CELL BIOLOGY, GENETICS AND EVOLUTION (DZOOO3) (MSC - ZOOGOLOGY)

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UNIT-I

LESSON-1.1

CELL BIOCHEMISTRY

CONTENTS

1.1.1 INTRODUCTION

The bulk of an organism is water. If the water is evaporated away, most of the remaining dry weight consists of molecules containing atoms of carbon. When first discovered, it was thought that carbon - containing molecules were present only in living organisms and thus were referred to as organic molecules to distinguish them from inorganic molecules found in the inanimate world. As chemists learned to synthesize more and more of these carbon-containing molecules in the lab, the mystique associated with organic compounds disappeared. The compounds produced by living organisms are called biochemicals.

1.1.2 OBJECTIVES

- To study different types of macromolecules
- To study the carbohydrate structures
- To study different primary, secondary, tertiary & quarternary structures of proteins
- To study different forms of lipids
- To study structure & function of nucleic acids

1.1.3 HYDROCARBONS

The nature of biological molecules can be better understand by starting with the simplest group of organic molecules, the hydrocarbons, which contain only carbon and hydrogen atoms. The molecule ethane (C_2H_6) is a simple hydrocarbon consisting of two atoms of carbon in which each carbon is bonded to the other carbon as well as to three atoms of hydrogen.

As more carbons are added, the skeletons of organic molecules increase in length and their structure becomes more complex. A hydrocarbon with the formula C_4H_{10} can exist as two different molecules.

These molecules have different properties because they have different structures. Two molecules having the same formula (e.g. C_4H_{10}) but different structures are said to be structural isomers of one another. Molecules made of larger numbers of atoms have increasingly greater number of structural isomers.

Functional Groups

Hydrocarbons do not occur in significant amounts within most living cells (though they constitute the bulk of the fossil fuels formed from the remains of ancient plants and animals). Many of the organic molecules that are important in biology contain chains of carbon atoms are replaced by various functional groups. Functional groups are particular groupings of atoms that often behave as a unit and give organic molecules their physical properties, chemical reactivity, and solubility in aqueous solution. Two of the most common linkages between functional groups are ester bonds, which form between carboxylic acids and alcohols, and amide bonds, which form between carboxylic acids and amines.

Most of the groups contain one or more electronegative atoms (N, P, O & S) and make organic molecules more polar, more water soluble, and more reactive. Many of the functional groups are capable of ionization and thus may become positively or negatively charged. The effect of the substitution of various functional groups is readily demonstrated. The hydrocarbon ethane (CH_3CH_3) is a toxic, flammable gas. Replace one of the hydrogens with a hydroxyl group (-OH) and the molecule (CH_3CH_2OH) becomes palatable – it is ethyl alcohol. Substitute a carboxyl group (-COOH) and the molecule becomes acetic acid (CH_3COOH), the strong-tasting ingredient in vinegar. Substitute a sulfhydryl group (-SH), and you have formed CH_3CH_2SH, a strong, foul-smelling agent, ethyl mercaptan, used by biochemists in studying enzyme reactions.

1.1.4 CLASSIFICATION OF BIOLOGICAL MOLECULES BY FUNCTION

The organic molecules commonly found within living cells can be divided into several categories based on their role in metabolism.

1.1.4.1 Macromolecules

The molecules that form the structure and carry out the activities of cells are huge, highly organised molecules called macromolecules, which contain anywhere from dozens to millions of carbon atoms. Because of their size and the intricate shapes that macromolecules can assume. Some of these molecular giants can perform complex tasks with great precision and efficiency. The presence of macromolecules, more than any other characteristic, endows organisms with the properties of life and sets them apart chemically from the inanimate world.

Macromolecules can be divided into four major categories: proteins, nucleic acids, polysaccharides and certain lipids. The first three types are polymers composed of a large number of low-molecular-weight building blocks, or monomers. These macromolecules are constructed from monomers by a process that

resembles coupling rail road cars onto a train. The basic structure and function of each type of macromolecule is similar in all organisms. It is not until you look very closely at the specific sequences of monomers that make up these various macromolecules that the diversity among organisms becomes apparent.

1.1.4.2 The building blocks of macromolecules

Most of the macromolecules with in a cell have a short life time compared with the cell itself; with the exception of the cell's DNA, they are continually broken down and replaced by new macromolecules. Consequently, most cells contain a supply (or pool) of low-molecular-weight precursors that are ready to be incorporated into macromolecules. These includes sugars, which are the precursors of polysaccharides; amino acids, which are the precursors of proteins; nucleotides, which are the precursors of nucleic acids; and fatty acids, which are incorporated into lipids.

1.1.4.3 Metabolic intermediates (metabolites)

The molecules in a cell have complex chemical structures and must be synthesized in a step-by-step sequence beginning with specific starting materials. In the cell, each series of chemical reactions is termed as a metabolic pathway. The cell starts with compound A and converts it to compound B, then to compound C, and so on until some end product (such as an amino acid building block of a protein) is produced that can be used in other reactions. The compounds formed along the pathways leading to the end products might have no function per se and are called metabolic intermediates.

1.1.4.4 Molecules of miscellaneous function

This is obviously a broad category of molecules but not as large as you might expect; the vast bulk of the dry weight of a cell is made up of macromolecules and their direct precursors. The molecules of miscellaneous function include substances like vitamins, which function primarily as adjuncts to proteins, certain steroid or amino acid hormones; molecules involved in energy storage, such as ATP or creatine phosphate; regulatory molecules such as cyclic AMP; and metabolic waste products such as urea.

1.1.5 FOUR TYPES OF BIOLOGICAL MOLECULES

The molecules can be divided into four types or classes of organic molecules: carbohydrates, lipids, amino acid and proteins and nucleotides and nucleic acids.

1.1.5.1 Carbohydrates

Carbohydrates include simple sugars (or monosaccharides) and all larger molecules constructed of sugar building blocks. Carbohydrates function primarily as stores of chemical energy and as durable building materials for biological construction. Most sugars have the general formula $(CH_2O)_n$. The sugars of importance in cellular metabolism have values of 'n' that range from 3 to 7. Sugars containing three carbons are known as trioses, those with four carbons as tetroses, those with five carbons as pentoses, those with six carbons as hexoses, and those with seven carbons as heptoses.

1.1.5.1.1 The structure of simple sugars

Each sugar molecule consists of a backbone of carbon atoms linked together in a linear array by single bonds. Each of the carbon atoms of the backbone is linked to a single hydroxyl group, except for one that bears a carbonyl (C=0) group. If the carbonyl group is located at an internal position (to form a ketone group), the sugar is a ketose, such as fructose. If the carbonyl is located at one end of the sugar, it forms an aldehyde group and the molecule is known as an aldose, as exemplified by glucose. The ring forms of sugars are usually depicted as flat (planar) structures lying perpendicular to the plane of the paper with the thickened line situated closest to the reader. The H and OH groups lie parallel to the plane of the paper projecting either above or below the ring of the sugar. In actual fact, the sugar ring is not a planar structure, but usually exists in a three dimensional conformation resembling a chair.

1.1.5.1.2 Stereoisomerism

A carbon atom can bond with four other atoms. The arrangement of groups around a carbon atom can be depicted with the carbon placed in the center of a tetrahedran and the bonded groups projecting into its four corners. In glyceraldehyde, which is the only aldotriose, the second carbon atom of glyceraldehyde is linked to four different groups (-H, $-OH_1$ –CHO and $–CH_2OH$). If the four groups bonded to a carbon atom are all different, as in glyceraldehyde, then two possible configurations exist that cannot be superimposed on one another. These two molecules (termed stereoisomers or enantiomers) have essentially the same chemical reactivities, but their structures are mirror images of one another (not unlike a pair of right and left human hands). By convention, the molecule is called D-glyceraldehyde if the hydroxyl group of carbon 2 projects to the right, and L-glyceraldehyde if it projects to the left. Because it acts as a site of stereoisomerism carbon 2 is referred to as an asymmetric carbon atom.

1.1.5.1.3 Linking sugars together (Fig. 1.1 to 1.8)

Sugar can be joined to one another by covalent glycosidic bonds to form larger molecules. Glycosidic bonds form by reaction between carbon atom C1 of one sugar and the hydroxyl group of another sugar, generating a – c – o – c - linkage between the two sugars. Molecules composed of only two sugar units are disaccharides. Disaccharides serve primarily as readily available energy stores. Sucrose, or table sugar, is a major component of plant sap, which carries chemical energy from one part of the plant to another. Lactase present in the milk of most mammals, supplies new born mammals with fuel for early growth and development. Lactose in the diet is hydrolysed by the enzyme lactase, which is present in the plasmamembrane of the cells that line the intestine. Many people lose this enzyme after childhood and find that eating-Lactose-containing dairy products causes digestive discomfort.

Sugars may also be linked together to form small chains called oligosaccharides (oligo=few). Most often such chains are found covalently attached to lipids and proteins, converting them into glycolipids and glycoproteins, respectively. Oligosaccharides are particularly important for the glycolipids and glycoproteins of the plasma membrane, where they project from the cell surface. Since oligosaccharides may be composed of many different combinations of sugar unit : hese carbohydrates can play an informational role; that is, they can serve to distinguish one type of cell fron. another and help mediate specific interactions of a cell with its surroundings.

