is stimulated by hypoglycemia, epinephrine, arginine, alanine, acetylcholine, and cholecystokinin. Inhibited by somatostatin, insulin, increased free fatty acids and keto acids into the blood, and increased urea production.
- Thyroid hormones (T_4 and T_3) are tyrosine-based hormones produced by the follicular cells of the thyroid gland.
- The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism.
- The free thyroid hormones in the circulation are the active form, and is of great diagnostic value.
- The diseases associated with the excess and deficiency of thyroid hormones are Graves disease and Hashimoto's thyroiditis, respectively.
- Parathyroid hormone (PTH), parathormone or parathyrin, is secreted by the chief cells of the parathyroid glands.
- PTH acts to increase the concentration of calcium (Ca^{2+}) in the blood.

- Growth hormone (GH or HGH), also known as somatotropin or somatropin, is secreted from anterior pituitary gland, is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans.
- A deficiency of this hormone produces dwarfism, and an excess leads to gigantism.

TERMINAL QUESTIONS

1. Classify Hormones
2. Write short notes on

   Insulin

   Thyroid Hormone

   Growth Hormone

ANSWERS TO INTEXT QUESTIONS

I. 1. (c) 2. (b) 3. (d) 4. (a) 5. (b)

II. 6. second messengers
7. GLUT2
8. Glucagon
9. Thyroxine binding globulin
10. calbindin,

III. 11. (e) 12. (a) 13. (d) 14. (c) 15. (b)

Notes

13

CLINICAL BIOCHEMISTRY

13.1 INTRODUCTION

Clinical Biochemistry deals with the study of biochemical events or parameters in the body.

OBJECTIVES

After reading this lesson, you will be able to:

- describe clinically important enzymes
- explain liver and renal functional test
- explain specimen collection & other pre-analytical variables.
- describe general laboratory techniques, procedures & safety measure

13.2 CLINICALLY IMPORTANT ENZYMES

Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiologic function in the blood. Examples of these **functional plasma enzymes** include lipoprotein lipase, pseudocholinesterase, and the proenzymes of blood coagulation and blood clot dissolution. The majority of these enzymes are synthesized in and secreted by the liver.

Enzymes	Principle Sources of Enzymes in Blood	Clinical Applications
Alanine aminotransferase	Liver	Hepatic parenchymal diseases

Alkaline phosphatase	Liver, bone, intestinal mucosa, placenta	Bone diseases, hepatobiliary diseases
Amylase	Salivary glands, pancreas	Pancreatic diseases
Aspartate aminotransferase	Liver, skeletal muscle, heart erythrocytes	Hepatic parenchymal disease, muscle disease
Cholinesterase	Liver	Organophosphorus insecticide, hepatic parenchymaldisease
Creatine kinase	Skeletal muscle, heart	Muscle diseases
γ- glutamyl transferase	Liver	Hepatobiliary diseases, marker of alcohol abuse.
Lactate dehydrogenase	Heart, liver, skeletal muscle, erythrocytes, platelets, lymph nodes	Hemolysis, hepatic parenchymal diseases, tumor marker
Lipase	Pancreas	Pancreatic disesases
5'- nucleotidase	Liver	Hepatobiliary diseases
Trypsin (Serine protease)	Pancreas	Pancreatic diseases

Alanine aminotransferase (ALT)

Clinical applications of ALT assays are mainly used for the evaluation of hepatic disorders. Higher elevations are found in hepatocellular disorders than in extrahepatic or intrahepatic obstructive disorders. In acute inflammatory conditions of the liver, ALT elevations are frequently higher than those of AST and tend to remain longer half-life of ALT in serum (16 hours and 24 hours, respectively). ALT levels have historically been compared with levels of AST to help determine the source of an elevated AST level and to detect liver involvement with myocardial injury.

Normal values of ALT:

Male: <45 U/L = <0.77 μkat/L

Female: <34 U/L = <0.58 μkat/L

Notes

Aspartate aminotransferase (AST)

The clinical use of AST is limited mainly to the evaluation of hepatocellular disorders and skeletal muscle involvement. In Acute myocardial infarction (AMI), AST levels begin to rise within 6-8 hours, peak at 24 hours, and generally return to normal within 5 days. However, because of the wide tissue distribution, AST levels are not useful in the diagnosis of AMI.

AST elevations are frequently seen in pulmonary embolism. Following congestive heart failure, AST levels also may be increased, probably reflecting liver involvement as a result of inadequate blood supply to that organ. AST levels are highest in acute hepatocellular disorders. Skeletal muscle disorders, such as the muscular dystrophies, and inflammatory conditions also cause increases in AST levels.

Normal values of AST:

Male: <35U/L = <0.60 µkat/L

Female: <31 U/L = <0.53 µkat/L

α-Amylase

It is an enzyme of the hydrolyase class that catalyzes the hydrolysis of 1,4-α-glycosidic linkages in polysaccharides. AMYs are calcium metaloenzymes, with the calcium absolutely required for functional integrity. AMYs normally occurring in human plasma are small molecules with molecular weights varying from 54 to 62 kDa. The enzyme is thus small enough to pass the glumeruli of the kidneys and AMY is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY) origin. If the plasma amylase activity fails to fall after an attack of acute pancreatitis there may be leakage of pancreatic fluid into the lesser sac (a pancreatic pseudocyst). Urinary amylase levels are high, differentiating it from macroamylasaemia. This is one of the few indications for estimating urinary amylase activity, which is inappropriately low relative to the plasma activity if there is glomerular impairment or macroamylasaemia.

Normal values of amylase: 28-100 U/L = 0.48-1.7 µkat/L

Lipase

It is a single–chain glycoprotein with molecular weight of 48 kDa. For full catalytic activity and greatest specificity the presence of bile salts and a cofactor called colipase, which is secreted by the pancreas, is required. LPS is a small molecule and is filtered through the glomerulus. It is totally reabsorbed by the

renal tubules, and it is not normally detected in urine. Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.

Normal values: 40-200 U/L

Gamma-glutamyl-transferase

It catalyzes the transfer of the γ–glutamyl group from peptides and compounds that contain it to an acceptor Gamma-glutamyl transferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

Causes of raised plasma GGT activity

- Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
- Hepatocellular damage, such as that due to infectious hepatitis: A patient should never be labeled an alcoholic because of a high plasma GGT activity alone.

Normal values for GGT

Male: <55 U/L = <0.94 µkat/L

Female: <38 U/L = <0.65 µkat/L

Lactate Dehydrogenase (LD)

It catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage.

It is increased in plasma in Myocardial Injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of isoenzymes in more useful in clinical diagnosis between hepatic disease and M.I.

Normal range of total LDH: 180-360 U/L= 3.1-6.1 µkat/L.

13.3 LIVER FUNCTION TESTS

Definition

Liver function tests, or LFTs, include tests that are routinely measured in all clinical laboratories. LFTs include bilirubin, a compound formed by the breakdown of hemoglobin; ammonia, a breakdown product of protein that is

Notes

normally converted into urea by the liver before being excreted by the kidneys; proteins that are made by the liver including total protein, albumin, prothrombin, and fibrinogen; cholesterol and triglycerides, which are made and excreted via the liver; and the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH).

Other liver function tests include serological tests (to demonstrate antibodies) and DNA tests for hepatitis and other viruses; and tests for antimitochondrial and smooth muscle antibodies, transthyretin (prealbumin), protein electrophoresis, bile acids, alpha-fetoprotein, and a constellation of other enzymes that help differentiate necrotic (characterized by death of tissues) versus obstructive liver disease.

The hepatic function panel evaluates:

Alanine aminotransferase (ALT)

Alkaline phosphatase (ALP)

Aspartate aminotransferase (AST)

Total bilirubin and direct bilirubin. Bilirubin is a byproduct of the normal breakdown of red blood cells. It usually passes through the liver and is excreted from the body. But if that doesn't happen due to a liver disease, bilirubin levels in the blood can rise and the skin can take on the yellow discoloration known as jaundice. Tests for bilirubin may be total (measuring the level of all of the bilirubin in the blood) or direct (measuring only bilirubin that has been processed by the liver and attached to other chemicals).

Albumin and total protein. Protein is needed to build and maintain muscles, bones, blood, and organ tissue. Sometimes when there's a problem with the liver, it can't make proteins as well, so protein levels decrease. Liver function tests measure albumin specifically (the major blood protein produced by the liver), as well as the total amount of all proteins in the blood.

Normal results

Reference ranges vary from laboratory to laboratory and also depend upon the method used. However, normal values are generally framed by the ranges shown below. Values for enzymes are based upon measurement at 37°C.

ALT: 5–35 IU/L. (Values for the elderly may be slightly higher, and values also may be higher in men and in African-Americans).

AST: 0–35 IU/L

ALP: 30–120 IU/LALP is higher in children, older adults and pregnant females

GGT: males 2–30 U/L; females 1–24 U/L

LDH: 12–60 years: 100–190 U/L

Bilirubin: (Adult, elderly, and child)

Total bilirubin: 0.1–1.0 mg/dL

Indirect bilirubin: 0.2–0.8 mg/dL

Direct bilirubin: 0.0–0.3 mg/dL. (Newborn)

Note: Critical values for adult: greater than 1.2 mg/dL

Critical values for newborn (requiring immediate treatment): greater than 15 mg/dL

Ammonia: 10–70 micrograms per dL (heparinized plasma). Normal values for this test vary widely, depending upon the age of the patient and the type of specimen.

Albumin: 3.2–5.4 g/L

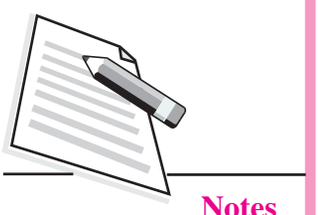
13.4 RENAL FUNCTION TEST

Definition

Kidney function tests are a collective term for a variety of individual tests and procedures that can be done to evaluate how well the kidneys are functioning. A doctor who orders kidney function tests and uses the results to assess the functioning of the kidneys is called a nephrologist.

Laboratory tests

There are a number of urine tests that can be used to assess kidney function. A simple, inexpensive screening test a routine urinalysis is often the first test conducted if kidney problems are suspected. A small, randomly collected urine sample is examined physically for things like color, odor, appearance, and concentration (specific gravity); chemically, for substances such a protein, glucose, and pH (acidity/alkalinity); and microscopically for the presence of cellular elements (red blood cells [RBCs], white blood cells [WBCs], and epithelial cells), bacteria, crystals, and casts (structures formed by the deposit of protein, cells, and other substances in the kidneys's tubules). If results indicate a possibility of disease or impaired kidney function, one or more of the following additional tests is usually performed to pinpoint the cause and the level of decline in kidney function.



Notes

Creatinine clearance test

This test evaluates how efficiently the kidneys clear a substance called creatinine from the blood. Creatinine, a waste product of muscle energy metabolism, is produced at a constant rate that is proportional to the individual's muscle mass. Because the body does not recycle it, all creatinine filtered by the kidneys in a given amount of time is excreted in the urine, making creatinine clearance a very specific measurement of kidney function. The test is performed on a timed urine specimen – a cumulative sample collected over a two to 24-hour period. Determination of the blood creatinine level is also required to calculate the urine clearance.

Urea clearance test

Urea is a waste product that is created by protein metabolism and excreted in the urine. The urea clearance test requires a blood sample to measure the amount of urea in the bloodstream and two urine specimens, collected one hour apart, to determine the amount of urea that is filtered, or cleared, by the kidneys into the urine.

Urine osmolality test

Urine osmolality is a measurement of the number of dissolved particles in urine. It is a more precise measurement than specific gravity for evaluating the ability of the kidneys to concentrate or dilute the urine. Kidneys that are functioning normally will excrete more water into the urine as fluid intake is increased, diluting the urine. If fluid intake is decreased, the kidneys excrete less water and the urine becomes more concentrated. The test may be done on a urine sample collected first thing in the morning, on multiple timed samples, or on a cumulative sample collected over a 24-hour period. The patient will typically be prescribed a high-protein diet for several days before the test and be asked to drink no fluids the night before the test.

Urine protein test

Healthy kidneys filter all proteins from the bloodstream and then reabsorb them, allowing no protein, or only slight amounts of protein, into the urine. The persistent presence of significant amounts of protein in the urine, then, is an important indicator of kidney disease. A positive screening test for protein (included in a routine urinalysis) on a random urine sample is usually followed up with a test on a 24-hour urine sample that more precisely measures the quantity of protein.

There are also several blood tests that can aid in evaluating kidney function. These include:

Blood urea nitrogen test (BUN)

Urea is a byproduct of protein metabolism. Formed in the liver, this waste product is then filtered from the blood and excreted in the urine by the kidneys. The BUN test measures the amount of nitrogen contained in the urea. High BUN levels can indicate kidney dysfunction, but because BUN is also affected by protein intake and liver function, the test is usually done together with a blood creatinine, a more specific indicator of kidney function.

Creatinine test

This test measures blood levels of creatinine, a by-product of muscle energy metabolism that, similar to urea, is filtered from the blood by the kidneys and excreted into the urine. Production of creatinine depends on an person's muscle mass, which usually fluctuates very little. With normal kidney function, then, the amount of creatinine in the blood remains relatively constant and normal. For this reason, and because creatinine is affected very little by liver function, an elevated blood creatinine level is a more sensitive indicator of impaired kidney function than the BUN.

Other blood tests

Measurement of the blood levels of other elements regulated in part by the kidneys can also be useful in evaluating kidney function. These include sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, protein, uric acid, and glucose.

Results

Normal values for many tests are determined by the patient's age and gender. Reference values can also vary by laboratory, but are generally within the following ranges:

Urine tests

Creatinine clearance. For a 24-hour urine collection, normal results are 90 mL/min–139 mL/min for adult males younger than 40, and 80–125 mL/min for adult females younger than 40. For people over 40, values decrease by 6.5 mL/min for each decade of life.

Urine osmolality. With restricted fluid intake (concentration testing), osmolality should be greater than 800 mOsm/kg of water. With increased fluid intake (dilution testing), osmolality should be less than 100 mOsm/kg in at least one of the specimens collected. A 24-hour urine osmolality should average 300–900 mosm/kg. A random urine osmolality should average 500–800 mOsm/kg.

Notes

Urine protein. A 24-hour urine collection should contain not more than 150 mg of protein.

Urine sodium. A 24-hour urine sodium should be within 75–200 mmol/day.

Blood tests

Blood urea nitrogen (BUN) should average 8–20 mg/dL.

Creatinine should be 0.8–1.2 mg/dL for males, and 0.6–0.9 mg/dL for females.

Uric acid levels for males should be 3.5–7.2 mg/dL and for females 2.6–6.0 mg/dL.

Low clearance values for creatinine indicate a diminished ability of the kidneys to filter waste products from the blood and excrete them in the urine. As clearance levels decrease, blood levels of creatinine, urea, and uric acid increase. Because it can be affected by other factors, an elevated BUN, alone, is suggestive, but not diagnostic for kidney dysfunction.

The inability of the kidneys to concentrate the urine in response to restricted fluid intake, or to dilute the urine in response to increased fluid intake during osmolality testing, may indicate decreased kidney function. Because the kidneys normally excrete almost no protein in the urine, its persistent presence, in amounts that exceed the normal 24-hour urine value, usually indicates some type of kidney disease.

13.5 COLLECTION OF SPECIMENS

Collection of Blood sample

Blood and separated serum are the most common specimens taken to investigate outbreaks of communicable disease. Venous blood can be used for isolation and identification of the pathogen in culture.

Venous blood samples

Materials for collection

- Skin disinfection: 70% alcohol (isopropyl alcohol, ethanol) or 10% povidone iodine, swabs, gauze pads, band aid
- Disposable latex or vinyl gloves
- Tourniquet, Vacutainer, Monovette, or similar vacuum blood collection devices, or disposable syringes and needles
- Vacutainer or sterile screw-cap tubes (or cryotubes if indicated), blood culture bottles (50ml for adults, 25ml for children) with appropriate media
- Labels and indelible marker pen.

Method of collection

- Place a tourniquet above the venepuncture site.
- Palpate and locate the vein. It is critical to disinfect the venepuncture site meticulously with 10% povidone iodine or 70% isopropyl alcohol by swabbing the skin concentrically from the centre of the venepuncture site outwards. Let the disinfectant evaporate. Do not repalpate the vein again. Perform venepuncture.
- If withdrawing with conventional disposable syringes, withdraw 5-10 ml of whole blood from adults, 2-5ml from children and 0.5-2ml for infants.
- If withdrawing with vacuum systems, withdraw the desired amount of blood directly into each transport tube and culture bottle.
- Remove the tourniquet. Apply pressure to site until bleeding stops, and apply sticking plaster (if desired).
- Using aseptic technique, transfer the specimen to relevant cap transport tubes and culture bottles. Secure caps tightly.
- Label the tube, including the unique patient identification number, using indelible marker pen.
- Discard the sharps into disposal container without recaping.
- Complete the case investigation and the laboratory request forms using the same identification number.

Handling and transport

- Blood culture bottles and blood sample tubes should be transported upright and secured in a screw cap container or in a rack in a transport box.
- Cushion or suspend bottles during transport over rough terrain to prevent lysis of red cells. They should have enough absorbent paper around them to soak up all the liquid in case of a spill.
- If the specimen will reach the laboratory within 24 hours, most bacterial pathogens can be recovered from blood cultures transported at ambient temperature.

Urine specimen collection

Materials for collection

- Sterile plastic cup with lid (50 ml or more)
- Clean, screw-top specimen transport containers (“universal” containers are often used)
- Gauze pads
- Soap and clean water (or normal saline) if possible.

Notes

Method of collection

- Give the patient clear instructions to pass urine for a few seconds, and then to hold the cup in the urine stream for a few seconds to catch a midstream urine sample. This should decrease the risk of contamination from organisms living in the urethra.
- To decrease the risk of contamination from skin organisms, the patient should be directed to avoid touching the inside or rim of the plastic cup with the skin of the hands, legs or external genitalia. Tighten the cap firmly when finished.
- For hospitalized or debilitated patients, it may be necessary to wash the external genitalia with soapy water to reduce the risk of contamination.
- Urine collection bags may be necessary for infants. If used, transfer urine from the urine bag to specimen containers as soon as possible to prevent contamination with skin bacteria. Use a disposable transfer pipette to transfer the urine and label it.

Handling and transport

- Transport to the laboratory within 2–3 hours of collection. If this is not possible, do not freeze but keep the specimen refrigerated at 4-8°C. Keeping the specimen refrigerated will decrease the risk of overgrowth of contaminating organisms.
- Ensure that transport containers are leak-proof and tightly sealed.

13.6 GENERAL LABORATORY TECHNIQUES

Laboratory services are an integral part of disease diagnosis, treatment, monitoring response to treatment, disease surveillance programmes and clinical research. Essential Health Technology as an important ingredient of Essential Clinical Services. Use of diagnostic techniques aid early diagnosis enabling appropriate and prompt intervention thereby reducing overall disease burden and promoting health. All laboratories are not equipped with facilities for carrying out complex investigations. The structure and function of a clinical laboratory varies according to the level of health care facility. Peripheral laboratories carry out simple tests like urine analysis and haemoglobin estimation whereas higher centers are equipped with sophisticated technology and trained manpower to carry out complex investigations. Establishing a network between peripheral and higher laboratories allows collection of specimen at periphery and their storage and transport for testing at higher centers and communicating report to the peripheral center efficiently without actually having to transfer the patient. In the event of patient transfer, the higher centers do not need to repeat

investigations carried out at the peripheral health center, thereby saving crucial time as well as cost and providing continuity in patient care. Networking between laboratories is also essential in disease surveillance programmes and outbreak investigations in order to obtain quick and reliable results.

Scope

Good Clinical Laboratory Practices should be used by all laboratories where tests are done on biological specimens for diagnosis, patient care, disease control and research such as:

- Microbiology & Serology
- Hematology & Blood Banking
- Molecular Biology and Molecular Pathology
- Clinical Pathology
- Clinical Biochemistry
- Immunology (Immunohematology and Immunobiochemistry)
- Histopathology/Pathology and Cytology

Equipment

- Each laboratory should prepare an exhaustive list of equipment and consumables required and available for general functioning of the laboratory and specialized equipment for special tests.
- Laboratory equipment should be of adequate capacity to meet work load requirement.
- Equipment should be suitably located in the laboratory so as to allow accessibility and sequential utilization thus minimizing the need for frequent movement of specimens or reagents.
- All equipment should be in good working condition at all times. Periodic inspection, cleaning, maintenance of equipment should be done. An equipment log book should be maintained for all major equipment. Laboratories should maintain necessary instructions for operation and maintenance of equipment in the form of Standard Operating Procedures (SOPs). A copy of SOP should be readily available.
- Maintenance contracts including warranty cards, telephone numbers of staff to be contacted in case of equipment malfunction should be kept safely. User manual should be available readily for reference. The staff should be aware of trouble shooting measures to be adopted for preventing equipment

malfunction. A format of the equipment log book provided in Annexure 1 can be used.

- New equipment should be calibrated and validated before routine use. AMR (Analytical Measurement range) should be verified, manufacturer can be consulted for verification and selection of range.
- Periodic performance check/calibration check for all equipment should be done using reference standard/reference material. The frequency of performance check should be based on the day-to-day performance of the equipment.
- Equipment performance should be verified from Internal Quality Control results and External Quality Assessment results. Outlier parameter trend analysis record should be maintained in respect of its effect on the equipment.
- All analytical equipment should be calibrated and calibration certificate provided by equipment company. Non-analytical equipment such as pipette, thermometer, weighing balance and centrifuge should be calibrated by accredited calibration laboratory or done in-house with traceability to National Physical Laboratory (NPL). For in-house calibration, laboratories should use :
    - Calibrated tachometer - for centrifuge
    - Calibrated digital temperature sensor - for checking temperature of refrigerator, incubator etc.
    - Calibrated glass thermometer- for temperature checking of oven, water bath etc.
    - Calibrated weights - for balance
    - National Institute of Science and Technology (NIST) buffer – for pH meter.Standard buffer solutions bought from reputed manufacturers with certifiable traceability can be used as alternative.

Standard Operating Procedure (SOP)

- SOP is a document, which contains detailed, written instructions describing the stepwise process and technique of performing a test or procedure in the laboratory.
- SOP helps to ensure uniformity, consistency and control over the processes carried out. It ensures that the procedures are done in exactly the same way each time irrespective of the operator.
- SOP should contain information on who can perform the test, their qualification and training, how to carry out the test including pre-analytical,

analytical and post-analytical stages of test/procedure, laboratory conditions required for the test/ procedure, routine care and maintenance of equipment, precautions and safety instructions, trouble shooting measures, waste disposal and linkage with reference laboratories.

- SOP should be simple and written in an easy to understand language.
- The procedure described in the SOP must be followed exactly by all staff members to ensure high quality results.
- It should be titled along with version number, dated and signed by an authorized person and updated regularly.
- It is important for the SOP document to be readily available in the working area and is therefore also referred to as **'laboratory bench work manual'.**
- SOPs are **controlled documents** and can be changed only with approval of the laboratory quality manager and/or Head of the laboratory.

Safety in Laboratories

Personnel working in laboratories may be exposed to risks from various chemicals, infectious materials, fire hazard, gas leak etc. The environment is also at risk of being contaminated by hazardous materials used and wastes generated in the laboratory. Safety in laboratories therefore includes **protection of both the staff and the environment** from hazardous materials.

General Safety Measures

- Documentation of Laboratory Safety Policies and Procedures.
- All laboratory personnel should be aware about the laboratory safety policies and procedures and follow these at all times.
- List of hazardous materials used in the laboratory should be prepared. All hazardous materials should be accounted for on a continuous basis.
- Laboratory personnel should follow safe hygienic practices which include hand washing, wearing protective clothing, gloves, eye protection etc.
- Eye wash facility should be available as "stand-alone" facility or attached to sink. Portable, sealed, refillable bottles should also be available.
- Biohazard symbol should be used on all container/equipment containing biohazardous material.
- Laboratories should ensure proper preservation and security of specimens.

- Destruction/disposal of hazardous material should be authorized, supervised and handled according to standard procedures.
- Laboratory personnel should be thoroughly trained in managing fire, and nonfire emergencies such as large spillage, gas leakage etc.
- Adequate fire extinguishers should be readily available in the laboratory
- Periodic checking of all safety equipment and accessories should be ensured.
- Accident/incident/injuries record of laboratory personnel should be maintained and reported to the designated authority. The report should include description of the event, factors contributing to the event and information on first aid or other health care provided. This information can be analyzed periodically towards effectively controlling and preventing future events. The records should be checked periodically by the laboratory safety officer even in the absence of fresh entries.

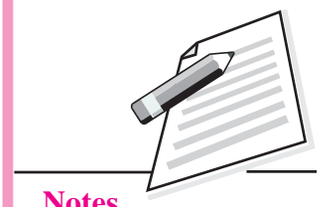
INTEXT QUESTIONS 13.1

1. Fill in the blanks:

1. Clinical applications of ALT assays are mainly used for the evaluation of ..... disorders.
2. The normal value of a-amylase in blood is .....
3. Bilirubin is a byproduct of the normal breakdown of .....
4. Urea is a waste product that is created by ..... metabolism.
5. ..... is a by-product of muscle energy metabolism that is similar to urea and is filtered from the blood by the kidneys.

TERMINAL QUESTIONS

1. Name some clinically important enzymes?
2. What is the normal value of ALT and AST?
3. What is Bilirubin?
4. What is Urea clearance test?
5. What is the normal range of creatinine in blood?



ANSWERS TO INTEXT QUESTIONS

13.1

1. hepatic
2. 28-100U/L
3. red blood cells
4. protein
5. creatinine

Notes

14

BODY WATER, OSMOLARITY AND IONIC COMPOSITION OF BODY FLUIDS

14.1 INTRODUCTION

Water is the solvent of life. It bathes our cells, dissolves and transports compounds in the blood, provides a medium for movement of molecules into and throughout cellular compartments, separates charged molecules, dissipates heat, and participates in chemical reactions. In spite of the variation in the amount of water we ingest each day and produce from metabolism, our body maintains a nearly constant amount of water that is approximately 60% of our body weight.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the fluid component in the body
- explain ionic composition of body fluids
- explain osmolarity and osmolality
- describe the buffer systems of human body

14.2 FLUID COMPARTMENTS IN THE BODY

Total body water is roughly 50 to 60% of body weight in adults and 75% of body weight in children. Because fat has relatively little water associated with it, obese

people tend to have a lower percentage of body water than thin people, women tend to have a lower percentage than men, and older people have a lower percentage than younger people.

Total body water is approximately around 42L. Approximately 40% ($1/3^{rd}$) of the total body water is intracellular fluid (ICF) and 60% ($2/3^{rd}$) is extracellular fluid (ECF).

ECF : The extracellular fluid includes

(i) *Plasma*: fluid part of blood after the blood cells have been removed. It constitutes about 25% of ECF.

(ii) *Interstitial water*: the fluid in the tissue spaces, lying between cells and also includes lymph. It constitutes about 75 % of ECF..

(iii) *Transcellular water* : a small, specialized portion of extracellular water that includes gastrointestinal secretions, urine, sweat.

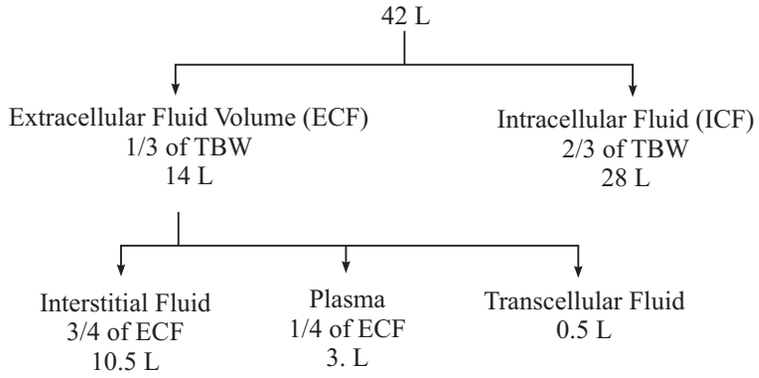


Fig. 14.1: Distribution of body fluid compartments

14.3 IONIC COMPOSITIN OF BODY FLUIDS

The ions are unequally distributed between the ECF and ICF compartments.

ECF

Major cation: Na^+

Major anion: Cl^- and HCO_3^-

ICF

Major cation: K^+

Major anion: Inorganic Phosphates

Notes

Table 14.1: Concentration of ions in body fluids

Ions mmol/L	ECF	ICF
Cations		
Na^+	145	12
K^+	4	150
Anions		
Cl^-	105	5
HCO_3^-	25	12
Inorganic Phosphates	2	100

14.4 OSMOLARITY AND OSMOLALITY

The number of molecules of a particular substance per unit volume of a particular body fluid is expressed in moles or osmoles.

A mole is the gram molecular weight of a substance i.e. the molecular weight of the substance in grams.

Osmolarity: The number of osmoles per litre of solution is called osmolarity.

Osmolality: The number of osmoles per kilogram of solvent is called osmolality

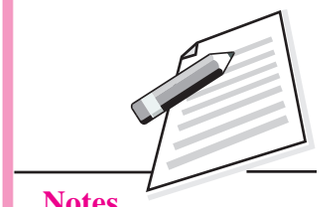
14.4.1 Body Fluid Osmolality

Normally, the osmolality of the ECF and ICF are at equilibrium in spite of difference in composition of electrolytes. The osmotic equilibrium between ECF and ICF is maintained by easy transfer of water between the compartments. Plasma osmolality can be easily measured which reflects the osmolality of ECF and ICF.

Normal Plasma osmolality ranges between 280 to 295 mosm/kg H_2O. Sodium and Chloride contributes to 90% of plasma osmolality and the rest is by glucose, urea, proteins and other ions. Chloride movement from one compartment to other always follows the movement of sodium. Therefore, it is mainly sodium that maintains the osmolality.

Hence Plasma osmolality is calculated as,

Plasma osmolality (mmol/kg) = 2 × Plasma Na^+ (mmol/L)



14.5 TONICITY OF FLUID

Isotonic fluid

Isotonic fluid is fluid having the same osmolality as that of plasma. Isotonic fluid do not change the volume of cells. Examples: 0.9% NaCl, 5% glucose, 10% mannitol, 20% urea.

Hypotonic fluid

Hypotonic fluid is fluid having osmolality less than that of plasma. The cells swell when placed in hypotonic solution.

Hypertonic fluid

Hypertonic fluid is fluid having osmolality more than that of plasma. The cells shink when placed in hypertonic solution.

14.6 DISORDERS OF FLUID VOLUME

Iso-osmotic dehydration

This occurs during loss of isotonic fluid from the body. Examples are diarrhea, vomiting, hemorrhage

Hypo-osmotic dehydration

This occurs during loss of salt in excess of water from the body. It's seen in Adrenocortical insufficiency.

Hyperosmotic dehydration

This occurs during loss of water in excess of solutes from the body. It's seen in Diabetes insipidus, excessive sweating.

Iso-osmotic volume expansion

This occurs during excessive infusion of isotonic fluid into the body.

Hypo-oosmotic volume expansion

This occurs during excessive water gain into the body. It's seen during ingestion of large volume of water.

Hyperosmotic volume expansion

This occurs during excessive infusion of hypertonic saline.

INTEXT QUESTIONS 14.1

1. Short notes

1. Classify the body fluid comparments
2. Write down the ionic composition of different fluid compartment in the body.
3. Define osmolality and give normal value of plasma osmolality. Give examples of isotonic, hypotonic and hypertonic fluid.

2. Fill in the blanks.

1. Plasma constitutes …… percentage of ECF.
2. Major cation present in the ECF is……..
3. Major cation present in TCF is ……
4. Plasma osmolality is determined by …… ion.
5. 20% urea is an example of …… fluid.

3. State if the following statements are true or false.

1. 0.9% saline is hypotonic fluid.
2. Cells swell when placed in hypotonic solution.
3. Diarrhoea causes hypotonic dehydration.
4. K^+ ion is the major ion contributing to plasma osmolality.
5. 60% of body fluid is comprised by ECF.

14.7 BUFFER SYSTEM IN BLOOD, ROLE OF LUNGS AND KIDNEYS IN ACID-BASE BALANCE

Acid – Base homeostasis is essential to the body. Many enzymatic activities and metabolic processes need an optimal pH. Hence, any disturbance in the acid-base balance leads to derangement in the vital functions of the body.

14.7.1 Definitions

Acid: chemical substance that can donate H^+

Base: chemical substance that can accept H^+

Examples : Strong acids (e.g. HCl, H_2SO_4)

Strong bases (e.g. NaOH, KOH)

Weak acids (e.g. H_2CO_3, CH_3COOH)

Weak bases (e.g. NH_3)

pH: The pH of the solution is defined as the negative logarithm of the hydrogen ion concentration in the solution.

pK and K_a : The pK is defined as the negative logarithm of the ionization constant of an acid (K_a) in a solution. pK is the pH at which concentration of acid and its conjugate base are in equal proportion.

Buffer: An aqueous solution of weak acid & its conjugate base or a weak base & its conjugate acid, that resist changes in pH to addition or removal of small amounts of acids/ bases.

$$HB \leftrightarrow H^+ + B^-$$

The efficiency of a buffer is given by Henderson-Hasselbach equation:

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

14.7.2 Concept of Acid–Base Balance

Normal pH of the body is maintained over a very narrow range of 7.35 to 7.45. Acids are continuously produced in the body. But there are mechanisms that buffer the acids.

Acidosis : decreased pH.

Alkalosis: increased pH.

Volatile acid: Carbonic acid (H_2CO_3) à dissociates into CO_2: excreted by lungs

Non-volatile/ fixed acids: H_2SO_4, H_3PO_4 & organic acids; excreted exclusively by kidneys

Acid load in the body :

- volatile acids
- non volatile acids ingested in diet
- non volatile acids produced from metabolism
- Bicarbonate excreted in feces also contributes to acid load

This acid load is excreted by both lungs and kidneys to maintain the homeostasis.

The reabsorption of the bicarbonate which is filtered by the kidneys also corresponds to the acid – base balance, apart from the excretion of the acid load.

14.7.3 Buffer Systems in the Body

There are four buffer systems in the body:

Notes

I. Bicarbonate-carbonic acid buffer system

II. Plasma protein buffer system

III. Phosphate buffer system

IV. Hemoglobin buffer system

Bicarbonate-carbonic acid buffer system

- Most important buffer system in plasma
- In lungs it is involved in removal of acid in the form of CO_2
- In kidneys it is involved in reabsorption of bicarbonates.

Plasma protein buffer system

- Accounts for 95 % of non bicarbonate buffer in plasma.
- Mainly is contributed by abumin.

Phosphate buffer system

- Is an important intracellular buffer
- Has organic and inorganic components
- Organic phosphate : ATP, AMP, ADP
- Inorganic phosphate : Disodium hydrogen phosphate, sodium dihydrogen phosphate

Hemoglobin buffer system

- Major buffer in RBCs, around 85%.
- The rest 15 % is by 2,3- Diphosphoglycerate.
- Histidine residue in Hb is responsible for buffering action.

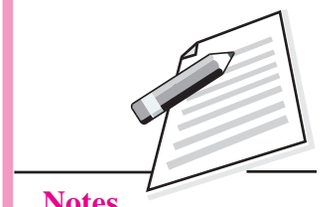
14.7.4 Role of Lungs in Acid – Base Balance

The respiratory mechanism gets mainly operated by changing the rate and depth of respiration. The bicarbonate buffer system is the major regulator.

The carbonic acid in the body reacts with H^+ and produces $CO_{2.}$.

$$HCO_3^- + H^+ \longrightarrow H_2O + CO_2$$

The level of CO_2 in the body in turn stimulates the chemoreceptors and increases ventilation. The increased ventilation causes removal of CO_2.



14.7.5 Role of Kidneys in Acid – Base Balance

Three major mechanisms operate in kidneys:

HCO_3^- reabsorption

Excretion of H^+ as titratable acid (with PO_4^{3-} buffer)

Excretion of H^+ as NH_4^+

14.7.5.1 HCO_3^- reabsorption

- Almost all the filtered of HCO_3^- reabsorbed (4320 mEq per day)
  - 80% in Proximal Convoluted Tubule
  - 10% in Thick Ascending Limb
  - 6% in Distal Convoluted Tubule
  - 4% in Collecting Duct
- Mechanisms: different in different sites

14.7.5.1.1 HCO_3^- reabsorption In PCT and TAL

1. H^+ secretion in the apical membrane using:
   (a) Na^+ - H^+ exchanger/ antiporter (NHE3):
      - predominant pathway
      - a form of secondary active transport
   (b) H^+-ATPase: a form of active transport
2. HCO_3^- exit at the basolateral membrane using:
   (a) Na^+-HCO_3^- cotransporter (NBC1)
   (b) Cl^--HCO_3^- antiporter

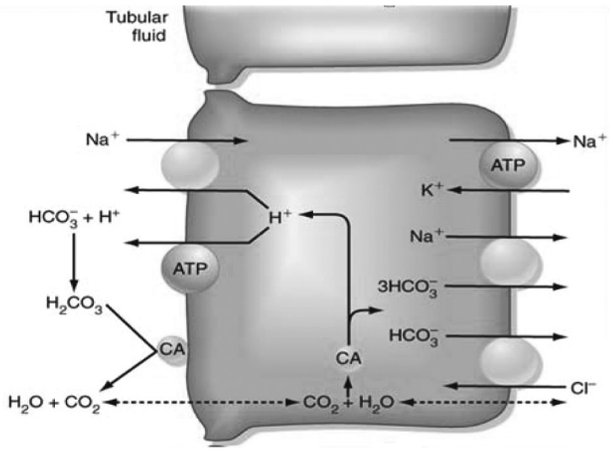


Fig. 14.2: Bicarconate reabsorption in PCT

14.7.5.1.2 HCO_3^- reabsorption In DCT and CD

1. H^+ secretion in the apical membrane:
   (a) H^+-ATPase: a form of active transport
   (b) H^+/K^+-ATPase: similar to that found in gastric parietal cell
2. HCO_3^- exit at the basolateral membrane:
   (a) by Cl^- - HCO_3^- antiporter (AE-1)

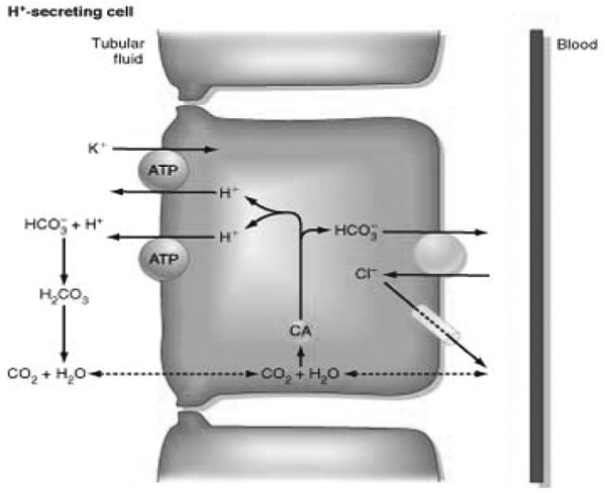


Fig. 14.3: Bicarconate reabsorption in DCT and CD

14.7.5.2 Role of phosphate buffer

H^+ excreted in urine binds with phosphate buffer (HPO_4^-)and gets excreted as $H_2PO_4^-$. There is formation of new bicarbonate.

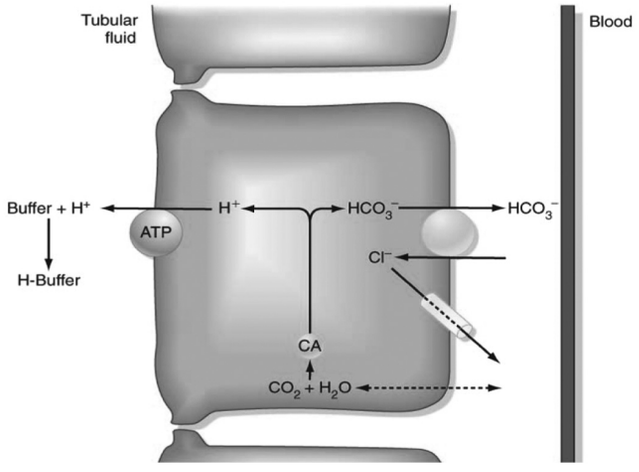


Fig. 14.4: Role of phosphate buffer

*There is no new bicarbonate formation in bicarbonate buffer system.

Titratable acid

- amount of base to be added to a sample of urine to bring its pH to that of plasma.
- reflects the amount of H^+ excreted by phosphate buffer.

14.7.5.3 Role of ammonium buffer

- NH_3 is synthesized in PCT by glutamine metabolism (Ammoniagenesis)
- This NH_3 gets excreted in collecting duct as NH_4^+ by combining with H^+ excreted in urine.
- There is also new bicarbonate formation during the process.