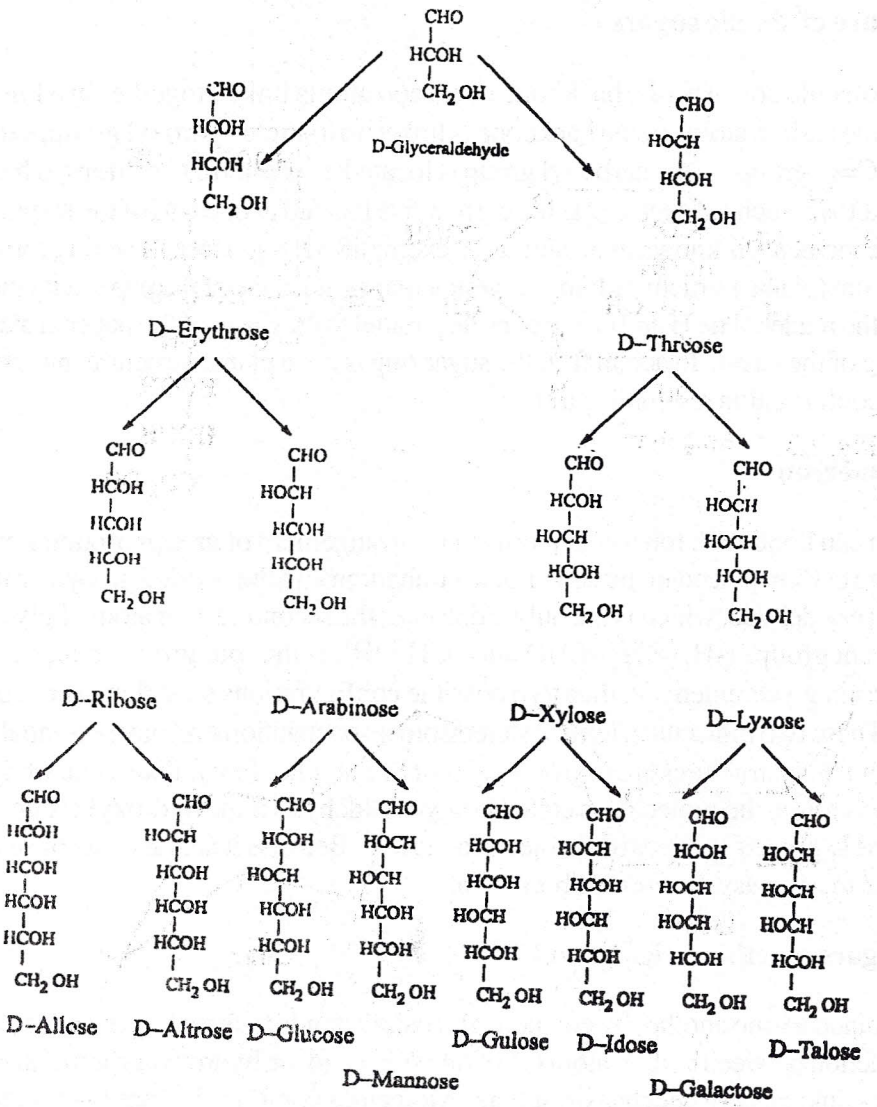


Fig. 1.1 Configurations of D-aldoses

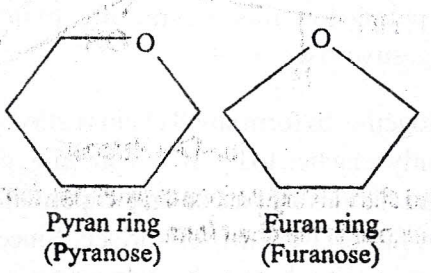


Fig. 1.2 Six-membered ring is a derivative of pyran, whereas five-membered ring is related to furan form.

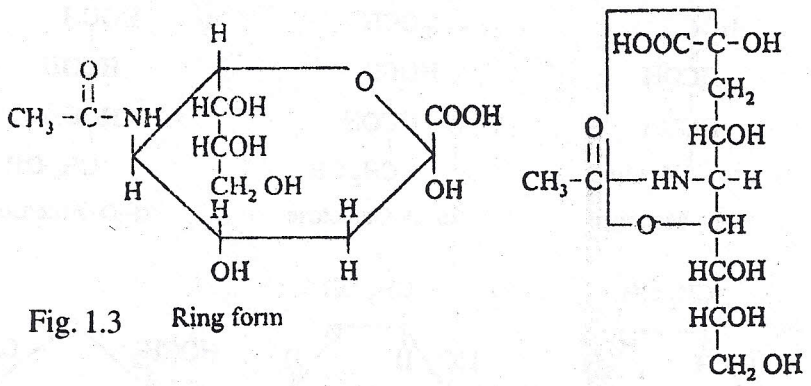


Fig. 1.3 Ring form

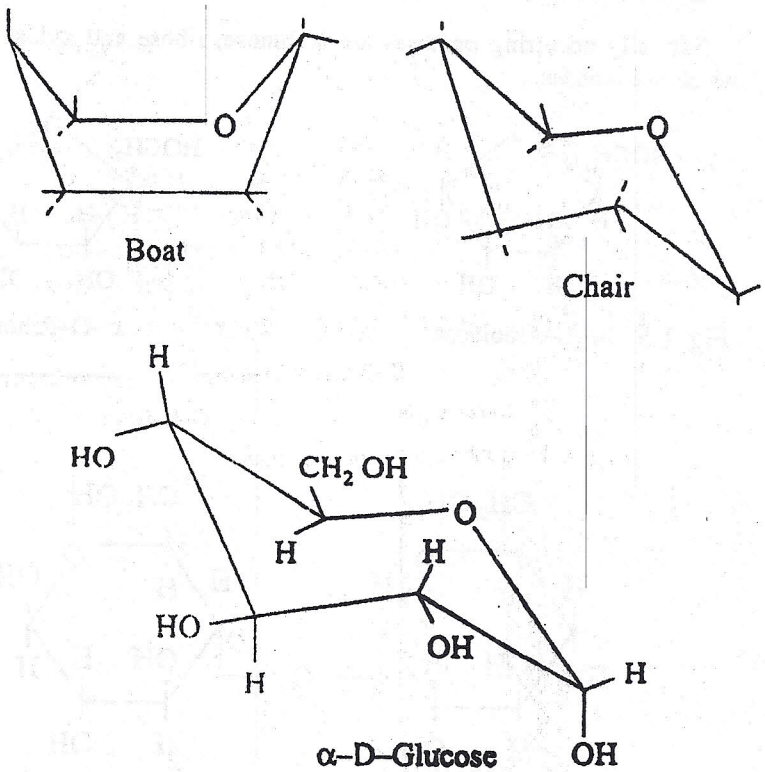
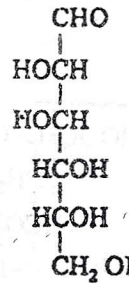
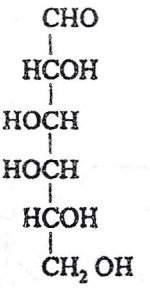


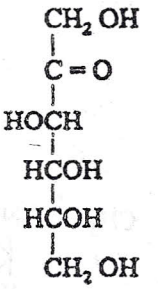
Fig. 1.4 Boat and chair configurations of pyranose form of monosaccharides. α–D-glucose in the chair form



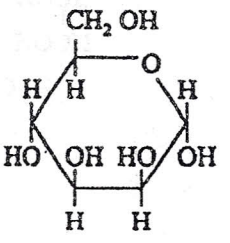
α–D–Mannose



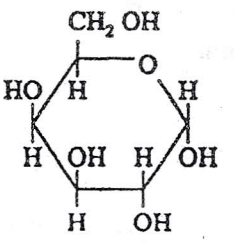
α–D–Galactose



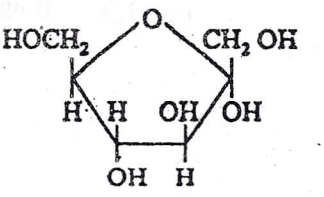
α–D–Fructose



α–D–Mannose



α–D–Galactose



α–D–Fructose

Naturally occurring pentoses are arabinose, ribose and xylose, and all of which are aldoses.

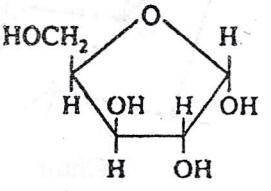
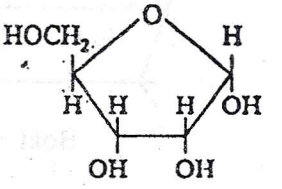