14.7.6 Acid-Base Disorders

14.7.6.1 Metabolic acidosis:

- Decreased pH
- Decreased ECF bicarbonate
- Increased metabolic acid production or reduced acid elimination
- Eg. diabetes mellitus

14.7.6.2 Metabolic alkalosis

- Increased pH
- Increased ECF bicarbonate
- Increased bicarbonate or increased acid elimination
- Eg. Excess vomiting

14.7.6.3 Respiratory acidosis

- Decreased pH
- Decreased CO_2 elimination
- Eg. Respiratory diseases

14.7.6.4 Respiratory alkalosis

- Increased pH
- Increased CO_2 elimination
- Eg. Hyperventilation

INTEXT QUESTIONS 14.2

1. Enumerate the role of kidneys in acid- base homeostasis.
2. Write briefly on the buffer systems of the body.
3. Give a brief description on various acid – base disorders.
4. Role of lungs in acid-base balance.
5. Fill in the blanks
    1. The important ECF buffer is .....
    2. Phosphate is an important ..... buffer.
    3. New bicarbonate formation occurs in ..... & ..... buffer.
    4. Normal pH of the body is .....
    5. Titrable acid is contributed by ..... buffer.

WHAT HAVE YOU LEARNT

- Water is solvent of life. It bathes our cells, dissolves and transposrts compounds in the blood, provides a medium for movement of molecules into and throughout cellular components, separates charged molecules, dissipates heat
- Total body weight is roughly 50 to 60% of body weight in adults and 75% of body weights in children
- Total body water is present as Extracellular and Intracellular fluids
- Extracellular fluids are plasma, Interstitial water and Transcellular water
- Osmolarity is the number of osmoles per litre of solution
- Osmolality is the number of osmoles per kilogram of solvent
- Normality, the osmolality of the ECF and ICF are at equilibrium in spite of difference in composition of electrolytes
- Based on the Osmolality fluids are classified as Isotonic, Hypotonic and Hypertonic
- Acid-Base homeostasis is essential to the body. Many enzymatic activities and metabolic processes need an optimal pH.

- pH of a solution is the negative logarithm of the hydrogen ion concentration in the solution
- pK is the negative logarithm of the ionization constant of an acid in a solution
- Buffer is an aqueous solution of weak acid and its conjugate base or a weak base and its conjugate acid, that resist changes in pH to addition or removal of small amounts of acids/bases
- Bicarbonate-carbonate acid buffer system, Plasma protein buffer system, Phosphte byffer system and Hemoglobin buffer system are the buffer sytems in the body

ANSWERS TO INTEXT QUESTIONS

14.1

2. 1. 25%
   2. Sodium (Na^+)
   3. Potassium (K^+)
   4. Sodium
   5. Isotonic Fluid

3. 1. False
   2. True
   3. False
   4. False
   5. True

14.2

5. 1. Bicarbonate carbonic acid buffer
   2. Intracellular
   3. Phosphate and Ammonium
   4. 7.35-7.45
   5. Phosphate

Notes

15

NUTRITION

15.1 INTRODUCTION

Nutrition is the requirement of the body from external sources to sustain life. The requirement comprises of foods that provide energy i.e. carbohydrates, fats, proteins, provide the building blocks such as proteins and amino acids or act as the functional molecules such as micro nutrients and vitamins acting as co enzymes and carrier molecules.

OBJECTIVES

After reading this lesson, you will be able to:

- define nutrition. basal metabolic rate
- explain factors affecting basal metabolic rate
- describe the nutritional aspects of carbohydrates proteins and fats
- enumerate the various essential amino acids
- explain the composition of food and what constitutes a balanced diet
- describe the causes, symptoms and treatment of protein energy malnutrition disease such as kwashiorkor and marasmus.

15.2 NUTRITION

As explained earlier nutrition is the requirement of the body from external sources to sustain life. Nutrition consists of intake of food that provides both macro as well as micro nutrients in the correct amount.

A reduced, unbalanced or excessive intake of nutrients can result in various health related problems. A reduced intake can cause deficiency disorders such as vitamin deficiency disorders

i.e scurvy, beri beri etc, or protein energy malnutrition disorders such as kwashiorkor, marasmus. Similarly over nutrition in general or of a certain nutrient can cause various diseases. Excessive intake of Vitamins can cause hypervitaminosis. Excessive intake of calories by any mean can cause obesity and associated disorders such as Diabetes mellitus, cardio vascular disease etc.

Hence we should have a complete knowledge of the nutritional requirement of the human body to prevent these disorders of over and under nutrition.

15.3 BASAL METABOLIC RATE (BMR)

Basal metabolic rate is the energy requirement of the body when the body as well as the brain is at rest i.e. Basal condition. It is the energy requirement of the body for its life sustaining processes such as respiration, circulation, peristalsis, maintenance of body temperature etc. It is expressed in K cal of energy consumed per hour by the person for ever square meter of the body's surface area. Its unit hence is Kcal/ hour/m^2.

BMR is calculated by measuring the consumption of oxygen by a person who is awake but at complete rest and at a comfortable room temperature. The oxygen consumption gives a good approximation of energy metabolism in our body. The oxygen consumption for 10 minutes is measured and then multiplied by 6 to get the oxygen use for an hour. This is done for 24 hours. For each litre of oxygen consumed it is calculated that and approximate amount of 4.825 Kcal of energy is used.

The body surface area can be calculated by the Du Bois formulae:

$$\text{Area } (m^2) = \frac{\{\text{Height (cm)}\ 0.725 \times \text{Weight (kg)}\ 0.425 \times 71.}{10{,}000}$$

Average BMR of an adult male is 40 Kcal / Hr/ sq m.

Average BMR of an Adult female is 36 Kcal/ Hr/ sq m.

BMR is not a static property of the body. It is dependent on various physiological and pathological states.

BMR is higher in 'hypermetabolic' states such as in pregnancy, fever etc. It is also higher in growing age groups such as children and adolescents. Athletes and individuals with higher muscle mass also have significantly higher BMR. So BMR is affected by age, sex, activity level, body mass and various pathological and physiological states.

Notes

15.4 THERMOGENESIS

It is the process of heat production in the body to maintain the body temperature in warm blooded animals. It is done through various methods. Thermogenesis can be done by muscle movements. A proportion of the energy produced to support muscle action is leaked from the electron transport chain and results in thermogenesis. Similarly shivering also results in thermogenesis by this mechanism.

Brown adipose tissue also plays an important role in thermogensis. It has a unique protein that uncouples the electron transport chain resulting in leaking of electrons from the chain. In this case the energy is lost ion form of heat instead of being stored as ATP.

15.5 NUTRITIONAL ASPECTS OF CARBOHYDRATES

Carbohydrates are the most important sources of energy. Even though their calorific value at 4 Kcal/g is less than half of fats they form the chief source of energy in our body because they are easiest to digest also the most abundant and cheap source of energy.

Brain uses glucose exclusively as a source of energy. Almost 20 to 25% of our total energy intake is used by the brain and almost 50% of our carbohydrate intake.

The main forms of carbohydrate that we consume are:

1. **Starch:** It is the form of carbohydrate we consume the most and hence forms the most important source of energy in our body. It is present in all food grains, pulses, tuberous vegetables etc.
2. **Sugars:** Sugar is a term generally used for disaccharides and monosaccharides. They are present in fruits and vegetables in form of glucose, fructose, sucrose. It is also found in milk in form of lactose. Glucose and fructose are examples of naturally occurring monosaccharides. Lactose, Sucrose, Maltose are naturally occurring disaccharides.
3. **Cellulose:** Cellulose is a polysaccharide which is present in the structural elements of plants. It cannot be digested by humans but it plays a very important role as roughage. Roughage provides bulk to our food. The bulk helps in the movement of food through our digestive system and also has a role to play in digestion. It traps water in the large intestine and helps in its absorption.

Carbohydrates have a protein sparing effect which means that when adequate carbohydrates are taken in diet the amino acids in diet are spared from energy metabolism and used for synthesis of proteins in the body.

All carbohydrates required by our body from other sources hence there are no essential amino acids.

15.6 NUTRITIONAL ASPECTS OF LIPIDS

Lipids play many roles in our body. They act as a storage of energy, structural elements of cells and tissues. They act as hormones and vitamins. As a source of energy it has very high calorific value 9kcal/g.

Lipids as a group is composed of hydrophobic non polar organic compounds that are insoluble in water and soluble in organic solvents. They are composed of trigycerides (fats and oils), cholesterol, cholesterol esters.

Lipids can be divided in to saturated, mono unsaturated and poly unsaturated lipids based on the number of double bonds present in the fatty acid. All poly unsaturated fatty acids cannot be synthesized in our body hence certain lipids that have essential fatty acids need to be present in our diet to prevent deficiency disease. Essential fatty acids are alpha-linolenic acid, linoleic acid and arachidonic acid. These essential fatty acids should make up for at least 3% of the energy requirement in adults and 5% to 6% of energy requirements in children. These essential fatty acids are present in plant oils rich in poly unsaturated fatty acids. Fish oil is also a good source of poly unsaturated fatty acids.

Our diet should be rich in poly unsaturated fatty acids and low in saturated fatty acids as saturated fatty acids are linked to atherosclerosis and cardiac disorders.

Lipids also act as carriers of fat soluble vitamins. Certain fats also act as hormones such as steroid hormones.

Cholesterol is also an important component of our diet. It is responsible for the structural integrity of the cell membrane and hence some amount needs to be taken exogenously. The yolk of an egg is a rich source of cholesterol. But excessive cholesterol intake has been linked to atherosclerosis and cardiovascular disorders. The RDA for cholesterol intake in adults and children above four years of age is 300 mg / day.

15.7 NUTRITIONAL ASPECTS OF PROTEINS

Proteins form the main building blocks of our body both functionally and structurally. Almost all of our body is composed of proteins. Our muscles, bones, cartilages, blood vessels, connective tissues, skin, hair, nails etc are all mainly composed of proteins. Apart from these almost all functional elements of our body are also composed of proteins such as the actin myosin involved in

Notes

movement, the enzymes responsible for all metabolic reactions in our body, the hormones, the cellular channels, the receptors for various signaling molecules etc. In cases of energy deprivation proteins can also be broken down as a source of energy. They have a calorific value similar to carbohydrates, i.e. 4 Kcal /g, but their metabolism is much less efficient than that of carbohydrates.

Proteins are composed of amino acids. They are polymers of the 20 amino acids that exist in our body. Of these 11 amino acids can be synthesized in the body from non protein sources and hence are deemed non essential in our diet. The rest 9 amino acids cannot be synthesized in the body and hence need to be taken in food to prevent any deficieny disorder from occurring. They are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

Not all proteins are nutritionally equivalent when it comes to food. Certain proteins such as keratin undergoes almost nil digestion while proteins like albumin present in the egg undergo almost complete digestion. Hence the nutritional quality of proteins has to be decided depending upon the balanced presence of all essential amino acids, their digestibility (% of protein ingested digested known as digestibility coefficient, DC), and the biological value, BV (which is the percentage of protein retained by the body after digestion and absorption).

Net protein utilization (NPU) is a measure of the utilization of ingested protein for protein synthesis inside the body. It is calculated by:

$$NPU = \frac{(DC \times BV)}{100}$$

Animal proteins are superior biologically as they contain all essential amino acids in the needed amount and are better digested and absorbed. They have a high biological value and are termed as first class proteins. While vegetable proteins have a low biological value and are termed as second class proteins as they are generally deficient in one amino acid or the other and they are also more difficult to digest and absorb. Whole egg protein is taken as a reference protein due to its excellent bioavailability and completeness as far as amino acids are concerned.

This short coming of plant proteins can be overcome are mutual supplementation i.e. taking two kinds of plant proteins together. A perfect example is rice and pulses. This results in a complete protein diet being taken by using two proteins that are deficient in two different amino acids.

Protein requirement is higher in growing years as well as in hyper metabolic states such as pregnancy, fever, infection etc. Protein requirement is expressed in

relation to body weight. In infants, upto 3 months of age, the protein requirement is about 2.5 gm per kg body weight. Then post this the protein requirement is between 2 to 1.5 gm/ kg body weight uptill adolescence. In adulthood, after 18 yrs of age, the protein requirement in about 1gm/ kg body weight. During pregnancy and lactation the requirement is higher nearly 2.5 to 2 gm / kg body weight.

15.8 BALANCED DIET

A balanced diet is a diet that provides all the nutrients in their correct proportion so as to fulfill the biological requirement of an individual. It neither creates a deficiency nor an excess of any of the nutrients. Balanced diet is different for different individuals and is dependent on the biological need of an individual. For example the biological demand for carbohydrates fats proteins as well of the vitamins required for their metabolism will be much higher in an athlete than in a person with a sedentary desk job.

A balanced diet should contain

(a) Cereals

(b) Pulses

(c) Vegetables and Fruits

(d) Milk and milk products

(e) Oils and fats

(f) Sugars

(g) In cases of non vegetarians it should also contain animal proteins and eggs on a regular bases.

A balanced diet should contain all the above said food groups in our daily diet in a balanced manner so that all the nutritional requirements of the body are fulfilled.

The cereals are energy rich and form a major source of carbohydrates in our diet. Their energy yield is about 350 kcal/100g.

They consist of staples such as wheat, rice, maize, sorghum, jowar, bajra etc. The cereals are deficient in proteins and the proteins present in them are generally deficient in one amino acid or another.

They contain adequate minerals but the absorption of minerals from them is inefficient.

Their outer covering is rich in vitamin B complex. But in most cases the outer covering is lost due to milling.

Pulses are a rich source of proteins among vegetarian food. But similar to other vegetable proteins they are deficient in certain amino acids but these deficiencies can be overcome by supplementing them with cereals. They have a energy density of about 350Kcal/100 gm and 20% to 25% of their mass is protein. Germinating pulses are an important source of vitamin C and vitamin B complex.

Vegetables and fruits are the most important source of water soluble and and certain fat soluble vitamins in our diet. Apart from that they are also an important source of fiber. They provide minerals and carbohydrates. Their contribution to proteins in our diet is negligible.

Dairy and its products form almost a complete diet. It contains almost all nutrients other than vitamin C and Iron. It is a specially good source of Calcium. Milk is rich in saturated fats.

The protein content of milk can be increased by converting to curd. Also curd can be eaten by those who are lactose intolerant as during the fermentation process most of the lactose is fermented.

Fats and oils form an essential part of our diet. They are obtained from the fats and oils we add to our food during cooking. Other than these lipids they are obtained also from lipids contained in food items such as dairy and its products, lipids present in cereals and pulses and fats present in meats and eggs. We should try to avoid saturated fats in our diet and include unsaturated lipids in form of oils in our diet. Refined sugars are used as flavoring agents in our diet but excess of these sugars should be avoided as they provide empty calories.

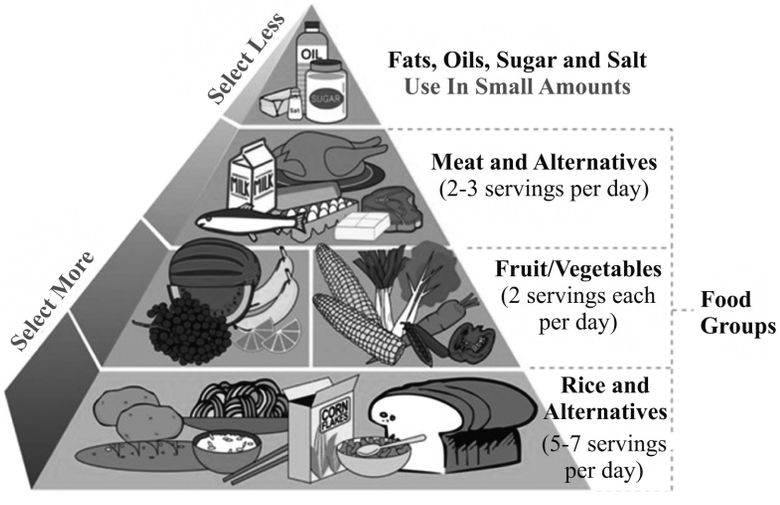


Fig. 15.1: Food pyramid showing the servings of various food items in a balanced diet

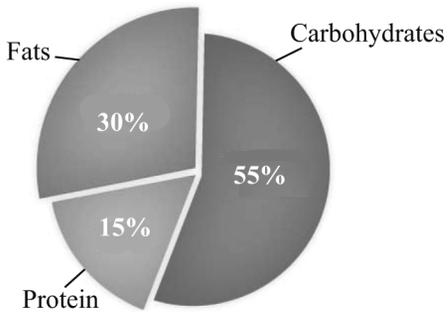


Fig. 15.2: This pie graph shows the percentage of energy that should be provided by the various macro nutrients in a balanced diet.

Eggs are an excellent source of protein and cholesterol in our diet. An average egg is about 100 k cal in energy value. Similarly non vegetarian diet such as meat, poultry and fish are an excellent source of high quality proteins. They are also a good source of fat soluble vitamins as well as vitamins of the B complex except for vitamin C.

A balanced diet should take care of the energy and nutrient requirement of the body and these should be provided by a mix of food items.

The energy requirement of an individual has to provide for the energy requirement for the various activities as well as growth and repair of wear and tear of the body. Hence the energy requirement is dependent on the sex, built as well as the life style of an individual. The following are the average energy requirements for various population groups

Group	Particulars	Body Wt.kg	Net EnergyKcal
Man	Sedentary	60	2320
	Moderate	”	2730
	Heavy work	”	3490
Woman	Sedentary	55	1900
	Moderate	”	2230
	Heavy work	”	2850
	Pregnancy	55kg + GWG	+350
	Lactation (0-6 m)	55kg + WG	+600
	Lactation (6-12 m)	”	**+520**

Notes

Daily energy requirement for an average Indian man and woman as per ICMR guideline (2010)

Energy requirement of infants and children according to ICMR (2010)

Group	Age	Weight	Energy requirement (Kcal)
Infants	0-6 months	5.4	500
	6-12 months	8.4	670
Children	1-3 y	12.9	1060
	4-6 y	18.1	1350
	7-9 y	25.1	1690
Boys	10-12 y	34.3	2190
Girls	10-12 y	35.0	2010
Boys	13-15 y	47.6	2750
Girls	13-15 y	46.6	2330
Boys	16-17 y	55.4	3020
Girls	16-17 y	52.1	2440

15.9 PROTEIN ENERGY MALNUTRITION

Protein energy malnutrition is generally seen in children being weaned off mother's milk. It presents as specific syndromes depending on whether the protein deficiency is more severe or carbohydrate deficiency.

(a) Kwashiorkor: It is a disorder seen in children weaned off milk onto a diet that is deficient in proteins but adequate in calories. It results in the child developing a protein deficiency. This results in muscle wasting and retarded growth. Patchy areas of hyper and hypo pigmented skin are seen and also brittle hypo pigmented hair with flagging sign.

A classical feature of kwashiorkor is a child with a distended belly and edema. The distended belly and edema are due to loss of plasma oncotic pressure and may completely mask muscle wasting. This loss is seen due to loss of plasma albumin. Fatty liver caused by deficiency of apo-lipoproteins also causes distention of abdomen. Also there is anaemia caused by decreased globin synthesis.

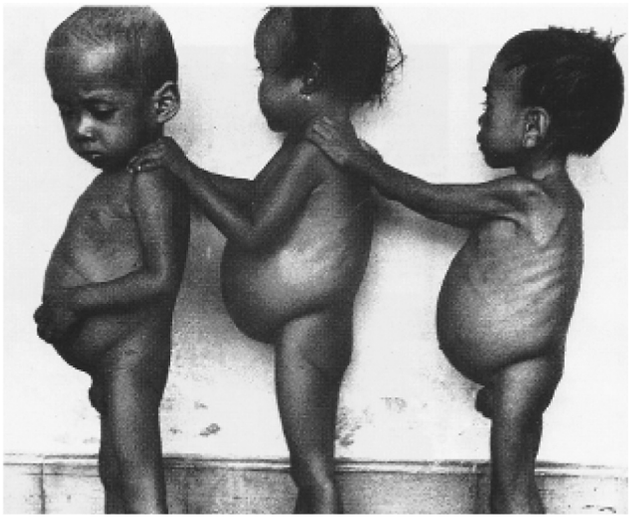


Fig. 15.3: A classical picture of kwashiorkor

Kwashiorkor can be easily prevented by adding protein rich food to the diet of the child being weaned off mother's milk. This can be done by add undiluted animal milk, eggs, pulses, groundnuts, bengal gram, cotton seed flour etc.

(b) Marasmus is a disorder of both protein as well as calorie intake. Because of deficiency in calorie intake most of the protein in diet is used for energy.

There is gross muscle wasting with loss of subcutaneous fat. The child shows growth retardation.

There are frequent infections that put a further stress on the metabolism. Diarrhea results in loss of further nutrients.

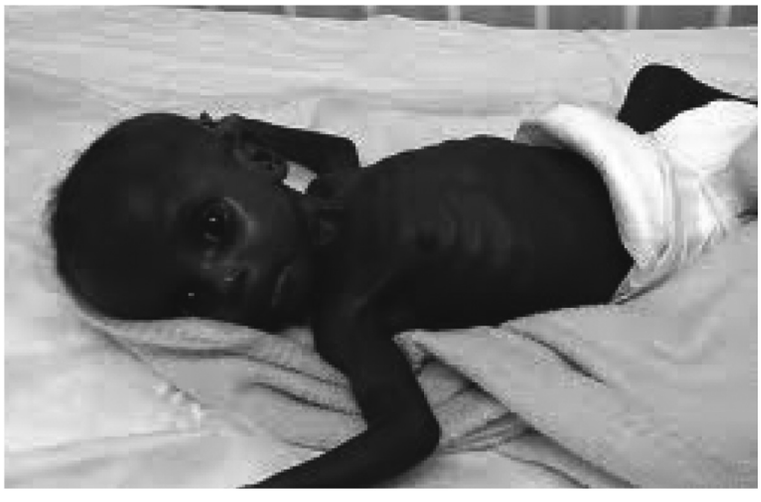


Fig. 15.4: A marasmic child

Notes

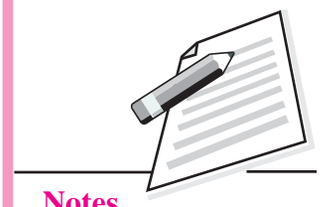
A marasmic child

Marasmus can be corrected by :

1. Increasing protein and energy intake.
2. Supplementing Vitamins and minerals.
3. Correcting dehydration.
4. Correcting the infections.

TERMINAL QUESTIONS

1. What is nutrition?
2. What is basal metabolic rate (BMR)?
3. How is Basal metabolic rate calculated?
4. What are the various factors that influence BMR?
5. Define thermogenesis. How does it happen?
6. What are nutritional aspects of carbohydrates?
7. What is the importance of roughage in diet?
8. What is the importance of protein in diet?
9. What are the essential amino acids? Enumerate the essential amino acids
10. What does the Bioavailability of a protein mean. How is it sued to calculate net protein utilization?
11. What is the nutritional importance of lipids?
12. What are essential fatty acids?
13. Define balanced diet. What are the factors that determine the balanced diet for an individual.
14. What is Kwashioko? What are its symptoms and how can it be prevented.
15. What is marasmus? What are its symptoms and how can it be corrected.



16

KIDNEY FUNCTION TEST

16.1 INTRODUCTION

The main function of the kidney is excretion of water soluble waste products from our body. The kidney has various filtration, excretion and secretary functions. Derangement of any of these function would result in either decreased excretion of waste products and hence their accumulation in the body or loss of some vital nutrient from the body. Based on the level of these excretory products and nutrients in the urine as well as in blood we can make an accurate calculation to decipher the efficieny of the kidney to undertake its various functions.

OBJECTIVES

After reading this lesson, you will be able to:

- explain the importance of kidney function test.
- describe the types of lesions detected by the renal function tests.
- describe the various components of the kidney function test.
- explain the importance of various components of the kidney function test.

16.2 THE FUNCTIONAL COMPONENTS OF A KIDNEY

The functional unit of the kidney is called a nephron. It consists of two main parts, the glomerulus and the tubular system.

The glomerulus is composed of a bowman's capsule and a tuft of leaky blood vessels encapsulated by the bowman's capsule. The primary purpose of the glomerulus is filtrations. The leaky vessels filter into the glomerulus almost all the water, electrolytes, small proteins, nutrients such as sugar etc and excretory

products such as urea etc. The filtrations is dependent on the size and charge of the particles. The average pore size is 8 nm hence particles of only smaller size will pass through. Also the basement membrane carries a negative charge hence preventing negatively charged particles from passing through.

The Tubular system is responsible for re absorption of most of the water, electrolytes, nutrients as well as excretion of the remaining nutrients by means of secretion into the tubules. These tubules are responsible for the concentration of urine.

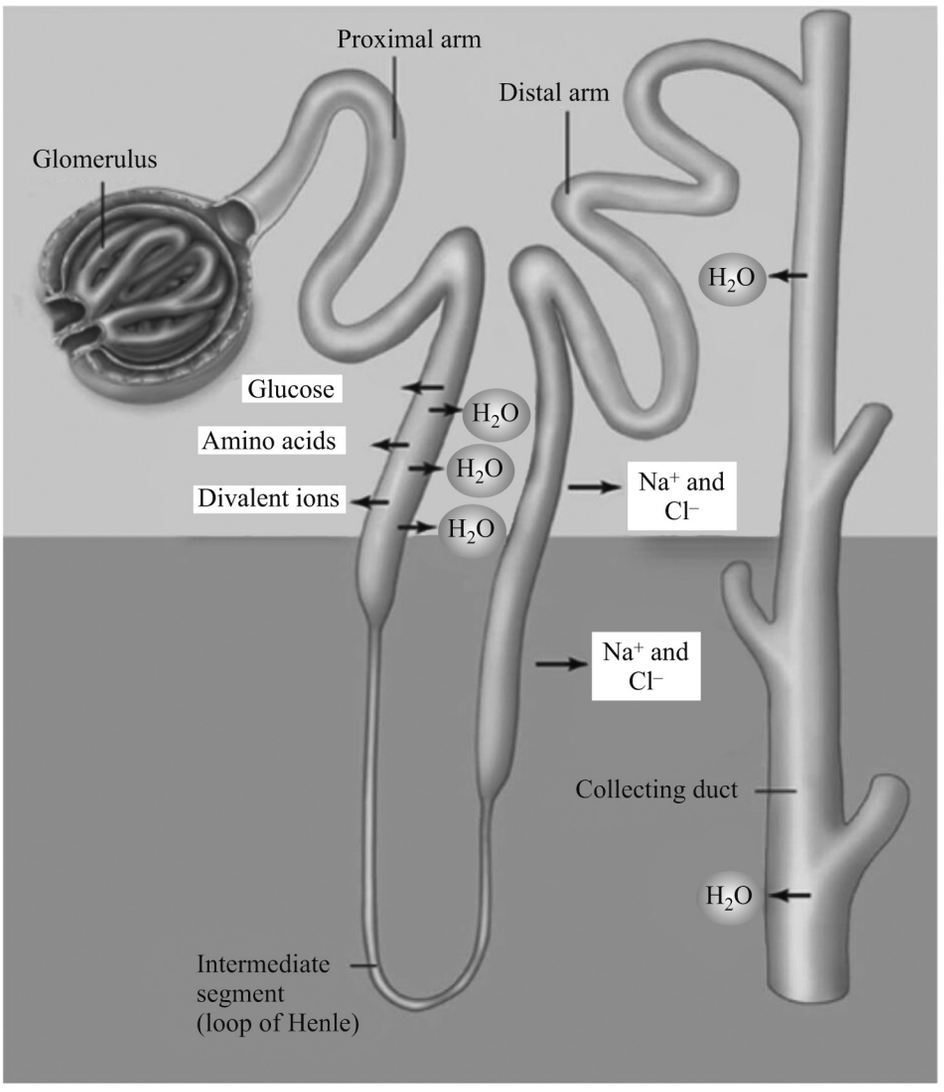


Fig. 16.1: A nephron showing the glomerulus as well as the tubules

16.3 COMPONENTS OF KIDNEY FUNCTION TEST

The components of the Kidney function test can be broadly divided into two categories.

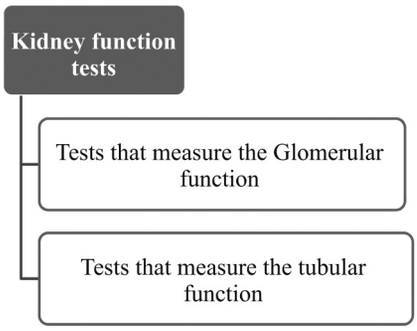


Fig. 16.2

The tests that are part of the Kidney Function test panel are:

(a) Urine examination
(b) Serum Urea
(c) Serum creatinine
(d) Blood urea nitrogen (BUN)
(e) Calcium
(f) Phosphorus
(g) Protein
(h) Albumin
(i) Creatinine clearance
(j) Urea clearance
(k) Inulin clearance
(l) Dilution and Concentration test
(l) Serum electrolyte levels

16.4 URINE EXAMINATION

Before we do a quantitative examination of urine a qualitative examination is necessary as it can provide excellent clues to the nature and location of the lesion in the renal system.

This examination consists of a physical examination where the colour, odour, quantity, specifc gravity etc of the urine is noted. Microscopic examination of urine is done to rule out any pus cells, Rbc casts, Crystals.

16.5 SERUM UREA

Urea is the end product of protein catabolism. The urea is produced from the amino group of the amino acids and is produced in the liver by means of the Urea cycle.

Notes

Urea undergoes filtrations at the glomerulus as well as secretion and re absorption at the tubular level. The rise in the level of serum urea is generally seen as a marker of renal dysfunction specially glomerular dysfunction. Urea level only rises when the glomerular function is reduced below 50%.

The normal serum urea level is between 20-45 mg/dl. But the level may also be affected by diet as well as certain non kidney related disorders. A high protein diet may increase the blood urea level. Similarly a low protein diet may decrease blood urea level. Other causes of protein catabolism such as any hyper metabolic conditions, starvation etc also cause increased blood urea levels. Similarly the level of urea may also be decreased in case of hepatic injury.

So even though blood urea is not an excellent marker of renal dysfunction as it rises quite late in the dysfunction and its rise is also not exclusive to kidney dysfunction, but for practical purposes serum urea level is still one of the most ordered test and forms an important part of the kidney function test.

Urea is measured in diagnostic labs either by UV kinetic method using á keto glutarate as an NH_3^+ acceptor in presence of enzyme glutamate dehydrogenase.

It is also measured calorimetrically by Berthelot's end point method and is read in visible range using a calorimeter.

16.6 BLOOD UREA NITROGEN (BUN)

Sometimes the Serum urea level is expressed as blood urea nitrogen. BUN can be easily calculated from the serum urea level. The molecular weight of urea is 60 and it contains two nitrogen atoms of combined atomic weight of 28. Hence the contribution of nitrogen to the total weight of urea in serum is 28/60 that is equal to 0.47. Hence the serum urea levels can be easily converted to BUN by multiplying it by 0.47.

A rise in blood nitrogen level is known as azotemia.

16.7 CALCIUM

This test measures the amount of Calcium in your blood, not the calcium in your bones. The body needs it to build and fix bones and teeth, help nerves work, make muscles contraction, help blood clot, and help the heart to work. The Calcium test screens for problems with the parathyroid glands or kidneys, certain types of cancers and bone problems, inflammation of the pancreas (pancreatitis), and kidney stones.

Normal Results: 8.5 to 10.2 mg/dl

16.8 PHOSPHORUS

Phosphorus is a mineral that makes up 1% of a person's total body weight. The body needs phosphorus to build and repair bones and teeth, help nerves function, and make muscles contract. The Kidneys help control the amount of phosphate in the blood. Extra phosphate is filtered by the kidneys and passes out of the body in the urine. It plays an important role in the body's utilization of carbohydrates and fats and in the synthesis of protein for the growth, maintenance, and repair of cells and tissues.

High levels of phosphorus in blood only occur in people with severe kidney disease or severe dysfunction of their calcium regulation. Excessively high levels of phosphorus in the blood, although rare, can combine with calcium to form deposits in soft tissues such as muscle.

Normal Results: Standard range not available

16.9 PROTEIN

Protein in urine is noticeably increased in renal disease of any etiology, except obstruction, and is therefore a very sensitive, general screening test for renal disease, though not specific. The extent of proteinuria also provides useful information. The greatest degree of proteinuria is found in the nephrotic syndrome (> 3 - 4 g/day). In renal disease with the nephritic syndrome, the urinary protein excretion rate is usually about 1 - 2 g/day. In tubulo-interstitial disease, urine protein is generally less than 1 g/day. Only in the nephrotic syndrome is the urine protein loss sufficiently great to result in hypoproteinemia.

Protein in serum can generally be maintained at concentrations above the lower limit of normal by increased hepatic protein synthesis so long as protein loss is less than about 3 g/day

16.10 SERUM CREATININE LEVEL

Creatine is a small tripeptide found in the muscles. It stays in its phosphorylated form and releases energy for any burst of muscular activity. It is released from the muscles during regular wear and tear and is converted to creatinine (its internal anhydride). It is to be remembered that unlike urea, creatinine is not a toxic waste. It is simply used as a marker of renal function.

Creatinine is freely filtered at the glomerulus and is also to a very small extent secreted into the tubules. So any problem with gromerular filtrations has a significant effect on the excretion of creatinine resulting in a much substantial rise in serum creatinine level.

Notes

Normal serum creatinine level is 0.6 to 1.5 mg/dl. Serum creatinine is a better indicator of renal function and more specifically glomerular function than urea. For a particular individual the creatinine level is dependent on the muscle mass and muscle wear and tear. There may be significant difference in creatinine level of individuals with vastly differing muscle mass. For example a body builder or athlete will have higher creatinine levels than a sedentary desk worker. Similarly creatinine level will also increase in case of any muscle trauma or excessive wear and tear as seems in athletes and people involved in hard physical labor.

Creatinine is most commonly measured in laboratories calorimetrically by Jaffe's method.

16.11 UREA CLEARANCE

Urea clearance is the hypothetical amount of blood from which kidney clears urea in one minute. This is measured by measuring the concentration of urea in blood, concentration of urea in urine and amount of urine excreted over a one hour interval.

Urea clearance is less than its glomerular filtration as some of the urea that is filtered at the glomerulus is reabsorbed at the tubules.

To measure urea clearance first the patient is made to void urine and then the made to drink two glasses of water. Then the urine is collected after an hour and a blood specimen is also collected at the same time. Then the patients urine sample is collected after another hour. The urea level in the two urine samples and the blood sample is measured. The urine volume is calculated as urine output per minute.

If the urine output is more than 2 ml/minute then urea clearance (in ml/ minute) is measured as:

$$\frac{(\text{Urine urea conc.} \times \text{Urine volume per minute})}{\text{Urea conc. in serum}}$$

If urine output is less than 2 ml/minute then urea clearance (in ml/min) is measured as:

$$\frac{(\text{Urine urea conc.} \times \sqrt{\text{urine volume}}\ \text{ml/min})}{\text{Urea conc. in serum}}$$

Maximum urea clearance of an average individual or body surface area of 1.73 sq m is 75 ml/ min and a standard urea clearance is 54 ml/min. A urea clearance below 60% of standard is considered impaired.

16.12 CREATININE CLEARANCE RATE

Creatinine is filtered at the glomerulus and its reabsorption at the tubular level is insignificant. Because of this creatinine clearance can be used to measure Glomerular Filtration Rate (GFR).

It is measured over a period of 24 hrs. For this urine is collected over a 24 hour period and blood sample is also collected. The concentration of creatinine is measured both in the urine as well as the serum sample.

Creatinine clearance is measured by the following method:

$$\frac{(\text{Conc. of creatinine in urine} \times \text{volume of urine})}{\text{Conc. of creatinine in serum}} \times 1440$$

The normal range of creatinine clearance is:

Males : 100 – 120 ml/ min

Females : 95 – 105 ml/min

This is very close to the glomerular filtration rate.

16.13 INULIN CLEARANCE

Inulin is a small polysaccharide of low molecular weight made up of fructose.

To measure glomerular filtrate the substance used should have the following qualities:

(a) It should be non toxic.

(b) Should not be metabolized in the body.

(c) Should be completely filtered at the glomerulus.

(d) Should neither be secreted or reabsorbed at the tubules.

Inulin meets all these criteria and hence makes for a suitable candidate to measure GFR. Inulin clearance hence equals to GFR. GFR is the amount of blood that passes though and is filtered through the glomerulus in a minute.

To measure Inulin clearance first Inulin is introduced in the blood by means of a slow continuous infusion to maintain a steady conc. of Inulin in the blood. This is done by first infusing 30 ml of 10% inulin in 250 ml of normal saline infused at a rate of 20 ml/ min to achieve desired concentration.

Then 70 ml of 10% inulin in 500 ml saline in infused at a rate of 4 ml/ min to maintain the desired concentration.

Notes

The patient is asked to micturate 20 minutes after the second infusion and the urine in discarded and the time noted. After exactly 60 minutes, take another sample of urine and blood is collected. Measure the volume of urine and the conc. of inulin in both the serum and urine.

Thereafter the inulin clearance is measured by the formulae:

$$\frac{\text{(Conc. of Inulin in urine} \times \text{volume of Inulin)}}{\text{Conc. of Inulin in serum}}$$

Normal inulin clearance is 120 to 130 ml/minute for an average person with a body surface area of 1.73 sq m. This is a close approximation of the GFR.

A below normal inulin clearance shows an impaired glomerular function.

16.14 CONCENTRATION TEST

In case of water shortage in the body the kidney is able to concentrate urine and conserve water. This is done by increasing the reabsoption of water from the glomerular filtrate at the tubular level. So in effect the measure of the ability of the kidney to conserve water and concentrate urine is a measure of tubular function.

For this test the patient is not allowed to take any food or water after the evening meal. The first three urine samples passed in the morning are collected and their specific gravity measured. In a normal person the specific gravity of atleast one of the samples should be above 1.025 or above. If the specific gravity remains below 1.025 then it is a sign of tubular dysfunction.

16.15 DILUTION TEST

Like the concentration test the dilution test is also a measure of functioning of the tubules. In cases of fluid overload of our body the tubules reabsorb lesser amounts of water resulting in excretion of diluted urine.

For this test the subject is put on overnight fast and then in the morning the subject is made to drink 1200 ml of water over a time period of 30 minutes. Then the urine samples are collected every hour for 4 hours. The specific gravity of the samples is measured and atleast one of the samples should have a specific gravity of 1.003 or less. If none of the samples have the specific gravity of 1.003 or less this is a sign of tubular dysfunction.

16.16 ELECTROLYTES

The purpose of the kidney is not just water balance and excretion but also to maintain the electrolyte balance of our body. Kidneys actively reabsorb or

excrete electrolytes to maintain the electrolyte balance of the body. Owing to their small size almost all electrolytes are filtered at the glomerulus. After filtration most of the electrolytes are absorbed back at the tubular level but any problem at the tubular level will result in non absorption and excessive loss of electrolytes in urine.

Serum electrolytes that are measured for this purpose are:

Serum Sodium levels (Na^+) : 135 to 145 mmols/liter

Serum Pottasium level (K^+) : 3.5 to 5 mmols/liter

Serum Chloride level (Cl^-) : 95 to 105 mmols/liter

INTEXT QUESTIONS 16.1

1. The functional unit of kidney is .....
2. Kidney functional test measure ..... & ..... function
3. Urea level rises when the flomerular function is reduced below .....
4. Normal serum urea level is .....
5. A rise in blood nitrogen level is known as .....
6. Normal serum creatinine level is .....
7. Creatinine is commonly measured in laboratories calorimetrically by ..... method
8. ..... rate can be used to measure Glomerular Filteration rate

WHAT HAVE YOU LEARNT

- The main function of the kidney is excretion of water soluble waste products from our body. The kidney has various filtration, excretion and secretary functions.
- Derangement of any of these function would result in either decreased excretion of waste products and hence their accumulation in the body or loss of some vital nutrient from the body.
- The functional unit of the kidney is nephron. It consists of two main parts, the glomerulus and the tubular system.
- Components of Kidney function test are tests that measure the Glomerular function and tubular function.

Notes

The tests that are part of the Kidney Function test panel are:

(a) Urine examination
(b) Serum Urea
(c) Serum creatinine
(d) Blood urea nitrogen (BUN)
(e) Urea clearance
(f) Creatinine clearance
(g) Inulin clearance
(h) Dilution and concentration test
(i) Serum electrolyte levels

- Urine examination consists of a physical examination where the colour, odour, quantity, specifc gravity etc of the urine is noted.
- Microscopic examination of urine is done to rule out any pus cells, Rbcs, casts, Crystals.
- Urea is the end product of protein catabolism and Urea undergoes filtrations at the glomerulus as well as secretion and re absorption at the tubular level.
- The normal serum urea level is between 20-45 mg/dl. But the level may also be affected by diet as well as certain non kidney related disorders.
- Blood Urea Nitrogen can be easily calculated from the serum urea level and the serum urea levels can be easily converted to BUN by multiplying it by 0.47.
- Creatine is a small tripeptide found in the muscles and is simply used as a marker of renal function.
- Normal serum creatinine level is 0.6 to 1.5 mg/dl. Serum creatinine is a better indicator of renal function and more specifically glomerular function than urea.
- Creatinine is filtered at the glomerulus and its reabsorption at the tubular level is insignificant. Because of this creatinine clearance can be used to measure Glomerular Filtration Rate (GFR).
- The purpose of the kidney is not just water balance and excretion but also to maintain the electrolyte balance of our body.

TERMINAL QUESTIONS

1. What is the functional unit of the kidney? Draw a diagram showing its various components.

2. What is the purpose of a Kidney Function Test?
3. What is urea? How is it excreted by the kidney?
4. What is urea clearance? How is it measured?
5. What is Creatinine? How is its clearance measured?
6. On what conditions other than renal function does the level of creatinine in blood depend upon?
7. What is GFR (Glomerular filtration rate)? How is it measured?
8. What are the ideal characteristics of a substance used to measure the GFR?

ANSWERS TO INTEXT QUESTIONS

16.1

1. Kidney
2. Glomerular & Tubular
3. 50%
4. 20-45 mg/dl
5. Azotemia
6. 0.6 - 1.5 mg/dl
7. Jaffe's
8. Creatinine Clearance

Notes

17

LIVER FUNCTION TESTS

17.1 INTRODUCTION

Liver function tests are a group of tests done to assess the functional capacity of the liver as well as any cellular damage to the liver cells. To assess all functional capabilities of the liver such as:

(a) Its Synthetic ability: By measuring the various plasma proteins such as albumin and prothrombin that are synthesized by the liver. Also lipids which are also synthesized in the liver.

(b) Its secretory/excretory abilities: By measuring the serum billirubin level

OBJECTIVES

After reading this lesson, you will be able to:

- enumerate the various tests undertaken to measure liver function and damage.
- describe the various tests are read to reach a conclusion as to the type of damage inflicted on the liver.

17.2 THE COMMON TESTS THAT FORM PART OF THE LIVER FUNCTION TEST PROFILE

(a) Serum Bilirubin: both conjugated and unconjugated.

(b) Total serum proteins and albumin globulin ratio.

(c) Liver enzymes : Transaminases :AST (SGOT), ALT (SGPT). Others: ALP, GGT, LDH.

(d) Prothrombin time

17.3 SERUM BILIRUBIN

Bilirubin is one of the end products of haem metabolism and is derived from the haem part of the hemoglobin molecule. It is a yellow coloured pigment.

Liver plays an important role in the metabolism of bilirubin. After the breakdown of haem portion of the hemoglobin molecule 'unconjugated bilirubin' is insoluble in water. It is transferred from the site of RBC and haem breakdown such as the spleen to the liver for 'conjugation' bound to albumin. At the liver it is conjugated with glucoronic acid with the help of enzyme glucuronyl transferase. This conjugation makes bilirubin water soluble and this conjugated bilirubin is excreted into the bile.

While measuring bilirubin we measure total and conjugated bilirubin (Direct bilirubin) and calculate the indirect bilirubin by substracting the direct from the total.

The normal range of bilirubin is:

Total Bilirubin: 0.2 to 1 mg/dl

Unconjugated Bilirubin: 0.1 to 0.6 mg/dl

Conjugated bilirubin: 0.1 to 0.4 mg/dl

A rise of bilirubin level to that of 2 mg/dl results in the symptoms of jaundice which is marked by deposition of bilirubin in the various mucous membranes.

Jaundice is divided into three types depending on its etiology:

(a) Pre hepatic jaundice: In this case the cause of jaundice is at the level of bilirubin processing before it reaches the liver. Most common cause is over production of bilirubin due to hemolytic disorders. In this case the rise in the level of unconjugated bilirubin is more than conjugated bilirubin hence there is a rise in total and indirect bilirubin.

(b) Hepatic jaundice: This is caused by cellular dysfunction of the liver hence is also called hepatocellular jaundice. It is caused by the inability of the liver cells to process and excrete the bilirubin in the system. It is seen in hepatitis, cirrhosis of liver etc. In this jaundice there is rise in total, direct as well as indirect bilirubin levels.

(c) Post hepatic jaundice: This is also known as obstructive jaundice as it is caused by obstruction to the outflow of bile resulting is reabsoption of conjugated bilirubin and it making an appearance in the serum. It cause by carcinoma of the mouth of gall bladder, stone in the bile duct etc. In this type of jaundice we see a rise in total as well as direct (conjugated) bilirubin.

The common method of measuring serum bilirubin level is the Diazo method using Diazotized sufanilic acid to convert bilirubin into a azobilirubin the color intensity of which is measured colorimetrically at a wavelength between 555 nm (550 to 580 nm).

The conjugated bilirubin reacts directly with the Diazo sulfanilic acid in an aqueous medium and hence also called direct bilirubin. For unconjugated or free bilirubin which is not water soluble we need to dissolve it into DMSO for the reaction to occur. This method is the 'indirect method' for measuring bilirubin levels and it measures both conjugated and unconjugated bilirubin i.e. total serum bilirubin.

INTEXT QUESTIONS 17.1

1. Liver function test assesses ..... of liver
2. The synthetic ability of liver is assessed by measuring .....
3. The secretory ability is assessed by measuring .....
4. Indirect bilirubin is calculated by substracting ..... from .....
5. Serum bilirubin is commonly measured by ..... method

17.4 SERUM ALBUMIN AND ALBUMIN GLOBULIN RATIO

Serum albumin is an important serum protein vital for maintaining the plasma oncotic pressure as well as acts as a carrier for various biological substances and drugs. Serum albumin is exclusively synthesized by liver and hence the level of serum albumin gives us a stock of the synthetic ability of the liver.

Another cause of fall of serum albumin maybe protein malnutrition but in that case the fall of all serum proteins including globulins will be seen.

The normal total protein level is 5 to 8.5 gm/dl. The total serum albumin level is 3.5 to 5 gm/dl. The total plasma globulin level is calculated by subtracting the plasma albumin from the total protein level and is normally in the range of 2 to 2.5 gm/dl.

The normal range for albumin : globulin ratio is 1.2 to 1.5. But with hepatic dysfunction this ratio recedes towards 1 as the synthetic function of liver is compromised. The reversal of the ratio i.e if the value recedes below 1, it is an ominous sign and may mark an infective/inflammatory pathology marked by rise in serum globulin level and fall in serum albumin levels.

To measure serum albumin the Bromocresol green method is used. Albumin in the presence of bromocresol green at a slightly acidic pH gives a yellow green to blue geen colour. The intensity of this colour is dependent on the concentration of albumin in the sample. This intensity is read at a wavelength of 630 nm.

To measure the total protein content of the sample the biuret method it used. In this method the cupric ions of copper (II) sulphate, present in the biuret reagent, form a violet coloured complex with the proteins in a slightly alkaline medium. The intensity of the colour formed is measured at a wavelength of 540 nm (530 to 550 nm).

INTEXT QUESTIONS 17.2

Match the following

1. Total Bilirubin	(a) 5 - 8.5 mg.dl
2. Conjugated Bilirubin	(b) Serum albumin
3. Total Protein	(c) 0.2 - 1 mg/dl
4. Total serum albumin	(d) Total protein
5. Bromocresol green method	(e) 0.1 - 0.4 mg/dl
6. Biuret method	(f) 3.5 - 5 gm/dl

17.5 PROTHROMBIN TIME

Prothrombin is a clotting factor (clotting factor II) and it forms an important part of both the intrinsic and extrinsic pathway. Its active form is Thrombin (also clotting factor IIa). It is a serine peptidase which converts fibrinogen to fibrin. Prothrombin is synthesized in the liver. And hence prothrombin activity in plasma is used to measure the synthetic function of liver.

Prothrombin time is measured by taking human plasma from blood that has been collected in tube containing citrate as an anticoagulant. The plasma in put in an automated machine which adds an excess of calcium to reverse the anticoagulant effects of citrates and measures the time taken for fibrinogen to be converted to fibrin hence measures the activity of thrombin in the plasma.

The prothrombin time differs in accordance to the analytical method used. Hence to compensate for this International normalized ratio (INR) is used. In this the manufacturers of the kit assign an International sensitivity index (ISI) value. This shows the amount of tissue factor present in the kit as against an internationally accepted standard. The ISI value is generally 1 to 2.

The following is the method to calculate INR:

$$INR = \left(\frac{\text{PT test sample}}{\text{PT control}}\right)^{ISI}$$

A ratio of 0.8 to 1.2 is considered normal for patients not on warfarin. For individuals on warfarin for any disorder an INR of 2.0 to 3.0 is the target.

17.6 LIVER ENZYMES

Liver enzymes along with bilirubin are the most commonly measured parameter measured in the liver function test. These enzymes are hepatic in origin and they are leaked into the serum with the destruction of hepatic cells. Liver enzymes are measured to get an idea of the cellular insult on the liver and are increased in a wide variety of conditions such as viral hepatitis, toxic hepatitis, cirrhosis of liver etc.

The commonly measured enzymes are:

(a) Transaminases: AST (SGOT), ALT (SGPT)

(b) Transpeptidases: GGT

(c) Phosphatase: ALP.

(a) Transaminases: They are a group of enzymes that transfer the amino group from an amino acid to α keto acid converting the α keto acid into an amino acid while converting the amino acid into a keto acid.

The transaminases that are measured in the liver function test are ALT and AST.

Alanine transaminase (ALT) catalyses the following reaction:

$$\text{Alanine} + \alpha\text{ keto Glutarate} \xrightarrow{ALT} \text{Pyruvate} + \text{Glutamate}$$

Aspartate transaminase (AST) catalyses the following reaction:

$$\text{Aspartate} + \alpha\text{ keto Glutarate} \xrightarrow{AST} \text{Oxaloacetate} + \text{Glutamate}$$

- The normal level of ALT in serum is 7 to 40 IU/L.
- The normal level of AST in serum is 8 to 40 IU/L.

An increase in AST or ALT levels hints at an insult to the liver parenchyma tissue. ALT is a more specific marker of hepatic injury than AST as AST elevation is also seen in cardiac tissue injury, haemolysis and muscle tissue.

To measure the level of transaminases the reaction catalysed by them is coupled to a reaction in which NADH is used up resulting in change in the photometric intensity when read in the UV range at 340 nm. It is a UV kinetic method.

For ALT (SGPT):

$$\text{Alanine} + \alpha\text{ Keto glutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{(Lactate dehydrogenase)}]{\text{LDH}} \text{Lactate} + NAD^+$$

For AST (SGOT):

$$\text{Aspartate} + \alpha\text{ Keto glutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow[\text{(Malate dehydrogenase)}]{\text{MDH}} \text{Malate} + NAD^+$$

(b) Alkaline Phosphatase: It is a hydrolase that removes phosphates from all kinds of molecules such as proteins, nucleotides etc.

It is found in cells lining the billiary system hence a rise in it level is indicative of damage to the billiary tree due to cholestasis. It maybe due to stone blocking the large ducts or intrahepatic obstruction, inflammation of the biliary channels.

Alkailine phosphatase is also found in placenta and bones. Hence the level is also increased in growing children in whom bones undergo remodeling and in Paget's disease in adults.

Normal level of alkaline phosphatase is between 45 to 115 IU/L.

The method for measuring the level of alkaline phosphatase is a kinetic method using p-nitrophenylphosphate as substrate for the enzyme and measuring rate of formation of the colored substrate (p- nitrophenol) formed from the reaction. This measurement of the color intensity is done colorimetrically at a wavelength of 405 nm.

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{p- Nitrophenol} + \text{Phosphate}$$

(c) Gamma glutamyl transpeptidase: It is another enzyme specific to the biliary tree and a more specific indicator of cholestasis and damage to the biliary tree. It is also a highly specific marker and is raised in even minute and subclinical damage to the biliary tree.

Its normal range is in between 0 to 42 IU/l.

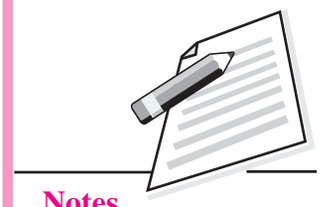
INTEXT QUESTIONS 17.3

Match the following

1.	AST	(a)	45 - 115 IU/L
2.	Alkaline Phosphatase	(b)	0 - 42 IU/L
3.	ALT	(c)	8 - 40 IU/L
4.	Gamma glutamyl transpeptidase	(d)	7 - 40 IU/L

WHAT HAVE YOU LEARNT

- Liver function tests are a group of tests done to assess the functional capacity of the liver
- Synthetic ability of the liver is assessed by measuring the various plasma proteins
- Secretory/excretory ability is assessed by measuring the serum billirubin level
- The common tests that form part of the liver function test profile are Serum Bilirubin both conjugated and unconjugated, Total serum proteins and albumin globulin ratio, Liver enzymes and Prothrombin time
- While measuring bilirubin we measure total and conjugated bilirubin (Direct bilirubin) and calculate the indirect bilirubin by substraction the direct from the total.
- The common method of measuring serum bilirubin level is the Diazo method
- The normal total protein level is 5 to 8.5 gm/dl. The total serum albumin level is 3.5 to 5 gm/dl and the normal range for albumin : globulin ratio is 1.2 to 1.5
- Serum albumin is measured by Bromocresol green method and total protein is measured by biuret method
- Prothrombin is a clotting factor (clotting factor II) and it forms an important part of both the intrinsic and extrinsic pathway
- International normalized ratio (INR) is used for measuring the Prothrombin time
- The commonly measured enzymes are Transaminases: AST (SGOT), ALT (SGPT), Transpeptidases: GGT, Phosphatase: ALP.



TERMINAL QUESTIONS

1. What are functional aspects of liver?
2. Write a short note on conjugated and unconjugated bilirubin.
3. Write short note on the laboratory method to measure serum bilirubin level both direct and indirect.
4. What is the method used to measure albumin in serum?
5. What is the method used to measure total protein in serum?
6. What is the importance of INR and how is it calculated?

ANSWERS TO INTEXT QUESTIONS

17.1

1. Functional capacity
2. Plasma protein
3. Serum bilirubin
4. Direct, total bilirubin
5. Diazo

17.2

1. (c) 2. (e) 3. (a) 4. (f) 5. (b) 6. (d)

17.3

1. (c) 2. (a) 3. (d) 4. (b)

Notes

18

SPECTROPHOTOMETRY, LIGHT EMISSION AND SCATTERING ANALYTICAL TECHNIQUE

18.1 INTRODUCTION

Measuring light emission, transmittance and scattering are few of the most important techniques used in modern biochemistry laboratory. They are used in spectrophotometer, colorimetry, chemiluminescence, ELISA etc. The ease and accuracy of measurement of light emission or transmittance makes it a method of choice in a large number of analytical techniques.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the functional aspects of a colorimeter.
- describe Spectrophotometry.
- explain the functional aspects of chemiluminescence.
- explain the concepts of incident, transmitted, absorbed and scattered light.
- describe the Beer Lambert's law.

18.2 EXPLAINING THE TERMS USED

As we study further we will be encountering some terms such as incident light, absorbed light or absorbance, transmitter light or transmittance, scattered light etc. Here we try to explain the terms:

(a) Incident light: Incident light refers to the beam of light that is directed at the cuvette (or reaction vessel) from a light source.

(b) Transmitted light: Transmitted light is the light that passes through the cuvette to emerge on the other side.

(c) Absorbed light: Absorbance is the term used to describe the light that is absorbed by the reaction mixture and it depends on the intensity of the color of a reaction product in the reaction vessel.

Absorbance (A) = Incident light (I) – Transmitted light (T)

In conditions where scatterence is zero.

(d) Scattered light: It is the light that is not absorbed but is scattered or reflected back by opaque substances in the reaction vessel. This is used to measure turbidity in reaction such as immuno turbidity reactions.

18.3 COLORIMETRY

Colorimetry is a method employing the measurement of absorbance of a reaction mixture. It is the simplest method used and is employed by a large number of instruments such as a colorimeter, semiauto analyzer, auto analyzer. The most important thing for this method is that the metabolite or chemical we intend to measure should undergo a reaction in the cuvette to produce a colored product or there should be certain change in absorbance before and after the reaction as in cases of UV kinetic methods. The intensity of the color developed or change in absorbance will depend on the concentration of the metabolite in the sample. The method is based on passing a beam of light of a certain wavelength through the reaction vessel or cuvette containing the colored product of the reaction. The incident light as well as the transmitted light of the particular wavelength is measured and the absorbance calculated. The absorbance is plotted on a graph which has been drawn by plotting a curve of absorbance of the reaction mixture as against a serial dilution of the metabolite or chemical the reaction mixture contains.

Absorbance numerically is the log of the ratio of incident light upon transmitted light.

$$A = \log_{10}(I/ T)$$

This same principle is used in all colorimeters as well as autoanalyzers, semi auto analyzers. The difference being that in auto analyzers the machine itself transfers a pre determined amount of the sample and reagents into a reaction vessel and reads the absorbance and then plots the absorbance on a graph pre plotted during calibration while in a semi auto analyzer the reagent and sample

are mixed in a test tube and fed into the semi auto analyzer to be read and the value is plotted on a pre stored curve plot during calibration. In colorimeter the reading is taken manually and then the concentration is calculated by manually plotting the absorbance on a calibration curve.

In all these instruments the basic architecture is the same. A source of light is used from which the light is passed through a filter which blocks all wavelengths except the wavelength at which we need the sample to be read. This light passes through a cuvette which holds the sample post the color producing reaction. After passing through the sample the light beam falls onto a photo voltaic cell. This photo voltaic cell produced a current the strength of which is directly proportional to the amount of light falling on it which will be inversely proportional to the absorbance. In reality the photo voltaic cell measures incident light and based on this calculated the absorbed light or absorbance. The absorbance will be directly proportional to the amount of the colored particles in the reaction mixture and hence directly proportional to the metabolite or chemical we are trying to evaluate in the sample.

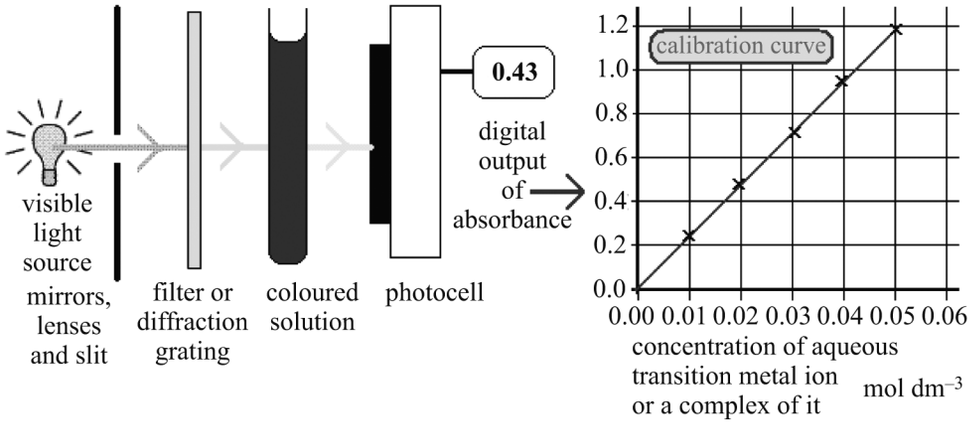


Fig. 18.1: A Simplified diagram of a colorimeter

18.4 BEER LAMBERT'S LAW

The Beer Lamberts law describes the co relation between the intensity of incident light, the path the light has to travel and the absorbance of the medium it passes through.

Absorbance is directly proportional to length of the path travelled by the light beam and the optical density of the colored solution. In a colorimeter the length of the path is fixed and this factor is negated when we set zero in the colorimeter

with only distilled water in the cuvette. Then the only factor affecting the absorbance is the optical density of the solution in the cuvette.

$A = \varepsilon b c$

A = absorbance (-)

ε = molar absorbtivity with units of L /mol/cm

b = path length of the sample (cuvette)

c = concentration of the compound in solution, expressed in mol / L

18.5 SPECTROPHOTOMETRY

The difference between colorimetry and spectrophotometry is that in spectrophotometry instead of taking reading at a set wavelength we read the absorbance over a range of wavelength taking readings at every 5 or 10 nm range. It instead of giving a specific absorbance reading gives a data stream which contain absorbance for every wavelength made incident on the sample. Or it can also give a spectra reading or a graph that plots absorbance against the increasing wavelength of incident light.

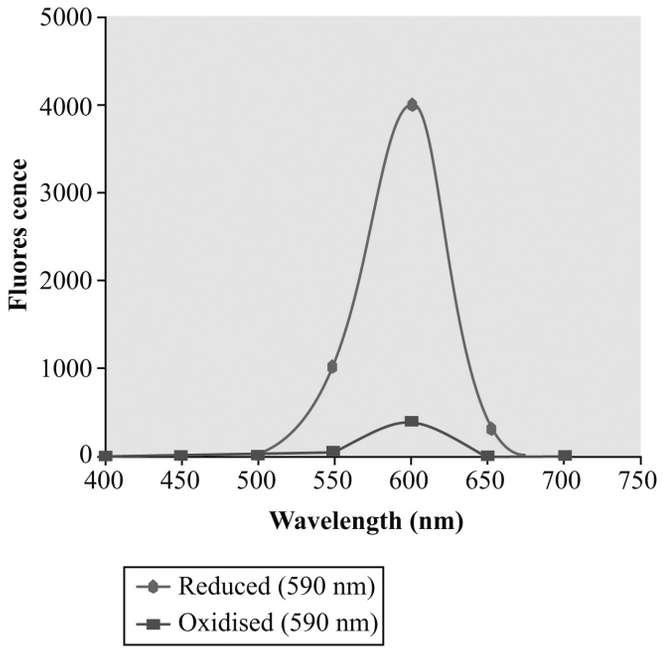


Fig. 18.2: Spectophotometry

This is an example of spectra reading where the absorbance of the sample is plotted over a wavelength between 400 to 750 nm.

18.6 LIGHT EMISSION TECHNOLOGIES OR CHEMILUMINESCENCE

Chemiluminescence is the production of light during a chemical reaction. This is seen in nature in various natural reactions such as the one happening in fire flies.

The chemical reaction can be displayed like this:

$$[A] + [B] \rightarrow [\blacklozenge] \rightarrow [\text{Products}] + \text{light}$$

One such reaction is the reaction between luminol and hydrogen per oxide:

$$\text{luminol} + H_2O_2 \rightarrow \text{3-APA}[\blacklozenge] \rightarrow \text{3-APA} + \text{light}$$

- where 3-APA is 3-aminophthalate
- 3-APA[♦] is the excited state fluorescing as it decays to a lower energy level.

Immunochemilumnisence is a method in which a chemiluminescence reaction is used by conjugating an enzyme, catalyzing the chemiluminescence, reaction to an antibody specific to another antibody which is bound to the antigen of interest. This enzyme converts a substrate to a product and during the process releasing light energy. This photon of light is detected by a sensor and an accurate measurement of the number of chemiluminescence reactions occurring during a specific time period can be made. This directly proportional to the number of enzyme molecules present which is directly proportional to the antigen molecules present in the sample.

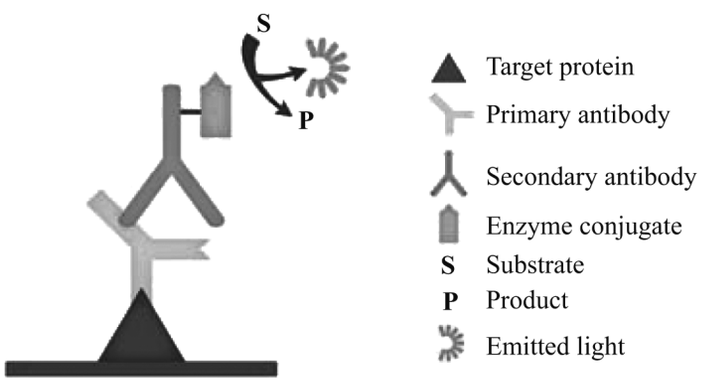


Fig. 18.3: Chemiluminescence

Chemiluminescence is an extremely accurate method with its accuracy reaching to the gold standard tests such as Radio immune assays (RIA). It is used to measure hormone levels in serum.

18.7 FLUORESCENCE

It is the ability of a substance to emit visible light after absorbing light from the visible or UV range. When these substances absorb light in the UV range of the spectra and emit light in the visible range, this property is called UV florescence and is responsible for certain substances like Vitamin B_2, quinine, certain minerals glowing in the dark.

The principle behind fluorescence is that of excitation of an electron. The incoming light commonly UV light provides the electron in an orbital energy hence helping it shift to a higher energy orbital. Some of the energy in the incident light is used up in this movement of electron. After sometime the electron returns to its original lower energy orbital and the remaining energy is released in form of light. This light has lesser energy than the incident light and hence has a larger wavelength. This results in the phenomenon of high energy UV light being absorbed by a substance and low energy visible light being emitted in its place.

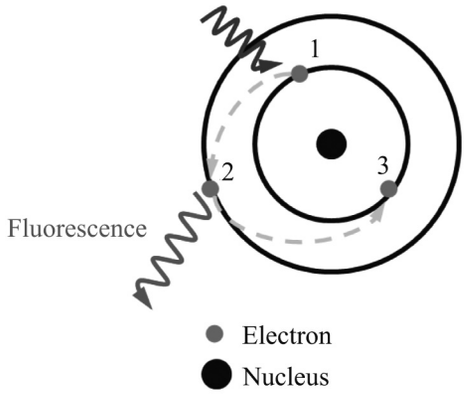


Fig. 18.4: Fluorescence

Principal of fluorescence

In laboratory fluorescence spectroscopy is used to analyze organic compounds. Tryptophan is an aromatic amino acid which displays fluorescence. It is present in a large number of proteins. The fluorescence of the tryptophan residue of any protein is effected by the environment of the tryptophan residue. Hence in cases of aberrant proteins or in cases of aberrant folding of the amino acid chain in any protein the tryptophan residue's immediate environment is changed and hence a measureable change in fluorescence is seen.

The fluorospectrometer consists of a light source, a filter, a monochromatizer, a sensor to measure the fluorescent light placed at 90% to the incident beam of

light to prevent reflected light from reaching the sensor. A quartz cuvette is used as a high grade quartz cuvette does not absorb the light in the wavelength range of fluorescence.

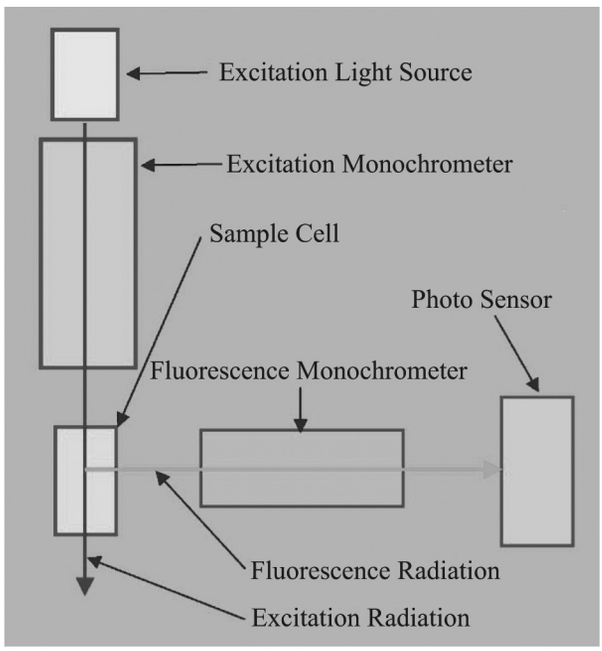
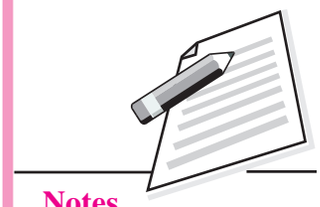


Fig. 18.5: Simple diagram of the architecture of a fluorescence spectrophotometer

Apart from this spectro photometer the flame photometer also works on the principal of excitation of an electron so as to move it out of its orbit and then read the visual spectra of the light emitted when the electron moves back to its original orbit. The only difference being that the excitation energy is provided by the flame into which the metal ions are sprayed into in form of a fine spary or mist.

INTEXT QUESTIONS 18.1

1. Beam of light directed to the cuvette is .....
2. Absorbance of a reaction mixture is measured by .....
3. ..... is used to measure turbidity in reaction
4. Absorbance over a range of wavelength is taken in .....
5. The production of light during a chemical reaction is .....



WHAT HAVE YOU LEARNT

- Measuring light emission, transmittance and scattering are few of the most important techniques used in modern biochemistry laboratory.
- They are used in spectrophotometer, colorimetry, chemiluminescence, ELISA etc.
- The ease and accuracy of measurement of light emission or transmittance makes it a method of choice in a large number of analytical techniques
- Incident light refers to the beam of light that is directed at the cuvette (or reaction vessel) from a light source.
- Transmitted light is the light that passes through the cuvette to emerge other side.
- Absorbance is the term used to describe the light that is absorbed by the reaction mixture and it depends on the intensity of the color of a reaction product in the reaction vessel.
- Scattered light is the light that is not absorbed but is scattered or reflected back by opaque substances in the reaction vessel and is used to measure turbidity in reaction such as immune turbidity reactions.
- Colorimetry is a method employing the measurement of absorbance of a reaction mixture. It is the simplest method used and is employed by a large number of instruments such as a colorimeter, semiauto analyzer, auto analyzer
- The difference between colorimetry and spectrophotometry is that in spectrophotometry instead of taking reading at a set wavelength we read the absorbance over a range of wavelength taking readings
- Chemiluminescence is the production of light during a chemical reaction
- Immunochemilumnisence is a method in which a chemiluminescence reaction is used by conjugating an enzyme, catalyzing the chemiluminescence, reaction to an antibody specific to another antibody which is bound to the antigen of interest
- Fluorescence is the ability of a substance to emit visible light after absorbing light from the visible or UV range

TERMINAL QUESTIONS

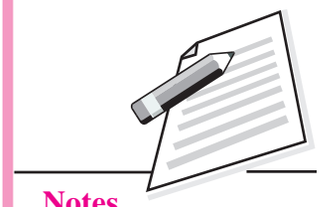
1. Enumerate the various diagnostic methods that use emitted, transmitted or generated light for measurement.

2. What is the beer lambert law? What are the correlations derived from it.
3. What is the principle of colorimetry? Draw a simple labelled diagram of a colorimeter.
4. Write a short note on spectrophotometry.
5. What is chemiluminescence? What are its uses in diagnostic?
6. What is fluorescence? How is it used in diagnostics?
7. What is the principal of a flame photometer?

ANSWERS TO INTEXT QUESTIONS

18.1

1. Incidence light
2. Colorimetry
3. Scattered light
4. Spectrophotometry
5. Chemiluminescence



19

BASIC PRINCIPLES OF RADIOACTIVE MEASUREMENTS

19.1 INTRODUCTION

Radioactivity is defined as the process in which unstable atomic nuclei loses energy by emitting radiation in the form particles or electromagnetic waves. These radiations are able to ionize the atoms and molecules along their track. These radiations are able to cause cancer and death. Therefore these radiations are of health and safety concern.

To understand radioactivity we should be able to understand certain basic concepts like atom and atomic stability.

OBJECTIVES

After reading this lesson, you will be able to:

- describe Atom
- explain radioactive delay
- describe units of radioactivity
- explain the characteristics of radioactive emissions

19.2 ATOM

An atom is defined as the smallest component of an element having the chemical properties of the element. It consists of positively charged nucleus surrounded by negatively charged electrons. The nucleus is made up of positively charged protons and uncharged neutrons.

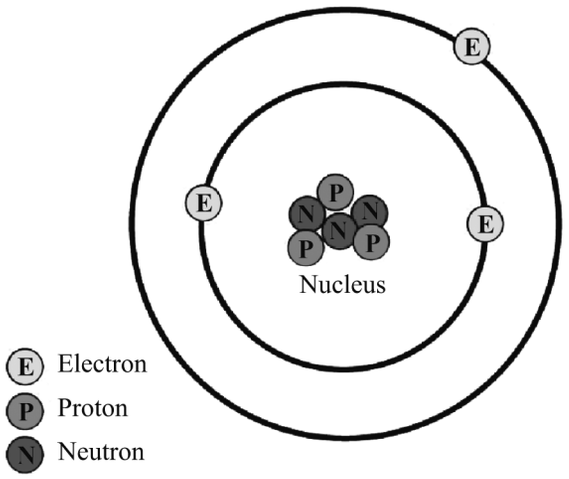


Fig. 19.1: Structure of an atom

In an atom the number of protons (P) and number of electrons (E) are equal. This number is termed as atomic number (Z).

Atomic number (Z) = (P) = (E)

The atomic number is independent of neutrons. Therefore it does not affect the chemical properties of an atom. The mass of the proton and neutron is equal and is1850 times more than that of electron. Hence the mass of an atom is concentrated in its nucleus. Mass number of an atom (A) is given by the sum of number of protons and number of neutrons in a given nucleus.

Mass number (A) = (P) + (N)

The number of neutrons for a given atom of an element may vary which gives rise to atoms with different mass number. Isotopes are defined as atoms with same atomic number but different mass number.

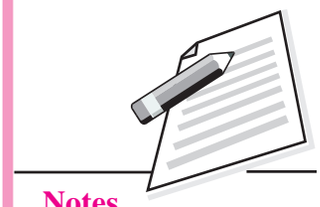
Eg: There are 3 isotopes for hydrogen- ^{1}H, ^{2}H, ^{3}H

19.2.1 Atomic stability and radiation

The stability of an atom depends upon the neutron to proton ratio (N: P)(also known as N:Z) in its nuclei. For elements with lower atomic number, N: P is 1. For elements with higher atomic number, N: P is greater than1. If this ratio is altered then the atom becomes unstable. By emitting particles or electromagnetic radiation these atoms tend to become stable. This process is known as Radioactivity or radioactive decay.

19.3 RADIOACTIVE DECAY

There are several types of radioactive decay. The most relevant to biochemistry are:



1. Decay by negatron emission
2. Decay by positron emission
3. Decay by alpha particle emission
4. Decay by emission of gamma rays
5. Decay by electron capture.

1. Decay by negatron emission

In this type of decay a neutron is converted into a proton by ejection of a negatively charged beta (β) particle called a negatron (β –ve)

$$\text{Neutron} \longrightarrow \text{Proton} + \text{Negatron } (\beta\ -\text{ve})$$

Negatron is nothing but an electron of nuclear origin. As a result of this emission the nucleus gains a proton but loses a neutron. Here N: P ratio decreases, Z increases by 1 and A remains constant. An isotope frequently used in biological work that decays by negatron emission is ^{14}C.

$$^{14}_{6}C \longrightarrow {}^{14}_{7}N + (\beta\ -\text{ve})$$

Negatron emission is important because most of the radioactive substances used in biochemistry decay by this mechanism.

^{3}H &^{14}C are used label any organic compound.^{35}S for methionine & ^{32} P for nucleic acid.

2. Decay by positron emission

In this type of decay a proton is converted into a neutron by ejection of a positively charged beta (β) particle called as positron (β +ve)

$$\text{Proton} \longrightarrow \text{Neutron} + \text{Positron } (\beta{+}\text{ve})$$

Positron is extremely unstable with transient existence. After losing their energy they interact with electrons and get destroyed. The mass and energy of these two particles gets converted into two gamma rays (γ) emitted at 180° to each other this is known as back to back emission.

As a result of this emission the nucleus gains a neutron but loses a proton. Here N: P ratio increases, Z decreases by 1and A remains constant. An isotope that decays by positron emission is 22 Na.

$$^{22}_{11}Na \longrightarrow {}^{14}_{7}N + (\beta\ -\text{ve})$$

Positron emission tomography (PET),a brain scanning technique is used in finding out the active and inactive areas of the brain.

Notes

3. Decay by alpha particle emission

Isotopes of elements with high atomic number usually decay by alpha (α) particle emission. As a result of this emission the nucleus loses 2 protons and 2 neutrons, which is similar to helium nucleus ($^{4}_{2}He$)

Here N: P ratio remains constant, Z decreases by 2 and A decreases by 4. Alpha emitters are rarely used in biological work they are highly toxic due to its large mass and ionizing power.

4. Decay by emission of gamma rays

This is an electromagnetic radiation as a consequence of electron excitation that accompanies alpha and beta particle emission. It does not lead to change in atomic number or mass. Gamma radiation has low ionizing but high penetrating power.

$$^{131}_{63}\text{ I} \longrightarrow {}^{131}_{64}\text{I} + (\beta - \text{ve}) + \gamma$$

5. Decay by electron capture

In this type of decay a proton captures an electron orbiting in the innermost k shell. The proton becomes a neutron and an electromagnetic radiation (X-ray) is given out.

$$\text{Neutron} + \text{Electron} \longrightarrow \text{Proton} + \text{X-rays}$$

$$^{125}_{53}\text{ I} \qquad\qquad ^{125}_{52}\text{ Te}$$

19.3.1 Radioactive decay energy

The radioactive decay energy is expressed as electron volt. One electron volt is the energy acquired by one electron accelerating through a potential difference of 1volt and is equivalent to 1.6×10^{-9} J. For isotopes million or mega electron volts is applicable. Alpha particles are more energetic falling in the range 4-8 Mev. Beta and gamma emitters have decay energies less than 3 Mev.

19.3.2 Rate of radioactive decay

Radioactive decay is a spontaneous process, which takes place in an exponential way. Different isotopes decay at different rate. The number of atoms disintegrating in a given time depends upon number of isotopes present (N) at that time (t) and λ is decay constant. This λ is specific for a given isotope and is defined as number of atoms disintegrating in unit time (t – 1).

$$dN/dt = - \lambda N$$

This equation can be converted into logarithmic form

$$\text{Ln } Nt/N0 = - \lambda t$$

Nt is the number of radioactive atoms present at time t,and N0 is the number of radioactive atoms present originally. In practice decay constant is expressed in terms of half life $t_{1/2}$.

The half-life of an radioactive material is defined as the time taken to become half of its original value

So Nt/No becomes ½ at t becomes $t_{1/2}$. Now the above said equation becomes

$$Ln_{1/2} = - \lambda t_{1/2}$$

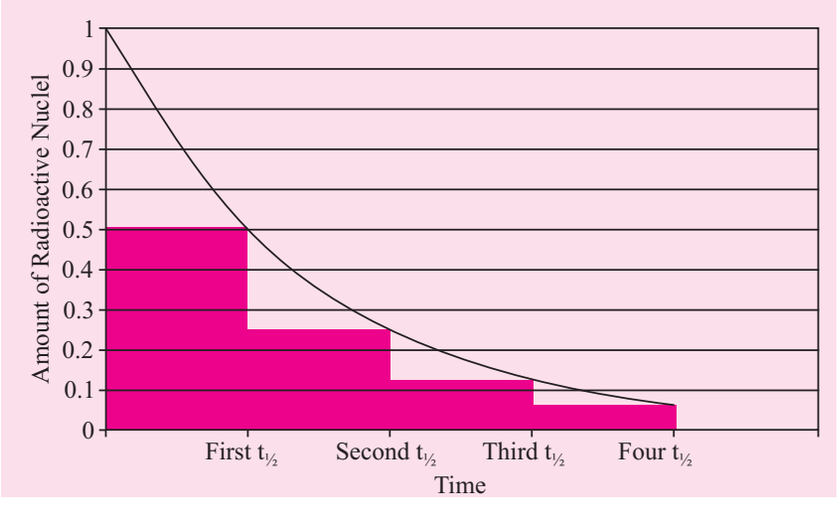


Fig. 19.2: Radioactive decay

Conversion of ln to log it becomes

$$2.303 \log_{10}(1/2) = - \lambda t_{1/2}$$

Finally, $$t_{1/2} = 0.693/ \lambda$$

The values of $t_{1/2}$ varies from 10^{19} years for lead (^{204} pb) and 3×10^{-7} s for polonium (^{212}Po).

Half life of some isotopes used in biological studies

Isotopes	Half-life
^{3}H	12.26 years
^{14}C	5760 years
^{32}P	14.20 days
^{35}S	87.20 days
^{125}I	60 days

Notes

19.4 UNITS OF RADIOACTIVITY

The radioactivity of a substance can be measured using different units. They are

- Becquerel is the SI unit for measurement of radioactivity. It is defined as the number of disintegration per second (d.p.s.)

  1 **Becquerel** (Bq) = 1 dps

  Terra Bq = 10^{12} Bq

  Giga Bq = 10^{9} Bq

  Mega Bq = 10^{6} Bq

- Curie is the commonly used unit. It is defined as the quantity of radioactive material having nuclear disintegration similar to that of 1g of radium i.e. 3.7×10^{10} dps (or 37 Giga Bq)

  1 **Curie** (Ci) = 3.7×10^{10} dps

  Milli Ci = Ci $\times 10^{-3}$

  Micro Ci = Ci $\times 10^{-6}$

- Specific activity of a substance is defined as activity per unit weight or volume (e.g., Bq/gm or B/l).
- Counts per minute (c.p.m) is disintegration detected by a radiation counter.

19.5 CHARACTERSTICS OF RADIOACTIVE EMISSIONS

19.5.1 Alpha Particles

- Alpha particles have helium nucleus with double positive charge
- These are high energy particles (3-8 Mev) with less speed
- They interact with matter in 2 ways

1. Excitation: In this electrons of nearby atoms are shifted to higher orbitals
2. Ionisation: It removes the orbital electron completely from nearby atoms

- Their penetrating power is less

19.5.2 Negatrons

- Negatrons are very small and rapidly moving particles
- They also cause excitation and ionization but lesser than alpha particle
- They have more penetrating power
- These are low energy particles (0.018-4.81 Mev)

19.5.3 Gamma Rays

- These are electromagnetic rays with no mass and charge.
- They have very high penetrating power
- They lead to production of secondary electrons which in turn cause excitation and ionization.