Fig. 1.5 α–L–Arabinose



α–D–Ribose

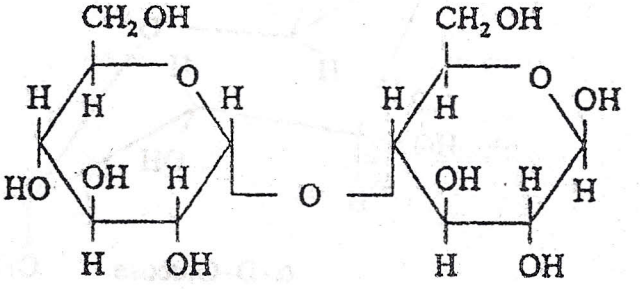


Fig. 1.7 β – Maltose

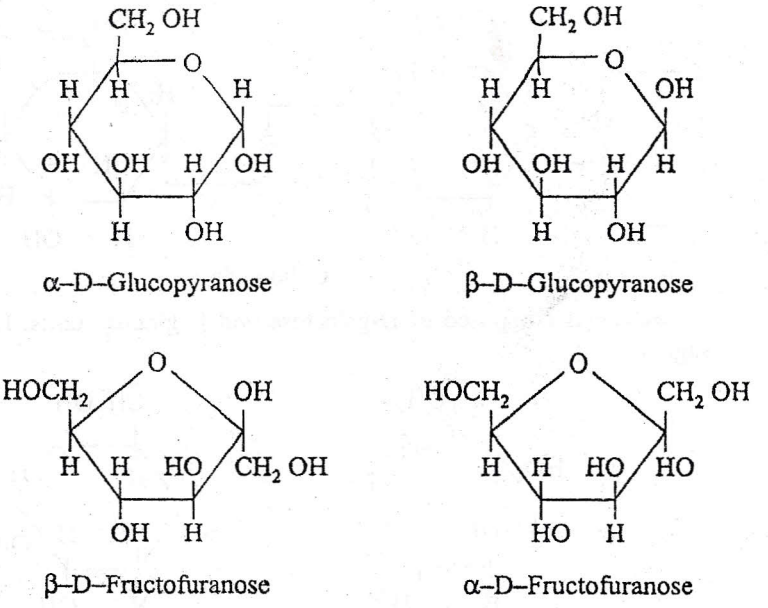


Fig. 3.5 Glucose and fructose sugars shown in pyranose and furanose forms

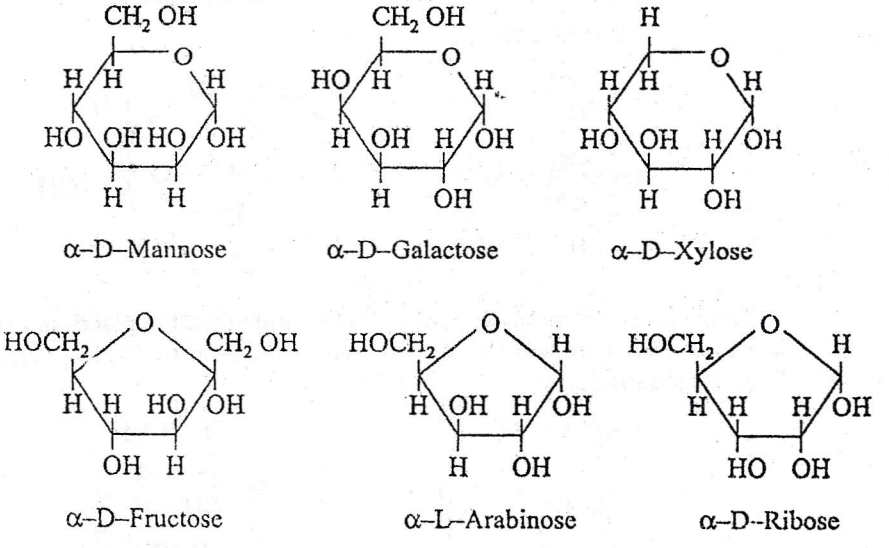
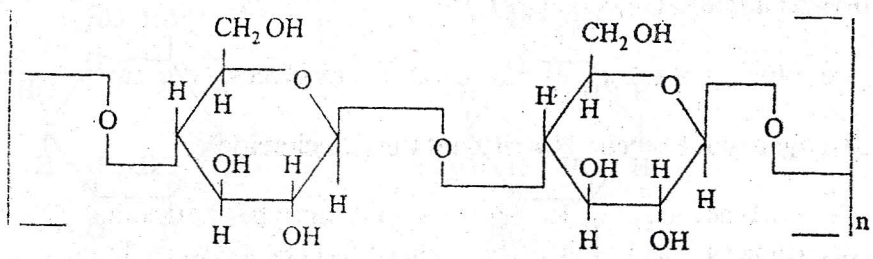
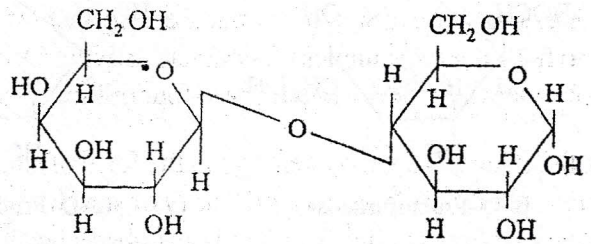


Fig. 1.6 Haworth formulas of some important monosaccharides



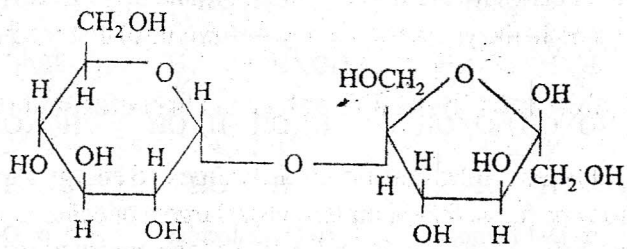
Cellobiose

Lactose is composed of D-galactose and D-glucose units. It is a reducing sugar.



Lactose

Sucrose, the common cane sugar is a non-reducing sugar and is composed of equimolar amounts of D-glucose and D-fructose units.



Sucrose

Trehalose is a naturally occurring disaccharide found in plants and in insects in which glycosidic bond exists between 1–1 carbon (α-D-glucopyranosyl-β-D-glucopyranoside).

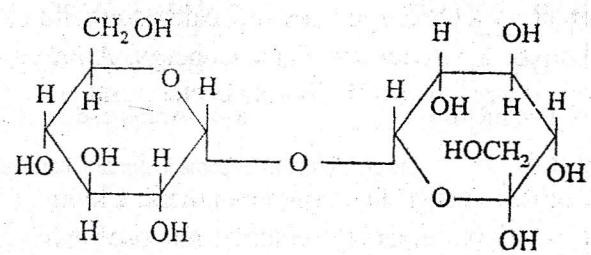


Fig. 1.8

1.1.5.1.4 Polysaccharides (Figs. 1.9 to 1.16)

They are polymers of sugar units joined together by glycosidic bonds.

1.1.5.1.4.1 Glycogen and Starch: Nutritional Polysaccharides

Glycogen is a branched polymer containing only one type of monomer : glucose. Most of the sugar units of a glycogen molecule are joined to one another by a (1 ® 4) glycosidic bonds. Branch points contain a sugar joined to three neighbouring units rather than to two, the extra neighbour, which forms the branch, is linked by an a (1 ® 6) glycosidic bond.

Glycogen serves as a store house of surplus chemical energy in most animals. Human skeletal muscles contain enough glycogen to fuel about 30 minutes of moderate activity. Depending on various factors, glycogen typically ranges in molecular weight from about one to four million daltons.

Most plants bank their surplus chemical energy in the form of starch, which like glycogen is also a polymer of glucose. Potatoes and cereals consist primarily of starch. Starch is actually a mixture of two different polymers, amylose and amylopectin. Amylose is an unbranched, helical molecule whose sugars are joined by a (1-4) linkages, where as amylopectin is branched. Amylopectin differs from glycogen in being much less branched and having an irregular branching pattern. Starch is stored as densely packed granules, or starch grains, which are enclosed in membrane bound organelle (plastids) within the plant cell. Although animals do not synthesize starch, they possess an enzyme (amylase) that readily hydrolyses starch molecules.

1.1.5.1.4.2 Celluose, Chitin and Glycosaminoglycans : Structural polysaccharides

Where as some polysaccharides constitute easily digested energy stores, others form tough, durable structural materials. Cotton and linen, for example, consist largely of cellulose, which is the major component of plant cell walls. Cotton textiles owe their durability to the long, unbranched cellulose molecules, which are ordered into side-by-side aggregates to form molecular cables that are ideally constructed to resist pulling (tensile) forces. Like glycogen and starch, cellulose consists solely of glucose monomers; its properties differ dramatically from these other polysaccharides because the glucose units are joined by b(1 ® 4) linkages. Ironically, multicellular animals lack the enzyme needed to degrade cellulose, which happens to be the most abundant organic material on earth and rich in chemical energy. Animals that make a living by digesting cellulose, such as termites and sheep, do so by harboring bacteria and protozoa that synthesize the necessary enzyme, cellulase.

Not all biological polysaccharides consist of glucose monomers. Chitin is an unbranched polymer of the sugar N-acetylglucosamine, which is similar in structure to glucose but has an acetyl amino group instead of a hydroxyl group bonded to the second carbon atom of the ring.

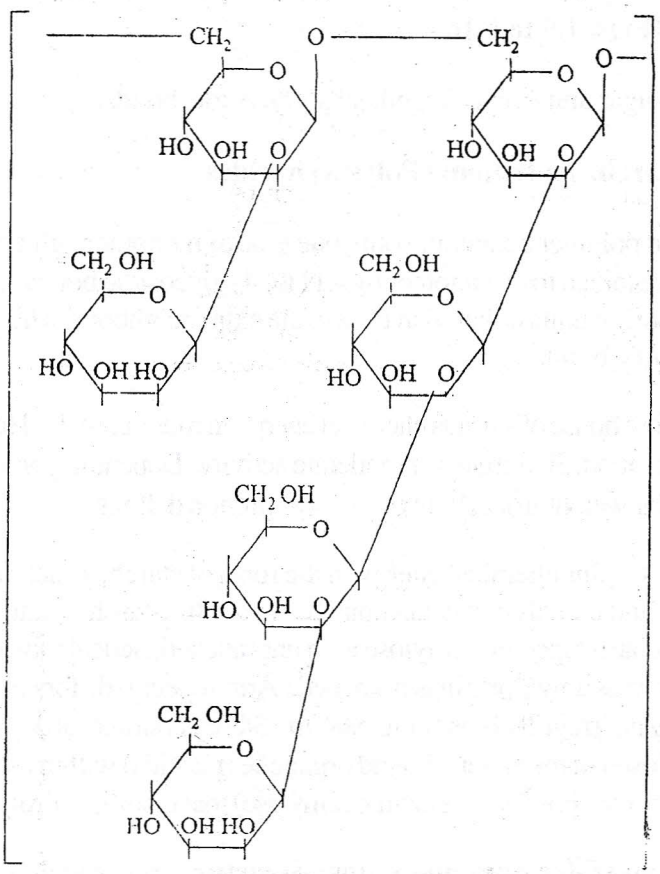
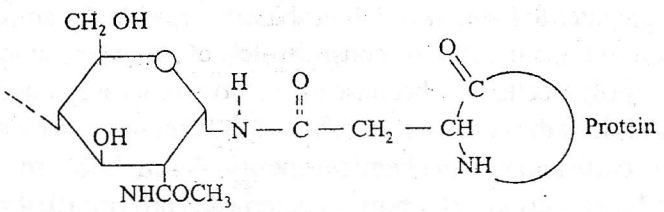