INTEXT QUESTIONS 19.1

1. Match the following:

1.	Alpha particles	(a)	Electromagnetic radiation
2.	Negatron	(b)	Electron capture
3.	Positron	(c)	High energy radiation
4.	Gamma rays	(d)	Low energy radiation
5.	X-rays	(e)	Extremely unstable radiation

2. True or false:

1. Curie is SI unit of radioactivity.
2. One Becquerel is number of disintegrations equal to 1 gram of Radium.
3. Alpha particles have Helium nucleus.
4. Negatrons have high penetrating power than gamma rays.
5. Positron emission tomography is used for studying active regions of the brain.

WHAT YOU HAVE LEARNT

- Radioactivity is defined as the process in which unstable atomic nuclei loses energy by emitting radiation in the form particles or electromagnetic waves
- The half-life of an radioactive material is defined as the time taken to become half of its original value
- Different types of radioactive decay are:
  1. Decay by negatron emission
  2. Decay by positron emission
  3. Decay by alpha particle emission
  4. Decay by emission of gamma rays

TERMINAL QUESTIONS

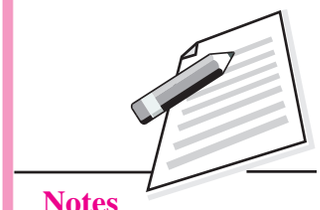
1. What is Radioactivity?
2. What are the different types of radioactive decay?
3. What is half-life?
4. What are the characteristics of various radioactive emissions?
5. Write a note on units of radioactivity.

ANSWERS TO INTEXT QUESTIONS

18.1

1. 1. (a) 2. (b) 3. (c) 4. (d) 5. (e)

2. 1. F 2. F 3. F 4. F 5. F



Notes

20

ELECTROCHEMISTRY

20.1 INTRODUCTION

Electrochemistry is the study of interchange between chemical energy and electrical energy. Many significant chemical reactions are electrochemical in nature. Electrochemical reactions are chemical reactions in which electrons are transferred. To understand electrochemical reactions, it is necessary to understand the terms and concepts of electricity and extend these to apply to electrochemical relationships.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the basic concepts of Oxidation and Reduction
- explain Electrochemical cells
- describe Electrochemical potentials
- describe the application of Electrochemistry

20.2 BASIC CONCEPTS OF OXIDATION AND REDUCTION

Electrons are always transferred from one atom or molecule to another in an electrochemical reaction. There are three different types of electrochemical reactions based on the changes in oxidation state that occur in them. They are

1. **Oxidation reactions:** atoms of the element(s) involved in the reaction lose electrons. The charge on these atoms must then become more positive.

   Eg: Fe^{2+} (aq) $\longrightarrow$ Fe^{3+}(aq) + e^-

Notes

2. **Reduction reactions:** In reduction reactions, atoms of the elements involved gain electrons.

   Eg: $Zn^{2+}(aq) + 2e^{-} \longrightarrow Zn(s)$

3. **Redox reactions :** A redox reaction is an electrochemical reaction in which both reduction and oxidation take place together. The electrons lost in an oxidation component are gained in a reduction component.

   Eg: $Fe^{3+} + Cu^{+} \longrightarrow Fe^{2+} + Cu^{2+}$

These redox reactions are of two types they are:

(a) **Direct redox reaction:** In a direct redox reaction, both oxidation and reduction reactions take place in the same vessel. Chemical energy is converted to heat energy in a direct redox reaction.

(b) **Indirect redox reaction:** In indirect redox reactions, oxidation and reduction take place in different vessels. In an indirect redox reaction, chemical energy is converted into electrical energy.

20.3 ELECTROLYTE AND ELECTRODES

Compounds, molecules, and atoms that do not carry any charge are referred to as neutral species. Eg KCl, O_2.

- **Ion** is an atom or molecule that has an electrical charge.
- **Cation:** An ion that carries a positive charge is called a cation.
- **Anion:** An ion that carries a negative charge is called an anion.
- **Electrolyte:** A solution that contains ions is called an electrolyte solution, or an electrolyte. They conduct electricity as charged ions can move through them.
- **Electrodes:** A solid electric conductor through which an electric current enters or leaves an electrolytic cell or other medium. In electrochemical reactions oxidation or reduction reaction takes place at the electrodes. These are called electrode reactions, or half-reactions.
- A half-reaction can be either a reaction in which electrons appear as products (oxidation) or a reaction in which electrons appear as reactants (reduction). A combination of two half reaction forms the complete reaction.
- **Cathode:** An electrode in which reduction takes place.
- **Anode:** An electrode where oxidation takes place.

20.4 ELECTROCHEMICAL CELLS

Electrochemical cell is a device which converts chemical energy into electrical energy in an indirect redox reaction.

An electrochemical cell can either drive an external electrical device (load) or be driven by it (power supply), depending upon the relative electromotive forces applied by the cell and the device. It is of three types galvanic, reversible, or electrolytic:

- A galvanic cell is a cell in which current flows, power is produced, and the cell reaction is proceeding spontaneously.
- An electrolytic cell is a cell in which current flows, power is consumed,and the cell reaction being driven is the reverse of the spontaneous cell reaction.
- A reversible cell is a cell in which no current flows (and therefore no power is involved, because $P = EI$. The cell reaction in a reversible cell is neither spontaneous nor nonspontaneous; it is called reversible because an infinitesimal change in the cell potential can cause it to proceed in either direction.

In an electrochemical cell, the half cell where oxidation takes place is known as oxidation half cell and the half cell where reduction takes place is known as reduction half cell. Oxidation takes place at anode which is negatively charged and reduction takes place at cathode which is positively charged. During this process transfer of electrons takes place from anode to cathode while electric current flows in the opposite direction. An electrode is made by dipping the metal plate into the electrolytic solution of its soluble salt. A salt bridge is a U shaped tube containing an inert electrolyte in agar-agar and gelatine.

A salt bridge maintains electrical neutrality and allows the flow of electric current by completing the electrical circuit.

20.4.1 Representation of an electrochemical cell

While representing an electrochemical cell Anode is written on the left while the cathode is written on the right.

Anode represents the oxidation half cell and is written as:

Metal/Metal ion (Concentration) Cathode represents the reduction half cell and is written as: Metal ion (Concentration)/Metal. Salt bridge is indicated by placing double vertical lines between the anode and the cathode. The physical state of all components like solid, liquid, gas, aqueous (s, l, g, aq, etc.) should be mentioned.

Example: An aqueous cell that operates spontaneously using the following reaction:

$$2Cu(s) + Zn^{2+} \longrightarrow 2Cu^{+} + Zn(s)$$

would be written in cell notation as

$$Cu(s)/Cu^{+}//Zn^{2+}/Zn(s)$$

The Zn^{2+} is being reduced at the cathode, so the Zinc electrode couple is written on the right.

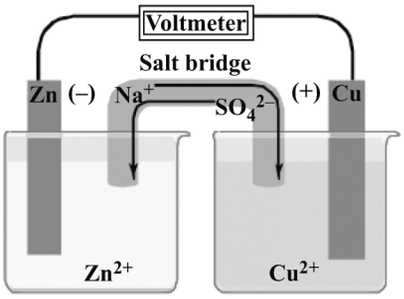


Fig. 20.1

20.5 ELECTROCHEMICAL POTENTIALS

It is the potential difference measured between two electrodes. Voltmeters can be used to measure the potential differences across electrochemical cells but cannot measure directly the actual potential of any single electrode.

20.5.1 Electrode potential

Electrode potential is the potential difference that develops between the electrode and its electrolyte. The separation of charges at the equilibrium state results in the potential difference between the metal and the solution of its ions. It is the measure of tendency of an electrode in the half cell to lose or gain electrons.

There are 2 types of electrode potentials: Oxidation potential and reduction potential. Oxidation potential is the tendency of an electrode to lose electrons or get oxidized. Reduction potential is the tendency of an electrode to gain electrons or get reduced. Oxidation potential is the reverse of reduction potential. The electrode having a higher reduction potential has a higher tendency to gain electrons. So, it acts as a cathode. The electrode having a lower reduction potential acts as an anode.

20.5.2 Standard electrode potentials

When the concentration of all the species involved in a half cell is unity, then the electrode potential is known as standard electrode potential. It is denoted as

$E^{\grave{E}}$. For solid or liquid compounds or elements, standard conditions are the pure compound or element; for gases they are 100 kiloPascal (kPa) pressure, and for solutes they are the ideal 1 molar (mol/liter) concentration.

The standard electrode potential of an electrode cannot be measured in isolation. Tables of standard electrode potentials can be obtained for all elements if any one electrode, operated under standard conditions, is designated as the standard electrode or standard reference electrode with which all other electrodes will be compared. According to convention, the Standard Hydrogen Electrode, abbreviated SHE is taken as a reference electrode and it is assigned a zero potential at all temperatures.

The potential difference across a reversible cell made up of any electrode and a SHE is called the reversible potential of that electrode, *E*. If this other electrode is also being operated under standard conditions of pressure and concentration, the reversible potential difference across the cell is the standard electrode potential E^{Θ} of that electrode.

In many practical potential measurements, the standard hydrogen electrode cannot be used because hydrogen reacts with other substances in the cell or because other substances in the cell react with the catalytic platinum electrode surface upon which the H^+/H_2 potential is established. It is often much more convenient to use alternative electrodes whose potentials are precisely known with respect to the SHE.

Two of the electrodes most commonly used as reference electrodes are the silver/silver chloride electrode with E^{Θ} = +0.2224 V, and the saturated calomel electrode (SCE) at 0.241 V. The effect of changing the reference electrode is to change the zero of a potential scale while leaving the relative positions of all of the potentials unchanged.

20.5.3 Standard hydrogen electrode

Standard hydrogen electrode consists of a platinum wire sealed in a glass tube and carrying a platinum foil at one end. The electrode is placed in a beaker containing an aqueous solution of an acid having 1 Molar concentration of hydrogen ions. Hydrogen gas at 1 bar pressure is continuously bubbled through the solution at 298 K. The oxidation or reduction takes place at the Platinum foil. The standard hydrogen electrode can act as both anode and cathode.

If the standard hydrogen electrode acts as an anode:

$$H_2\,(g) \longrightarrow 2H^+\,(aq) + 2e^-$$

If the standard hydrogen electrode acts as a cathode:

$$2H^+\,(aq) + 2e^- \longrightarrow H_2\,(g)$$

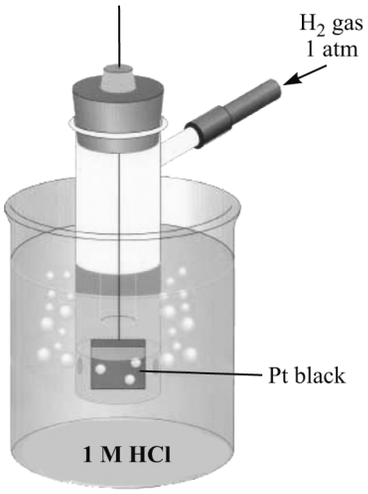


Fig. 20.2

In the electrochemical series, various elements are arranged as per their standard reduction potential values. A substance with higher reduction potential value means that it has a higher tendency to get reduced. So, it acts as a good oxidising agent. A substance with lower reduction potential value means that it has a higher tendency to get oxidised. So, it acts as a good reducing agent. The electrode with higher reduction potential acts as a cathode while the electrode with a lower reduction potential acts as an anode. The potential difference between the 2 electrodes of a galvanic cell is called cell potential and is measured in Volts.

The cell potential is the difference between the reduction potential of cathode and anode.

$$E_{cell} = E_{cathode} - E_{anode}$$

Cell potential is called the electromotive force of the cell (EMF) when no current is drawn through the cell.

20.5.4 Electrode potential at Non-Standard conditions

Nernst studied the variation of electrode potential of an electrode with temperature and concentration of electrolyte. Nernst formulated a relationship between standard electrode potential E^{Θ} and electrode potential E.

When cell is not at standard conditions, use **Nernst Equation**:

$$E = E^{\circ} - \frac{RT}{nF} \ln Q$$

R = Gas constant 8.315 J/K•mol

F = Faraday constant

Q = reaction quotient [products] $^{\text{coefficient}}$/[reactants] $^{\text{coefficient}}$

E = Energy produced by reaction

T = Temperature in Kelvins

n = number of electrons exchanged in BALANCED redox equation

Electrode potential increases with increase in the concentration of the electrolyte and decrease with decrease in temperature.

Nernst equation when applied to a cell:

$$E_{cell} = E^{\Theta}_{cell} - \frac{2.303\ RT \log}{nF} \frac{[\text{Anode ion}]}{[\text{Cathode ion}]}$$

This helps in calculating the cell potential. At equilibrium, cell potential E_{cell} becomes zero

20.6 APPLICATION OF ELECTROCHEMISTRY IN BIOCHEMISTRY

Based on the electrochemical principle several analytes of biochemical importance can be detected. Some of the devices are explained below

20.6.1 Ion selective electrodes: (I.S.E.)

Principle

An ideal I.S.E. consists of a thin membrane across which only the ion to be measured can be transported. The transport of ions from a high conc. to a low one through a selective binding with some sites within the membrane creates a potential difference. This forms the basis of ion selective electrode.

Types of I.S.E.

A wide variety of analytes can be detected using ISE by varying the membrane as given below:

1. Glass membrane (i.e. H^+ electrode)
2. Solid-state electrode (e.g. F- electrode uses a Eu2 +-doped LaF3 crystal)
3. Liquid-based electrode (e.g. Ca2+ electrode uses a liquid chelator)
4. Compound electrode (e.g. CO_2 gas sensing electrode)

Notes

Advantages and limitations of I.S.E.

Advantages

1. Linear response: It shows linear response for wide concentration range of the analyte.
2. Non-destructive: The analyte is not consumed at the end of the reaction.
3. Non-contaminating.: The sample remains unchanged at the end of the reaction and can be used for other analysis
4. Short response time: The analyte can be determined within seconds or minutes
5. Unaffected by color or turbidity: Presence of color and turbidity does not affect the analysis as in spectrophotometry.

Limitations

1. Precision is rarely better than 1%.
2. Electrodes can be fouled by proteins or other organic solutes.
3. Interference by other ions.
4. Electrodes are fragile and have limited shelf life.
5. Electrodes respond to the activity of uncomplexed ion. So ligands must be absent or masked.

20.6.2 pH Electrodes

Principle: The H+ ions either diffuse out or into the gel layer of the glass membrane depending on the pH value of the measured solution. Since the glass electrode has an internal buffer with a constant pH value, the potential at the inner surface of the membrane is constant during the measurement. The total membrane potential is a result of the difference between the inner and outer charge. From the potential the H+ ions concentration can be determined using Nernst equation.

Instrumentation

The whole pH measuring circuit consists of a measuring electrode (glass electrode) and a reference electrode, which are both immersed in the same solution. In order to obtain a definite pH value the reference electrode must have a defined stable potential which is independent of the measured solution. Every reference electrode consists of a reference element which is immersed in a defined electrolyte.

This electrolyte must be in contact with the measured solution. This contact most commonly occurs through a porous ceramic junction. Of the many reference

systems, only the mercury/calomel and the silver/silver chloride systems, along with certain modifications of them, have attained practical importance.

Due to environmental considerations, however, the mercury electrode is rarely used today. The potential of the reference electrode system is defined by the reference electrolyte and the reference element (e.g. silver/silver chloride). Here it is important that the reference electrolyte has a high ion concentration which results in a low electrical resistance. Ideally no reaction between the reference electrolyte and the measuring solution should occur over a wide temperature range.

Combination electrodes

Since the combination electrode is much easier to handle than the separate electrodes, the former is used almost exclusively today. In the combination electrode the glass electrode is concentrically surrounded by the reference electrolyte.

Only when the different parts of the electrode are expected to have very different life expectancies is the use of separate electrodes recommended instead of a single combination electrode.

Three-in-one electrodes A recent innovation is the addition of a temperature sensor to the pH combination electrode. By housing the temperature sensor in the same body as the pH and reference elements, temperature compensated readings can easily be made with a single probe.

Application

Most of the biochemical reactions are pH dependent.

Eg. Enzyme assays

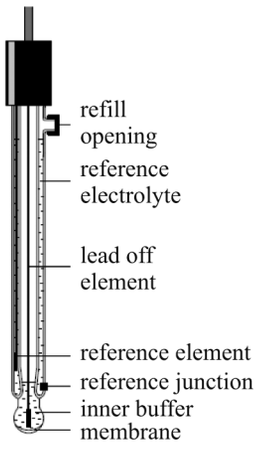


Fig. 20.3

20.6.3 pO_2 (Clark) Electrode

Principle

Binding of oxygen to the polypropylene membrane creates a potential which is directly propotinal to pO_2

Instrumentation

This electrode is known as "Clark Type" after their inventor, Dr. Leland Clark. The Clark electrode consists of an anode and cathode in contact with an electrolyte solution. It is covered at the tip by a semi-permeable membrane usually polypropylene membrane, which is permeable to gases but not contaminants and reducible ions of the sample. The cathode is in a glass envelope in the body of the electrode. The anode has a larger surface that provides stability and guards against drift due to concentration of the pO_2 electrolyte (usually potassium chloride, 0.1 M). This silver/ silver chloride (Ag/ AgCl) anode provides electrons for the cathode reaction. The Clark (pO_2) electrode measures oxygen tension amperometrically. That is the pO_2 electrode produces a current, at a constant polarizing voltage (usually -0.6 V vs. Ag/AgCl) which is directly proportional to the partial pressure of oxygen (pO_2) diffusing to the reactive surface of the electrode. Silver at the anode becomes oxidized.

$$\text{Ag anode} : 4Ag + 4\ Cl^- \ 4AgCl + 4e^-$$

$$\text{Pt cathode} : O_2 + 4H^+ + 4e^- \ 2H_2O$$

Reduction of oxygen occurs at the surface cathode which is exposed at the tip of the electrode. Oxygen molecules diffuse through the semi-permeable membrane and combine with the KCl electrolyte solution. The current produced is a result of the following reduction of oxygen at the cathode.

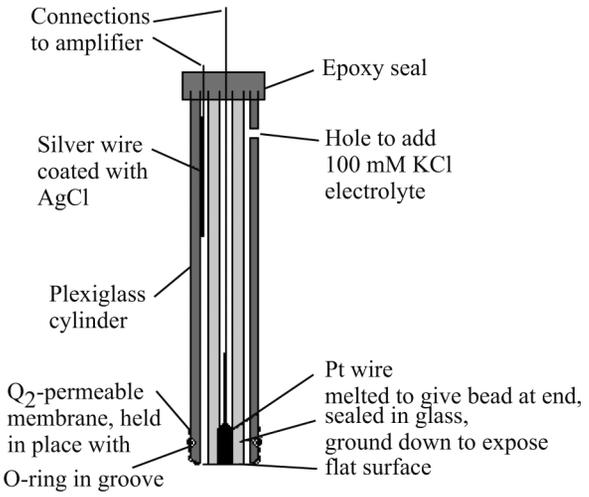


Fig. 20.4

Production of four electrons accompanies each molecule reduced. The pO_2 channel measures this flow of electrons and the resulting microvoltage is displayed as pO_2. Therefore, pO_2 is measured amperometrically; the pO_2 electrode produces a current at a constant polarizing voltage (0.6 V) which is directly proportional to the partial pressure of oxygen diffusing to the reactive surface of the electrode.

20.6.4 Biosensors

Principle

Biosensors are analytical instruments that convert biochemical signal into quantifiable electrical signal.

Instrumentation

It consists of a biocatalyst which can be an enzyme, cell or tissue. This is immobilized on a membrane or gel. It is held in close contact with a transducer which converts biochemical signal into quantifiable electrical signal.

Advantages

1. Highly specific & sensitive
2. Detect wide range of molecules

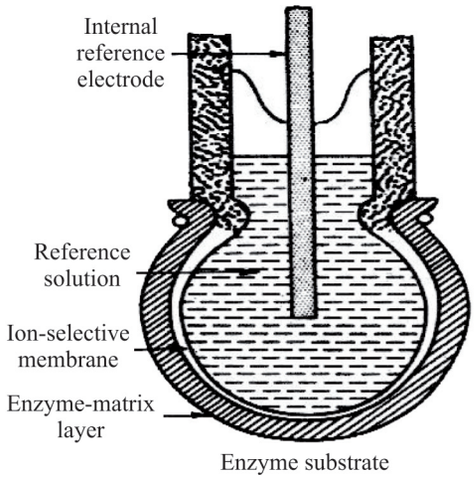


Fig. 20.4

Uses

1. It has wide range of uses in clinical, environmental and Industrial fields

   Eg. Glucose sensors used in the detection of blood glucose for diabetes management

Notes

Types

Based on the intimacy between the biocatalyst and the transducer. They are classified into

1. First generation
2. Second generaion
3. Third generation sensors

In first generation, the biocatalyst and transducer can be separated easily. Both can function separately without the other.

In second generation, the biocatalyst and transducer cannot function separately.

In third generation, biochip is used.

Analyte	Biocatalyst	Transducer
Alcohol	Alcohol oxidase	O_2
Glucose	Glucose oxidase	O_2
Urea	Urease	NH_4^+
Glutamate	Glutamate decarboxylase	CO_2

20.6.4.1 Micro-potentiometric sensors

- These sensors are very small and are used to measure analytes in micro litre volumes of sample.
- These are initially developed for studying pH in living cells.
- These units are also used for monitoring organ perfusion.
- It can measure ions like H^+, Na^+, K^+, Ca^{2+} in 10 µL volumes as in 96-well microtiter plates
- It can be used in flow-through systems, such as HPLC detector with 50 µL volumes.

20.6.5 Electrochemical Detectors

Introduction

Electrochemical detection (ECD) technique used for High Performance Liquid chromatography (HPLC) is an extremely selective and sensitive detection technique.

Detection principle

In amperometric electrochemical detection the electrical current is measured resulting from oxidation or reduction reactions. A sample is introduced in HPLC

and separated on the chromatographic column. The column is connected to an ECD cell, an electrochemical sensor where a reaction takes place at an electrode. Electrochemically active substances that elute from the column undergo an electrochemical reaction, electrons are transferred resulting in an electrical current. The electrodes are connected to an electronic circuitry with a powerful -low noise- amplifier that converts a pico- or nanoampere current in a signal in the range of ± 1 Volt which is commonly used in data acquisition.

Uses

- It is useful in analyses of several compounds such as aromatic substances, drugs, catechol amines, neurotransmitters etc.
- Concentrations as low as 50 pmole/L and as high as 100 µmole/L or more can be detected using this technique.

INTEXT QUESTIONS 20.1

1. Fill in the blanks
    1. Oxidation is ..... of electrons
    2. Reduction is ..... of electrons
    3. Both reduction and oxidation takes place in ..... reactions.
    4. The potential of Standard Hydrogen electrode is .....
    5. In cathode ..... reaction takes place.
2. State True or false:
    1. In pH electrode polypropylene membrane is used.
    2. Silver/ Silver chloride electrode can be used as standard electrode.
    3. Micropotentiometric sensors use larger volume for detection of ions.
    4. Glutamate detection biosensor uses CO_2 as transducer
    5. First generation biosensors, the biocatalyst and the transducer cannot be separated.

WHAT YOU HAVE LEARNT

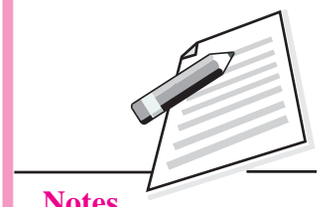
- Oxidation is defined as a loss of electrons while reduction is defined as a gain of electrons.

Notes

- In a redox reaction, both oxidation and reduction reaction takes place simultaneously.
- In an electrochemical cell: Oxidation takes place at anode which is negatively charged and reduction takes place at cathode which is positively charged. Transfer of electrons takes place from anode to cathode while electric current flows in the opposite direction. An electrode is made by dipping the metal plate into the electrolytic solution of its soluble salt. A salt bridge is a U shaped tube containing an inert electrolyte in agar-agar and gelatine.
- Electrode potential is the potential difference that develops between the electrode and its electrolyte. The separation of charges at the equilibrium state results in the potential difference between the metal and the solution of its ions. It is the measure of tendency of an electrode in the half cell to lose or gain electrons.
- According to convention, the Standard Hydrogen Electrode is taken as a reference electrode and it is assigned a zero potential at all temperatures. Standard calomel electrode can also be used as a reference electrode.
- The cell potential is the difference between the reduction potential of cathode and anode. $E_{cell} = E_{cathode} - E_{anode}$
- Ion selective electrodes are useful for detecting wide range of bioanalytes.

TERMINAL QUESTIONS

1. What is Redox reaction?
2. Write short notes on redox potential.
3. Write about electrode potential and Nernst equation
4. Write about biosensors, its types and applications
5. What is an electrochemical cell and how to represent it?
6. Write a note on Standard hydrogen electrode
7. Write about different types of ion sensitive electrodes and its uses
8. Write a note on Clarks oxygen electrode
9. Write a note on pH electrode
10. What are electrochemical detectors and its applications?



21

ELECTROPHORESIS

21.1 INTRODUCTION

The movement of particles under spatially uniform electric field in a fluid is called **electrophoresis**. In 1807, Ferdinand Frederic Reuss observed clay particles dispersed in water to migrate on applying constant electric field for the first time. It is caused by a charged interface present between the particle surface and the surrounding fluid. The rate of migration of particle depends on the strength of the field, on the net charge size and shape of the molecules and also on the ionic strength, viscosity and temperature of medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It is used analytically to study the properties of a single charged species and as a separation technique. It provides the basis for a number of analytical techniques used for separating molecules by size, charge, or binding affinity, example- for the separation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein molecules using an electric field applied to a gel matrix. Gel matrix used mainly is polyacrylamide and agarose. DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.

OBJECTIVES

After reading this lesson, you will be able to:

- define the electrophoresis
- describe the principle and important types of electrophoretic methods
- explain the principle and components of a electrophoresis
- explain various uses of electrophoresis

Notes

21.2 PRINCIPLE

The surface adsorbed sample strongly affects suspended particles by applying electric surface charge, on which an external electric field exerts an electrostatic coulomb force. According to the double layer theory, all surface charges in fluids are screened by a diffuse layer of ions, which has the same absolute charge but opposite sign with respect to that of the surface charge. The electric field also exerts a force on the ions in the diffuse layer which has direction opposite to that acting on the surface charge This force is not actually applied to the particle, but to the ions in the diffuse layer located at some distance from the particle surface, and part of it is transferred all the way to the particle surface through viscous stress. This part of the force is also called electrophoretic retardation force. When the electric field is applied and the charged particle to be analyzed is at steady movement through the diffuse layer, the total resulting force is zero:

$$F_{toto} = 0 = F_{el} + F_f + F_{ret}$$

Considering the drag force on the moving particles due to the viscosity of the dispersant, in the case of low turbulence and moderate electric charge strength E, the velocity of a dispersed particle ν is simply proportional to the applied field, which leaves the electrophoretic mobility μ_e defined as:

$$\mu_e = \frac{\nu}{E}$$

The most known and widely used theory of electrophoresis was developed in 1903 by Smoluchowsky

$$\mu_e = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta},$$

where ε_r is the dielectric constant of the dispersion, ε_0 is the permittivity of free space ($C^2\ N^{-1}1\ m^{-2}$), η is dynamic viscosity of the dispersion medium (Pa s), and ζ is zeta potential (i.e., the electrokinetic potential of the slipping plane in the double layer).

The Smoluchowski theory is very powerful because it works for dispersed particles of any shape at any concentration. Unfortunately, it has limitations on its validity. It follows, for instance, from the fact that it does not include Debye length κ^{-1}. However, Debye length must be important for electrophoresis, as follows immediately from the Figure on the right. Increasing thickness of the double layer (DL) leads to removing point of retardation force further from the particle surface. The thicker DL, the smaller retardation force must be.

Detailed theoretical analysis proved that the Smoluchowski theory is valid only for sufficiently thin DL, when particle radius a is much greater than the Debye length :

$$a\kappa >> 1$$

This model of "thin Double Layer" offers tremendous simplifications not only for electrophoresis theory but for many other electrokinetic theories. This model is valid for most aqueous systems because the Debye length is only a few nanometers there. It breaks only for nano-colloids in solution with ionic strength close to water.

The Smoluchowski theory also neglects contribution of surface conductivity. This is expressed in modern theory as condition of small Dukhin number.

$$Du << 1$$

In the effort of expanding the range of validity of electrophoretic theories, the opposite asymptotic case was considered, when Debye length is larger than particle radius:

$$a\kappa < 1$$

Under this condition of a "thick Double Layer", Huckel predicted the following relation for electrophoretic mobility:

$$\mu_e = \frac{2\varepsilon_r \varepsilon_0 \zeta}{3\eta}$$

This model can be useful for some nanoparticles and non-polar fluids, where Debye length is much larger than in the usual cases. There are several analytical theories that incorporate surface conductivity and eliminate the restriction of a small Dukhin number pioneered by Overbeek and Booth. Modern, rigorous theories valid for any zeta potential and often any $a\kappa$ stem mostly from Dukhin-Semenikhin theory. In the **thin Double Layer** limit, these theories confirm the numerical solution to the problem provided by O'Brien and White.

INTEXT QUESTIONS 21.1

1. Define electrophoresis.
2. The gel matrix widely used in electrophoresis is mainly ..... and .....
3. The widely used theory of electrophoresis was developed in ..... by .....
4. The part of the force that is transferred to the particle surface through viscous stress is called .....

21.3 PREPARING AND RUNNING STANDARD AGAROSE GEL

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple (Fig 21.1) and include:

1. An electrophoresis chamber and power supply
2. Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the Gel is being cast, then removed prior to electrophoresis.
3. Sample combs, around which molten agarose is poured to form sample wells in the gel.
4. Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
5. Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
6. Ethidium bromide, a fluorescent dye used for staining nucleic acids. *NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.*
7. Transilluminator (an ultraviolet light box), which is used to visualize Ethidium bromide-stained DNA in gels. *NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.*

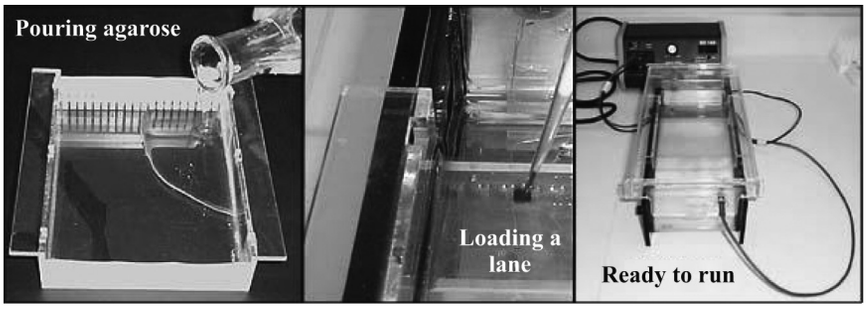


Fig. 21: Gel Electrophoresis unit

To pour a gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and then heated in a microwave oven until completely melted. Most commonly, Ethidium bromide is added to the gel (final concentration 0.5 µg/ml) at this point to facilitate visualization of DNA after electrophoresis. After

cooling the solution to about 6°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.

After the gel has solidified, the comb is removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer are then pipeted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied. You can confirm that current is flowing by observing bubbles coming off the electrodes. DNA will migrate towards the positive electrode, which is usually colored red.

The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes. Bromophenol blue and xylene cyanol dyes migrate through agarose gel at roughly the same rate as double-stranded DNA fragments of 300 and 4000 bp, respectively.

When adequate migration has occurred, DNA fragments are visualized by staining with Ethidium bromide. This fluorescent dye intercalates between bases of DNA and RNA. It is often incorporated into the gel so that staining occurs during electrophoresis, but the gel can also be stained after electrophoresis by soaking in a dilute solution of Ethidium bromide. To visualize DNA or RNA, the gel is placed on a ultraviolet transilluminator.

Fragments of linear DNA migrate through agarose gel with a mobility that is inversely proportional to the $\log_{10}$ of their molecular weight**.** In other words, if you plot the distance from the well that DNA fragments have migrated against the $\log_{10}$ of either their molecular weights or number of base pairs, a roughly straight line will appear.

21.3.1 Requirements

Electrophoretic unit, conical flask, measuring cylinder, power pack, micropipette, micro tips (1X) TAE buffer, Gel loading dye, EtBr, Agarose.

21.3.2 Steps

1. For preparing 0.8% agarose gel, 0.14g of Agarose was dissolved in 20ml of TAE (1X).
2. Mixture was boiled till a clear solution was obtained.
3. Left at room temperature till suspension reaches 40-45°C.
4. Then 2µl of 1% EtBr was added.
5. Seal the casting tray properly and placed the combs at appropriate place.

6. Pour the gel and leave at room temperature for 45-50 minutes to solidify the gel.
7. Fill the buffer tank with TAE (1X) so that the gel was dipped.
8. 5 µl of the sample was loaded into the well by mixing with 1µl of 6X loading dye containing Bromophenol blue.
9. Switch on the power supply at the rate of 5V/cm (Fig. 21.2)
10. When the electrophoretic front reaches bottom of the gel power supply was switched off. The gel was placed over transilluminator and observed under UV light.

Fig. 21.2: Gel Electrophoresis tank with power unit

INTEXT QUESTIONS 21.2

1. The fluorescent dye used in the gel electrophoresis is .....
2. Give the function of a transilluminator.
3. Which two dyes migrate through the gel at same rate to help in visualizing the sample that is loaded in the gel?

   ..... and .....
4. The electrophoresis buffer used is mostly .....or .....

21.4 TYPES OF ELECTROPHORESIS

21.4.1 Affinity electrophoresis

The methods include the mobility shift electrophoresis, charge shift electrophoresis and affinity capillary electrophoresis. The methods are based on changes in the electrophoretic pattern of molecules (mainly macromolecules) through biospecific interaction or complex formation. The interaction or binding of a molecule, charged or uncharged, will normally change the electrophoretic properties of a molecule. Membrane proteins may be identified by a shift in mobility induced by a charged detergent. Nucleic acids or nucleic acid fragments may be characterized by their affinity to other molecules. The methods has been used for estimation of binding constants, as for instance in lectin affinity electrophoresis or characterization of molecules with specific features like glycan content or ligand binding. For enzymes and other ligand-binding proteins, one dimensional electrophoresis similar to counter electrophoresis or to "rocket immunoelectrophoresis", affinity electrophoresis may be used as an alternative quantification of the protein. Some of the methods are similar to affinity chromatography by use of immobilized ligands.

21.4.2 Capillary electrophoresis

Capillary electrophoresis (CE), can be used to separate ionic species by their charge and frictional forces and hydrodynamic radius. In traditional electrophoresis, electrically charged analytes move in a conductive liquid medium under the influence of an electric field (Fig. 21.3). Introduced in the 1960s, the technique of capillary electrophoresis (CE) was designed to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte.

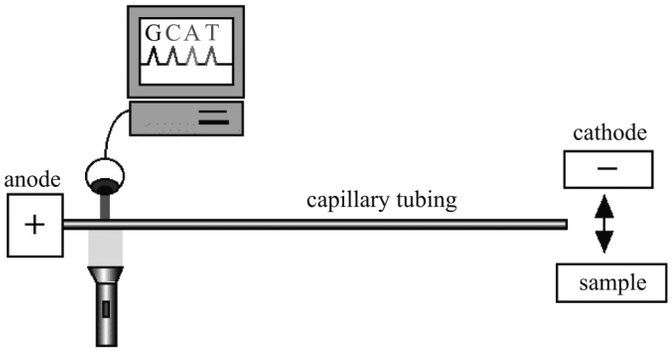


Fig. 21.3: Capillary Electrophoresis

Notes

21.4.3 Immunoelectrophoresis

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies reacting with the proteins to be separated or characterized.

1. **Rocket immunoelectrophoresis** is one-dimensional quantitative immunoelectrophoresis
2. **Fused rocket immunoelectrophoresis** is a modification of one-dimensional quantitative immunoelectrophorsis used for detailed measurement of proteins in fractions from protein separation experiments.
3. **Affinity immunoelectrophoresis** is based on changes in the electrophoretic pattern of proteins through specific interaction or complex formation with other macromolecules or ligands.

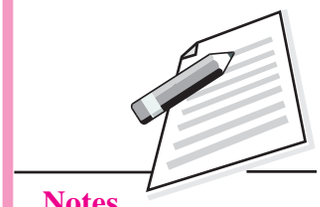
21.4.4 Pulsed field gel electrophoresis

Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

While in general small fragments can find their way through the gel matrix more easily than large DNA fragments, a threshold length exists above 30–50 kb where all large fragments will run at the same rate, and appear in a gel as a single large diffuse band. However, with periodic changing of field direction, the various lengths of DNA react to the change at differing rates. That is, larger pieces of DNA will be slower to realign their charge when field direction is changed, while smaller pieces will be quicker. Over the course of time with the consistent changing of directions, each band will begin to separate more and more even at very large lengths. Thus separation of very large DNA pieces using PFGE is made possible.

21.4.5 SDS-PAGE

One of the most common means of analyzing proteins by electrophoresis is by using **Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis**. SDS is a detergent which denatures proteins by binding to the hydrophobic regions and essentially coating the linear protein sequence with a set of SDS molecules. The SDS is negatively charged and thus becomes the dominant charge of the



complex. The number of SDS molecules that bind is simply proportional to the size of the protein. Therefore the charge to mass ratio should not change with size. In solution (water), in principle all different sized proteins covered with SDS would run at about the same mobility. However, the proteins are not run through water. Instead they are run through an inert polymer, polyacrylamide. The density and pore size of this polymer can be varied by just how you make it (concentration of monomer and of cross-linking agent). Thus, the size of molecules that can pass through the matrix can be varied. This determines in what molecular weight range the gel will have the highest resolving power.

21.4.6 Native Gels

It is also possible to run protein gels without the SDS. These are called native gels in that one does not purposely denature the protein. Here, the native charge on the protein (divided by its mass) determines how fast the protein will travel and in what direction.

21.4.7 Electrofocusing Gels

Another variation of gel electrophoresis is to pour a gel that purposely has a pH gradient from one end to the other. As the protein travels through this pH gradient, its various ionizable groups with either pick up or lose protons. Eventually, it will find a pH where its charge is zero and it will get stuck (focused) at that point.

21.4.8 DNA Agarose Gels

A simple way of separating fairly large fragments of DNA from one another by size is to use an agarose gel. Agarose is another type of matrix used for many purposes (such as the support for the growth of bacteria on plates). DNA does not need a detergent, since it already has a large under of negative phosphate groups evenly spaced. Thus, as with SDS-PAGE, the charge to mass ratio is constant. Also like SDS-PAGE, the separation results from the matrix itself. The range of size sensitivity can be varied by changing the density of the agarose.

DNA denaturing polyacrylamide gels (often called sequencing gels). To look at smaller DNA molecules with much higher resolution, people generally denature the DNA via heat and run it through a thin polyacrylamide gel that is also kept near the denaturing temperature. These gels usually contain additional denaturing compounds such as Urea. Two pieces of DNA that differ in size by 1 base can be distinguished from each other this way.

Notes

INTEXT QUESTIONS 21.3

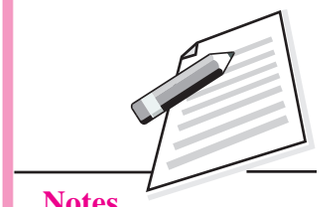
1. Expand the following abbreviations:
   - SDS
   - PAGE
   - CE
2. DNA denaturing gels are also called as .....
3. Mention the correct type of electrophoresis for the following:
   - Gel has a pH gradient
   - Separate large DNA fragments
   - Separate protein without SDS
   - Separate proteins based on electrophoresis and reaction with antibodies
   - separate ionic species by charge, friction force and hydrodynamic radius

21.5 APPLICATIONS

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

WHAT HAVE YOU LEARNT

- Electrophoresis plays a vital role in the separation of nucleic acids and proteins in the field of genomics and proteomics
- The techniques are very simple but has its role in advanced studies
- The electrophoretic devices are economical and can be explored to the core to analyze the complexity of biomolecules



ANSWERS TO INTEXT QUESTIONS

21.1

1. The movement of particles under spatially uniform electric field in a fluid is called **electrophoresis**.
2. Polyacrylamide and agarose
3. 1903, Smoluchowsky
4. electrophoretic retardation force

21.2

1. ethidium bromide (Et Br)
2. It is used to visualize the Et Br stained DNA or RNA in gel through ultraviolet light of specific wavelength.
3. Bromophenol blue and xylene cyanol
4. TAE (tris Acetate EDTA) or TBE (Tris Borate EDTA)

21.3

1. SDS – Sodium dodecyl sulphate

   PAGE – polyacrylamide gel electrophoresis

   CE – capillary electrophoresis
2. Sequencing gels
3.

• Gel has a pH gradient	electrofocusing gels
• Separate large DNA fragments	Agarose gels
• Separate protein without SDS	Native gels
• Separate proteins based on electrophoresis and reaction with antibodies	Immunoelectrophoresis
• separate ionic species by charge, friction force and hydrodynamic radius	capillary electrophoresis

Notes

22

CHROMATOGRAPHY AND MASS SPECTROMETER

22.1 INTRODUCTION

We know that the biochemistry or biological chemistry deals with the study of molecules present in organisms. These molecules are called as biomolecules and they form the basic unit of every cell. These include carbohydrates, proteins, lipids and nucleic acids. To study the biomolecules and to know their function, they have to be obtained in purified form. Purification of the biomolecules includes many physical and chemical methods. This topic gives about two of the commonly used methods namely, chromatography and mass spectrometry. These methods deals with purification and separation of biomolecules namely, protein and nucleic acids.