Fig. 1.9 A structural homopolysaccharide, mannan, common in baker's yeast. It is formed by repeating units of mannose as indicated. Mannan is covalently linked to protein forming a stable glycan



Mannan – asparaginyl bond

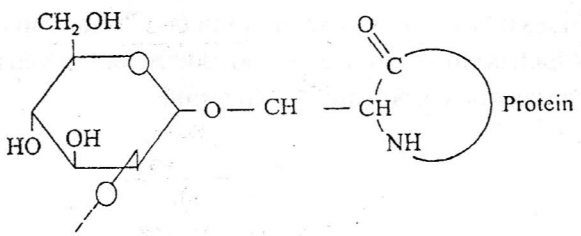
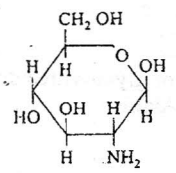
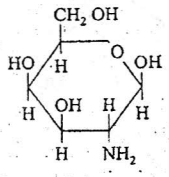


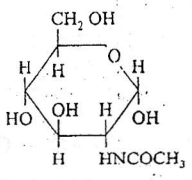
Fig. 1.10 Attachment of mannan to protein is possible in two ways as indicated



β – D – Glucosamine



β – D – Galactosamine



N – Acetylglucosamine

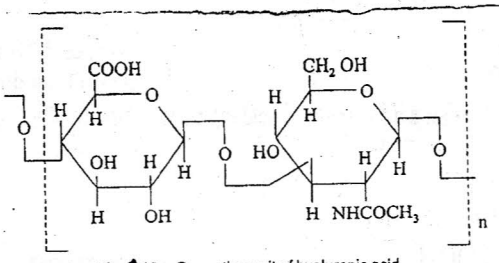


Fig. 1.12 Repeating unit of hyaluronic acid

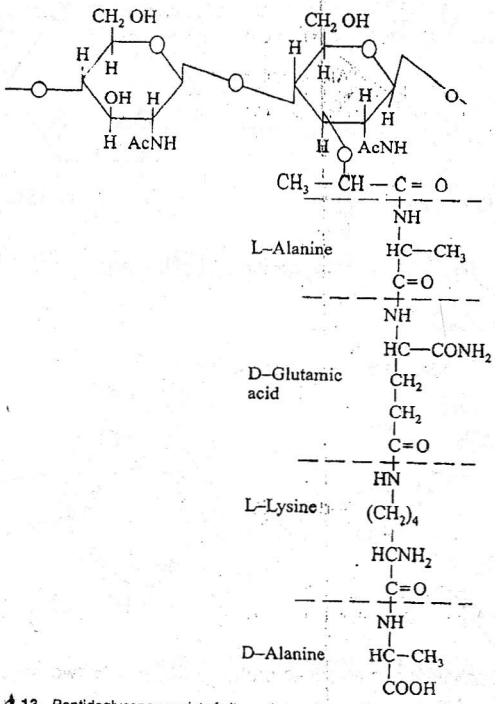
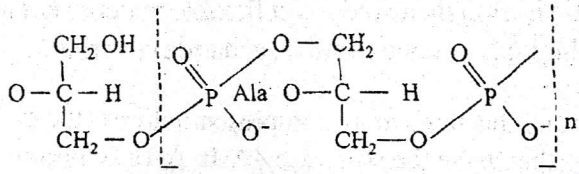
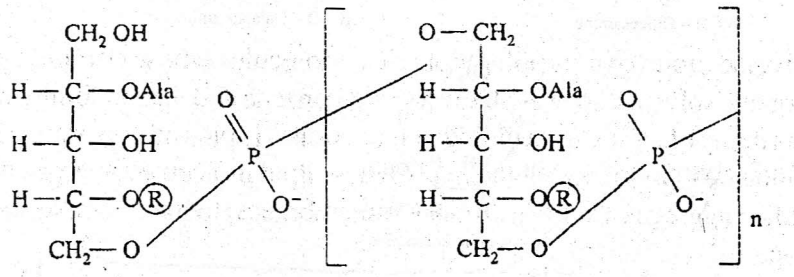


Fig. 1.13 Peptidoglycans consist of alternating residues of N–acetylglucosamine and N–acetylmuramic acid. The parallel glycan chains are cross linked through tetrapeptide bridges. The cell wall of Staphylococcus aureus has a tetrapeptide sequence (L-alanine–D–glutamic acid–L–lysine–D–alanine)



(a)

R = H or glycosyl residue
Ala = Alanine



(b)

R = α or β-N- acetylglucosamin
Ala = D-alanine

Fig. 1.14 Teichoic acid (a) glycerol type (b) ribitol type

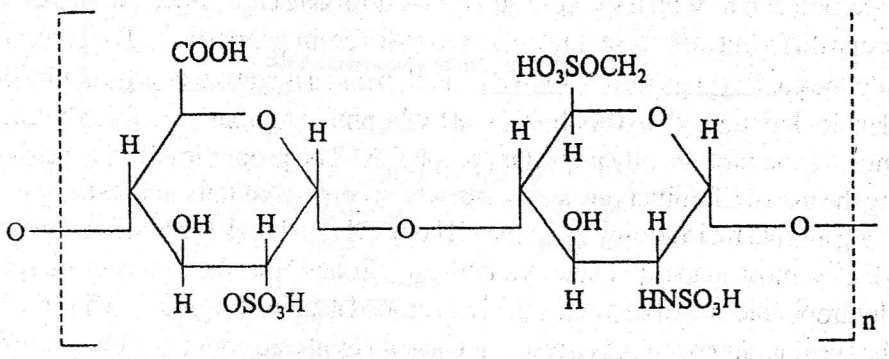


Fig. 1.15 Repeating unit of heparin

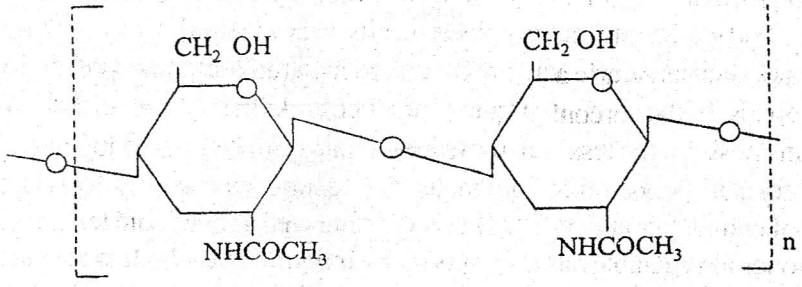


Fig. 1.16 Repeating unit of chitin

Chitin is widely used as a structural material among invertebrates, particularly in the outer covering of insects, spiders and crustaceans. Chitin is a tough, resilient, yet flexible material not unlike certain plastics. Insects owe much of their success to this highly adoptive polysaccharide covering.

Another group of polysaccharides that has a more complex structure is the glycosaminoglycans (or GAGs) unlike other polysaccharides, they have the structure –A-B-A-B-, where A and B represent two different sugars. These polysaccharides are found primarily in the spaces that surround cells.

1.1.5.2 Lipids

Lipids are a diverse group of non-polar biological molecules whose common properties are their ability to dissolve in organic solvents, such as chloroform or benzene and their inability to dissolve in water - a property that explains many of their varied biological functions. Lipids of importance in cellular function include fats, steroids and phospholipids. While none of these lipid molecules is large enough to be called a macromolecule, they often aggregate (as in fat droplets or membranes) to form complexes large enough to be seen under a microscope.

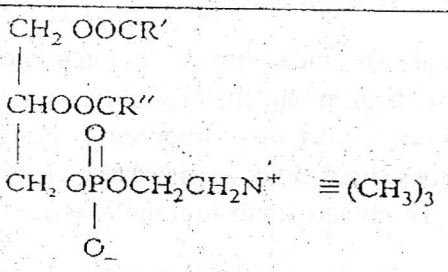
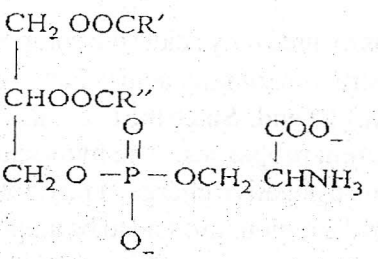
1.1.5.2.1 Fats (Figs. 1.17 to 1.23)

Fats consist of a glycerol molecule linked by ester bonds to three fatty acids; the composite molecule is termed a triacylglycerol. We will begin by considering the structure of fatty acids. Fatty acids are long, unbranched hydrocarbon chains with a single carboxyl group at one end. Since the two ends of a fatty acid molecule have a very different structure, they also have different properties. The hydrocarbon chain is hydrophobic, while the carboxyl group (-COOH), which bears a negative charge at physiological pH, is hydrophillic. Molecules having both hydrophobic and hydrophillic regions are said to be amphipathic; such molecules have unusual and biologically important properties. The properties of fatty acids can be appreciated by considering the use of a familiar product; soap, which consists of fatty acids. In past centuries, soaps were made by heating animal fat in strong alkali (NaOH or KOH) to break the bonds between the fatty acids and the glycerol. Today, most soaps are made synthetically. Soaps have their grease –dissolving capability to the fact that the hydrophobic end of each fatty acid can embed itself in the grease, while the hydrophilic end can interact with the surrounding water. As a result, greasy materials are converted into complexes (micelles) that can be dispersed by water.