OBJECTIVES

After reading this lesson, you will be able to:

- define the chromatography and mass spectrometry
- describe the principle and important types of chromatographic methods
- describe the principle and components of a mass spectrometer
- enlist types of mass spectrometer
- describe various uses of mass spectrometry

22.2 CHROMATOGRAPHY

When we have a mixture of colored small beads, it is easily separated by visual examination. The same holds true for many chemical molecules. In 1903,

Mikhail, a botanist (person studies plants) described the separation of leaf pigments (different colors) in solution by using solid adsorbents. He named this method of separation called chromatography. It comes from two Greek words:

chroma – colour

graphein – to write/detect

Modern separation methods are based on different types of chromatographic methods. The basic principle of any chromatography is due to presence of two phases:

- Mobile phase – substances to be separated are mixed with this fluid; it may be gas or liquid; it continues moves through the chromatographic instrument
- Stationary phase – it does not move; it is packed inside a column; it is a porous matrix that helps in separation of substances present in sample. It works on simple physical process called **adsorption**.

22.2.1 Components of Chromatography

The equipment used for separation of mixture in a sample is called a chromatograph. The major components of a chromatograph:

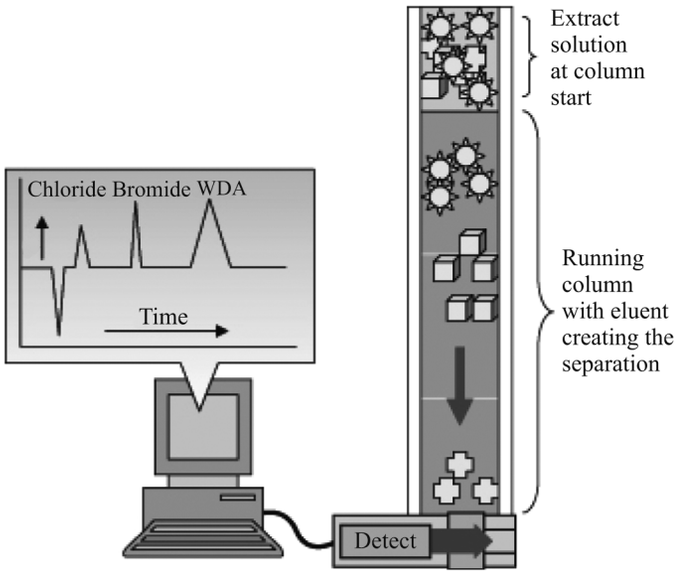


Fig. 22.1

- Mobile phase source – separate chambers or containers having either gas or liquid
- Analyte – sample to be separated
- Sample injection – chamber to inject the sample
- Separating column – having the stationary phase

- Detector – identify the change in separation of a molecule; this calculates the time between injection and detection of a sample, which is called as **retention time.**
- Recorder and analyser – usually a computer attached to the chromatograph. This records the response of detector as a graph called chromatogram

22.2.2 Types

There are many types of chromatographic methods present today. Some of the important methods are described as follows:

- Gas chromatography
- Liquid chromatography
- Gel filtration chromatography
- Ion exchange chromatography
- Affinity chromatography
- Others- hydrophobic interaction chromatography (HIC), Isochromatic focusing (ICF), etc.

22.2.2.1 Gas Chromatography

The gas chromatography principle involves separation of the components of the sample due to separation in between the gaseous mobile phase and stationary phase (usually silica). The components separated into gas comes out first while other comes later. It detects compounds like fatty acids, essential organic solvents, flavored oils, etc.

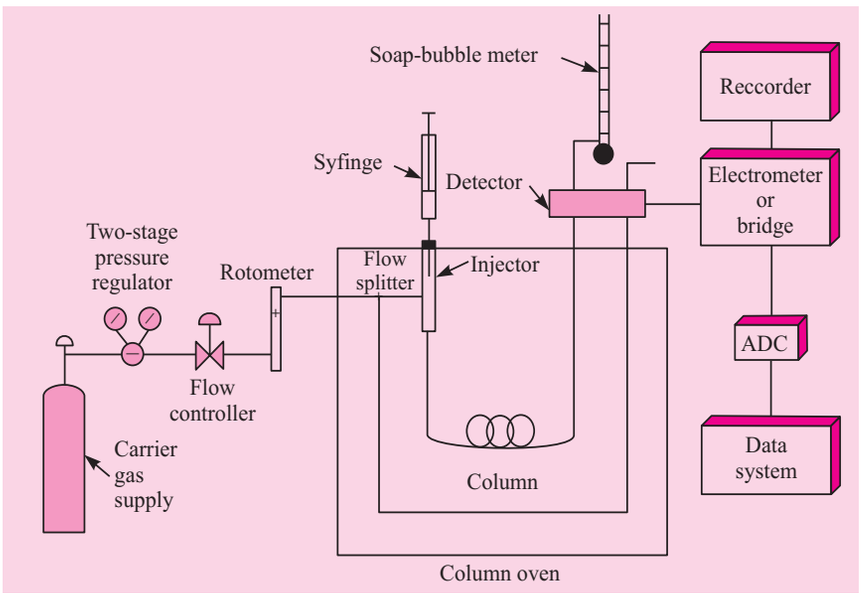


Fig. 22.2: Gas chromatography

In GC, a liquid sample is injected into the column. The GC column is usually coated with stationary phase and placed inside an oven chamber. The sample is vaporized as it passes the column which is more than its boiling point. The sample compounds are carried to column by gas (usually helium or nitrogen) and then to a detector. The detector signals a chart recorder for chromatogram (Fig.22.2)

22.2.2.2 Liquid Chromatography

It is separation technique where the mobile phase is a liquid. It can be performed in a column or a plane surface. This has been advanced to many types :

- HPLC – high performance liquid chromatography (Fig.22.3)
- FPLC – fast protein liquid chromatography

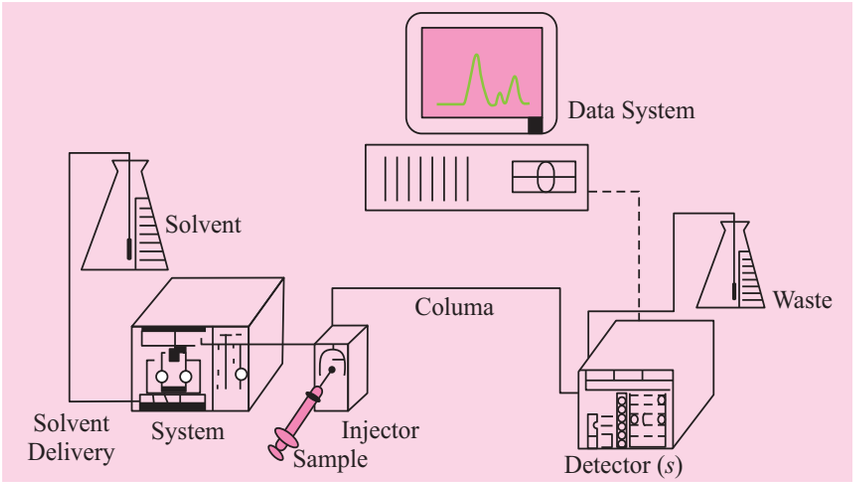


Fig. 22.3: HPLC

22.2.2.3 Gel Filtration Chromatography

This chromatographic technique is also known as size exclusion chromatography or molecular sieve chromatography. It involves the separation of molecules based on their molecular weight and size. (Fig.22.4) It involves:

- Mobile phase – liquid mixed with sample mixture
- Stationary phase – gel matrix containing a particular pore size to allow smaller molecules to easily pass through or vice versa.

It well suitable for separation of biomolecules that are sensitive to environmental conditions like temperature, pH, etc.

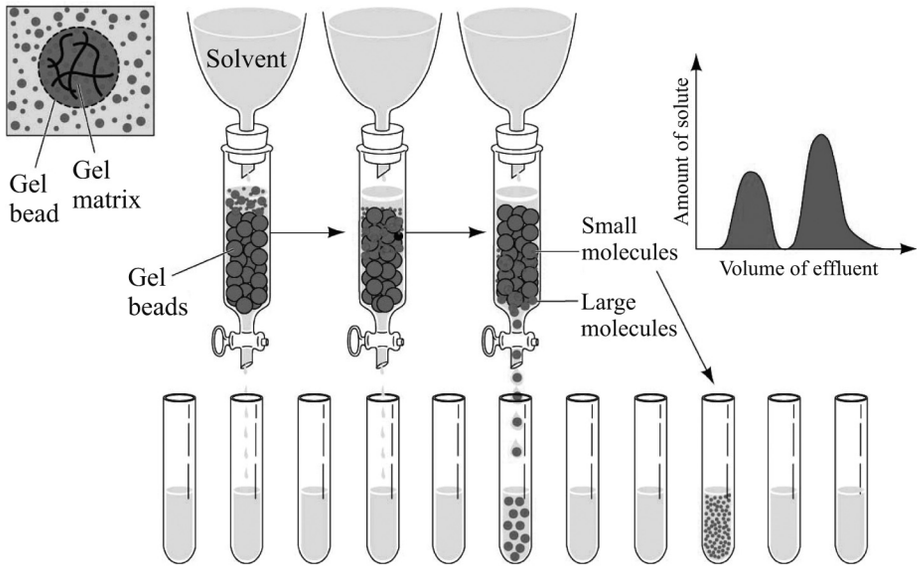


Fig. 22.4: Gel Filtration Chromatography

22.2.2.4 Ion Exchange Chromatography

Every chemical molecule that is capable of forming into ions (different charges – positive, **cations** and negative, **anions**) is separated by using this chromatography (Fig. 22.5). In this:

- Mobile phase – liquid
- Stationary phase – matrix of beads with either positive charge (cationic) to separate anionic sample or negative charge (anionic) to separate cationic sample.

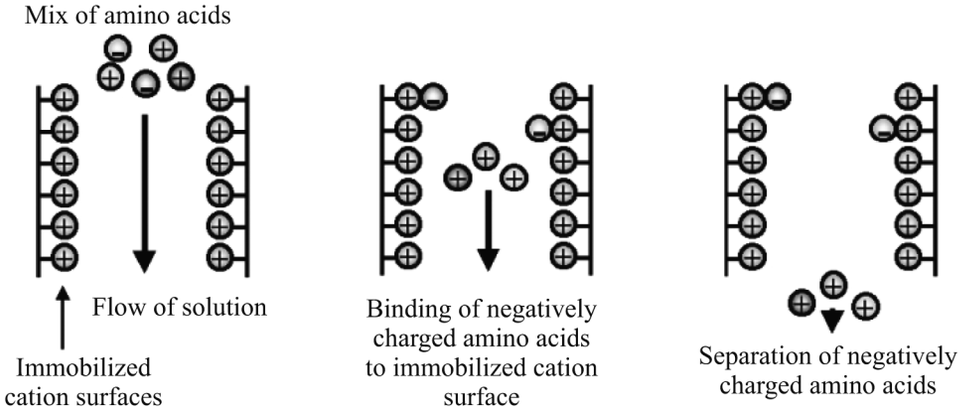


Fig. 22.5: Ion Exchange Chromatography

It is used to separate charged proteins and peptides in a given sample.

22.2.2.5 Affinity Chromatography

Affinity chromatography separates proteins on the basis of a specific interaction between the molecules in the sample with a compound called ligand in the column (Fig.22.6). Some of the ligands used in matrix are:

- enzymes
- antibody
- metal ions like Nickel,etc.

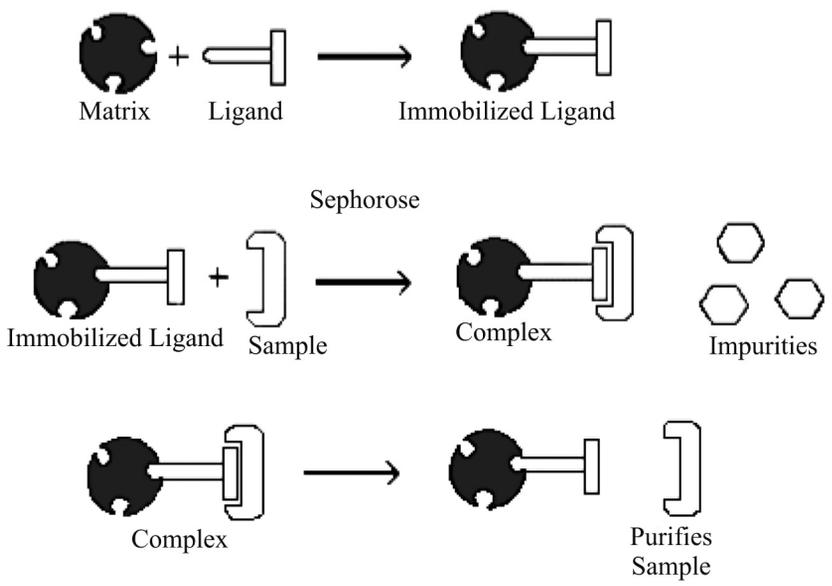


Fig. 22.6: Affinity Chromatography

This technique is highly specific and is used mostly for purification of proteins and peptides.

22.3 MASS SPECTROMETRY

The mass spectrometer is an instrument that measures the masses and relative concentrations of atoms and molecules. It makes use of the basic magnetic force or velocity on a moving charged particle.

22.3.1 Basic Principle

Suppose you had a cannonball travelling past you and you wanted to deflect (change of direction) it. All you've got is a jet of water from a hose-pipe. But, it's not going to make a lot of difference! Because the cannonball is so heavy, it will hardly be deflected at all from its original course.

If suppose instead of water, you try to deflect the table tennis ball travelling at same speed using jet of water. Because the ball is so light, it will be deflected easily.

Thus from the above examples, we can understand that amount of deflection depends on the weight (mass) of the object. The same principle holds true for atom-sized molecules.

22.3.1.1 Working Principle

"The technique for studying the masses of atoms or molecules or fragments of molecules separated by their mass-to-charge ratio (m/z)".

22.3.2 Components

There are many different kinds of mass spectrometers, but all use magnetic and/or electric fields to exert forces on the charged particles produced from the chemicals to be analyzed (Fig. 22.7). A basic mass spectrometer consists of three parts:

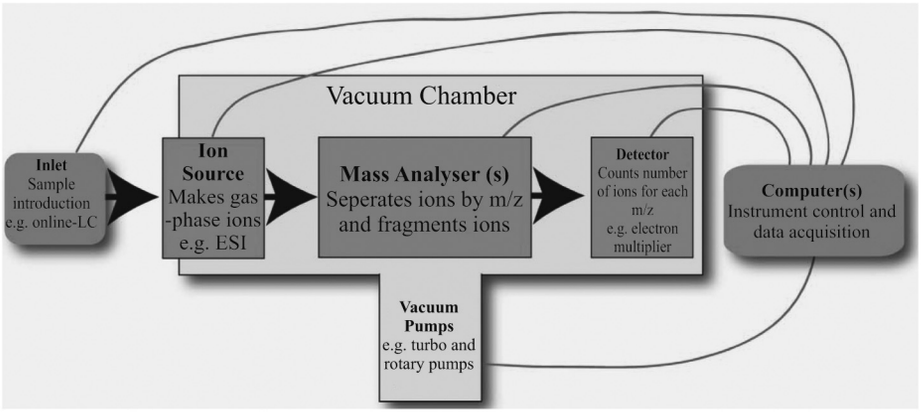


Fig. 22.7: Mass Spectrometry - components

1. **Source** - in which ions are produced from the chemical substances to be analyzed.
2. **Analyzer** - in which ions are separated according to mass.
3. **Detector** - which produces a signal from the separated ions.

A magnetic field or electric field separates ions according to their momentum (mass x velocity)

22.3.3 Types of Mass Spectrometry

There are many different kinds of mass spectrometers based on the type of ion source, mass analyzer and detector used. Let us see the various types of ion source and mass analyzers:

22.3.3.1 ESI – TQ Mass Spectrometer

The basic workflow of ESI tandem MS is as follows:

- the proteins pass through the source become ions;
- then the ions pass through the first analyzer and some specific ions are selected;
- then these selected ions are broken up by a procedure called collision-induced dissociation (CID);
- finally the second analyzer is used to catch the ions produced after CID.

ESI – Electro Spray Ionization – helps in ionizing all molecules of a given sample (Fig. 22. 8)

Sample flow $\xrightarrow{\text{Small Capillary}}$ Droplets with ions $\xrightarrow{\text{dry}}$ ions $\longrightarrow$ Mass analyzer

Fig. 22.8: Work flow of ESI ion source

Mass Analyzer – it may be of:

- Triple Quadrapole – consists of three single quadrupole mass analyzers Q1, Q2 and Q3.
- Ion Trap – traps specific molecules/ions with a particular m/z ratio and leaves all other to pass through; voltage is then suddenly increased leading to colliding of molecules/ions and then detection (Fig. 22.9)

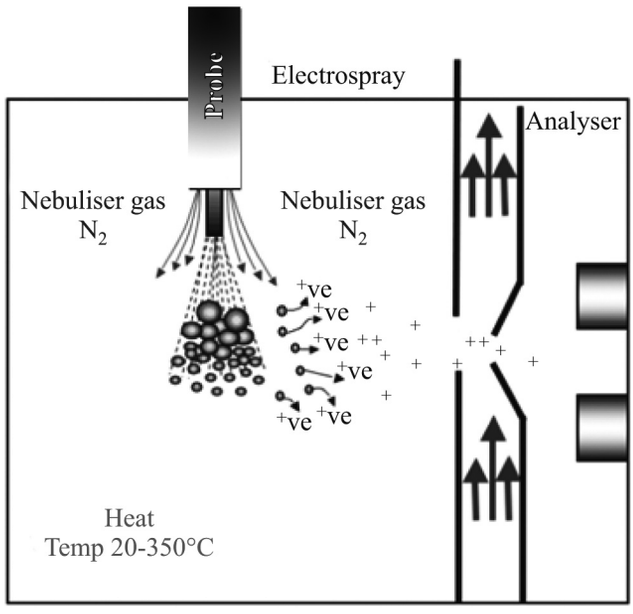


Fig. 22.9: ESI Tandem Mass Spectrometer

22.3.3.2 MALDI TOF Mass Spectrometer

MALDI – Matrix Assisted Laser Desorption and Ionisation, is a soft ionization technique used for analysis of biomolecules (Fig.22.10)

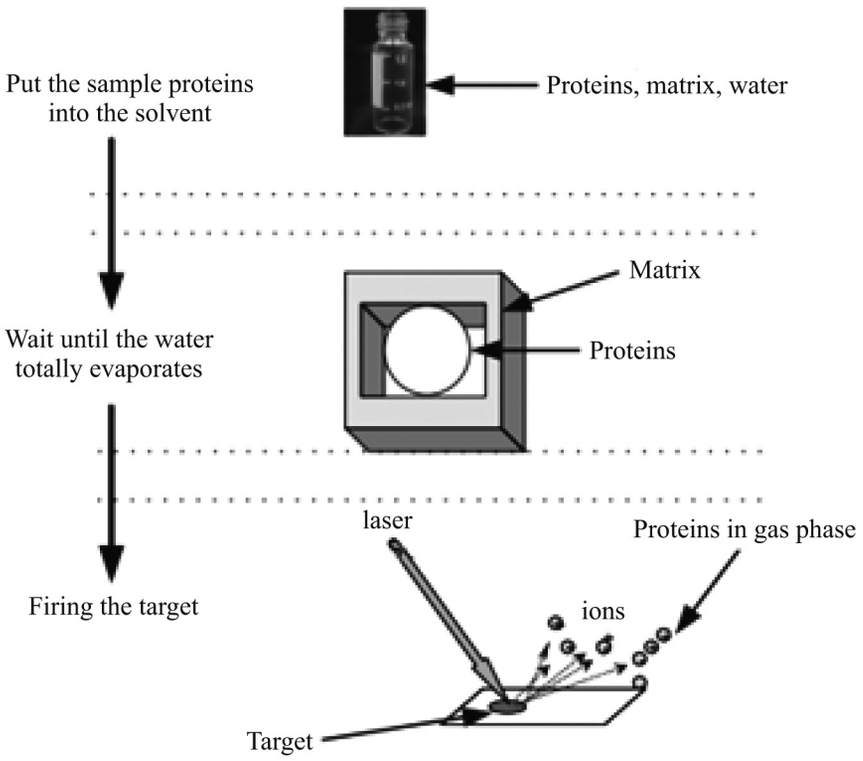


Fig. 22.10: Ion source of MALDI

TOF – Time Off Flight analyzer - An ion of known electrical charge and unknown mass enters a mass spectrometer and is accelerated by an electrical field of known strength. This acceleration results in any given ion having the same kinetic energy as any other ion given that they all have the same charge. The velocity of the ion will depend however on the m/z ratio (Fig.22.11).

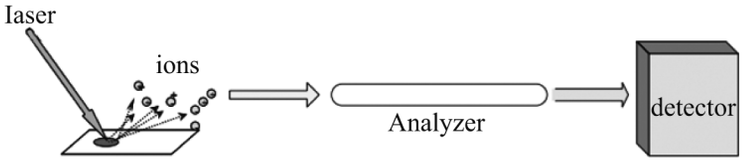


Fig. 22.11: TOF Analyzer and Detector

The result given by the detector is in the form of a graph called as a mass spectrum based on the different molecules present in the sample (Fig. 22.12).

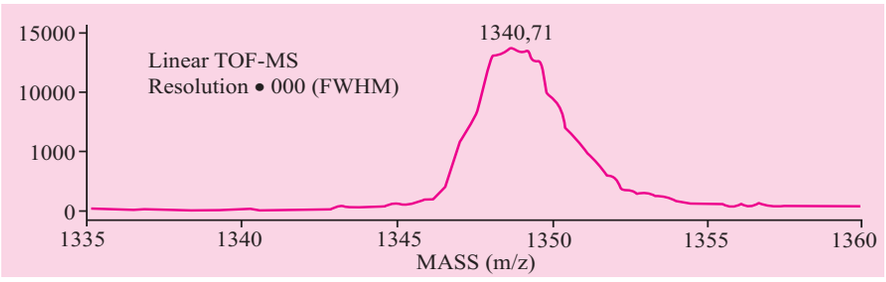


Fig. 22.12: Mass Spectrum

22.3.4 Uses

- Helps in identification of similar fragments of a molecule
- It can easily combine different ion source and mass analyzers.
- Has high resolution, friendly and robust; highly sensitive
- It is used for all kinds of chemical analyses, ranging from environmental analysis of petroleum products, trace metals and biological materials.

INTEXT QUESTIONS 22.1

1. Phases of chromatography are ..... & .....
2. Types of liquid chromatography are ..... & .....
3. Gel filtration chromatography separates molecules based on ..... & .....
4. The instrument that measures the masses and relative concentration of atom and molecules is .....

WHAT HAVE YOU LEARNT

- Among the different methods to separate large and complex biomolecules, chromatography and mass spectrometry are important today.
- Each method can be used either individually or together to find out the composition of a large molecule.
- Based on the different properties and characters of molecules, differences in chromatography and mass spectrometry are discussed in the text.
- Finally, the need to study and importance of each of the methods are analyzed.

TERMINAL QUESTIONS

1. Why is it necessary to separate and study molecules?
2. Define chromatography and the principle on which it works.
3. Discuss the major components of a chromatographic system.
4. What are the different chromatographic techniques?
5. Explain the basic principle of mass spectrometry.
6. Discuss the difference between TQ and TOF mass analyzers.

ANSWERS TO INTEXT QUESTIONS

22.1

1. Mobile and Stationary
2. High performance & Fast Protein
3. Molecular weight and Size
4. Mass Spectrometry

23

CLINICAL ENZYMOLOGY

23.1 INTRODUCTION

Enzymes are catalysts that increase the rate or velocity of physiologic reactions. Each and every reaction in our body takes place with the help of an enzyme. In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood. This forms the basis of clinical enzymology. Thus clinical enzymology refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

OBJECTIVES

After reading this lesson, you will be able to:

- describe plasma enzymes
- explain about the assessment of cell damage and proliferation
- describe the role of enzymes in health and diseases

23.2 PLASMA ENZYMES

Enzymes present in plasma can be classified into 2 types, they are

- Functional Plasma enzymes and
- Non-functional plasma enzymes

Functional plasma enzymes:

- Present in plasma at higher concentration than tissues

Notes

- They function in plasma.
- Mostly synthesized by the liver
- Usually decreased in disease conditions
- Eg. Clotting enzymes, lipoprotein lipase

Non-functional plasma enzymes:

- Present in plasma at lower concentration than tissues
- Do not have any function in plasma
- Mostly synthesized by liver, skeletal muscle, heart, brain etc
- Usually increased in disease conditions
- Eg. Creatine kinase, Alanine transaminase etc
- Measurement of these enzymes in plasma can be used to assess cell damage and proliferation i.e. diagnosis of disease.

23.2.1 Assessment of Cell Damage and Proliferation

Plasma enzyme activities can be used in the diagnosis of disease and prognosis of treatment.

Plasma enzyme levels depend on balance between the rate of influx of active enzyme into the circulation and its eventual clearance from the blood. The rate of influx is determined by the rate of release from damaged cells and altered rate of enzyme synthesis.

23.2.2 Localization of Damage

Enzymes used to measure tissue damage are present in nearly all cells with varying concentration. So the measurement may indicate an abnormality, but the specific diagnosis cannot be made. For example if there is circulatory failure after a cardiac arrest very high plasma levels of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance: the raised plasma levels of 'cardiac' enzymes do not necessarily mean that a myocardial infarct caused the arrest.

The diagnostic precision of plasma enzyme analysis may be improved by

1. Estimation of more than one enzyme. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For eg. Alanine and aspartate transaminases are abundant in the liver, the concentration of aspartate transaminase is much greater than that of alanine transaminase in heart muscle

2. Isoenzyme determination. Some enzymes exist in more than one form: these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues such identification will give more information than the measurement of plasma total enzyme activity: for example, creatine kinase may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium
3. Serial enzyme estimations. The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or occasionally of impaired clearance. The distribution of enzymes within cells may differ. Alanine transaminase and lactate dehydrogenase are predominantly located in cytoplasm and glutamate dehydrogenase in mitochondria, whereas aspartate transaminase occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities

23.2.3 Isoenzymes

- Isoenzymes (also known as isozymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction
- Believed to be originating from closely linked genes or from multiple gene loci
- Evolution from a single form possibly due to long-term mutations
- They vary with respect to their kinetic parameters, electrophoretic mobility, and localization
- They all have independent action
- Eg.Lactate dehydrogenase have 5 isoenzymes (LDH1, LDH2, LDH3, LDH4 & LDH5)
- They can be used to identify the specific affected tissues
- They can be differentiated from each other and can be clinically quantified in the lab

23.3 ENZYMES IN HEALTH AND DISEASES

Estimation of enzymes activities in the serum has many applications in the diagnosis, differential diagnosis (e.g. in myocardial infarction both AST and LDH are increased in the serum but in case of pulmonary embolism AST is normal but LDH is increased), assessing prognosis of diseases, and early detection of disease (e.g. increase level of ALT in serum in viral hepatitis before

Notes

the occurrence of jaundice). Some important enzymes of clinical significances are discussed below:

Distribution and application of clinically important enzymes

Enzymes	Tissues	Clinical applications
Alanineamino transferase	Liver	Hepato parenchymal diseases
Alkaline phosphatase	Liver, bone, intestinal mucosa, Placenta	Liver and bone diseases
Amylase	Salivary glands, Pancreas	Pancreatic diseases
Aspartate amino transferase	Liver, Skeletal muscle, Heart, Erythrocytes	Hepatic parenchymal disease, Muscle disease
Cholinesterase	Liver	Organophosphorus insecticide poisoning, Hepatic parenchymal diseases
Creatine kinase	Skeletal muscle,Heart	Muscle diseases
Gamma glutamyl transferase	Liver	Hepatobiliary diseases, Marker of alcohol abuse
Lipase	Pancreas	Pancreatic diseases
Lactate dehydrogenase	Heart, liver, skeletal muscle erythrocytes, lymph nodes, Platelets	Hepatic parenchymal diseases, muscle diseases Hemolysis, tumor marker
5'nucleotidase	Liver	Hepatobiliary diseases
Trypsin	Pancreas	Pancreatic diseases

23.3.1 Pancreatic enzymes

23.3.1.1. a-Amylase: (EC3.2.1.1; 1,4- a-D-glucan glucanohydrolase; AML) belongs to hydrolyase class that catalyzes the hydrolysis of 1,4- a-glycosidic linkages in polysaccharides. They are low molecular weight proteins (54 to 62 kDa) that can pass the glomeruli of the kidneys. It is the only plasma enzyme physiologically found in urine. The AMY activity present in normal serum and urine is of pancreatic (P-AMY) and salivary gland (S-AMY)origin.

Clinical Significance

Normal values of amylase: 28-100 U/L = 0.48-1.7 ì kat/L

Causes of Raised Plasma Amylase Activity

1. Marked increase (five to 10 times the upper reference limit):
   - Acute pancreatitis
   - Severe glomerular impairment
2. Moderate increase (up to five times the upper reference limit):
   - Perforated peptic ulcer
   - Acute cholecystitis
   - Intestinal obstruction
   - Salivary gland disorders like mumps, salivary calculi

23.3.1.2 Lipase: (EC 3.1.1.3; triacylglycerol acylhydrolase; LPS) is a single –chain glycoprotein with molecular weight of 48 kDa.

Clinical Significance

Normal values: 40-200 U/L

- Plasma lipase levels are elevated in acute pancreatitis and carcinoma of the pancreas.
- serum amylase is increased in mumps, pancreatic disease or due to some other cause, whereas lipase is increased only in pancreatitis. Therefore, the determination of both amylase and lipase together helps in the diagnosis of acute pancreatitis.

23.3.1.3 Trypsin: (EC 3.4.21.4; no systemic name; TRY) is a serine proteinase that hydrolyze the peptide bonds formed by the carboxyl groups of lysine arginine with other amino acids.

Clinical Significance

Normal values of trypsin: 25 ± 5.3 μ g/L

Increased in pancreatic disease. But as there is no distinct role of trypsin estimation in the routine management of patients with acute pancreatitis, this test is therefore considered of limited clinical value.

23.3.2 Liver enzymes

The assay of serum enzymes is very useful for the differential diagnosis and monitoring of various heptobiliy disorders.

There are three types of enzymes:

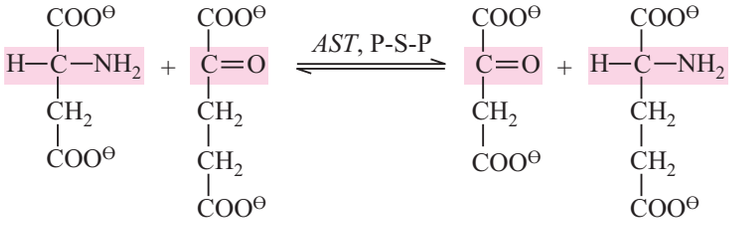
1. Enzymes which are normally present inside the hepatocytes released into the blood when there is a hepatocellular damage= markers of hepatocellular damage.
2. Enzymes which are primary membrane bound (plasma membrane or side of hepatocytes) = markers of cholestasis
3. Enzymes which are synthesized in the hepatocyte = indicates disturbances in the hepatocellular synthesis.

23.3.2.1 Markers of hepatocellular damage

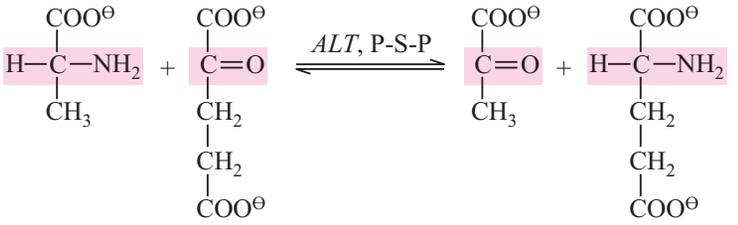
1. Aminotransferases/Transaminases

The transaminases are enzymes involved in the transfer of an amino group from a 2-amino- to a 2-oxoacid: they need the cofactor, pyridoxal phosphate for optimal activity. They are widely distributed in the body.

The 2-oxoglutarate/L-glutamate couple serves as one amino group acceptor and donor pair in all amino-transfer reactions; the specificity of the individual enzymes derives from the particular amino acid that serves as the other donor of an amino group. Thus AST catalyzes the reaction:



ALT catalyzes the analogous reaction:



The reactions are reversible, but the equilibrium of AST and ALT reactions favor formation of aspartate and alanine respectively.

- In the liver, the concentration of ALT per unit weight of the tissue is more than AST.
- AST and ALT enzymes are more important in assessing and monitoring the degree of liver cell inflammation and necrosis.
- Elevated plasma ALT are considered to be relatively specific for liver disease.
- AST may be elevated in other forms of tissue damage, such as myocardial infarction, muscle necrosis and renal disorders.
- In liver disease, the ALT level is increased markedly compared to AST.

  In acute viral hepatitis there is a 100-1000 times increase in both ALT and AST but ALT level is increased more than that of AST

(a) Aspartate Transaminase (EC 2.6.1.1; L-aspartate:2-oxoglutarate aminotransferase; AST)

Clinical Significance

Normal values of AST: Male: <35 U/L = <0.60 mkat/L

Female: <31 U/L = <0.53 mkat/L

(b) Alanine Transaminase (EC 2.6.1.2; L-alanine:2-oxoglutarate aminotransferase; ALT)

Clinical Significance

Normal values of ALT: Male: <45 U/L = <0.77 mkat/L

Female: <34 U/L = <0.58 mkat/L

23.3.2.2 Markers of cholestasis

I. Alkaline phosphatase (EC 3.1.3.1; orthophosphoric-monoester phosphohydrolase

[alkaline optimum]; ALP). Half-life= 10 days

Clinical Significance

The alkaline phosphatases are a group of enzymes that hydrolyse organic phosphates at high pH. They are present in most tissues but are in particularly high concentration in the osteoblasts of bone and the cells of the hepatobiliary tract, intestinal wall, renal tubules and placenta. The exact metabolic function of ALP is unknown but it is probably important for calcification of bone In adults plasma ALP is derived mainly from bone and liver in approximately equal proportions: the proportion due to the bone fraction is increased when there is increased osteoblastic activity that may be physiological

Notes

Causes of raised Plasma ALP activity

1. Physiological: There is a gradual increase in the proportion of liver ALP with age: in the elderly the plasma bone isoenzyme activity may increase slightly.
2. Bone ailment: rickets and osteomalacia
3. Liver disease:
4. Malignancy bone or liver involvement or direct tumor production.

Possible Causes of Low Plasma ALP Activity

- Arrested bone growth
- Hypophosphatasia: an autosomal recessive disorder, associated with rickets or osteomalacia.

Isoenzymes of Alkaline Phosphatase

Bone disease with increased osteoblastic activity, or liver disease with involvement of the biliary tracts, are the commonest causes of an increased total alkaline phosphatase activity. The isoenzymes originating from cells of bone, liver, intestine and placenta may be separated by electrophoresis, but interpretation may be difficult if the total activity is only marginally raised.

Assays for ALP isoenzymes are needed when:

I. The source of an elevated ALP in serum is not obvious and should be clarified.

II. The main clinical question is concerned with detecting the presence of liver or bone involvement

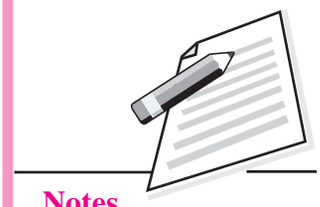
III. In the case of metabolic bone disorders, to ascertain any modifications in the activity of osteblastes to monitor the disease activity and the effect of appropriate therapies.

2. Gamma-glutamyl-transferase (EC 2.3.2.21; γ-glutamyl-peptide: amino acid γ-glutamyletransferase; GGT): catalyzes the transfere of the γ–glutamyl group from peptides and compounds that contain it to an acceptor Gamma-glutamyl transferase occurs mainly in the cells of liver, kidneys, pancreas and prostate. Plasma GGT activity is higher in men than in women.

Clinical Significance

Normal values for GGT Male: <55 U/L = <0.94 μkat/L

Female: <38 U/L = <0.65 μkat/L



Causes of raised plasma GGT activity

- Induction of enzyme synthesis, without cell damage, by drugs or alcohol.
- Hepatocellular damage, such as that due to infectious hepatitis:

23.3.2.3 Other enzymes

1. **Cholinesterase** (EC 3.1.1.7, acetylecholine acetylhydrolase), which is called true cholinesterase or choline esterase I. found in:
   (a) erythrocytes
   (b) lung and spleen
   (c) nerve endings
   (d) the gray matter of the brain.

Normal values for CHE: 4.9-11.9 U/mL

Measurements of CHE activity in serum are used:

1. as a test of liver function
2. as an indicator of possible insecticide poisoning

Causes of decreased plasma cholinesterase activity

1. Hepatic parenchymal disease (reduced synthesis)
2. Ingestion or absorption through the skin, of such anticholinesterases as organophosphates.

Causes of increased plasma cholinesterase activity

1. Recovery from liver damage (actively growing hepatocytes)
2. Nephrotic syndrome

2. **Glutamate dehydrogenase** (EC 1.4.1.3; L-glutamate: NAD(P)+ oxidoreductase, deaminating; GLD) is a mitochondrial enzyme found mainly in the:
   (a) liver
   (b) heart muscle
   (c) kidneys but small amounts occur in other tissue, including
   (d) brain
   (e) skeletal muscle tissue
   (f) leukocytes

Notes

Clinical significance

GLD is increased in serum of patients with hepatocellular damage offering differential diagnostic potential in the investigation of liver disease, particularly when interpreted in conjunction with other enzyme test results. The key to this differential diagnostic potential is to be found in the intraorgan and intracellular distribution of the enzyme. As an exclusively mitochondrial enzyme, GLD is released from necrotic cells and is of value in estimation of the severity of liver cell damage. GLD activity in serum is stable at 4ºC for 48 hours and at -20ºC for several weeks. The GLD upper reference limits are 6U/L (women) and 8U/L (men), when a method optimized at 37ºC is used.

23.3.3 Muscle enzymes

23.3.3.1 Creatine Kinase (EC 2.7.3.2; adenosine triphosphate: creatine Nphosphotransferase CK) CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.The concentration gradients between some human tissues and serum for creatine kinase. The concentration gradient is logarithmic

Clinical significance

Normal range for total CK: Male : 46-171 U/L= 0.78-2.90 µkat/L

Female: 34-145 U/L= 0.58-2.47 µkat/L

Serum CK activity is greatly elevated in all types of muscular dystrophy. In progressive muscular dystrophy (particularly Duchenne sex-linked muscular dystrophy), enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent. Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease. About 50%-80% of the asymptomatic female carriers of Duchenne dystrophy show threefold to six-fold increase of CK activity. Quite high values of Ck are noted in viral myositis, polymyositis and similar muscle disease. However in neurogenic muscle disease, such as:

(a) Myasthenia gravis

(b) Multiple sclerosis

(c) Polimyeltis

(d) Parkinsonism

Serum enzyme activity is normal

Isoenzymes of CK

CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3). CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.

CK-MB accounts for about 35 per cent of the total CK activity in cardiac muscle and less than five per cent in skeletal muscle: its plasma activity is always high after myocardial infarction. It may be detectable in the plasma of patients with a variety of other disorders in whom the total CK activity is raised, but this accounts for less than six per cent of the total. CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal. Approximate concentrations of tissue CK activity (expressed as multiple activity concentrations in serum and cytoplasmic isoenzyme composition

23.3.3.2 Lactate Dehydrogenase (EC 1.1.1.27; L-lactate: NAD+ oxidoreductase; LD) catalyses the reversible interconversion of lactate and pyruvate. The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes: measurement of plasma total LD activity is therefore a non-specific marker of cell damage.

LD has a molecular weight of 134 kDa and is composed of four peptide chains of two types:

M (or A)

H (or B)

Each under separate genetic control

The subunit compositions of the five isoenzymes are listed below in order of their decreasing anodal mobility in an alkaline medium.

LD-1 (HHHH; H4) = migrates fastest towards the anode

LD-2 (HHHM; H3M)

LD-3 (HHMM; H2M2)

LD-4 (HMMM; HM3)

LD-5 (MMMM; M4)

Notes

Clinical significance

Normal range of total LDH: 180-360 U/L= 3.1-6.1 μkat/L

It is increased in plasma in Myocardial injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in clinching diagnosis between hepatic disease and Myocardial.Injury.

Causes of Raised Plasma Total LD Activity

- Artefactual:

  Due to in vitro haemolysis or delayed separation of plasma from whole blood.
- Marked increase (more than 5 times the upper reference limit in adults):
- Circulatory failure with 'shock' and hypoxia:
- Myocardial infarction
- Some haematological disorders. In blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas. very high levels (up to 20 times the upper reference limit in adults) may be found.
- Moderate increase. viral hepatitis: malignancy of any tissue: skeletal muscle disease: pulmonary embolism: infectious mononucleosis.

Isoenzymes of LD

LD1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.