Fatty acids differ from one another in the length of their hydrocarbon chain and the presence or absence of double bonds. Fatty acids present in cells typically vary in length from 14 to 20 carbons. Fatty acids that lack double bond, such as stearic acid are described as saturated; those possessing double bonds are unsaturated. Double bonds (of the cis configuration) produce kinks in a fatty acid chain. The more double bonds that fatty acid chains possess, the less well these long-chains can be packed together. This lowers the temperature at which a fatty acid – containing lipid melts. Tristeorate, whose fatty acids lack double bonds, is a common component of animal fats and remains in a solid state well above room temperature. In contrast, the profusion of double bonds in vegetable fats accounts for their liquid state – both in the plant cell and on the grocery shelf – and for their being labelled as 'polyunsaturated'. Fats which are liquid at room temperature

are described as oils. A molecule of fat can contain three identical fatty acids or it can be a mixed fat, containing more than one fatty acid species. Most natural fats, such as olive oil or butter fat, are mixtures of molecules having different fatty acid species.

Fig. 1.17 Structures of some phospholipids

Phospholipid		*Non-polar*
	Phosphatidylcholine (lecithin)	R′-Sı palm R″-p ted
	Phosphatidylserine	R′-sa acid R″-pc ted
	Phosphatidylethanol amine (Cephalin)	R′-sat

Structures of some phospholipids

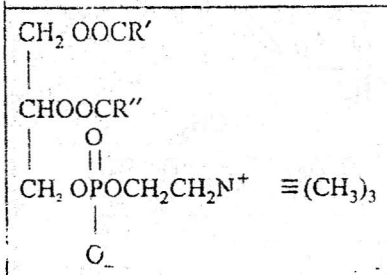
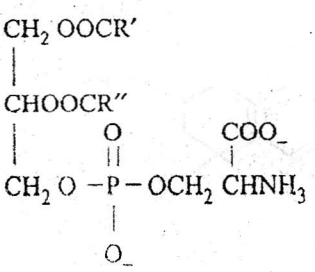
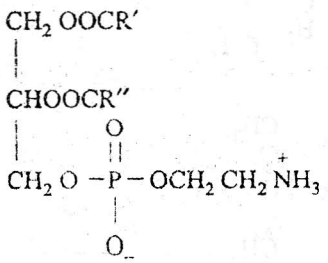
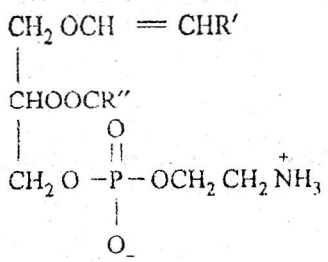
Phospholipid		*Non-polar component*
	Phosphatidylcholine (lecithin)	R′-Stearic or palmitic R″-polyunsaturated
	Phosphatidylserine	R′-saturated fatty acid R″-polyunsaturated
	Phosphatidylethanol amine (Cephalin)	R′-saturated R″-polyunsaturated
	Phosphital-aminoethanol (plasmalogen)	R′-unsaturated ether R″-polyunsaturated

Fig. 1.17 (contd.)

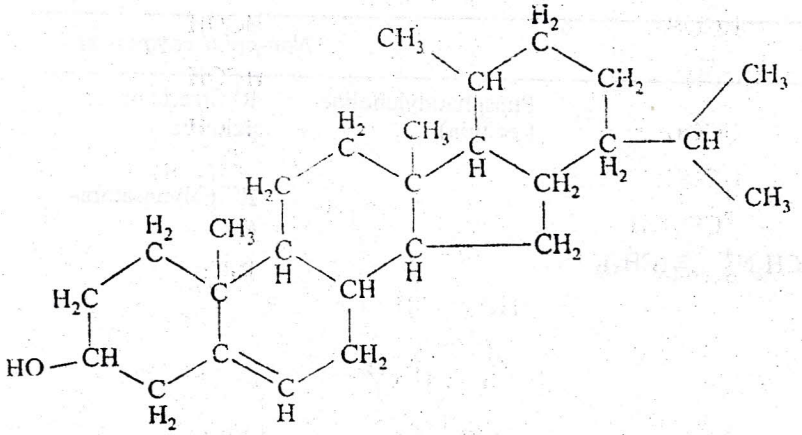
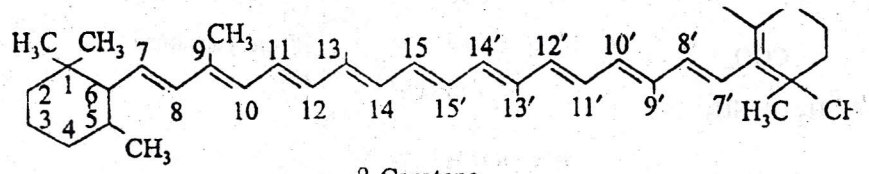
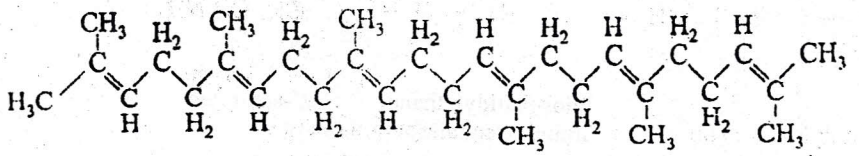


Fig. 1.18 Cholesterol



β-Carotene



Squalene

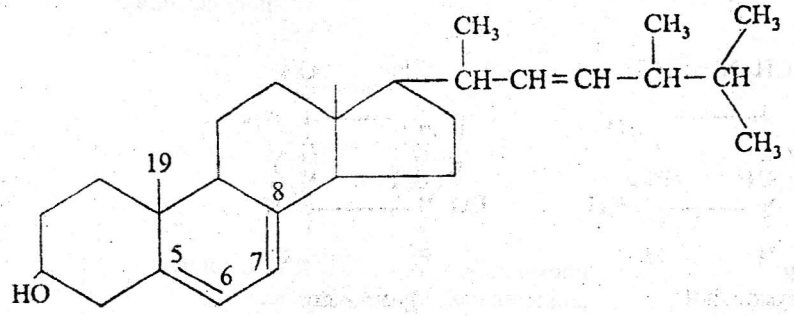
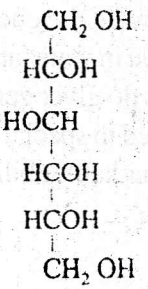
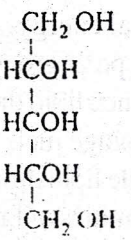


Fig. 1.19 Ergosterol



Sorbitol



Ribitol

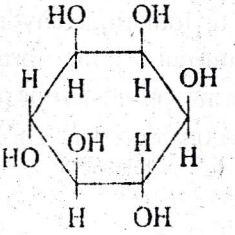


fig. 1.20 Myoinositol

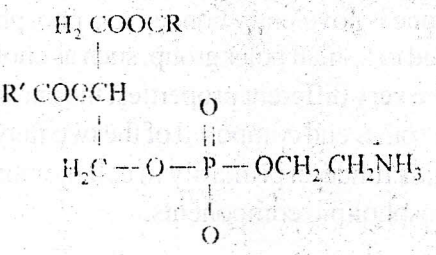
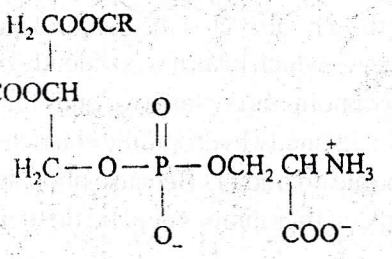


fig. 1.21 Phosphatidylethanolamine



Phosphatidylserine

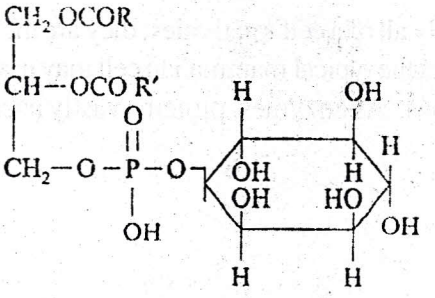
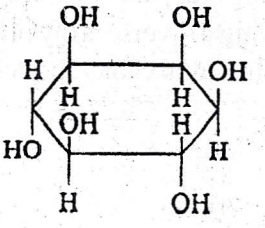


fig. 1.22 Phosphatidylinositol



Myoinositol

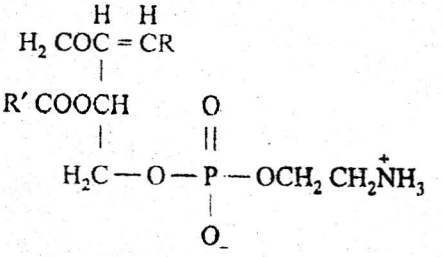


fig. 1.23 A Plasmalogen (L – Phosphatidylethanolamine)

Fats are very rich in chemical energy: a gram of fat contains over twice the energy content of a gram of carbohydrate. Since fats lack polar groups, they are extremely insoluble in water and are stored in cells in the form of dry lipid droplets. Since lipid droplets do not contain water as do glycogen granules, they represent an extremely concentrated storage fuel. In many animals, fats are stored in special cells (adopocytes) whose cytoplasm is filled with a single lipid droplet. Adipocytes exhibit a remarkable ability to change their volume to accommodate varying quantities of fat.

1.1.5.2.2 Steroids

Steroids are build around a characteristic four-ringed hydrocarbon skeleton. One of the most important steroids is cholesterol, a component of animal cell membranes and a precursor for the synthesis of a number of steroid hormones, such as testosterone, progesterone and estrogen. Cholesterol is largely absent from plant cells, that is why vegetable oils are considered as 'cholesterol-free'.

1.1.5.2.3 Phospholipids

The molecule resembles a fat (triacyl glycerol), but has only two fatty acid chains rather than three; it is a diacylglycerol. The third hydroxyl of the glycerol backbone is covalently bonded to a phosphate group (rather than a third fatty acid), which in turn is covalently bonded to a small polar group, such as choline. Thus unlike fat molecules, phospholipids contain two ends that have very different properties; the end containing the phosphate group has a distinctly hydrophillic character, the other end composed of the two fatty acid tails has a distinctly hydrophobic character. Because phospholipids function primarily in cell membranes, and because the properties of cell membranes depend on their phospholipid components.

1.1.5.3 Proteins (Fig. 1.24 to 1.30)

Proteins are the macromolecules that carry out virtually all of a cell's activities; they are the molecular tools and machines that make things happen. It is estimated that the typical mammalian cell may have as many as 10,000 different proteins having a diverse array of functions. As enzymes, proteins vastly accelerate the rate of metabolic reactions; as structural cables, proteins

Fig. 1.24 Structure of à-amino acids commonly found in proteins

1. Amino acid with nonpolar R groups

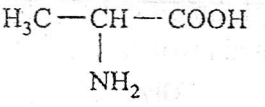
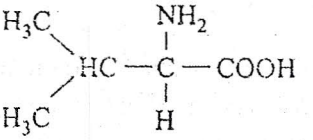
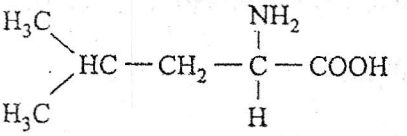
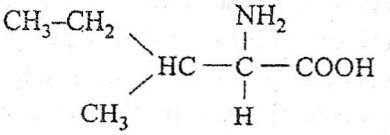
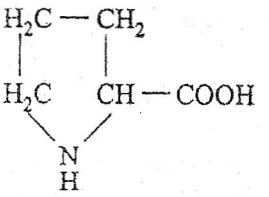
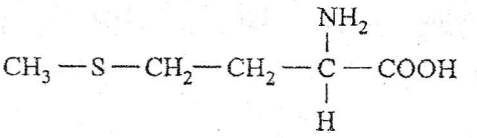
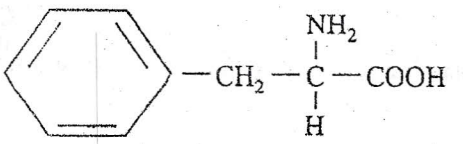
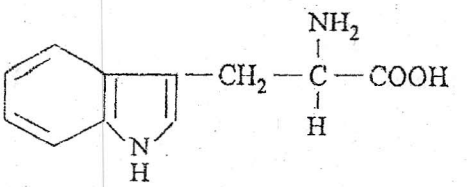
Name	*Abbreviation*	*Chemical structure*
Alanine	Ala	
Valine	Val	
Leucine	Leu	
Isoleucine	Ile	
Proline	Pro	
Methionine	Met	
Phenylalanine	Phe	
Tryptophan	Trp	

Fig. 1.2.4 (contd.)

2. Amino acid with polar but uncharged R groups

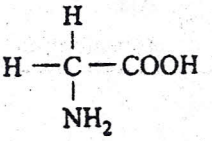
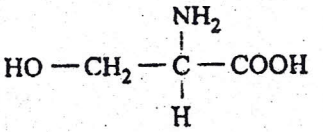
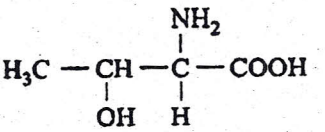
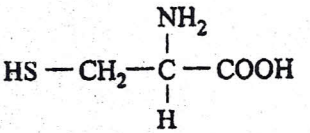
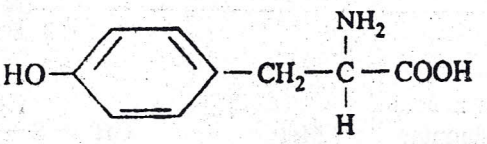
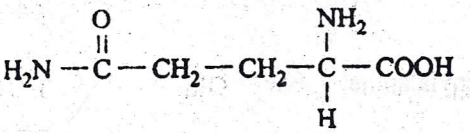
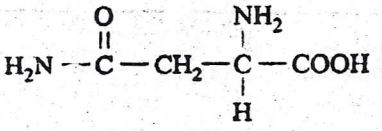
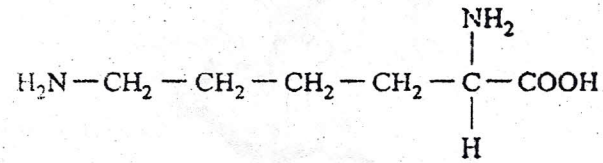
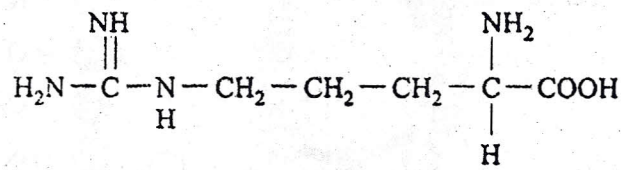
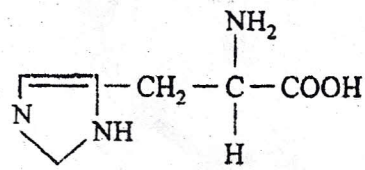
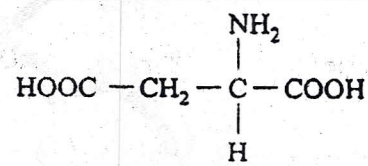
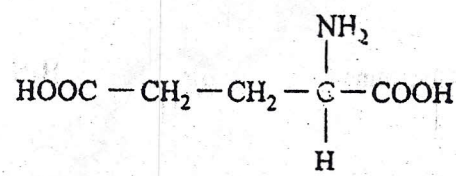
Name	*Abbreviation*	*Chemical structure*
Glycine	Gly	
Serine	Ser	
Threonine	Thr	
Cysteine	Cys	
Tyrosine	Tyr	
Glutamine	Gln	
Asparagine	Asn	

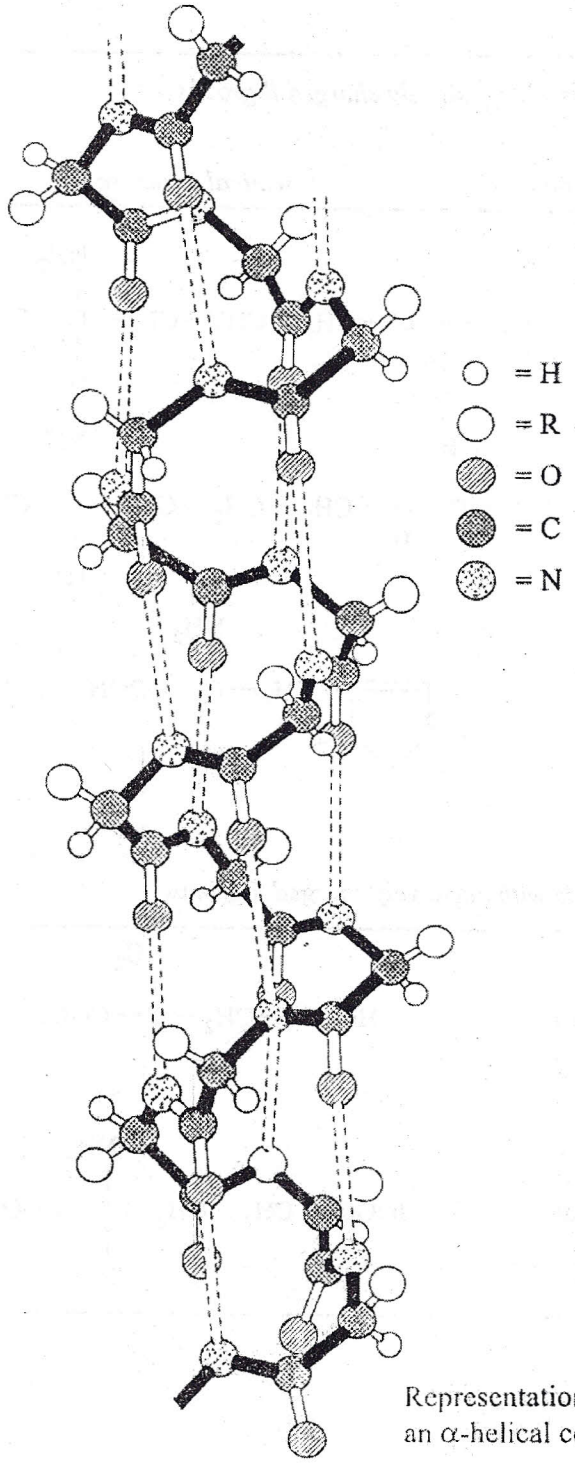
Fig. 1.2.4 (contd.)