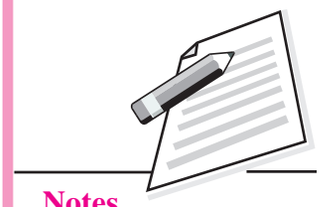
LD5 is the most abundant form in the liver and in skeletal muscle. \Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value.

- Predominant elevation of LD1 and LD5. (LD1 greater than LD5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.
- Predominant elevation of LD2 and LD3 occurs in acute leukaemia: LD3 is the main isoenzyme elevated due to malignancy of many tissues.
- Elevation of LD5 occurs after damage to the liver or skeletal muscle.

Other clinically important enzymes

23.3.3.3 Acid Phosphatase (EC 3.1.3.2; orthophosphoric acid-monoester phosphohydrolase [acid optimum]; ACP)

Acid phosphatase is present in lysosomes, which are organelles present in all cells with the possible exception of erythrocytes. Extra lysosomal ACPs are also present in many cells:



(a) prostate,

(b) bone (osteoclasts),

(c) spleen

(d) platelets

(e) erythrocytes.

The lysosomal and prostatic enzymes are strongly inhibited by d-tartrate ions (tartrate-labile ACP), whereas the erythrocyte and bone isoenzymes are not (TR-ACP)

Normal range of TR-ACP: 1.5-4.5 U/L= 0.03-0.08 ì kat/L

Elevated TR-ACP

(a) Paget disease

(b) Hyperparathyroidism with skeletal involvement

(c) Presence of malignant invasion of bones by cancers

The only nonbone condition in which elevated activities of TR-ACP are found in serum is Gaucher disease of the spleen, a lysosome storage disease.

The main indications for estimation are to help diagnose prostatic carcinoma and to monitor its treatment. The estimation is gradually being replaced by the measurement of plasma prostate specific antigen (PSA) a protein derived from the prostate. This test is more specific and sensitive for diagnosis and monitoring treatment. However, it may be raised in similar circumstances to those affecting prostatic ACP and is more expensive to estimate. ACP is more useful for monitoring the treatment of a known case of disseminated prostatic carcinoma than for making the diagnosis.

3.3.4 Glucose -6-phosphate Dehydrogenase (EC 1.1.1.49); D-Glucose -6-phosphate:

NADP+ oxidoreductase; G6PD) is expressed in all cells and catalyzes the first step in the hexose monophosphate pathway, the conversion of glucose-6-phosphate to 6-phosphogluconate, generating NADPH. G6PD deficiency is the most common ezymeopaty , affecting 400 million people worldwide. More than 400 different types of G6PD variants have been described, leading to different enzyme activities associated with a wide range of biochemical and clinical phenotypes.

The majority of G6PD – deficient individuals develop hemolysis only when oxidative stress occurs, as with infections and after ingestion of certain drugs or fava beans. Outside these periods, they are usually asymptomatic; however,

Notes

G6PD deficiency also leads to mild to sever chronic hemolysis, exacerbated by oxidative stress.

The reference interval for G6PD on erythrocytes is 8-14U/g Hb. Values >18 U/g Hb are often encountered in any condition associated with younger than normal RBCs but are of no clinical significance

INTEXT QUESTIONS 23.1

1. Fill in the blanks

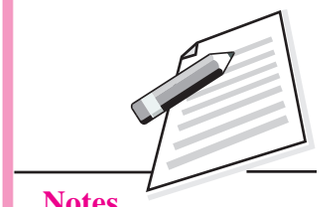
1. Clotting enzymes are plasma ..... enzymes.
2. Lactate dehydrogenase has ..... isoenzymes.
3. ..... isoenzyme is increased in myocardial infarction.
4. Specific marker for liver damage is .....
5. Acid phosphatase is a marker for .....

2. Match the Following:

1.	Lactate dehydrogenase	(a)	Muscle disease
2.	Creatine kinase	(b)	Cholestasis
3.	Alanine transaminase	(c)	Hemolysis
4.	Lipase	(d)	Hepatitis
5.	Alkaline phosphatase	(e)	pancreatitis

WHAT HAVE YOU LEARNT

- Enzyme concentrations are high in cells. Enzymes are released into the plasma due to cellular damage.
- Assays of selected enzymes may help to identify the damaged tissues and isoenzyme studies may increase the specificity. In general, knowledge of the patterns of enzyme changes, together with the clinical and other findings, are needed if a useful interpretation is to be made.
- Non-specific causes of raised enzyme activities include peripheral circulatory insufficiency, trauma, malignancy and surgery.
- Artefactual increases may occur in haemolysed samples.



- Enzyme estimations may be of value in the diagnosis and monitoring of:
  (a) Myocardial infarction (CK. LD and its isoenzymes and sometimes AST);
  (b) Liver disease (transaminases. ALP and sometimes GGT):
  (c) Bone disease (ALP):
  (d) Prosratic carcinoma (tartrate-labile ACP);
  (e) Acute pancreatitis (á-amylase):
  (f) Muscle disorders (CK)
- Plasma enzyme patterns in diseases
  (a) Muscle Disease

  In the muscular dystrophies -Plasma levels of the muscle enzymes, CK and the transaminases are increased, probably because of leakage from the diseased cells.

  (b) Hematological Disorders

  Very high activities of LD (HBD) may he found in megaloblastic anaemias and leukemias and in other conditions in which bone marrow activity is abnormal. Typically there is much less change in the plasma AST than in the LD (HBD) activities.

  (c) Myocardial Infarction

  All plasma enzyme activities (including that of CK-MB) may be normal until at least four hours after the onset of chest pain due to a myocardial infarction; blood should not be taken for enzyme assay until this time has elapsed. The simultaneous measurement of plasma CK-MB activity, which is shown to exceed six per cent of the total CK activity, may occasionally help in the early diagnosis: a raised plasma CK-MB activity or concentration alone is not diagnostic of an infarction.Most of the CK released after a myocardial infarction is the MM isoenzyme, which is found in both skeletal and myocardial muscle and has a longer half-life than the MB fraction. After about 24 hours the finding of a high MM and undetectable MB does not exclude myocardial damage as a cause of high total CK activities:by this time the plasma HBD activity is usually raised. In most cases of suspected myocardial infarction measurement of plasma total CK and LD1 (HBD) activities, together with the clinical and ECG findings, are adequate to make a diagnosis. Plasma total CK activity alone can be very misleading.

A raised plasma total CK activity, due entirely to the MM isoenzvme, may follow recent intramuscular injection, exercise or surgery, this is more likely if associated with normal plasma LDl (HBD) or AST activity.

Newer markers for myocardial infarctions: troponin T and troponin I are regulatory proteins involved in myocardial contractility. both being evaluated as an early and specific marker of acute myocardial infarction. Elevated serum troponins are more predictive of adverse outcomes in unstable angina or myocardial infarction than the conventional assay of CK2.

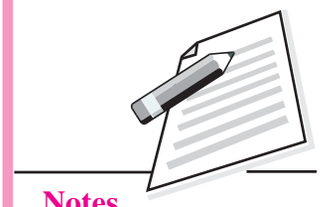
(d) Enzymes in Malignancy

Plasma total enzyme activities may be raised or an abnormal isoenzyme detected, in several neoplastic disorders.

- Serum prostatic (tartrate-labile) acid phosphatase activity rises in some cases of malignancy of the prostate gland.
- Any malignancy may be associated with a non-specific increase in plasma LD1 (HBD) and occasionally, transaminase activity.
- Plasma transaminase and alkaline phosphatase estimations may be of value to monitor treatment of malignant disease. Raised levels may indicate secondary deposits in liver or of alkaline phosphatase, in bone. Liver deposits may also cause an increase in plasma LD or GGT.
- Tumors occasionally produce a number of enzymes, such as the 'Regan' ALP isoenzyme.'LD (HBD) or CK-BB. assays of which may be used as an aid to diagnosis or for monitoring treatment.

TERMINAL QUESTIONS

1. Write a note on plasma enzyme types
2. What are isoenzymes. Add a note on its clinical significance
3. Write short notes on the following enzymes:
   (a) Lactate dehydrogenase
   (b) Creatine kinase
   (c) Alanine transaminase
   (d) Alkaline phosphatase
4. Explain in detail about the enzyme changes in liver diseases
5. Explain in detail about the enzyme changes in myocardial infarction



ANSWERS TO INTEXT QUESTIONS

23.1

1. 1. Functional
   2. Five
   3. CK-MB
   4. ALT
   5. Prostatic carcinoma
2. 1. (c)
   2. (a)
   3. (b)
   4. (e)
   5. (d)

Notes

24

IMMUNOCHEMICAL TECHNIQUES

24.1 INTRODUCTION

All vertebrates have advanced immune system. The more complex the organism the more advanced the immune system. The immune system of mammals has evolved over a million years; it provides an incredible protection system capable of responding to infective challenges that arise in the body. Immunity in our body is monitored or controlled by specific cells from stem cells in bone marrow. The most important cell types are B and T lymphocytes, which have the ability to act against bacterial and other viruses. B-cells releases **antibody** against stimulation of a foreign substance into the body. An **antigen** is a foreign substance capable of an immune response leading to the production of antibodies. They are the targets to which antibodies bind. So antibodies are antigen specific (bind to the antigen that initiated its production). This unit focuses on the identification and diagnostic chemical techniques on the response of an antibody to a specific antigen. **Immunochemical methods** are based on the selective, reversible and non-covalent binding of antigens by antibodies. These methods are employed to detect or quantify either antigens or antibodies.

OBJECTIVES

After reading this lesson, you will be able to:

- define immunochemical techniques
- explain the characteristic features and roles of antigen – antibody reactions
- describe and distinguish the types of immunochemical techniques

24.2 FACTORS CONTROLLING THE IMMUNOCHEMICAL TECHNIQUES

All the techniques cannot be used for the identification of a specific antigen or antibody. In order to perform the immunochemical techniques, several controlling criteria are appropriate:

- Experimental conditions – nature and the place of work, type of sample collected.
- Nature of Reagents – the quality is studied, standardized and analyzed
- Sensitivity and selectivity of technique to the particular sample

24.3 IMMUNOCHEMICAL TECHNIQUES

Immunochemistry is an advanced area of immunology. It deals with the chemical components and chemistry (chemical reactions) of immunological phenomena, that is of antibody and antigen. Immunochemical methods are processes utilizing the highly specific affinity of an antibody for its antigen. It detects the distribution of a given protein or antigen in tissues or cells. The methods used for the immunochemical analysis are called Immunochemical techniques; they are highly important in diagnostic and clinical context, as now even normal cell with many proteins are altered in diseased state (in cancer).

24.3.1 Characteristics and Role

24.3.1.1 Characteristics

- Simple, rapid and robust
- Highly sensitive
- Easily automated – applicable to regular clinical diagnostic laboratories
- Does not require extensive and easily destructible sample preparation
- Do not require expensive instrumentation
- Mostly based on simple photo-, fluoro- and luminometric detection
- Measurements may be either qualitative or quantitative.

24.3.1.2 Roles

The characteristic features of these techniques are helpful for the following:

- Function of newly identified or novel proteins can be identified
- Importance of uncharacterized protein in their natural environment can be analyzed

- Determination of species or tissues in which the protein or residues is expressed
- The cell type or sub-cellular compartment in which the protein can be found
- Detect whether there is any variation in expression of protein during development of the organism
- Some alteration in the normal expression pattern of a particular protein may indicate a particular disease state; Gives information that may be useful for diagnosis and treatment.

24.4 METHODS OF ANALYSIS

All immunochemical methods are based on a highly specific and sensitive reaction between an antigen and an antibody. Antibodies are immunoglobins, belonging to a family of glycoproteins – IgG, IgA, IgD, IgM and IgE. Structurally, antibodies are often visualized as Y-shaped molecules, each containing 4 polypeptides – 2 identical polypeptide units called heavy chains and another 2 called light chains. It has a domain called Fab, the site where it binds to an antigen. The region of an antigen binding to an antibody is called an epitope (Fig. 24.1).

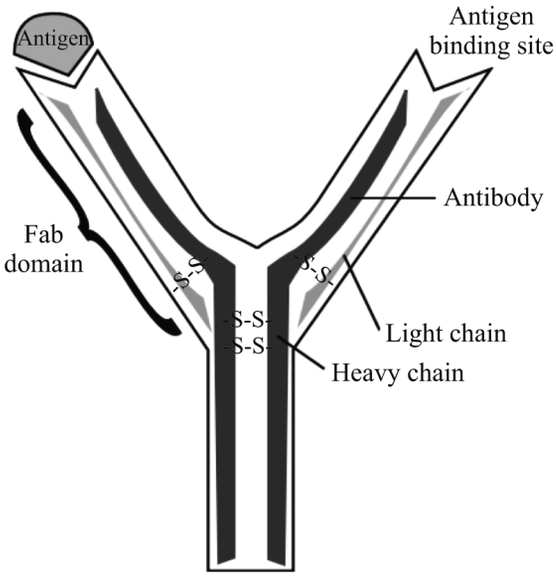


Fig. 24.1: Antigen-Antibody reaction

The measure of the strength of the binding is called **affinity**, and it is usually expressed in terms of the concentration of an antibody-antigen complex measured at equilibrium. It is measured by **quantitative precipitin curve** (basis for many immunochemical techniques) proposed by Heidelberger and Kendall in 1935.

Quantitative precipitin curve: it describes the relationship between the antigen concentration and the amount of precipitate for a constant quantity of an antibody. Three zones can be distinguished from the precipitin curve: (Fig. 24.2)

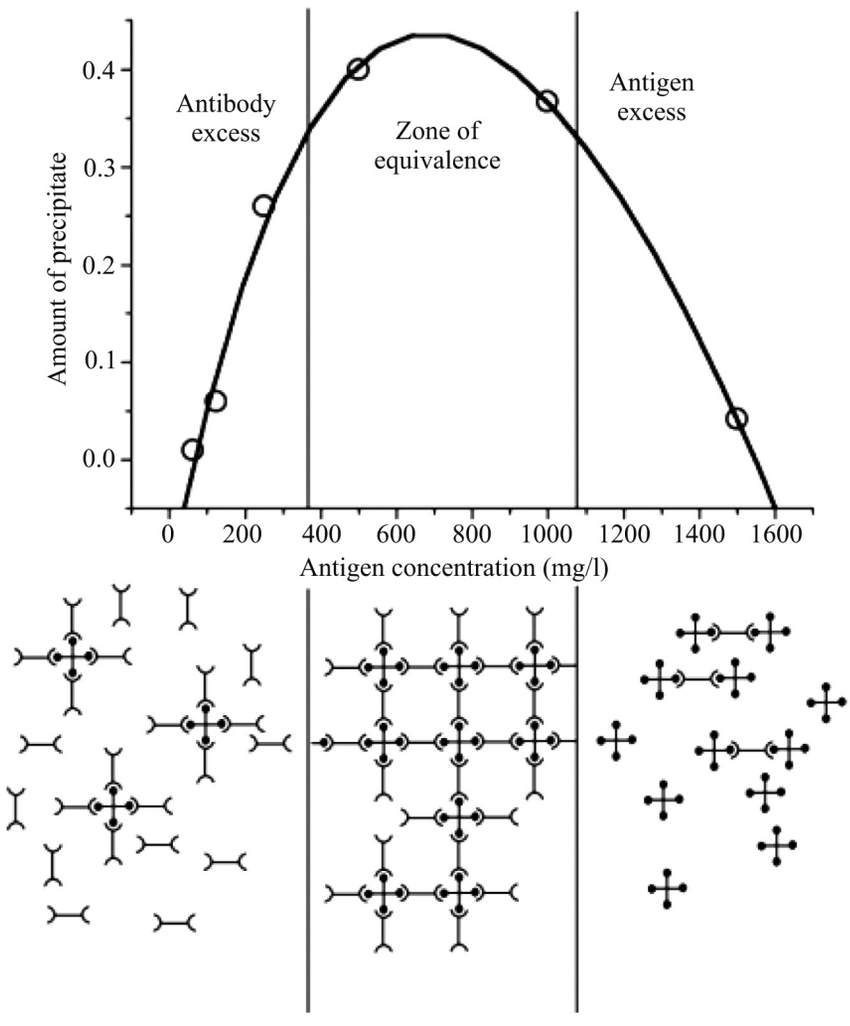


Fig. 24.2: Precipitin curve

(i) antibody excess zone – first phase where less antigen is present in sample

(ii) equivalence zone – both antigen and antibody are cross-linked forming precipitate; no free antigen or antigen is present

(iii) antigen excess zone – amount of precipitate reduces due to high antigen concentration

The precipitin curve forms the basis of most of the immunochemical techniques that can be performed in clinical laboratories.

INTEXT QUESTIONS 24.1

1. ..... cells releases antibody against stimulation of a foreign substance into the body
2. ..... is a foreign substance capable of inducing the production of antibodies
3. ..... methods detect or quantify either antigen or antibodies
4. The measure of the strength of the binding is called .....

24.4.1 Types of Antibody Used

The antibodies used for the methods are produced by various ways:

1. **Monoclonal antibody** – products of single clone of plasma cells by B-lymphocytes; mostly prepared in laboratory. They are directed against single epitope – identical copies with same structure and antigen specificity. They have excellent specificity but poor ability to precipitate antigen.
2. **Polyclonal antibody** – they are conventional, i.e., produced by immunization of animals with antigen. Thus antibody consists of mixture of monoclonal antibodies having specificity for complex antigens.

Sometimes monoclonal is called as "monovalent" and polyclonal as "polyvalent" which indicates the antigen specificity.

24.4.2 Types

Based on the type of reaction performed, reagents and samples used, the techniques are categorized as follows:

- Particle methods – where the antigen-antibody interaction is observed. It includes:
  - Agglutination
  - Immunoprecipitation
  - Immunoelectrophoresis
  - Immunofixation
  - Immunoturbidimetry
  - Immunonephlometry
- Label methods – either antigen or antibody is labeled and through the label concentration, the antigen-antibody reaction is observed. This includes:

- Immunoassay
- Competitive binding

- Other methods – includes immunofluoroscence, immunoelectron microscopy, etc.

24.4.2.1 Agglutination

Agglutination (from Latin, *agglutino* – to glue/ attach) is a process of formation of clumping of cells; it occurs due to reaction of antibody on a particulate antigen (Fig. 24.3). Among all other antibodies, IgM is a good agglutinin, since it has high affinity to different antigens.

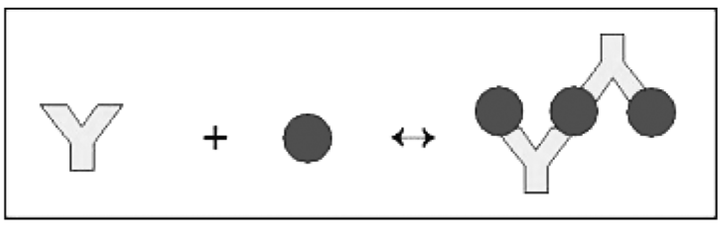


Fig. 24.3: Agglutination reaction

They may be either:

- Qualitative test – to identify the presence of an antigen or an antibody.
- Quantitative test – to measure the level of antibodies to particulate antigens; serial dilutions of sample is used.

There are many variations in agglutination method:

- Direct – antigen present on cell surface is directly agglutinated by specific antibodies.

  Eg: in hematology – determination of blood group (ABO typing) diagnosis of *Salmonella typhi – Widal Test*

- Indirect (reverse agglutination) – uses particles already coated with antigens (antibodies) to determine the antibody (antigen) in given sample. Eg: Pregnancy test – using hormone secretion (human chorionic gonadotropin), diagnosis of rheumatoid arthritis
- Agglutination inhibition – where competitive binding of antigen occurs; determine the concentration of soluble antigen in sample.
- Haemagglutination – involve reactions using red blood cells; detection of diseases and other viruses, blood typing, etc.

INTEXT QUESTIONS 24.2

1. Types of antibody used are ..... & .....
2. ..... is a process of formation of clumping cells
3. Example of Direct agglutination is ..... typing
4. Example of Indirect agglutination is ..... test

24.4.2.2 Immunoprecipitation

Immunoprecipitation methods include flocculation and precipitation reactions. When a solution of an antigen is mixed with its corresponding antibody under suitable conditions, the reactants form flocculating or precipitating aggregates. They can be assessed visually by the formation of precipitin line at the region of equivalence – where equivalent amount of antigen and antibody are present.

It may be either:

- Simple – reaction of one antigen and an Precipitin line antibody
- Complex – when many unrelated reactants are used

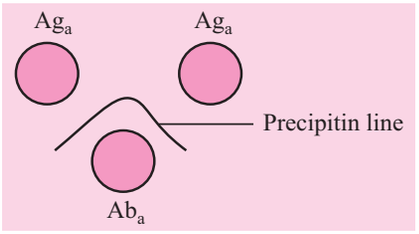


Fig. 24.4: Precipitation reaction

In *simple methods*, a concentration gradient is established between the antigen and antibody; it includes:

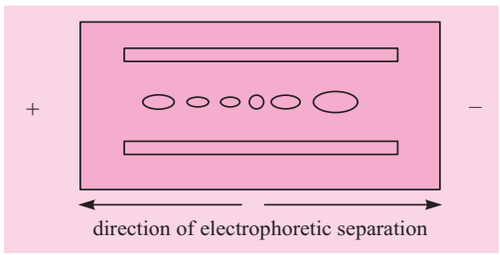
- Single radial immune diffusion (SRID) – developed by Mancini; simple technique where the circular precipitin area originating from antigen well in gel is equal to amount of antigen concentration.
- Double immune diffusion – developed by Orjan Ouchterlony; both antigen and antibody diffuse from separate wells in a gel to form precipitin line.

In *complex methods*, different antigens are compared with an antibody and vice versa. The result is based on the presence or absence of precipitin line.

24.4.2.3 Immunoelectrophoresis

Immunoelectrophoresis is a qualitative technique that combines of two methods one after another:

- Gel electrophoresis – separation of components by charge (Fig. 24.5a)
- Immunodiffusion – diffusion of both antibody and antigen toward each other produces precipitin line (Fig. 24.5b)



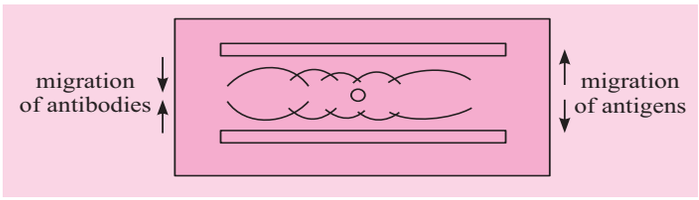


Fig. 24.5: (a) electrophoresis, (b) immunodiffusion

It is strictly *qualitatively* technique to detect antibody and antigen in sample. But also a *quantitative* technique exist called **rocket immunoelectrophoresis** to measure the antigen level in a sample.

Another variation called as counter-current immunoelectrophoresis where it is similar to double immunodiffusion.

24.4.2.4 Immunofixation

Immunofixation is used for detection and identifying antibody in serum, urine and other body fluids (Fig. 24.6). The principle is similar to immunoelectrophoresis, involves two stages:

- Electrophoresis separation of antibody proteins
- Immunoprecipitation separation with specific antibodies.

It is easy to perform, more sensitive and easily evaluated. In clinical laboratories, is used for detection for cancerous myeloma through specific antibody.

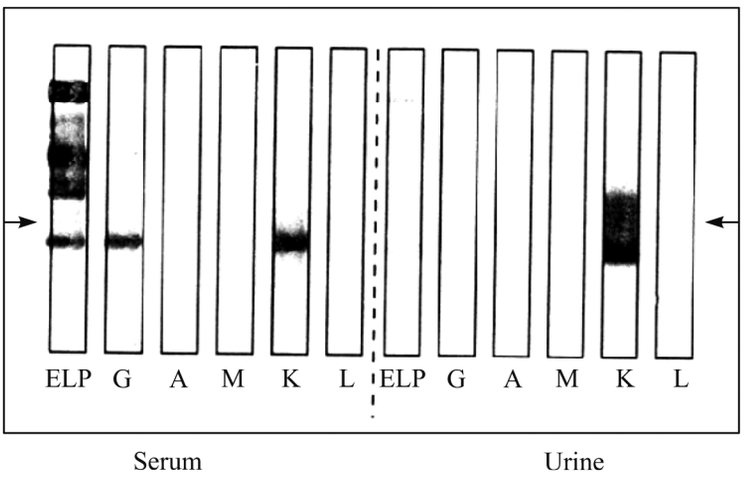


Fig. 24.6: Immunofixation – serum and urine samples

24.4.2.5 Immunoturbidimetry

The precipitin line or curve that forms in a gel, can also be formed in a solution. When an antigen solution is combined with a specific antibody solution, the clumping formed results in a cloudy appearance. This is called turbidity; it forms the principle of immunoturbidimetry. It is measured based on the intensity/ amount of light that pass through the sample. It is performed by an instrument called spectrophotometer (Fig. 24.7-A).

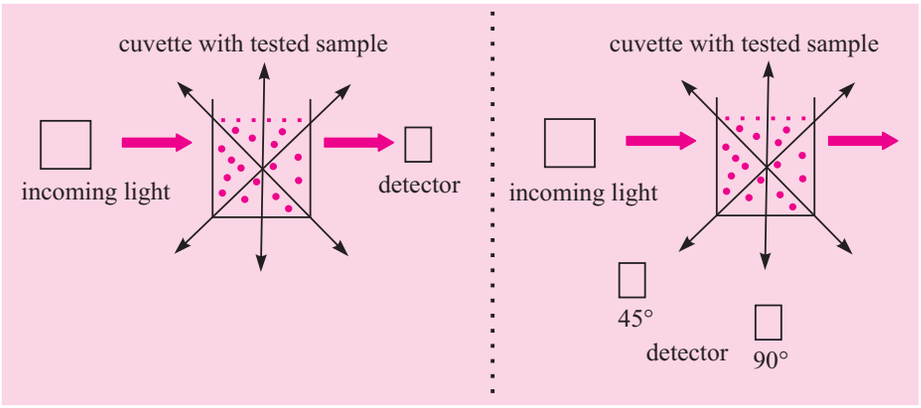
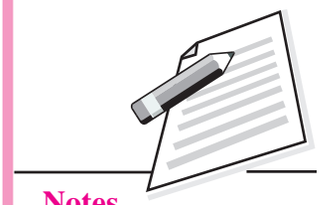


Fig. 24.7: A – turbidimetry, B – nephelometry

24.4.2.5 Immunonephelometry

Immunonephelometry works on the principle similar to turbidimetry, but this method measures the intensity of scattered light from sample directly. It uses laser light and is measured by using a nephelometer (Fig. 24.7-B).



Both the methods are faster but are more expensive. Increased sensitivity of antigen and antibody can be got by automation of these techniques.

24.4.2.7 Immunoassay

Immunoassay technique works on the principle of labeling either the antigen or antibody before the reaction. This increases the sensitivity of detection in the experiment than the formation of immunoprecipitate.

The label can be of:

- Radio isotope – called as Radio Immunoassay (RIA)
- Enzyme – reaction known as Enzyme Immunoassay (EIA); it also includes ELISA (Enzyme Linked Immuno Sorbent Assay)
- Fluorescent substance
- Chemilumniscent substance

RIA – first immunoassay technique; based on the measurement of radioactivity associated with antigen-antibody complexes.

ELISA – safer and efficient method; based on the measurement of an enzyme action associated with antigen-antibody complexes (Fig. 24.8) Most commonly used enzymes – biotin, alkaline phosphatase, etc.

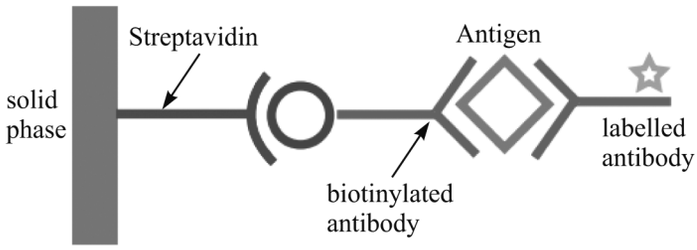


Fig. 24.8: ELISA – sandwich type

24.4.2.8 Competitive Binding

Another method to measure the amounts of antigen is competitive binding; in this technique, it works on the principle of competitive binding:

- Antibody is first incubated in solution having antigen
- This mixture is added to antigen coated reaction well

The more the antigen present in sample, the less the antibody will bind to the coated antigen. To this, when labeled antibody is added, we can measure the amount of antigen present in sample.

Notes

24.4.2.9 Other Methods

- **Immunofluorescence** – an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an antigen in or on a cell or tissue by the fluorescence emitted by the bound antibody.
- **Immunoelectron microscopy** – uses the specificity of antibody to view tisssues and its components through a microscope.
- **Immunostaining** – use of antibody labeled with enzyme or dye to detect specific protein in a sample. Most important staining method is **Western Blot** method that includes three processes:
- Electrophoretic separation of proteins in sample
- Immunoblotting – transfer of proteins from gel to a membrane
- Immunodetection – identification using labeled antibody

24.5 DETECTION

In order to detect and diagnose diseases and also certain body conditions properly, there are many fast immunochemical techniques. Some examples are:

- Detection of *Salmonella typhi* (causing typhoid) in blood
- Detection of narcotics in urine and saliva
- Detection of protein hormones like insulin, thyroxine, etc.
- Pregnancy test for level of human choriogonadotropic hormone (hCGH)

WHAT HAVE YOU LEARNT

- Immunity in our body is monitored or controlled by specific cells from stem cells in bone marrow.
- The most important cell types are B and T lymphocytes, which have the ability to act against bacterial and other viruses.
- B-cells releases antibody against stimulation of a foreign substance into the body.
- An antigen is a foreign substance capable of an immune response leading to the production of antibodies
- Immunochemical methods are based on the selective, reversible and non-covalent binding of antigens by antibodies

- The methods used for the immunochemical analysis are called Immunochemical techniques
- All immunochemical methods are based on a highly specific and sensitive reaction between an antigen and an antibody
- Antibodies are immunoglobins, belonging to a family of glycoproteins – IgG, IgA, IgD, IgM and IgE
- The measure of the strength of the binding is called affinity
- Quantitative precipitin curve describes the relationship between the antigen concentration and the amount of precipitate for a constant quantity of an antibody
- Types of antibodies used are Monoclonal antibody and Polyclonal antibody
- Based on the type of reaction performed, reagents and samples used, the techniques are categorized as follows:
- Particle methods – where the antigen-antibody interaction is observed. It includes:
  - Agglutination
  - Immunoprecipitation
  - Immunoelectrophoresis
  - Immunofixation
  - Immunoturbidimetry
  - Immunonephlometry
- Label methods – either antigen or antibody is labeled and through the label concentration, the antigen-antibody reaction is observed. This includes:
  - Immunoassay
  - Competitive binding
- Other methods – includes immunofluoroscence, immunoelectron microscopy, etc.

TERMINAL QUESTIONS

1. Give an account of characteristics of immunochemical techniques.
2. What are the different methods of analysis?

3. Discuss the various methods of agglutination.
4. Enumerate the differences between immunoturbidimetry and immunonephlometry.
5. Compare immunoprecipitation and immunoelectrophoresis.
6. Give the importance of immunoassay. Mention its types.

ANSWERS TO INTEXT QUESTIONS

24.1

1. B
2. Antigen
3. Immunochemical
4. Affinity

24.2

1. Monoclonal & Polyclona
2. Agglutination
3. ABO
4. Pregnancy

Notes

25

AUTOMATION IN CLINICAL LABORATORY

25.1 INTRODUCTION

The word automation is inspired by word automatic. Automatic means exercising control without interference. So automation means getting work done by machines which can run on their own without our continuous monitoring. Automation refers to machines with intelligence and adaptability which reduces our workload and need for nonstop supervision.

OBJECTIVES

After reading this lesson, you will be able to:

- define automation
- list the uses of automation in clinical lab
- discuss automation at each step of analysis
- describe different types of auto analyzers
- enlist the advantages and disadvantages of automation

25.2 AUTOMATION IN CLINICLA LABORATORY

There are several individual steps in the analysis process as a whole in a laboratory such as:

1. Identifying the patient
2. Getting the correct sample
3. Identifying and proper labeling of the sample
4. Delivery of sample in proper storage condition and within time

Notes

5. Preparation of sample for test
6. Sample loading/aspirating
7. Analysis
8. Reporting
9. Entering in register

Imagine if you are asked to add from no 1 to 100 and write the result throughout the day. How would you feel? Can you assure that after 2 hours of repeating the same task you will not be bored and make mistakes? But an automated machine will never feel tired nor make mistake as often as you will.

Automation has a lot of benefits for the laboratory personnel.

1. Reduces the workload
2. Increases turnaround time (Saves time used per analysis)
3. Increases total number of tests done in less time
4. Eliminates repetition and monotony from human life so decreases human error, improves accuracy
5. Improves reproducibility (repeatability)
6. Uses minimum amount of sample and reagent

In a clinical laboratory set up automation is useful in routine chemistry, hematology, immunological assay, and daily processing of large number of samples etc. Usefulness of automation in advanced and well equipped clinical laboratory can be also extended to

1. Transport of specimen
2. Processing of specimen
3. Loading of specimen into auto analyzer
4. Assessment of results of performed tasks

However, if above steps are not automated due to lack of finance and infrastructure, still any ordinary laboratory at least go for automation in its analysis step. The routine techniques and procedures that are done manually by technicians are replaced by automated analyzers called AUTOANALYZER.

INTEXT QUESTIONS 25.1

State true or false

1. One of the benefits of automation is that it saves reagent
2. One of the benefits of automation is that it reduces turnaround time

3. The total number of tests is decreased by automation
4. Automation improves reproducibility
5. Automation is has no use in hematology
6. Transport of sample is not part of automation of laboratory

In the beginning of this lesson we enlisted 9 number of steps involved in entire analytical process for a laboratory. Let us see the possibility of use of automation in some of these steps.

25.2.1 Sample collection

The use of glucometer is one such example where just by pressing a button on finger tip, the finger is pricked with least pain and analysis is also done then and there.

However, not all tests can be done in glucometer. So, automation in sample collection mainly refers to improved, faster and least discomfort causing techniques of collection. Such as: robotic system, vacutainers etc.

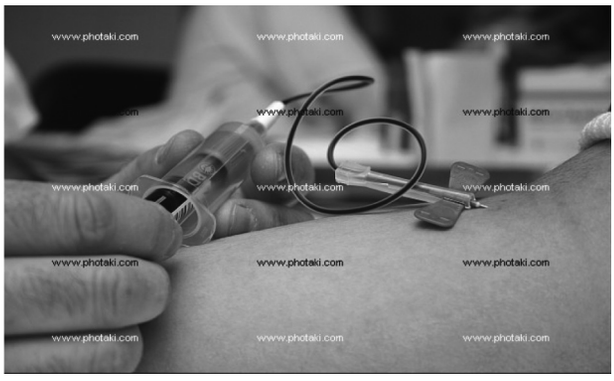


Fig. 25.1: Blood collection with vacutainer

Blood collected using a vacutainer. Here the phlebotomist need not pull the syringe, blood gets sucked in due to negative pressure filling the vaccum

Fig. 25.2: Vacutainer

Different types of vacutainer for serum collection and for plasma collection using different types of anticoagulants. The identification is done with the colour of caps used.

25.2.2 Sample identification by Labeling and bar coding

The laboratory information system comes into take part here. It first generates a unique identity or hospital number for each new patient. A record is maintained thereafter for him/her. All sample collected have to bear the name and details of the patient along with this unique identity (hospital number). The same is used while entering the details into auto analyzer software and result is also published with this number and other details.

Some advanced labs are using computer generated bar coding technology for labeling samples. It has the advantage that it can be scanned and read by bar code reader accurately so transcriptional error (mistake in writing manually) is avoided.

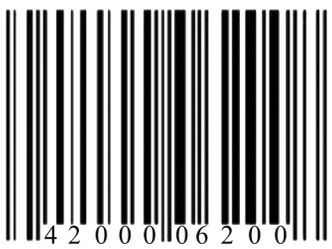


Fig. 25.3: An example of stickers with bar code

Fig. 25.4: Different samples with different bar codes pasted on the tube

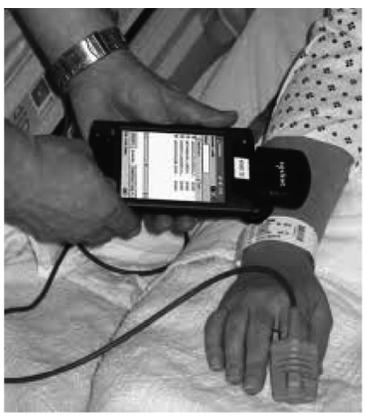


Fig. 25.5: Bar coding of patient

Bar coding of the patient,unique sample identity is attached to his/her wrist. Even when patient is sleeping or unconscious, nurse can read the code for patient identification.

Fig. 25.6: Bar code in Blood collection

Bar coding of blood bag in hematology, stores entire detail of type of blood, its components, storage condition, time of storage etc just by bar codes

25.2.3 Sample delivery

Most of the laboratories rely on human pick up system or conveyer belt system. Though cheaper, it may lead to human error, delays etc.

Pneumatic tube systems (use of pressurized gas to move the tubes containing samples) are used in some laboratories. However, care has to be taken that acceleration and deceleration should not damage any sample.

In very advanced laboratory mobile robots are used.

Fig. 25.7: A sealed sample container at left

Fig.25.8: A container to transport blood culture

The pneumatic tube systems at right sample which can fit into all pneumatic tube systems

25.2.4 Sample preparation

Most of the labs depend on technicians for sample processing (such as serum separation) as soon as sample arrives. However introduction of automation can reduce the workload on technicians and save their time and expertise for analysis purpose. Therefore, now days many semi automated devices are developed which can analyze whole blood itself. For example, automated ion selective electrode, use of dry chemistry etc.

The automated sample processors come in 2 types:

- Stand alone automated sample processors and
- Independent automated sample processors.

These automated sample processors can do the following tasks: sorting of samples, removing caps, separating samples, bar coding etc. having an automated sample processor solves the task of bar coding and sample delivery via conveyer belt system.

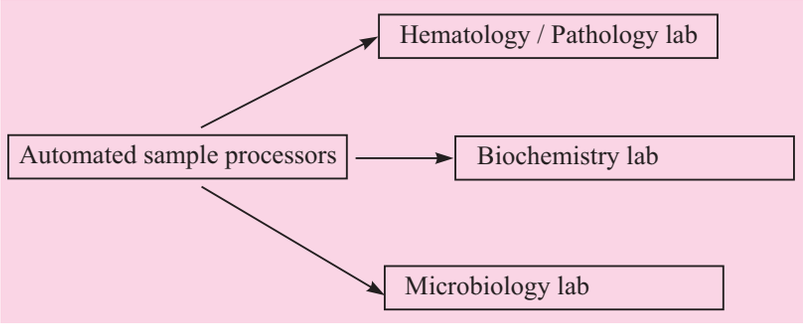


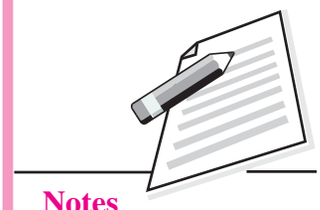
Fig. 25.9

The arrow mark represents pneumatic tube system or Conveyer belt

INTEXT QUESTIONS 25.2

Match the following

1. Sample collection	(a) Pneumatic tube systems
2. Sample identification	(b) Autoanalyzer
3. Sample delivery	(c) Different coloured caps
4. Plasma	(d) Bar coding
5. Sample analysis	(e) Vacutainer



25.3 SAMPLE ANALYSIS

Now let us study briefly about each aspect of automation in the analysis process itself which is the minimum essential aspect of automation for any laboratory. For automation in analysis process we have invented auto analyzer.

25.4 TYPES OF AUTO ANALYZERS

Auto analyzers based on above principle can be divided into two types

- Open system
- Closed system

25.4.1 Open system

In this system, the operator has the advantage that he can purchase reagents from any company so he can save money by buying reagents which are cheaper thereby reducing the cost per test.

In a modular design: An auto analyzer is designed by assembling pulling together of all parts. This increases the flexibility of machine according to customer's demand.

For example: Ion selective electrode. Due to modular approach, the facility for analysis of sodium, potassium and chloride can be built into the system or can be added later on.

25.4.2 Closed system

In this system the operator has to buy chemicals only from a particular company, because reagents from different companies will simply not be accepted by the machine, so machine will not run. So operator can not reduce the cost per test.

Next, reagents have to be provided in unique containers or format as prescribed by its manufacturer. This too adds to cost per test.

However, it allows high degree of automation.

Laboratory can be managed by just one or two well trained technical assistants.

The automated machines are designed to function as either a modular system or an integrated system.

Modular system in auto analyzer

Compare it with modular kitchen where there are many smaller cabinets fitted according to its use. So if there is a problem with any part, only that part is

replaced without disrupting the rest of the kitchen. Similarly, in a lab when whole automated system is subdivided into multiple useful parts it becomes a modular designed automation. Each part is created separately in such a way that they can fit into different systems. It is useful because if one part of machine is out of order, it can be replaced without affecting the entire machine. One such example is: Roche Modular P auto analyzer.