3. Amino acids with positively charged R groups

Name	*Abbreviation*	*Chemical structure*
Lysine	Lys	
Arginine	Arg	
Histidine	His	

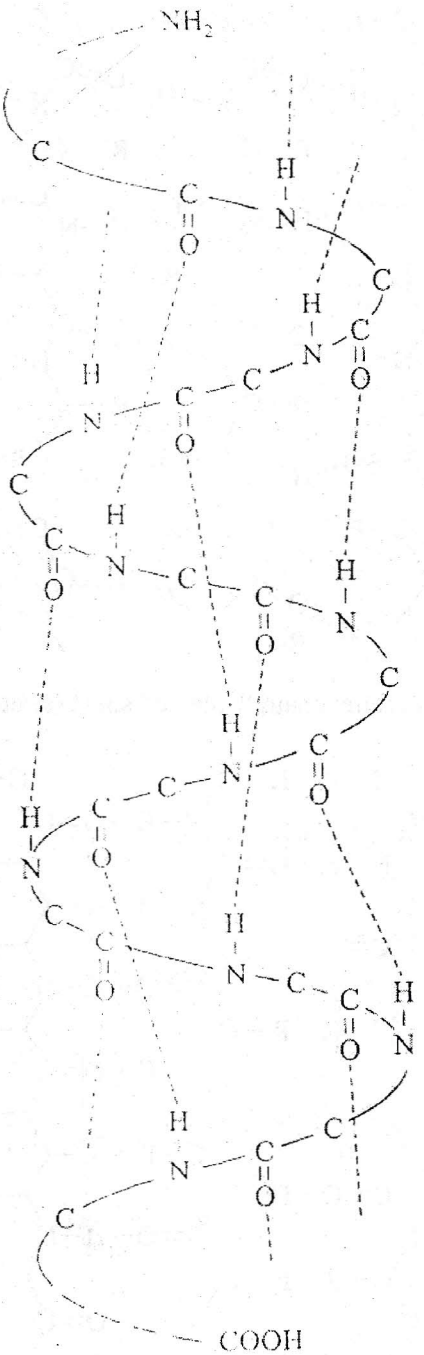
4. Amino acids with negatively charged R groups

Aspartic acid	Asp	
Glutamic acid	Glu	



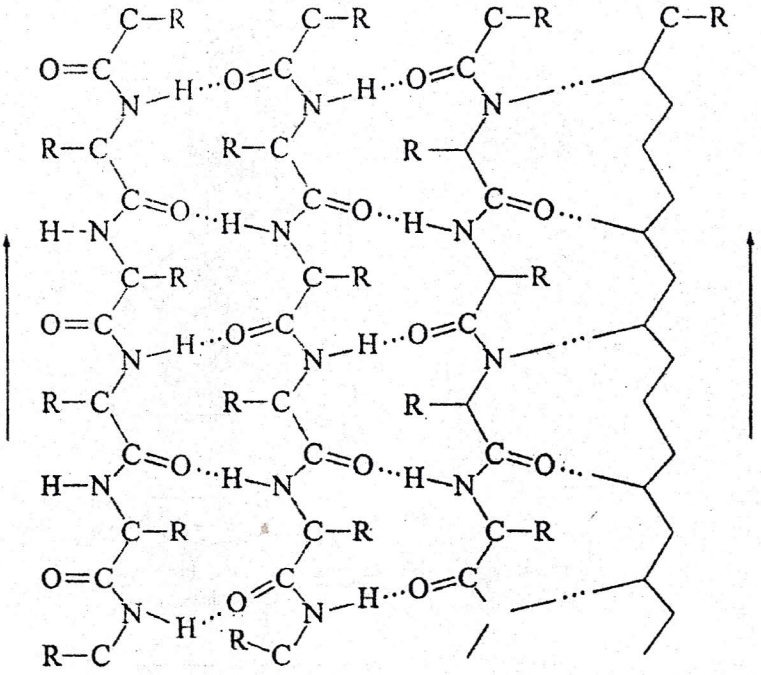
Representation of a polypetide chain in an α-helical configuration

Ball and stick model of α-helix

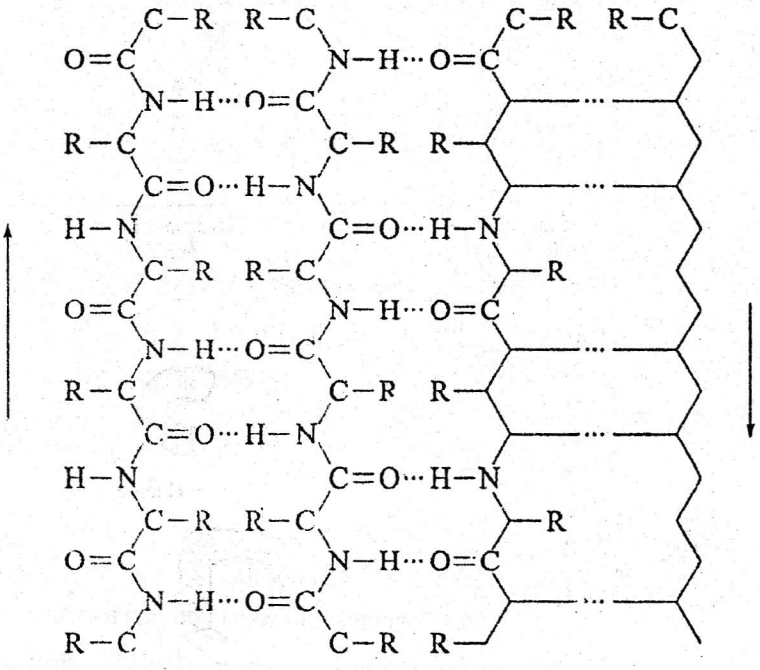


Left handed helix with hydrogen bonds

as storehouse of oxygen in the muscle which has 153 amino acid residues (MW ≈ 17500). There are no SH groups, hence disulphide bonds are absent. As shown in Fig. 6.5, the myoglobin chain is folded back on itself in a complex

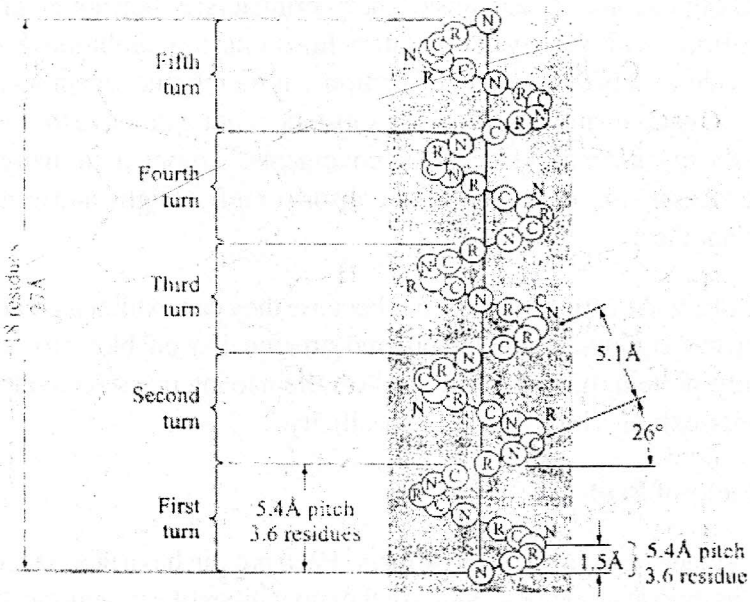


(a) Parallel chain β-pleated sheet (stretched keratin)

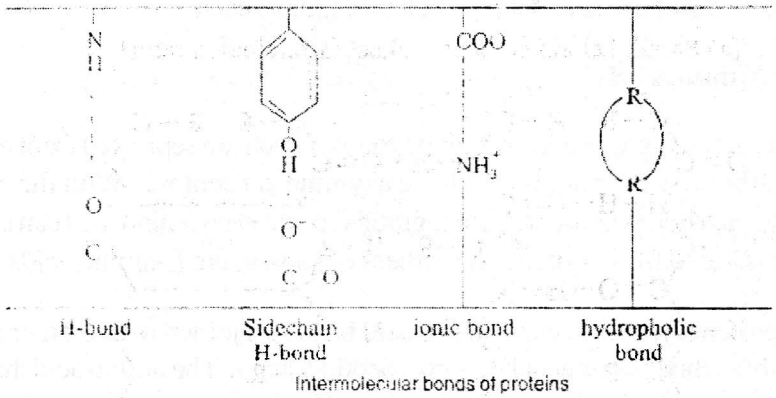


(b) Antiparallel β- pleated sheet (silk)

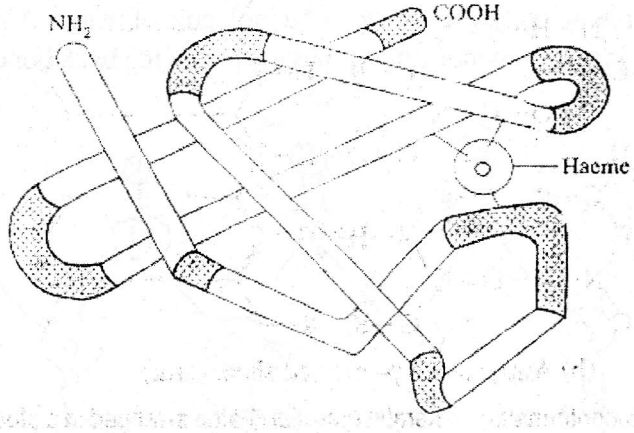
(a) β-conformation of keratin (parallel chains arranged in a pleated



α-helix showing dimensions of the helical turns. Each turn has a pitch of 5.4 Å consisting of 3.6 amino acid residues



Intermolecular bonds of proteins



1.30 Tertiary structure of myoglohin

provide mechanical support both within cells and outside their perimeters; as hormones, growth factors and gene activators, proteins perform a wide variety of regulatory functions; as membrane receptors and transporters, proteins perform a wide variety of regulatory functions; as membrane receptors and transporters, proteins determine what a cell reacts to and what types of substances enter or leave the cell; as contractile elements, protein constitute the machinery for biological movements. Among their many other functions, proteins act as antibodies, serve as toxins, form blood clots, absorb or refract light, and transport substances from one part of the body to another.

Proteins are capable of a wide variety of activities because they can exhibit a great variety of structures. Each protein, however, has a unique and highly ordered structure that enables it to carry out a particular function. Most importantly, proteins have shapes that allow them to interact selectively with other molecules. Proteins in other words, exhibit a high degree of specificity.

1.1.5.3.1 The Building Blocks of Proteins

Proteins are polymers made of amino acid monomers. Each protein has a unique sequence of amino acids that gives the molecule its unique properties. Many of the capabilities of a protein can be understood by examining the chemical properties of its constituent amino acids. Twenty different amino acids are commonly found in proteins, whether from a virus or a human. There are two aspects of amino acid structure to consider: that which is common to all of them, and that which is unique to each.