Fig. 25.10: A modular kitchen

Fig. 25.11: A modular kitchen see the different types of cabinets

Integrated system in auto analyzer

In integrated system, the entire equipment is designed in such a way that every part in an essential part of the machine. Basically it is possible due to the merging of the functionality of different softwares into one integrated solution.

It also comes with the disadvantage that service and maintainance may be difficult and expensive too. We have to rely on company engineers every time even for a minor service.

However, it offers a lot of advantages such as

- Improved communication
- Improved data exchange between different softwares and the machine

Now let us understand different types of processing by auto analyzer. It is broadly of 2 types:

(a) **Continuous flow processing:** Based on this principle a continuous flow analyzer (**CFA**) is **made of different modules**, such as:

Sampler

Pump

Mixing coil

Heater/incubator

Sample treatment chamber (dialysis, distillation etc)

Signal detector

Read out device (data generator)

This provides one analysis per analyte for one sample at a time. **The flowing carrier solution passes through small tubes continuously. This is the main principle of Continuous flow processing.** Here sample is injected into a flowing carrier solution. The sample mixes with diluents and reagent and it is sent through the tubing and mixing coils. **The machine prevents carry over effect between different samples by injecting bubbles of air**. The air bubbles literally create separate space for different reactions to take place inside the tubing and mixing coils.

The tubing passes the samples from one apparatus to the other. There are different apparatus for different functions, such as ion exchange, heating, incubation, and finally recording of the signal.

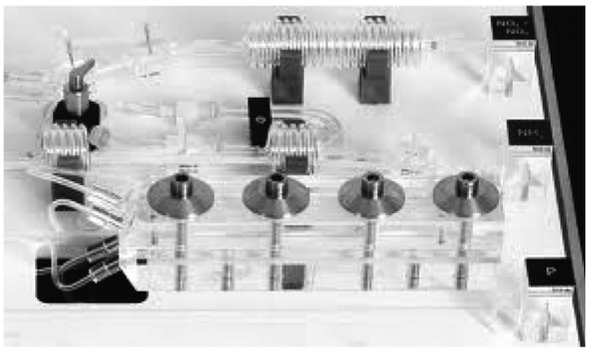


Fig. 25.12: Note the complex pattern of tubing system in a CFA

The flow conditions are regulated. When reaction is taking place, the optical density of the colour formed is read and results are obtained. So we do not have to wait till the reaction ends. It has separate heater for promoting enzymatic reaction and colour development.

Let us take one example for better understanding. In a nephrotic syndrome patient you want to analyze total protein, albumin and creatinine. In case of continuous flow processing analyzer the patient sample will be sucked by the instrument and injected into the tubing with reagents for protein, and diluents

(if needed). Next air bubbles will be injected and patient sample will be sucked again. This time instrument will inject reagent for albumin. Mixing will be done inside the tubing and mixing coils. Again the process will be repeated for creatinine estimation. The 3 reactions will occur inside the same long tubing but they will remain separate due to air bubbles in between.

As the sample and standard are treated in the same manner, mixed in same condition, travel the same length of tubing, it removes the difference between the two. So the difference in reading for test-tube from that of standard gives the answer.

Even though originally, CFA was designed to process only colorimetric reactions, later on CFA were designed to read reactions based on ion selective electrode, flame photometry, flurometry etc. depending on the need of the laboratories.

The major uses is for batch analysis such as liver profile, lipid profile, renal profile assay etc.

There is certain disadvantage in this system:

- **Even when there is no test to be done, reagents are drawn to maintain the flow**. This adds to the cost per test.
- Maintainance of instrument is required more frequently.
- The probe and internal tubings must be free of colgs. **When there is no sample the probe must be dipped in distilled water to avoid blockage or precipitation.**
- The machine itself occupies large space.

Fig. 25.13: Image of an aut analyzer based on continuous flow analysis

(b) **Discrete processing:** in this type of auto analyzer, each sample is provided a discrete space. It means **each analysis even for same analyte or sample takes place at different cups. This is the main principle of discrete processing**.

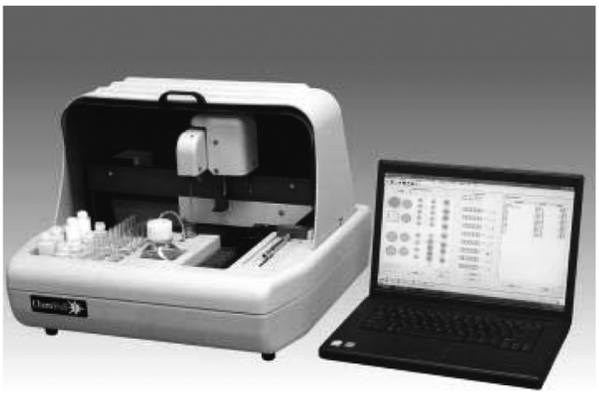


Fig. 25.14

Let us take the previous example of the nephrotic syndrome patient again. You want to analyze the same 3 parameters: total protein, albumin and creatinine. Now, in case of discrete processing analyzer the same patient sample will be sucked by the instrument and poured into 3 different cups. Then reagents for protein, albumin and creatinine and diluents (if needed) will be added. Mixing will be done. Cups will be read at different times to give results.

Exact amount of sample and reagent is aspirated and mixed. So there is no loss of excess reagents used for flow as in continuous flow processing. As each analysis is done in different cups and read in different cuvetes, **there is no carry over effect at all**. So literally each analysis is discrete from each other.

- This is more useful
- Saves reagent cost and hence popular than continuous flow analysis.
- No sample carry over effects

Based on this principle the auto analyzers developed into two different varieties such as centrifugal analyzer and random access analyzer.

25.4.2.1 Centrifugal analyzer

Sample and reagents is pipette into different chambers on a rotor. The centrifugal force is used for transfer and mixing of sample and reagents. Rotor moves the final product upto the optical system for final reading. This is time saving for batch analysis because all cuvets can be read at a time. But its disadvantage is that only one test can be performed at a time.

25.4.2.2 Random access analyzer

Each sample can be analyzed for multiple tests, and multiple samples for one test also can be done by giving appropriate commands to computer software. It

is the **most versatile of all type analyzers**. Let us say we have 3 different samples. First one needs renal profile, second one needs only glucose and urea and third one need triglyceride, albumin and calcium. So the technician has to simple take 3 different sample cups and loads 3 samples. Then he has to enter sample number, cup number and the tests required. And when he presses the start button tests will be done automatically.

Fig. 25.15

INTEXT QUESTIONS 25.3

Match the following

1. Modular design	(a) No carry over effect of samples
2. CFA	(b) Air bubbles separate samples
3. Integrated design	(c) Most versatile analyzer
4. Discrete analyzer	(d) Removal of parts easy
5. Random access analyzer	(e) Improved communication

25.5 REPORTING

The entire hospital information system can be linked to automated instruments and reporting authorities' desktops. So as soon as a sample report is ready, information goes to reporting officer's computer.

After he/she approves the result, the same is displayed in all computers in the hospital. So the clinician or nurses awaiting the report can check at regular intervals for the report and after matching the patient's unique hospital number they can directly take a print out of the report.

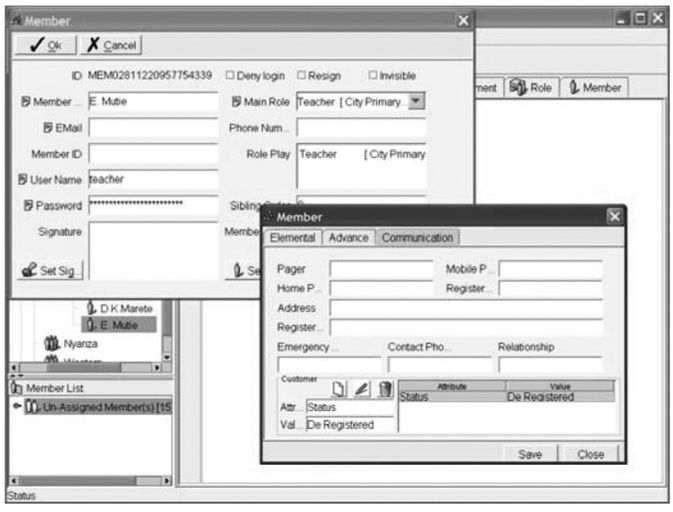


Fig. 25.16

This minimizes the time delay in reporting and manual report delivery.

Some problems faced in automation in lab

1. Lack of trained personnel at every aspect of automation
2. Automation may not work in a system where administration wants to reduce cost at every step
3. Hidden costs: trained personnel, supply and maintainance, system upgrading, service

Therefore, till now in many labs of our country you will not find entire system automated. However, most of the labs use automation in the form of automated analyzers discussed in this chapter for this purpose.

INTEXT QUESTIONS 25.4

1. An open system analyzer saves money by reducing .....
2. A modular system is flexible because its ..... can be replaced without affecting entire machine.
3. The main feature of CFA is that, a flow carrier solution runs through it .....
4. The main feature of discrete analyzer is that each reaction takes place discretely .....
5. Which analyzer is associated with minimum carry over effect? Choose between CFA and discrete analyzer.
6. One disadvantage of automation is ..... cost What have you learnt

WHAT HAVE YOU LEARNT

- Analysis in lab can be automated right from the step of patient identification upto report delivery. However, due to the hidden cost and demand of trained personnels at each stage most of the labs restrict their automation only at the laboratory analysis level. This is mainly done by auto analyzer. Modern auto analyzers run mostly on the principle of discrete analysis where each analysis takes place in different cuvets, avoiding carry over effect. Random access analyzers are the most versatile type where multiple tests can be run at any time. Integrated system in auto analyzers improve efficiency but increases maintenance tasks and cost per test.

TERMINAL QUESTIONS

1. What are the individual steps in the analysis process as a whole in a lab?
2. How can you introduce automation in patient identification?
3. Discuss the role of automation in sample identification and delivery.
4. Discuss the disadvantages of CFA.
5. How is discrete analyzer better than CFA?

ANSWERS TO INTEXT QUESTIONS

25.1

1. True 2. False 3. False 4. True 5. False

25.2

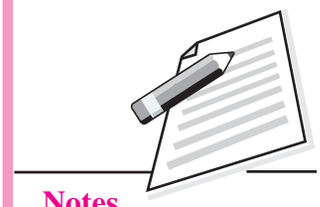
1. (e) 2. (d) 3. (a) 4. (c) 5. (b)

25.3

1. (d) 2. (b) 3. (e) 4. (a) 5. (c)

25.4

1. Cost per test 2. Damaged parts 3. Continously
4. In different cups 5. Discrete analyzer 6. Hidden



26

LABORATORY QUALITY MANAGEMENT

26.1 INTRODUCTION

Have ever accompanied your family members to nearby market for buying vegetables? Have you noticed how many customers lift few vegetables and either press or break it into two? For example, to know whether a lady's finger is supposed to be good if it is found to be firm while breaking off its tail. Many vegetables and fruits such as brinjal, apples etc. are supposed to be good if they are felt as firm but not hard at our hands. So what exactly is the customer doing by checking one or two vegetable or fruit? And is it sufficient if they check just one or two randomly? Why don't they touch and check each and every piece? Well, what they are doing is they are doing a quality check. And yes, most often if a random piece is good then mostly the whole bunch is usually almost good. Similarly, in a laboratory set up if you want to know whether the tests that are being done are of good standard or not you have to run a quality check. And this is slightly different from random checking of vegetables. It is a methodical process with certain rules and regulations which is known as **Quality control**. Besides the process, laboratory quality control has to be managed following regularity and policies. This is otherwise known as **Laboratory quality management**.

Fig. 26.1

Notes

OBJECTIVES

After reading this lesson, you will be able to:

- define quality control, accuracy and precision
- define laboratory quality management
- differentiate between precision and accuracy

26.2 QUALITY MANAGEMENTCONTENT

The main objective laboratory quality management is to

(a) Identify errors,

(b) Decrease errors

(c) Correct the root cause of errors

(d) Increase the efficiency of the lab

by regular checking of the analytical process at each step sticking to quality control material or calibration material (internal and external), before releasing the patients' reports .

So what is basically quality control?

Quality control otherwise known as QC is a measure of precision.

Precision means how often the measurement system produces **the same result** again and again over time and under different operating conditions.

As we are discussing precision you must also know what accuracy is. Accuracy is how often the measurement system produces **the correct result** over time and under different operating conditions.

For example according to internal quality control (level 2; secondary standard) of the laboratory, the value of creatinine is 2 mg/dl in the quality control material. If your laboratory measurement system measures this for 6 times by instrument A and 5 times by instrument B.

Instrument A produced results as:

(a) 4.1

(b) 4.2

(c) 4.1

(d) 4

(e) 4.1

(f) 4.1

And Instrument B produced results as:

(a) 4

(b) 0.9

(c) 2.1

(d) 2

(e) 1.9

(f) 2

Then your Instrument A is precise but not accurate. Why? This is so because, it 4 out of 6 times produced result close to 4. So it is always producing values close to one result. But it was supposed to produce result close to 2! Serum creatinine normal range is roughly 0.5 to 1.1 mg/dl in females and 0.6 to 1.2 mg/dl in males. And our level 2; secondary standard which corresponds to higher level standard, value should be close to 2. So it is not accurate and this type of inaccurate results will make laboratory reports unreliable.

On the other hand, Instrument B is accurate but not precise. Why? This is so because, 4 out of 6 times, it produced result close to 2. So it is always producing values close to standard value. But it also produces result far away from that is 0.9 and 4. So it is good for the laboratory to produce accurate result 3 times out of 5. But also in 2 cases out of 6 if value is so far from correct value, laboratory cannot be sure whether it's the right value or one of its precision errors.

For extra information on standards and internal quality control refer chapter on Primary and Secondary standards.

INTEXT QUESTIONS 26.1

1. The main objective laboratory quality management is to decrease errors
2. Accuracy means how often the measurement system produces the same result
3. Precision means how often the measurement system produces the correct result

26.3 LABORATORY QUALITY MANAGEMENT

The management of quality in a laboratory follows an order which works in a cycle. The various parts are

(a) The goal and objectives set by the management to maintain quality in laboratory

(b) To provide initial requirements such as instruments, calibration materials, trained manpower, a competent lab manager etc.

(c) To set the quality control processes

(d) To do quality assessment at required interval and identify error

(e) To take steps to eliminate error and also to improve quality

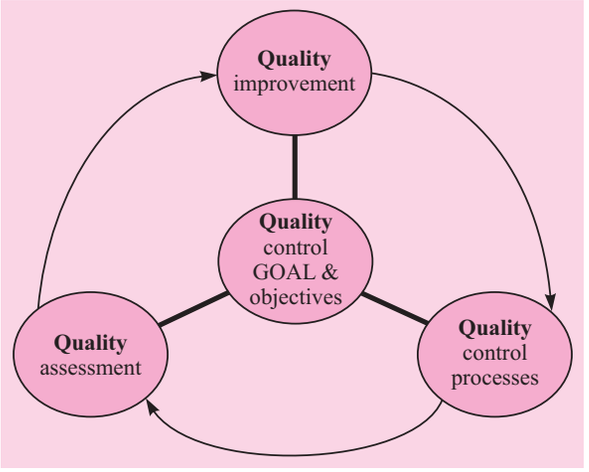


Fig. 26.2 : The management of quality in a laboratory

INTEXT QUESTIONS 26.2

1. Arrange the followings in order.
   (a) Set the quality control processes
   (b) Provide initial requirements
   (c) Take steps to eliminate error
   (d) Perform the quality assessment at required interval
   (e) Set your goals and objectives

26.4 SETTING OF GOAL AND OBJECTIVES FOR QC

This depends from laboratory to laboratory. This depends on the vision of management and facilities provided. The objectives for QC is guided by the keeping the following points in mind

- To set the analytical error limit
- To set the critical range for analytes to make medical decisions

So we should understand the types of errors that can occur in a laboratory. Basically all types of errors associated with analysis and final reporting of a patient's sample can be broadly divided into 3 categories.

1. Pre-analytical error
2. Analytical error
3. Post analytical error

26.4.1 Pre-analytical error

It consists of all errors that occur before the patient's sample reaches laboratory.

One should be aware that most of the errors (around 75%) come under this group. Therefore, even though being a laboratory personnel we are responsible for analytical error, we should not ignore pre-analytical error. Laboratory personnel **must be aware** of all types of pre-analytical error and be concerned with it. Because if we can decrease pre analytical error then the total load of analysis by us will also decrease. It means automatically the percentage of correct reports generated by our laboratory also will increase. Apart from that it will save the time, money and our energy spent on processing of wrong sample. This in turn will improve patient care service by laboratory. Some of the common preanalytrical errors are described below.

(a) Order the test:

- Inappropriate test: Thyroid hormones should not be ordered within 2 weeks of starting therapy
- Wrong patient identification: It can happen if clinician does not verify the patient his/her name and other details
- Handwriting not readable etc: One of the most common error laboratory personnel have to deal with! For example if FBS (Fasting blood sugar) is not written properly can look like RBS (Random blood sugar). So a value of 130 for FBS (should be <126mg/dL) that should have drawn attention for RBS (should be <140mg/dL) would go unnoticed.

(b) Sample collection

- Wrong patient identification: It can happen if clinician does not verify the patient his/her name and other details before collecting sample
- Wrong tube: Blood sample for serum should not be collected in anticoagulated tubes
- Inappropriate volume collected: First the phlebotomist should calculate the total amount of sample needed. This is done by adding the amount of sample needed for each test ordered. In this way sample collected would neither be wasted nor be less for any test.

- Inappropriate sample collected: For example if sample is hemolyzed due to incorrect way of collection, it should not be sent for potassium estimation.
- Collection at wrong time: Sample for cortisol should always be collected before 8am.
- Delayed transport: Delay in transport can decrease the value of sugar in blood sample at 7% rate. This happens because sugar gets utilized by RBC and WBC.

If the laboratory personnel identify pre-analytical error, sample should be rejected.

INTEXT QUESTIONS 26.3

(Being an efficient lab personnel, do you agree or not with the following statements. Answer with yes or no)

1. The clinicians are so busy. They need not write the diagnosis or patients name on requisition form
2. We should not fuss over simple mistakes like writing FBS which looks like RBS
3. Clinicians and nurses are directly dealing with patients and not by us who are sitting in laboratory. They can never make mistake in identifying their on patient
4. If a patient who has come from far distance, for giving blood for serum cortisol at 12 pm I should not feel pity. I should tell him to come again next day
5. I do not have a serum tube but there are enough heparinized tube with me. It is alright if I collect in serum tube, later on I can transfer into a heparinized tube

26.4.2 Analytical error

Most common analytical errors are

- Faulty calibration of instrument or errors not corrected in instrument
- Interfering substance in sample
- Sample mix up **(very dangerous and can lead to legal problems)** etc

The errors that occur while analyzing samples can be decreased by using standard equipments such as:

- Using standard quality of water
- Calibrating the pipettes, glasswares etc

- Maintaining the instrument within their proper working condition etc
- Checking the quality of analysis (most important) by comparing the values with quality control materials (external and internal QC material).

The QC sample has to be stable, should be divided into different vials. So we can analyzes the same QC sample from time to time. Either it has to be bought from laboratories supplying secondary standards as lyophilized powder for internal calibration. It can also be prepared by pooling of sera and storing kept in different aliquots at -20 degree for repeated use. The most common method for comparing the laboratory value observed for true value of standard is by drawing control charts or by statistics.

Laboratory quality control material is run at the beginning of each shift or preferably in the morning and in the evening, also, after an instrument is serviced, or when a new reagent box is opened etc and of course whenever patient results seem inappropriate.

The easiest chart is **Levey Jenings chart**. This chart is named after its inventors Sir Levey and Sir Jennings in 1950. later it was improved by Henry and Segalove. Here, the days are plotted in X-axis and observed values for standard are plotted on Y-axis. The mean and one standard deviation (1SD), two standard deviation (2SD), and three standard deviation (3SD) limits are plotted on the Y-axis. Comparing the pattern of plotted points gives the simple way to detect the

- Increased random error
- Any shifts in the trends of calibration

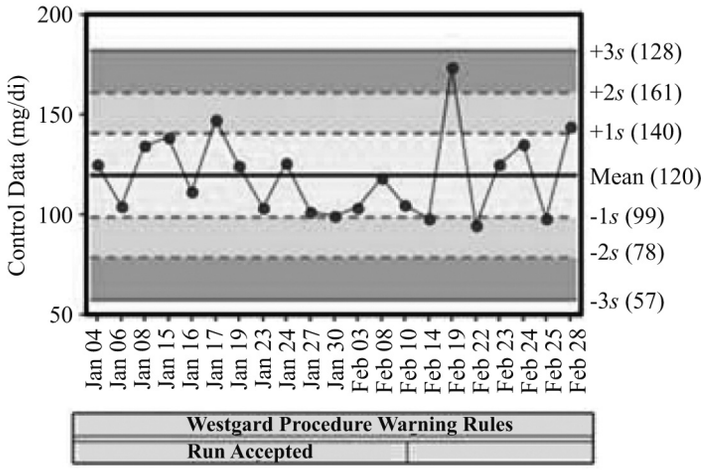


Fig. 26.3: Levey-Jennings chart/Graph

In order to draw a Levey Jenings chart, the QC material has to be analyzed for 20 consecutive days. Then calculate its mean and standard deviation. Now draw the points on the y axis on a graph sheet with no of days in X axis. Now join the

Notes

points. Next draw the mean as a line parallel to X axis. Next draw the lines on both sides of mean for range of ± 1SD then ± 2SD. Likewise, ± 3SD then ±42SD also can be drawn. However, the **control limit is taken as mean ± 2SD range**.

If any observed value falls out of this mean ± 2SD range, on any day, all laboratory procedure has to be stopped, error has to be corrected. Till then patient report should not be given out.

Shewhart chart on the other hand is a statistical process. Here, the short term estimate of sigma is used unlike Levey-Jennings chart where long term estimate of sigma (standard deviation) is used.

There is a **Westgard multirule procedure** which helps us decide which observed values for QC material is to be rejected. For example,

- If one control value exceeds ± 1SD then it's a warning.
- If one control value exceeds ± 3SD; or if one value exceeds + 2 SD and the other – 2SD then these are **random errors**, so test should be rejected. Go for error detection. Withhold patients' results.
- If 2 consecutive values exceed ± 2SD; or 4 consecutive values exceed ± 1SD then these are **systematic errors**, so test should be rejected. Go for error detection. Withhold patients' results.

And so on…..

Here we should know what a random error is. It is an error that occurred by chance. For example, sudden loss of power leading to decreased incubation temperature. So this would affect test run only at that time and not to any other test. So error happens randomly.

So what is a systematic error? It is an error that is occurring systematically to all tests. For example: use of a faulty pipette. So, an incorrect volume of sample or reagent will be equally added to all tests. So error happens systematically.

INTEXT QUESTIONS 26.4

1. The pipetting error is a type of ..... error
2. Sample mix up is an analysis error which can lead to ..... problems
3. Westgard rule is for ..... of a test.
4. Levey Jennings chart is drawn after minimum ..... days.

26.4.3 Post analytical error

Most common post-analytical errors are

- Wrong patient identification so wrong reporting
- Transcription error (wrong entry, writing not clear)
- Delayed reporting
- Previous values not available for comparison etc.

You should also know that delayed reporting is a post analytical error. Still the lab is held responsible for it because laboratory personnel are responsible for its reporting.

The laboratory is controlled by a laboratory manager who is the link between lab technicians and the management. The laboratory manager is responsible for satisfying customers' quality requirements and also to meet the regulations set by the management as well.

In any organization, the people working behind the machines are most important and not the machine itself. Therefore, irrespective of the quality of instruments and other facilities provided quality in laboratory can be maintained to its best possible standard by the dedication of laboratory personnel. Therefore, a positive attitude among all laboratory personnel, respect for each other and a sincere desire to solve problems will improve the over all quality of the laboratory service.

WHAT HAVE YOU LEARNT

- As the laboratory deals with patients' samples based on which medical decisions aret taken it is important to run a quality check and control of quality. The laboratory has to manage control of quality by certain rules in order to decrease pre analytical, analytical and post analytical errors which can occur at random or systemically. For error detection labs rely on various statistical methos or graphs such as Levey Jennings chart with Westgard's rules etc. after error detection the source of error has to be removed, till then no patient report should be given out.

TERMINAL QUESTIONS

1. What are the steps for management of laboratory quality?
2. Discuss various types of pre analytical errors

Notes

3. Describe Levey Jennigs chart and role of Westgard's rules in it.
4. Describe various types of errors that can be detected by Levey Jennigs chart.

ANSWERS TO INTEXT QUESTIONS

26.1

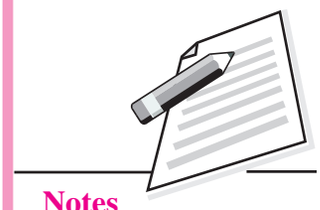
1. True
2. False
3. True

26.2

(e) Set your goal and objectives
(b) Provide initial requirements
(a) Set the quality control processes
(d) Perform the quality assessment at required interval
(c) Take steps to eliminate error

26.3

1. No
2. No
3. No
4. Yes
5. No



Notes

27

ELECTROLYTES AND BLOOD GASES

27.1 INTRODUCTION

Electrolytes are charged particles (ions) dissolved in the various fluid compartments of the body (intravascular, interstitial, and intracellular) and perform a variety of functions in the human body. The electrolytes of importance at this point in the course are: (1) Sodium; (2) Potassium; (3) Calcium; (4) Hydrogen; and (5) Bicarbonate. Since hydrogen and bicarbonate ions are primarily involved in pH balance, they are discussed separately.

Sodium, potassium and calcium will be considered here. Sodium is primarily an extracellular ion, that potassium occurs primarily intracellularly, and that calcium performs a variety of functions.

27.2 SODIUM

Sodium is the major extracellular cation. In general, sodium balance defines water balance.

- Na+ is the most abundant electrolyte in the body Transmission and conduction of nerve impulses
- Responsible for osmolality of vascular fluids
- Regulation of body fluid levels
- Sodium shifts into cells and potassium shifts out of the cells (sodium pump)
- Assists with regulation of acid-base balance by combining with Cl^- or $HCO3^-$ to regulate the balance

Notes

Abnormalities

Hyponatremia: Excessive sodium loss or H_2O gain

Causes

- Prolonged diuretic therapy
- Insufficient Na intake
- GI losses – suctioning, laxatives, vomiting
- Administration of hypotonic fluids
- Alcoholism

Hypernatremia: occurs with excess loss of H_2O or excessive retention of Na. Can lead to death if not treated

Causes

- Vomiting/diarrhea
- Inadequate AntiDiuretic Hormone
- Major burns

27.3 POTASSIUM

Potassium is the most abundant cation in the body cells. About 97% is found in the intracellular fluid. Normal extracellular K+ is 3.5-5.3mEq/L. A serum K+ level below 2.5 or above 7.0 can cause cardiac arrest

Functions

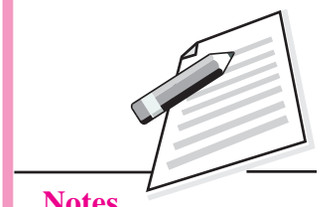
- Essential for normal membrane excitability for nerve impulse
- Promotes conduction and transmission of nerve impulses
- Contraction of muscle
- Promotes enzyme action
- Assist in the maintenance of acid-base

Abnormalities

Hypokalemia

Causes

- Prolonged diuretic therapy



- Inadequate intake
- Gastric suctioning, laxative use, vomiting
- Excess insulin
- Hepatic disease
- Lower Potassium concentration dramatically affects excitable membrane function of muscle and nerve. Levels < 3 produce marked neuromuscular symptoms

Hyperkalemia

Causes

- Shock, severe hemolysis, tumor lysis, burns (cellular release)
- Renal failure (decreased excretion)
- Endocrine diseases with mineralocorticoid deficiency
- "Potassium sparing" diuretics
- Acidosis (K exits cells in exchange for H)
- Artifactual hemolysis of blood specimens; K leak in chilled or unprocessed specimens

Symptoms

- Greater then 5.0, EKG changes, decreased pH
- Acts as myocardial depressant; decreased heart rate, cardiac output
- Symptoms: muscle weakness, nausea, lethargy, arrhythmias, characteristic EKG changes, cardiac arrest, GI hyperactivity
- levels > 7.5 produce symptoms and levels > 10 are lethal

27.4 CHLORIDE

The major extracellular anion (expected, 118 - 132 meq/l in serum)

Function

- Important in water distribution, osmotic pressure, and anion-cation balance
- Cl^-: regulates osmotic pressure and assists in regulating acid-base balance. Maintains serum osmolality along with Na^+
- Helps to maintain acid/base balance

Notes

- Combines with other ions for homeostasis; sodium, hydrochloric acid, potassium, calcium
- Closely tied to Na^+
- Decreased level is most commonly due to GI losses
- Found in ECF
- Changes the serum osmolality
- Along with Na^+ causes retention of water
- Assists with regulation of acid-base balance
- Cl^- combines with hydrogen ion to form hydrochloric acid in the stomach

Abnormalities

Hypochloremia

Causes

- Fluid volume expansion (dilution)
- Renal diseases (reabsorption failure)
- Metabolic acidosis with increased "unmeasured anions" (e.g. DKA)
- SIADH
- GI loss (protracted vomiting)

Hyperchloremia

Causes

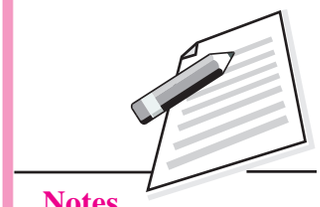
- Dehydration
- Renal tubular acidosis, acute renal failure
- Bicarbonate loss (diarrhea)
- Mineralocorticoid excess (Cushing's syndrome and hyperaldosteronism)
- Diabetes insipidus

27.5 CALCIUM

Ca^{2+}: usually combined with phosphorus to form the mineral salts of bones and teeth, promotes nerve impulse and muscle contraction/relaxation.

Abnormalities

Hypocalcemia:



Causes

- Abnormalities of the parathyroid gland
- Inadequate intake
- Excessive losses.

Symptoms

- Can cause skeletal and neuromuscular abnormalities
- Impairs clotting mechanisms
- Affects membrane permeability
- Diagnostic findings:
  - EKG changes
  - Serum Ca++levels < 8.5 mg/dL
  - Prolonged PT and PTT

Hypercalcemia

- Increased serum levels of Ca^{++}
- Symptoms are directly related to degree of elevation
- Clients with metastatic cancer are especially at risk

Causes

- Excessive intake
- Excessive use of antacids with phosphate-binding
- Prolonged immobility
- Excessive vitamin D intake
- Thiazide diuretics
- Cancer
- Thyrotoxicosis

27.6 MAGNESIUM

- Mg^{2+} plays role in carbohydrate and protein metabolism, storage and use of intracellular energy and neural transmission.
- Important in the functioning of the heart, nerves, and muscles

At a glance

- Hyponatremia (sodium deficit < 130mEq/L)
- Hypernatremia (sodium excess >145mEq/L)
- Hypokalemia (potassium deficit <3.5mEq/L)
- Hyperkalemia (potassium excess >5.1mEq/L)
- Chloride imbalance (<98mEq/L or >107mEq/L)
- Magnesium imbalance (<1.5mEq/L or >2.5mEq/L)

27.7 BLOOD GAS TESTING

- Blood gas analysis may be carried out in a main laboratory, in an ER or OR lab or using POC devices.
- Provides accurate measurement of PO2, PCO2, pH, hemoglobin saturation, bicarbonate and hematocrit (some analyzers also offer electrolytes, lactate, glucose, Hgb, CO-Hgb, met- and sulfHgb).
- Arterial blood, drawn in appropriate equipment and transported quickly (10 - 15 min) on ice to the lab.
- Check ulnar artery patency before withdrawing blood from radial artery .
- "Arterialized capillary blood" (warmed heel or earlobe) may be acceptable in some Cases.
- Specimen is accompanied by history with FIO2 and clinical status of patient, and patient temperature (all are important for interpretation and are included in the returned report).
- Erroneous results from room temp specimens and specimens with air bubbles or improper capping.

TERMINAL QUESTIONS

1. Enumerate in detail the functions of the electrolytes Sodium and Potassium and also describe the related abnormalities.
2. What is hypochloremia? Write down the causes.
3. What is hypokalemia? List out the causes and symptoms.
4. What is hypocalcemia? Write briefly on causes and symptoms of hypocalcmia.
5. List out the uses of blood gas analysis.
6. Normal levels of serum Calcium is…….
7. Hyponatremia is defined as when levels of sodium falls below…….
8. …….. artery blood sample is used for blood gas analysis.

28

CENTRIFUGATION

28.1 INTRODUCTION

A **centrifuge** is the equipment generally driven by an electric motor that puts an object to rotate around fixed axis, and a perpendicular force is applied to axis. The particles get separated according to their size, shape, density, viscosity of the medium and rotor speed.

OBJECIVES

After reading this lesson, you will be able to

- describe centrifugation and its principle
- describe centrifugal force
- practice safety measures in the laboratory

28.2 PRINCIPLE

The centrifuge involves principle of sedimentation, where the acceleration at centripetal force causes denser substances to separate out along the radial direction at the bottom of the tube. By the same concept lighter objects will tend to move to the top of the tube; in the rotating picture, move to the center. In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it float to the top. The greater the difference in density, the faster they move. If there is no difference in density (isopycnic conditions), the particles stay steady. To take advantage of even tiny differences in density to separate various particles in a solution, gravity can be replaced with the much more powerful "centrifugal force" provided by a centrifuge.

What happens to a particle (macromolecule) in a centrifugal field?

Consider a particle m in a centrifuge tube filled with a liquid.

The particle (m) is acted on by three forces:

F_C: the centrifugal force

F_B: the buoyant force

F_f: the frictional force between the particle and the liquid

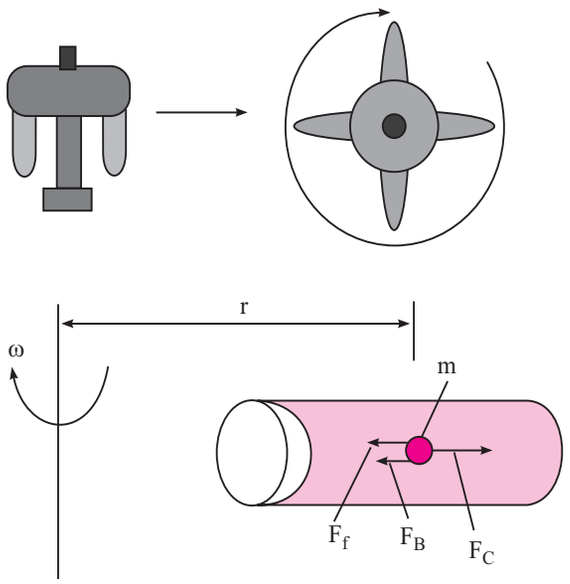


Fig. 28.1: Centrifugal field

28.3 SEDIMENTATION PRINCIPLE

Sedimentation is the tendency for particles in suspension to settle out of the fluid in which they are entrained, and come to rest against a barrier. This is due to their motion through the fluid in response to the forces acting on them: these forces can be due to gravity, centrifugal acceleration or electromagnetism. **Settling** is the falling of suspended particles through the liquid, whereas sedimentation is the termination of the settling process.

28.3.1 Svedberg Equation

The single most important advance in the use of centrifugal force to separate biologically important substances was the coupling of mechanics, optics and mathematics by T. Svedberg and J.W. Williams in the 1920's. They initiated the mathematics and advanced the instrumentation to a point where it was possible to prove that proteins were large molecules that could be weighed in a centrifuge. In honor of that work, the value for a molecule's (or organelle's) sedimentation

velocity in a centrifugal field is known as its Svedberg constant or S value for short.

The instrumentation has progressed quite far since the early work of Svedberg and Williams. Today, any technique employing the quantitative application of centrifugal force is known as ultracentrifugation. The design of the basic instruments, the rotors and the optical systems for measurement are too extensive to cover in this volume. For our purposes, we will concentrate on two types of rotor, and a few selected parameters to be measured.

Calculation of S:

$$S = \frac{v}{\omega^2 r} = \frac{M(1-\bar{v}\rho_{sol})}{N_{AV} f}$$

M = molecular weight ($m \times N_{AV}$)

s = svedberg coefficient

vρ = partial specific volume of the molecule

N = Avogadro's number

f = frictional coefficient

s = sedimentation coefficient (units: 1 Svedberg = 10-13 sec)

The above equation depends on the size of the molecule (M), however the shape of the molecule plays an important role in its behavior under centrifugal force so it is appropriate to take this (f) into account.

This is the Svedberg equation and is used to describe the motion of the particle in terms of molecular weight (a size term) and frictional coefficient (a shape term). The equation also relates the motion to the solvent density.

The Svedberg coefficients are not additive. That is, 40S plus 60S does not equal 100S. This is the case for the ribosomal subunits, where the combination of a 40S small subunit and a 60S large subunit produces an 80S complete ribosome.

28.4 CENTRIFUGAL FORCE

Centrifugal force, word from Latin ***centrum***, meaning "center", and ***fugere***, means "to flee", is the apparent force that draws a rotating body away from the center of rotation. It is caused by the inertia of the body as the body's path is continually redirected. In Newtonian mechanics, the term *centrifugal force* is used to refer to one of two distinct concepts: an inertial force (also called a "fictitious" force) observed in a non-inertial reference frame, and a reaction force corresponding to a centripetal force.

Notes

The term is also sometimes used in Lagrangian mechanics to describe certain terms in the generalized force that depend on the choice of generalized coordinates.

The concept of centrifugal force is applied in rotating devices such as centrifuges, centrifugal pumps. The two different forces are equal in magnitude, but centrifugal forces is opposite in direction to the centripetal force.

A centrifuge is used to separate particles or macromolecules:

1. Cells- biological components in tissues and cells are separated by centrifugation and this principle is widely used in biological laboratory, in fact it is one of the most essential instrumentation in design of a laboratory.
2. Sub-cellular components- substances like cytoplasmic fluid, nucleus, mitochondria, golgi bodies are separated by this principle.
   (a) Proteins- based on density protein in cells and tissues is separated using high speed centrifugation.
   (b) Nucleic acids- DNA, RNA, snRNA, etc., are separated by this method.

28.4.1 Basis of separation

- **Size:** size of the particle matters a lot while application of this principle. It has the basis that as much lesser the size will be, more the particle will be towards the base.
- **Shape**: the shape of particle ex- circular particles will settle down easily as compared to polygonal shape particles.
- **Density**: this component is main play of centrifugation principle, denser the object, lower the settling.

INTEXT QUESTIONS 28.1

1. The centrifuge works on the simple working principle of .....
2. What are the two distinct forces that come under the “centrifugal force”?
3. The ....., ... is used to describe the motion of a particle on the basis of molecular weight and frictional coefficient.
4. 1 Svedberg = ... unit .....
5. What is the difference between settling and sedimentation?

28.4.2 Methodology

- Utilizes density difference between the particles/macromolecules and the medium in which these are dispersed
- Dispersed systems are subjected to artificially induced gravitational fields.

28.4.2.1 Principle

Equipment

The acceleration achieved by centrifugation is expressed as a multiple of the earth's gravitational force (g = 9.81 m s^{-2}). Bench-top centrifuges can reach acceleration values of up to 15000 g, while high speed refrigerated centrifuges can reach 50000 g and ultra-centrifuges, which operate with refrigeration and in a vacuum, can reach 500000 g. Two types of rotor are available in high-powered centrifuges: *fixed angle rotors* and *swing-out rotors* that have movable bucket containers. The tubes or buckets used for centrifugation are made of plastic and have to be very precisely adjusted to avoid any imbalances that could lead to accidents.

$$\text{Relative centrifugal force } F = M\ r\ \omega^2$$

M: mass of particle

r: radius of rotation (cm) (ie.distance of particle from axis of rotation)

ω: Average angular velocity (radians/sec), ω = 2π revolutions/60 minutes

Theory

The velocity (v) of particle sedimentation during centrifugation depends on the angular velocity ω of the rotor, its effective radius (ref, the distance from the axis of rotation), and the particle's sedimentation properties. These properties are expressed as the Sedimentation

Coefficient S (1 Svedberg, = 10–13 s). The sedimentation coefficient depends on the mass M of the particle, its shape (expressed as the coefficient of friction, f), and its density (expressed as the reciprocal density v, "partial specific volume"). At the top right, the diagram shows the densities and sedimentation coefficients for biomolecules, cell organelles, and viruses. Proteins and protein-rich structures have densities of around 1.3 g cm^{-3}, while nucleic acids show densities of up to 2 g cm^{-3}. Equilibrium sedimentation of nucleic acids therefore requires high-density media – e.g., concentrated solutions of cesium chloride (CsCl). To allow comparison of S values measured in different media, they are usually corrected to values for water at 20°C ("S20W").

28.5 DENSITY GRADIENT CENTRIFUGATION

Density gradient centrifugation is used to separate macromolecules that differ only slightly in size or density. Two techniques are commonly used. In **zonal centrifugation,** the sample being separated (e. g., a cell extract or cells) is placed on top of the centrifugation solution as a thin layer. During centrifugation, the particles move through the solution due to their greater density.

The rate of movement basically depends on their molecular mass. Centrifugation stops before the particles reach the bottom of the tube. Drilling a hole into the centrifugation tube and allowing the contents to drip out makes it possible to collect the different particles in separate fractions.

During centrifugation, the solution tube is stabilized in the tube by a **density gradient**. This consists of solutions of carbohydrates or colloidal silica gel, the concentration of which increases from the surface of the tube to the bottom. Density gradients prevent the formation of convection currents, which would impair the separation of the particles. **Isopycnic centrifugation**, which takes much longer, starts with a CsCl solution in which the sample material (e. g., DNA, RNA, or viruses) is homogeneously distributed. A density gradient only forms *during* centrifugation, as a result of sedimentation and diffusion processes. Each particle moves to the region corresponding to its own *buoyant density*. Centrifugation stops once equilibrium has been reached. The samples are obtained by fractionation, and their concentration is measured using the appropriate methods.

28.6 MOVING BOUNDARY/ZONE CENTRIFUGATION

In moving boundary (or differential centrifugation), the entire tube is filled with sample and centrifuged. Through centrifugation, one obtains a separation of two particles but any particle in the mixture may end up in the supernatant or in the pellet or it may be distributed in both fractions, depending upon it size, shape,

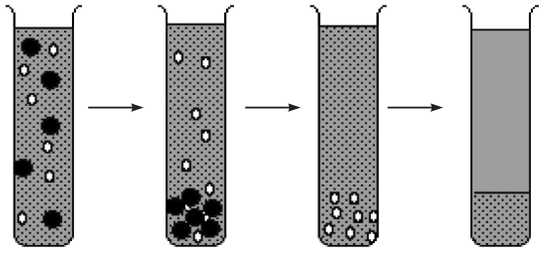


Fig. 28.2: Differential Centrifugation

density, and conditions of centrifugation. The pellet is a mixture of all of the sedimented components, and it is contaminated with whatever unsedimented particles were in the bottom of the tube initially. The only component which is purified is the slowest sedimenting one, but its yield is often very low. The two fractions are recovered by decanting the supernatant solution from the pellet. The supernatant can be recentrifuged at higher speed to obtain further purification, with the formation of a new pellet and supernatant.

28.7 RATE ZONAL CENTRIFUGATION

Particles of the same size (M) but different shapes (e.g., linear versus globular) will separate - the particle with the greater frictional coefficient (f) will move slower (rod shaped moves slower than globular). This technique is called velocity gradient centrifugation (a gradient of sucrose is used to linearize the motion of the particles).

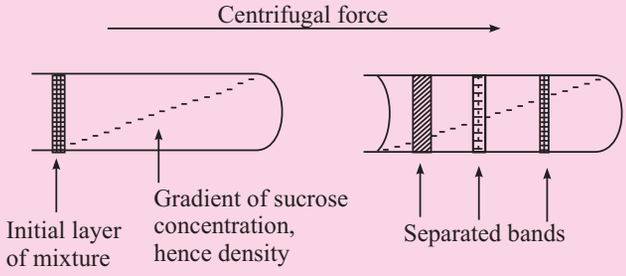


Fig. 28.3: Velocity Gradient Centrifugation

In rate zonal centrifugation, the sample is applied in a thin zone at the top of the centrifuge tube on a density gradient. Under centrifugal force, the particles will begin sedimenting through the gradient in separate zones according to their size shape and density. The run must be terminated before any of the separated particles reach the bottom of the tube.

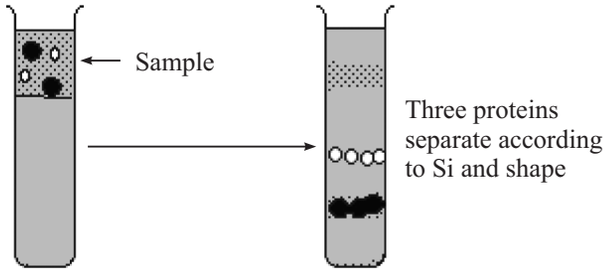


Fig. 28.4: Rate Zonal Centrifugation

Particles can be separated by density. When the density in the solvent equals the density of the particle, the denominator of the equation equals zero and therefore

velocity equals zero - the particle reaches its equilibrium density in the solvent this is called equilibrium density gradient centrifugation or isopycnic banding.

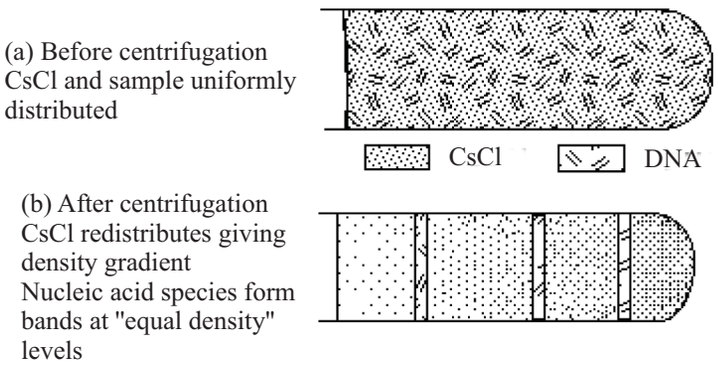


Fig.28.5: Before and After Centrifugation

28.8 ISOPYCNIC CENTRIFUGATION

In isopycnic technique, the density gradient column encompasses the whole range of densities of the sample particles. The sample is uniformly mixed with the gradient material. Each particle will sediment only to the position in the centrifuge tube at which the gradient density is equal to its own density, and there it will remain. The isopycnic technique, therefore, separate particles into separate zones solely on the basis of their density differences, independent of time. In many density gradient experiments, particles of both the rate zonal and the isopycnic principles may enter into the final separations. For example, the gradient may be of such a density range that one component sediments to its density in the tube and remains there, while another component sediments to the bottom of the tube. The self generating gradient technique often requires long hours of centrifugation.

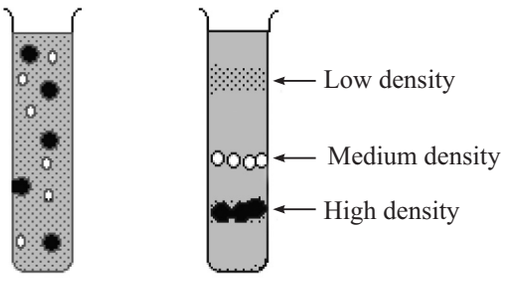
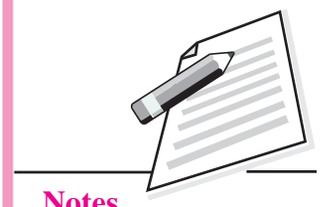


Fig. 28.6: Isopycnic separation with self-generating gradient - the sample is evenly distributed throughout the centrifuge tube

Isopycnically banding DNA, for example, takes 36 to 48 hours in a self-generating cesium chloride gradient. It is important to note that the run time



cannot be shortened by increasing the rotor speed; this only results in changing the position of the zones in the tube since the gradient material will redistribute farther down the tube under greater centrifugal force.

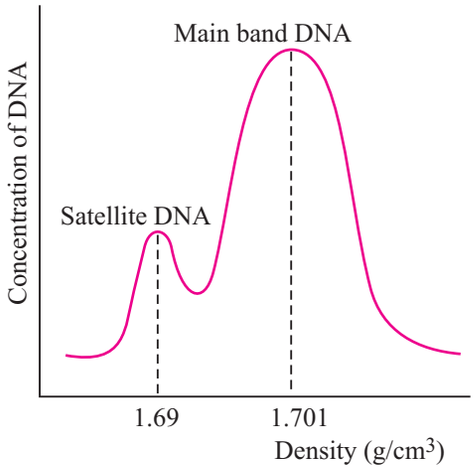


Fig. 28.7: Isopycnic banding of DNA

INTEXT QUESTIONS 28.2

1. The relative centrifugal force, F = ...
2. What are the two types of rotors found in high-powered centrifuges?
3. Write the type of centrifugation based on the given characteristics:
   - separate particles into separate zones solely on the basis of their density differences, independent of time .....
   - particles with same size but different shapes separate .....
   - separate macromolecules that differ only slightly in size or density .....
4. True/False.
   - Moving boundary centrifugation is also called differential centrifugation
5. Equilibrium density gradient centrifugation is also called as .....

28.9 SAFETY MEASURES

28.9.1 Safety while using centrifuge

1. The work surface must be level and firm. Do not use the centrifuge on an uneven or slanted work surface.

Notes

2. Balance the tubes in the rotor! If you want to run a tube with 10 ml of liquid, put another tube with 10 ml of water in the opposing hole on the rotor. If the liquid has a higher or lower density than water, you must balance the tubes by mass, not volume.
3. Do not open the lid while the rotor is moving. Even though many centrifuges have a "safety shutoff" if the lid is opened, the only thing this does is stop powering the rotor. The rotor will still spin due to its own inertia for a while until friction slows and eventually stops it.
4. If you see it wobbling or shaking, turn it off or pull the plug. A little vibration is normal, but excessive amounts can mean danger. FIRST, double check that you have correctly balanced the tubes. If the answer is yes and the wobbling still happens, contact the manufacturer or dealer and get the unit serviced. Do NOT continue to run a centrifuge that wobbles visibly when the rotor is spinning.
5. Wear a face shield and / or safety goggles if you have to work anywhere near a centrifuge that's in use.
6. Do not bump, jar, or move the centrifuge while the rotor is spinning. Make sure you don't have the cord dangling from a table edge where someone could catch their foot in it and pull down the centrifuge.

28.9.2 Precautions – working with bio-hazardous materials

The following procedures for centrifugation shall be used when working with bio-hazardous materials:

- Examine tubes and bottles for cracks or stress marks before using them. Discard any centrifuge tubes that have cracks in them.
- When working with bio-hazardous materials, wipe outside of tubes with disinfectant prior to removal from the biological safety cabinet and before placing in safety cups or rotors.
- Place all tubes in safety buckets or sealed rotors when centrifuging infectious materials.
- Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Open safety buckets or rotors in a biological safety cabinet.
- If any spills or leakage are apparent in the centrifuge rotor should be cleaned with a mild detergent, rinsed thoroughly with distilled water, and allowed to air dry completely (while in bio-safety cabinet).
- Clean the rotor and centrifuge well after each use.

28.9.3 Maintenance of Centrifuge

- Quality control
- Initial installation
- Initial calibration should be performed only by a qualified service technician.

28.9.3.1 Daily maintenance

- Wipe the inside of the bowl with disinfectant solution and rinse thoroughly.
- The centrifuge must not be used if the interior is hot, if unusual vibrations or noises occur, or if deterioration (corrosion of parts) is detected. A qualified service technician should be contacted.
- Most vibrations are due to improper balancing and can be corrected by re-balancing the buckets and tubes.

28.9.3.2 Monthly maintenance

- Clean the centrifuge housing, rotor chamber, rotors and rotor accessories with a neutral cleaning agent.
- Clean plastic and non-metal parts with a fresh solution of 5% sodium hypochlorite (bleach) mixed 1:10 with water (one part bleach plus nine parts water).

28.9.3.3 Annual maintenance

- The centrifuge must be serviced annually by a qualified service technician who must ensure that the unit operates safely and properly.
- The service should include cleaning condenser coils, fans, screens and filters, checking the centrifuge brushes, bearings, timer, temperature and speed, and checking for electrical integrity.
- The service technician must issue an inspection certificate indicating compliance with safety and proper operation. The most recent inspection certificate must be displayed close to the centrifuge.

28.9.3.4 Rotor safety

- It is an unavoidable fact that rotors age and become fatigued with repeated use. For the highest speed centrifuges, ultracentrifuges, rotors must be "**derated**" (their maximum rpm reduced gradually over time) and then eventually retired after a period of continuous use. This is due to the high stress on the titanium or aluminum from the ultra high gravitational forces, which will eventually lead to failure. Further, equipment that lasts 10–15 years can have multiple users, and rotors are often passed down over the life of the centrifuge. It is therefore important to manage all the rotors;

Notes

modern centrifuges have many built-in features that assist in this process, even for multiple user environments:

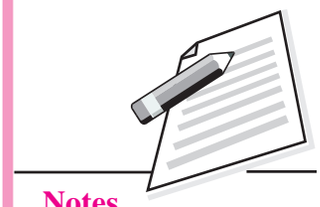
1. Rotor-life management allows end users to manage ultracentrifuge usage by rotor serial number, total number of hour's used or total number of cycles. This enables the centrifuge to alert the end user when it is time to derate or retire a rotor. Furthermore, once a serial number is denoted as derated, the centrifuge will not allow the rotor to be used in excess of the new reduced speed.
2. Automatic rotor ID prevents the running of non specified rotors in a given centrifuge. Rotor ID is achieved in a variety of ways, such as a magnet configuration on the bottom of the rotor, which is read by a sensor in the bottom of the chamber. Sometimes rotors are identified by inertia readings taken during acceleration, which compare the amount of energy being used to turn the rotor by the motor to the on-board database, which is programmed with the correct amount of energy required for all rotors usable in that unit.
3. Over speed protection and rotor ID are closely related. During an inertia check, the on-board system confirms that the programmed speed does not exceed the maximum rpm for the specified rotor. In the case of ultracentrifugation, speed disks on the bottom of the rotor are read by an optical eye, which limits the maximum speed by the number of black segments on the disk.
4. Rotor inspection/clinics can be conducted in the laboratory by most service technicians. Trained service technicians can inspect and educate laboratory staff on warning signs to look for that indicates imminent rotor failure.

INTEXT QUESTIONS 28.3

1. What is the meaning of the word "derated"?
2. True/False.
   - The work surface must not be level and firm.
   - Automatic rotor ID prevents the running of non specified rotors in a given centrifuge.
3. Match the following.

1. Daily maintenance	(a) Qualified service technician
2. Monthly maintenance	(b) Derated
3. Annual maintenance	(c) Disinfectant solution
4. Rotor safety	(d) Nueral cleaning agent

4. ..... and ..... are closely related in rotor safety



WHAT YOU HAVE LEARNT

- Centrifuge plays a vital role in the separation of biomolecules
- The techniques are very simple but has its role in advanced studies
- Different types of centrifuges are available for different purposes

ANSWERS TO INTEXT QUESTIONS

28.1

1. Sedimentation
2. • Inertial force
   • Reaction force
3. Svedberg equation
4. 10 -13 ; Unit – seconds
5. Settling is the falling of suspended particles through the liquid, whereas sedimentation is the termination of the settling process.

28.2

1. $F = Mr^2$
2. Fixed angle rotors and Swing-out rotors
3. • Isopycnic centrifugation
   • Rate zonal centrifugation
   • Density gradient centrifugation
4. True
5. Isopycnic banding

28.3

1. The meaning of the word "derated" is the rotor's maximum rpm reduces gradually over time.
2. • False • True
3. 1. (c) 2. (d) 3. (a) 4. (b)
4. Over speed protection and rotor ID

Notes

29

PRIMARY AND SECONDARY STANDARDS

29.1 INTRODUCTION

Often in our life we come across the word 'standard'. Whether it is the standard of bus service or behaviour or education, we use this term very commonly in our life. So why are we obsessed with standard? Is standard necessary in all spheres of our life? If the answer is yes then should we not follow a standard in our laboratory too? If yes, then next question arises how to prepare a standard in our lab.

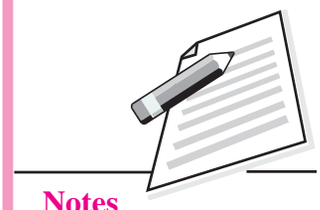
OBJECTIVES

After reading this lesson, you will be able to:

- define standard
- explain its uses
- classify standard with proper example
- explain various properties of standards

29.2 STANDARDS

In Biochemistry, the word standard means a material containing a substance of our interest with a known concentration. We can express this with definite numbers with proper units. By using this standard we can find out the concentration of that substance in a new material. Therefore, primary standard serves the purpose of being the primary calibrator or primary reference material.



1. Functions

Therefore, standard has the following uses in Biochemistry lab

(a) To provide a reference using which we can determine unknown concentration

(b) To standardize volumetric solutions

(c) Preparation of secondary standard

(d) To calibrate an instrument.

2. Types

Standards can be divided into two types:

1. Primary standard
2. Secondary standard

29.2.1. Primary standard

From the name itself it is obvious that this is a standard which comes first. That's why the name is primary.

A primary standard is a chemical or reagent which has certain properties such as

(a) It is extremely pure,

(b) Highly stable

(c) It is anhydrous

(d) It is less hygroscopic

(e) Has very high molecular weight

(f) Can be weighed easily

(g) Should be ready to use and available

(h) Should be preferably non toxic

(i) Should not be expensive

Having said that lets us understand each point one by one.

- A primary standard material should be extremely pure which means that it should be a chemical of high grade of purity, preferably 99.98%. In a chemistry lab you will come across chemicals of different grade of purity. If you check the label you will notice a number with percentage termed as purity. So when a chemical has purity of 99.98% or more it is a suitable material to be considered for primary standard.

Notes

Usually those chemicals that exceed the requirement of American chemical society (ACS) are extremely pure and can be used for making primary standard. It is an analytical reagent of extreme purity which is specially manufactured for the purpose of being used as primary standard.

- It should be highly stable which means it usually does not react easily when kept in its pure form. Or in other words it should have very low reactivity. This is important because if a reagent reacts easily with atmospheric oxygen or water or changes its property over time then it is unreliable. We can never use such unstable and unreliable chemicals as standard.
- It should be anhydrous which means that it does not contain any water molecule in its molecular structure. For example, in a chemistry laboratory you will come across same chemical with different number of water molecules attached with it. Let us see the example of Epsom salt. The chemical name is magnesium sulphate. So we write the formula is MgSO4. But the chemical Epsom salt which is found in grocery or drug store is a chemical with formula MgSO4.7H2O. Therefore if you want to prepare a primary standard of magnesium sulphate you should purchase an anhydrous MgSO4 preferably an analytical reagent grade chemical and with purity greater than 99.98%. Remember such reagents are usually available with common vendors.
- Just being anhydrous is not sufficient. The chemical preferably should be less hygroscopic that is on opening the container it should not absorb water molecules from atmosphere. Why water should not enter into chemical? This will be clearer in the following point where it is explained how presence of water molecule can affect the simple calculation of standard concentration making the entire standardization procedure unreliable.
- Has very high molecular weight compared to its other similar forms. If we take the same example of Epsom salt you can understand this statement. Let us take 1 gram of $MgSO_4$ fit for making a primary standard and we call it salt A. Now take 1 gram of $MgSO_4.7H_2O$ fit for common uses and name it salt B. Now you compare the actual weight of magnesium sulphate to make a standard solution for both chemicals. The molecular weight of MgSO4 is 108 but for $MgSO_4.7H_2O$ it is 234.

  In case of 1 molecule of salt A the weight of actual $MgSO_4$ will be 108 atomic mass unit. But In case of 1 molecule of salt B the weight of actual MgSO4 will be 108 out of its total weight of 234 atomic mass unit.

  108 gram salt A ($MgSO_4$) will give 108 gram of $MgSO_4$

  So 1 gram $MgSO_4$ salt will give = 108/108 = 1 gram of $MgSO_4$

  But 234 gram salt B ($MgSO_4.7H_2O$) will give 108 gram of $MgSO_4$

  So 1 gram $MgSO_4.7H_2O$ salt will give = 108/234 = 0.461 gram of $MgSO_4$

Therefore if you by mistake make a standard out of salt B, you will actually be taking 0.461 gram of MgSO4 and calculating it as 1 gram. So with this faulty standard estimation of MgSO4 in other unknown solution will give less result than the actual concentration

Therefore it is important that primary standards must be anhydrous and of high molecular weight.

- It can be weighed easily because it is so pure that its weight is in fact a true representative of number of moles present in its actual weight.
- One of the uses of primary standard is to standardize a volumetric solution. That means they are used for standardization of titration of solutions. It can be used for titration of acids as well as bases. Let us see how a primary standard is used for titration.
  - In a biochemistry laboratory the most common standards for acid titration we use basic chemical such as sodium carbonate ($Na_2CO_{3)}$, Tris which is also known as trisaminomethane [$(CH_2OH)_3CNH_2$] etc. you should know that Tris is a very commonly used chemical in Biochemistry and Molecular biology lab.
  - For base titration we use potassium hydrogen phthalate [(KHP): $KHC_8H_4O_4$] etc.
  - Another type of titration is redox titration which is very common in biochemistry lab. For this we frequently use potassium dichromate ($K_2Cr_2O_7$) very often as primary standard. Sodium oxalate ($Na_2C_2O_4$) is also used for this purpose.
- The primary standard is used for calibration of secondary standard or for method validation against a definitive method and it corresponds to the true value of the substance analyzed.

29.2.2 Secondary standard

From the name itself it is obvious that this is a standard which comes second. That's why the name is secondary.

A secondary standard is used by standard laboratories such as companies involved in preparation of reagents, kits or laboratories responsible for producing quality control material for other labs. They use primary standard as the primary calibrator or primary reference material. Secondary standard in turn is used for the purpose of calibration of control material in smaller lab for analysis of unknown concentration of a substance. So basically, secondary standard serves the purpose of external quality control for smaller labs. This makes it essential that the secondary standard must first be standardized against the primary standard.

Notes

- There are other points one has to remember. For preparing the secondary standard solution one must use aquous solution of high grade purity. It must be deionized if aquous solvent used is water. Without pure solvent, the standard solution prepared will be worthless (These points are also applicable for preparing primary standard solution).
- Similarly before using high grade chemicals one should also be vigilant and check for date of manufacture, expiry date, date of receipt of chemical, whether the conditions for its transport was followed or not, if the seal is not tampered with, its purity, standard reference material used etc.

  A secondary standard is a chemical or reagent which has certain properties such as

  (a) It has less purity than primary standard

  (b) Less stable and more reactive than primary standard

  (c) But its solution remains stable for a long time

  (d) Titrated against primary standard

  Usually a chemical fit for being a standard chemical yet does not meet the requirements of a primary standard
- The best example is anhydrous sodium hydroxide (NaOH). It is extremely hygroscopic. As soon as the bottle is opened NaOH starts absorbing moisture from atmosphere and within no time it becomes moist. You can experiment it in your lab. Take the NaOH bottle near an analytical balance. Place a Petridis and make its weight as zero (by using the tare button). Now open the container and place little NaOH crystal on it and quickly note the weight. Now keep the glass windows of the analytical balance open for few minutes and notice the gradual increase in its weight in terms of mg units. This is because the NaOH crystals absorb water molecule from air. (Remember to use a glass container such as a Petridis to weigh the chemical. Because, NaOH is a corrosive for metal panel of balance).
- Another example is potassium permanganate ($KMnO_4$) very often as secondary standard. It is a good oxidizing agent or in other words it is reactive so less stable. More often due to its reactivity, its own oxidized product manganese oxide (MnO_2) contaminates the content. That's why it is unsuitable for being a primary standard. But it can be used very well as a secondary standard.
- Next question is why is secondary standard called still a standard? This is so because secondary standard is used as a calibrator by smaller laboratories involved in actual analysis of unknown samples.

Primary and Secondary Standards

In our text we have used the term calibration. So what is calibration? This is the process by which we compare the measurements by a standard or an instrument (primary) with another standard or an instrument (secondary). By doing so, we try to eliminate any variation or difference in measurement by the secondary standard or an instrument. The other term for calibration is standardization. Have you noticed how the shopkeeper puts a standard weight on his balance to his right and adds potatoes or tomatoes etc to the left? In this case the weight is the standard and the vegetables are being standardized with equal measurement of for their weight. So if there is a mistake in the weight he uses (let us think it is less than what is written on it) on his right side we may be cheated and get less vegetable than we are paying for

- Calibration using titration: take the example of vitamin C estimation from lemon. For this the standard is ascorbic acid which is available in your laboratory. Take ascorbic acid and prepare a standard solution of known concentration (for example 40mg/dl). Next prepare a diluted solution of indicator called 2 Di -chloro indophenol (2- DCIP) and add ascorbic acid drop by drop till it is completely decolourized. Let us say we used 20 ml.

Fig. 29.1: Original colour of 2 DCIP indicator

100 ml ascorbic acid standard solution has 40 mg of ascorbic acid in it

1 ml of standard solution has = 40/100 = 0.4 mg ascorbic acid

Now 20 ml of standard solution has = 0.4 ×20 = 8 mg ascorbic acid

This says that our 2- DCIP requires 8 mg of pure ascorbic acid for its complete neutralization or titration.

Fig. 29.2: Lemon

Notes

Next let us use our lemon. Weigh the lemon. Let us say it weighs 4 g. add lemon juice drop by drop. Let us say it took several drops of lemon juice for complete neutralization of 2- DCIP. Now weigh lemon and now it weighs 2 g. So it proves that 2g of lemon juice present in 4 g of lemon has 8 mg of ascorbic acid. Why 8 mg of ascorbic acid? Simple. This is so because; 8 mg of ascorbic acid is required to completely neutralization 2 DCIP. So from this we came to know that

4 g of lemon has 8 mg of ascorbic acid

So 100 g of lemon would contain = 50 mg of ascorbic acid

So concentration of ascorbic acid in our variety of lemon is 50 mg/100 g that is equal to 50 mg%.

- The secondary standard is used for calibration of control materials in a lab using a reference method and it is closer to the true value of the substance. So it is used for external quality control programme.
- Whereas in a clinical laboratory unknown samples are analyzed against control materials using a routine/ field method

Let us compare the situation in biochemistry laboratory dealing with human samples. Here, we often use the term primary and secondary calibrator instead of calling it primary and secondary standard. A primary calibrator is a preparation with a stated purity (> 99.98%). The reference measurement procedure is calibrated with primary calibrator. Using this reference measurement procedure the **Institute for Reference Materials and Measurements (IRMM)** determines the value of secondary reference materials.

Even the companies, which sell their kits and reagents to us, use these secondary reference materials. So their calibrator products are basically secondary reference materials. We use these secondary calibrators as reference in our laboratories. The primary calibrator is used only by institutes like IRMM which are involved in quality assurance at National level.

Preparing a primary standard out of a pure chemical is relatively easier (Remember? It is one of the criteria to be a primary standard.). For **biological analytes**, we can not prepare the secondary standard by dissolving the analyte in water. It is so because in our blood they are dissolved in plasma and not in water. For example, for plasma proteins, there is a **human reference serum material** which comes with a certificate of concentrations present for 12 serum proteins from IRMM.

Primary standard — **At the National level**

↓

Reference measurement procedure/Definitive method

↓ **At the Company level**

Secondary standard (prepared and supplied by company)

↓

Company measurement procedure/ Reference method

↓

Product calibrator = Secondary standard supplied by company (external quality control)

↓ **At the End-User level**

Routine procedure in lab/ Field method (standardization against Product calibrator)

↓

Value assignment/Internal quality control/analysis of sample etc.

Flow chart summarizing the use of primary and secondary standards in a clinical laboratory set up

INTEXT QUESTIONS 29.1

1. Standard can be of ..... types.
2. A primary standard should be ..... pure.
3. Between primary and secondary standards, ... standard is more reactive.
4. We can calibrate a ..... or ..... using primary standard.
5. Secondary standard can be prepared by ..... using primary standard.

WHAT HAVE YOU LEARNT

- A standard also called a calibrator is a material containing a substance of our interest with a known concentration, which can be expressed with definite numbers and units. It is of 2 types primary and secondary. Primary standard is purer, more stable, less reactive, anhydrous and less hygroscopic

Notes

compared to secondary standard. Secondary standard is titrated or calibrated against primary standard and used as a reference material in all labs across country for the purpose of analysis. Preparation of standard solution should use pure, de-ionized aqueous solvent to keep the quality of standard intact. In a clinical lab set up, primary standard acts as a primary reference material. Secondary standard is used as an external quality control for calibration of internal quality control in labs. Unknown sample is analyzed after both external and internal quality check is complete and satisfactory.

TERMINAL QUESTIONS

1. What are the criteria of a primary standard?
2. What are the criteria of a secondary standard?
3. What are the differences between primary and secondary standards?
4. What are the uses of primary standard?
5. Why should the primary standard be of high molecular weight?
6. Why can't you use NaOH as a primary standard?
7. Why can't you use $KMnO_4$ as a primary standard?

ANSWERS TO INTEXT QUESTIONS

29.1

1. Two
2. >99.8%
3. Secondary
4. Secondary standard or an instrument
5. Titration

30

RADIOACTIVE ISOTOPES

30.1 INTRODUCTION

In the previous chapter we have seen about radioactivity and its types. In this topic we are going to see how different radioactive radiations can be measured. Based on its ability to ionize and excite molecules radioactivity can be detected and measured.

OBJECTIVES

After reading this lesson, you will be able to:

- describe the measurement of Radioactivity
- describe about tracer technique
- explain clinical and biochemical uses of radioactive substances
- describe health effects of radiations

30.2 MEASUREMENT OF RADIOACTIVITY

The 3 commonly used methods are

1. Ionisation of gases
2. Excitation of solids and liquids
3. Ability to expose photographic emulsions i.e.autoradiography

Notes

1. Ionization of gases

Principle: Radiation causes ionization of gaseous particles in its path. When this takes place between electrodes in a closed chamber, an electric pulse is generated. The magnitude of it depends upon applied potential and number of radiation particles entering the chamber.

Instrumentation: Ionization chamber consists of a gas chamber with insulated base. It consists of an anode wire and metallic cathode. The circuit is closed by connecting to a battery and Resistor. The electric pulse produced is measured by amperometer.

Types

30.2.1 Ionization Chambers or simple ionization

In this type only one ion pair is produced per collision

Disadvantages:

- Current production is low
- Sensitive devices are needed for detection

30.2.2 Proportional Counters or gas amplification

At high voltage ionized electrons moves with high speed. This causes secondary and tertiary ionization. Finally, large number of electrons reaches the anode. This is known as Townsend avalanche effect.

Advantages

- Current production is high

Disadvantages

- Constant voltage is needed as it affects ionization and amplification

Uses

- To detect alpha emitting isotopes

30.2.3 Geiger-Mueller Counters

All the isotopes including beta emitters induce complete ionisation. Current flow is independent of primary ions. Maximal gas amplification is seen. The output pulse is same in considerable voltage range. Number of times the pulse produced will give the radioactivity. Dead time is the time taken by the ion pairs to reach their respective electrodes. This is usually 100-200μS.

Advantages

- Can detect beta radiation

Disadvantages

- Ionising particles entering into the tube during the dead time cannot be detected.
- Some ions escape from the electrodes and gives continuous discharge

Uses

- Routine check of radioactive laboratory for contamination
- Qualitative analysis of radioactivity.
- Quick screening of gels & chromatographic fractions for labeled components

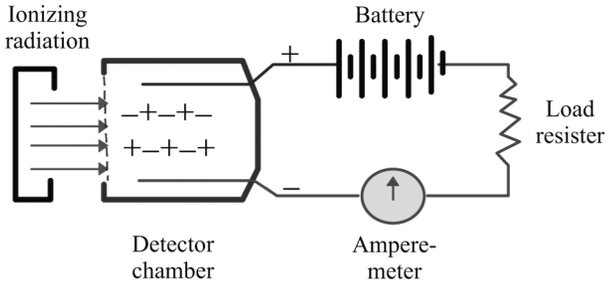


Fig. 30.1: Key Component in a Simple Ionization Chamber

30.3 EXCITATION OF SOLIDS AND LIQUID

Principle: Radioactive isotopes can excite a compound called fluor. This emits photons of light which can be detected and quantified using a photomultiplier tube. This is called scintillation counting.

Types: There are 2 types of scintillation counting

1. Solid scintillation counting and
2. Liquid scintillation counting

30.3.1 Solid Scintillation counting

Instrumentation: In solid scintillation counting the fluorescing substance is held in a light tight aluminium chamber. Radiations can penetrate through the aluminum and can cause excitation of solid crystals to emit photons of light. This fluorescence can be detected and quantified using a photomultiplier tube.

In photomultiplier tube, the electric pulse that results from the conversion of light energy to electrical energy. This is directly proportional to the radioactive event.

Crystal used for alpha isotopes: Zinc sulfide

Crystal used for beta isotopes: Anthracene

Crystal used for gamma isotopes: Sodium iodide

Advantages

- Gamma isotopes have weak penetrating power hence it rarely collides with molecules to cause excitation. In solid scintillation the atoms are densely packed in h crystal so the probability of collision is more

Disadvantages

- Cannot detect weak beta emitters like ^{3}H, ^{14}C & ^{35}S as they are not able to pass through the wall of the counter.

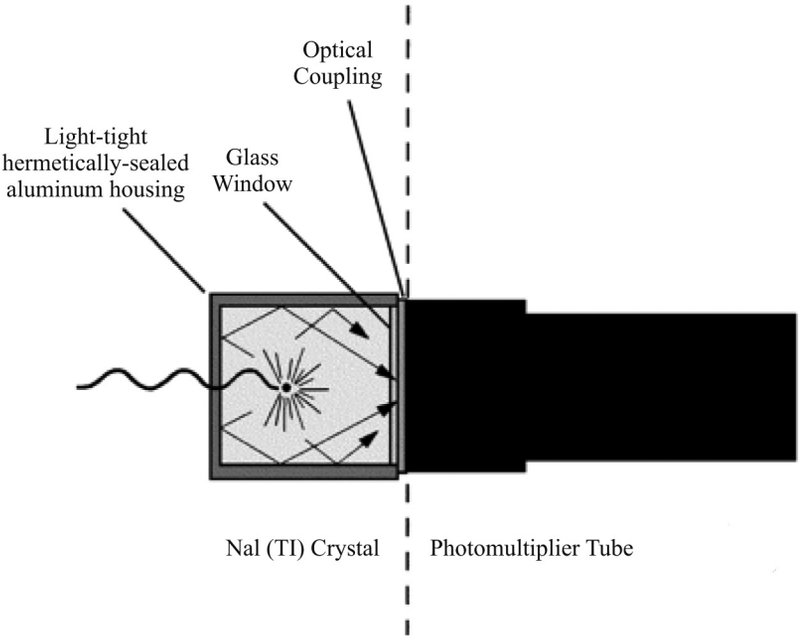


Fig. 30.2: Solid scintillation counter

30.3.2 Liquid scintillation counting

In liquid scintillation counting the sample is mixed with a scintillation cock tail containing solvent and one or more fluors

Instrumentation

In liquid scintillation counting, the radioactivity cause fluorescence of solvent. This emitted light in turn causes fluorescence of another compound called primary fluor. This emitted light in turn causes fluorescence of another compound called secondary fluor. This wavelength can be detected efficiently by the photomultiplier tube.

Advantages

- Can detect weak beta emitters like ^{3}H, ^{14}C & ^{35}S
- More than one isotope can be detected at a time
- Counting efficiency is high

Disadvantages

- Costly
- Quenching is the interference given by several substances in detecting actual fluorescence

30.4 ABILITY TO EXPOSE PHOTOGRAPHIC FILMS (AUTORADIOGRAPHY)

Principle: Ionising radiations acts upon photographic emulsions to produce a latent image

Instrumentation: A radiation source and photographic emulsion consisting of silver halides embedded in solid phase such as gelatin are needed. Radiation converts silver halide to metallic silver which forms a latent image. This can be developed as a blackening of the film and can be stored as a permanent image.

Uses: Distribution of radioactivity in biological specimens.

30.5 TRACER TECHNIQUE

It is technique in which one or more atoms of a chemical compound have been replaced by a radio isotope to trace the metabolic pathways. A radioactive tracer can also be used to track the distribution of a substance within a natural system such as a cell or tissue. Radioactive tracers form the basis of a variety of imaging systems, such as, PET scans, SPECT scans and technetium scans.

Notes

Principle

It is based on the principle that a stable isotope is replaced by a radioisotope. The radioisotope is capable of emitting radiations that can be detected and analysed. It is powerful than chemical reactions as they can be detected even in lower concentration as seen inside a cell. In general, isotopes of hydrogen, carbon, phosphorus, sulphur, and iodine have been used extensively to trace the path of biochemical reactions.

Uses

1. Tracing Metabolic pathways

When a labeled chemical compound undergoes reaction inside the cell ,one or more of the products will contain the radioactive label. Analysis of it provides detailed information on the mechanism of the chemical reaction.

2. Distribution of the compound

Radioactive compound provides a means to construct an image showing the way in which gets distributed in the organism

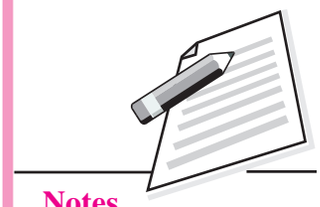
Application

1. In metabolism research, Tritium(^{3}H) and ^{14}C-labeled glucose are commonly used to measure rates of glucose uptake, fatty acid synthesis, and other metabolic processes.
2. In medicine, tracers are applied in a number of tests, such as ^{99m}Tc in autoradiography and nuclear medicine, including single photon emission computed tomography (SPECT), positron emission tomography (PET) and scintigraphy.
3. The urea breath test for helicobacter pylori commonly used a dose of ^{14}C labeled urea to detect H. pylori infection

30.6 CLINICAL AND BIOMEDICAL USES OF RADIOACTIVE SUBSTANCES

The Radioactive substances can be used to study various metabolic pathways. They are also used to diagnose and treat various diseases (eg cancer). Medical uses of few radioactive substances are given in the table below.

Clinical and biomedical uses of radioactive substances.



Radioactive isotope	Uses
Calcium- 47	Studying cellular function & bone formation
Carbon-14	Metabolism
Cesium-137	Cancer treatment
Chromium-51	RBC studies
Cobalt-57	Diagnosis of pernicious anemia
Cobalt-60	Sterilization of surgical instruments
Copper-67	Treat cancer
Iodine-123,125	Diagnose thyroid disorders
Iodine- 131	Treat thyroid disorders
Phosphorus-32,33	Molecular biology & genetic research
Selenim-75	Protein studies
Sulphur-35	Molecular biology & genetic research
Technetium-99m	Organ imaging & blood flow studies
Tritium	Drug metabolism
Xenon-133	Lung ventilation & blood flow

30.7 HEALTH EFFECTS OF RADIATIONS

We already know that radioactive radiations are able to ionize & excite the molecules they encounter. The effects of radiation depend upon the duration and its type.

30.7.1 Effects

The effects of radiation are

- *Generalised effect:* Biological effects are due to the ionization process that causes cell mutation. A given total dose will cause more damage if received in a shorter time period. A **fatal dose is (600 R)**
- *Acute Somatic Effects*: Relatively immediate effects to a person acutely exposed. Severity depends on dose. Death usually results from damage to bone marrow or intestinal wall. Acute **radio-dermatitis** is common in radiotherapy; chronic cases occur mostly in industry.

Notes

- *Critical Organs*: Organs generally most susceptible to radiation damage include: Lymphocytes, bone marrow, gastro-intestinal, gonads, and other fast-growing cells. The central nervous system is relatively resistant. Many nuclides concentrate in certain organs rather than being uniformly distributed over the body, and the organs may be particularly sensitive to radiation damage, e.g., isotopes of iodine concentrate in the thyroid gland. These organs are considered "critical" for the specific nuclide.

30.7.2 Basic Radiation Exposure Control Methods

The three basic ways to decrease the exposure to radiation are

- Decrease Time
- Increase Distance
- Increase Shielding
- **Time**: Minimize time of exposure to minimize total dose. Rotate employees to restrict individual dose.
- **Distance**: Maximize distance to source to maximize attenuation in air. The effect of distance can be estimated from equations.
- **Shielding**: Minimize exposure by placing absorbing shield between worker and source.

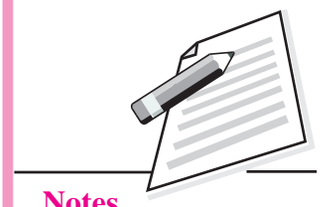
INTEXT QUESTIONS 30.1

1. Match the following:

1.	Carbon-14	(a)	Thyroid disorders
2.	Iodine-125	(b)	RBC studies
3.	Sulfur-35	(c)	Treat cancer
4.	Copper-67	(d)	Metabolism
5.	Chromium-51	(e)	Molecular biology

2. Fill up:

1. Gamma radiations can be detected ..... counting.
2. Weak beta emitters can be detected by ..... counting.
3. Dead time is seen ..... counters.
4. Fatal dose of radiation is .....
5. The ability to expose photographic films is called .....



WHAT HAVE YOU LEARNT

- Radiations can be measured by methods based upon radiation, excitation and ability to expose photographic films
- In methods based on radiation Geiger muller counter is important
- In scintillation methods gamma radiations can be detected by solid scintillation counting
- In liquid scintillation counting more than one radioactive substance can be detected at a time
- Autoradiography can be used to study whole animal samples
- Effects of radiation can be minimized by increasing shielding and distance from the radioactive substance and decreasing the exposure time.

TERMINAL QUESTIONS

1. What are different types of measurement of radioactivity?
2. Write about ionization method of detection of radioactivity.
3. Write short notes on Geiger-muller counters
4. What is scintillation counting. and what are its types
5. Write short notes on solid scintillation counting
6. Write short notes on liquid scintillation counting
7. Writ about autoradiography
8. Write about different radio isotopes used clinically
9. Write about the deleterious effect of radiation
10. What are the different methods to control external radiation?

ANSWERS TO INTEXT QUESTIONS

30.1

1. 1. (d) 2. (a) 3. (e) 4. (c) 5. (b)

30.2

2. 1. Solid Scintillation
   2. Liquid Schintillation
   3. Geiger - mueller
   4. 600R
   5. Autoradiography