1.1.5.3.2 The Structures of Amino Acids

All amino acids have a carboxyl group and an amino groups, which are separated from each other by a single carbon atom, the a carbon. Amino acids also have asymmetric centers. With the exception of glycine, the a-carbon of amino acids bonds to four different groups so that each amino acid can exist in either a D or an L form. Amino acid isolated from proteins, regardless of source, are L-amino acids.

During the proces of protein synthesis, each amino acid becomes joined to two other amino acids forming a long, continuous, unbranched polymer called a polypeptide chain. The amino acid that make up a polypeptide chain are joined by peptide bonds that result from the linkage of the carboxyl group of one amino acid to the amino group of its neighbor, with the elimination of a molecule of water. A polypeptide chain composed of a string of amino acids joined by peptide bonds has the following back bone:

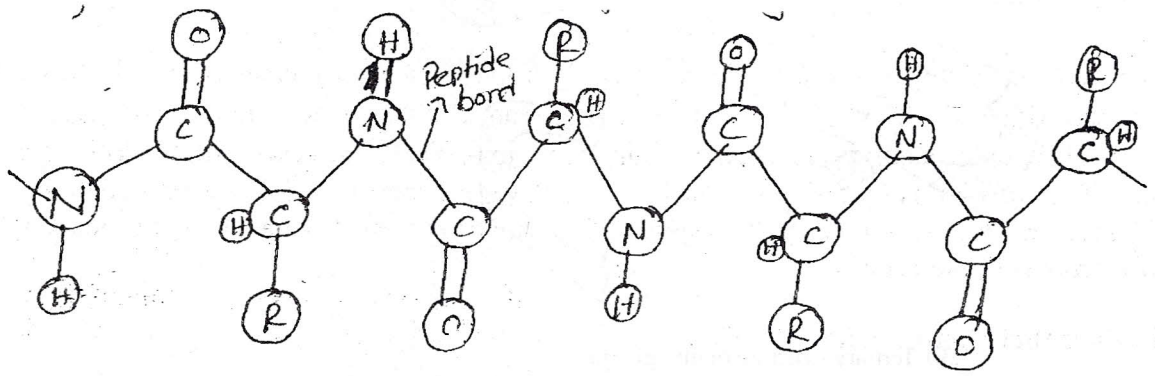


Fig. 1.1.1 Peptide bond

Once incorporated into a polypeptide chain, amino acids are termed residues. The residue on one end of the chain, the N-terminus, contains an amino acid with a free (unbonded) a-amino group, while the residue at the oppostie end, the C-terminus, has a free a-carboxyl group, while the residue at the opposite end, the C-terminus, has a free a-carboxyl group.

1.1.5.3.3 The Properties of the side chains

The backbone of the polypeptide chain is composed of that part of each amino acid that is common to all of them. The remainder of each amino acid, the side chain or R group, is highly variable among the 20 building blocks, and it is this variability that gives proteins their versatility. If all the amino acids are considered together, there is a large variety of organic reactions in which they can participate and a great many types of bonds they can form. The assorted characteristics of the side chains of the amino acids are important in both intramolecular interactions, which determine the structure of the molecule, and intermolecular interactions, which determine the activities the protein can perform.

Amino acids are conveniently classified on the character of their side chains. They fall roughly into four categories: Polar and charged, polar and uncharged, non-polar, and those with unique properties.

1.1.5.3.3.1 Polar, Charged

Amino acids of this group include aspartic acid, glutamic acid, lysine and arginine. These four amino acids contain side chains that become fully charged; that is, the side chains contain relatively strong organic acids and bases. At physiologic pH, the side chains of these amino acids are almost always present in the fully charged state. Consequently, they are able to form ionic bonds with other charged species in the cell.

1.1.5.3.3.2 Polar, uncharged

The side chains of these amino acids are only weakly acidic or basic. Although these groups are not fully charged at physiologic pH, they do contain atoms that have a partial negative or positive charge and thus can form hydrogen bonds with other molecules including water. These amino acids are often quite reactive. Included in this category are asparagine and glutamine (the amides of aspartic acid and glutamic acid), threonine, serine and tyrosine.

1.1.5.3.3 Non-polar

These are the amino acids whose side chains are hydrophobic and are unable to form electrostatic bonds or interact with water. The amino acids of this category are alanine, valine, leucine, isoleucine, tryptophan, phenylalanine, and methionine. The side chains of the non-polar amino acids generally lack oxygen and nitrogen. They vary primarily in size and shape, which allows one or another of them to pack tightly into a particular space within the core of a protein, associating with one another as the result of vanderwaals forces and hydrophobic interactions.

1.1.5.3.4 Other three amino acids

Glycine, Proline and Cysteine – have unique properties that separate them from the others. The side

chain of glycine consists of only a hydrogen atom, and glycine is a very important amino acid for this reason. Owing to this, lack of a side chain, glycine residues provide a site where the backbones of two polypeptides (or two segments of the same polypeptide) can approach one another very closely. Proline is unique in having its a-amino group as part of a ring (making it an imino group) proline is a hydrophobic amino acid that does not readily fit into an ordered secondaryestructure. Cysteine contains a reactive sulfhydryl (-SH) group and is often covalently linked to another cysteine residue, as a disulfide (-SS-) bridge.

In addition to amino acids, many proteins contain other types of components; these are called conjugated proteins. Conjugated proteins include those linked covalently or non-covalently to nucleic acids, the nucleoproteins; to lipids, the lipoproteins; to carbohydrates, the glycoproteins; or to various low-molecular weight materials, including metals and metal-containing groups.

1.1.5.3.4 The Structure of Proteins

Proteins are huge, complex molecules, but their structure in any given environment is completely defined and predictable. Each amino acid in a protein is located in a specific site within these giant molecules, giving the protein the structure and reactivity required for the job at hand. Protein structure can be described at several levels of organization, each emphasizing a different aspect and each dependent on different types of interactions. Customarily, four such levels are described: primary, secondary, tertiary and quaternary. The first, primary structure, concerns the amino acid sequence of a protein, while the latter three levels concern the organization of the molecule in space.

1.1.5.3.4.1 Primary structure

The primary structure of a polypeptide is the specific linear sequence of amino acids that constitute the chain. With 20 different building blocks, the number of different polypeptides that can be formed is 20^n, where **n** is the number of amino acids in the chain. Since most polypeptides contain over 100 amino acids, variety of possible sequences is essentially unlimited.

The first amino acid sequence of a protein was determined by Frederick Sanger and coworkers of Cambridge University in the early 1950s using the protein hormone insulin. Beef insulin was chosen for the work because of its availability and its small size - two polypeptide chains of 21 and 30 amino acids each. The sequencing of insulin was a momentous feat in the newly emerging field of molecular biology. It revealed that proteins, the most complex molecules in cells, had a specific definable substructure that was neither regular nor repeating, as was that of polysaccharides. With the advent of techniques for rapid DNA sequencing, the primary structure of a polypeptide can be deduced from the nucleotide sequence of the encoding gene. As a result, the sequences of tens of thousands of proteins are now known, providing invaluable information about their structures their mechanism of action and their evolution.

1.1.5.3.4.2 Secondary structure

Secondary structure describes the conformation of portions of the polypeptide chain. The early studies on protein secondary structure were carried out by Linus Pauling and Robert Corey of the California Institute of Technology. By studying the structure of simple peptides consisting of a few amino acids linked together, Pauling and Corey concluded that polypeptide chains exist in preferred conformations that provide the maximum possible number of hydrogen bonds between neighbouring amino acids.

Two conformations were proposed. In one conformation, the back bone of the polypeptide assumed the form of a cylindrical, twisting spiral called the alpha (a) helix. The backbone lies on the inside of the helix and the side chains project outward. The helical structure is stabilized by large numbers of hydrogen bonds between the atoms of one peptide bond and those situated just above and below it along the spiral. The x-ray diffraction patterns of actual proteins during the 1950s bore out the existence of the a helix. First in the protein Keratin found in hair and later in various oxygen-binding proteins, such as myoglobin and hemoglobin. Surfaces on opposite sides of an a helix may have contrasting properties. In water soluble proteins, the outer surface of an a-helix often contains polar residues in contact with the solvent, while the surface facing inward contains non-polar side chains. The arrangement of polar and non-polar residues is often reversed in the a-helices of membrane proteins that pass through the hydrophobic lipid bilayer.

Because it is coiled and held together by weak, non-covalent bonds, an a helix can be extended in length if subjected to pulling forces. This is illustrated by wool, whose protein fibers consist largely of a-helices.

The other conformation proposed by Pauling and Corey was the beta (b)- pleated sheet, which consists of several segments of a polypeptide lying side by side. Unlike the coiled, cylindrical form of the a helix, the backbone of each segment of polypeptide (or b strand) in a b sheet assume as folded or pleated conformation. Like the a helix, the b sheet is also characterised by a large number of hydrogen bonds, but these are perpendicular to the long axis of the polypeptide chain and project across from one part of the chain to another. Like the a helix, the b-sheet has also been found in many different proteins. Because b-strands are highly extended, the b-sheet resists pulling (tensile) forces. Silk is a protein consisting largely of b sheets; silk fibers owe their strength to this architectural feature.

1.1.5.3.4.3 Tertiary structure

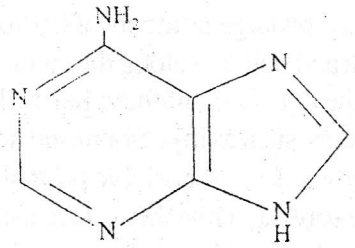
The next level after secondary structure is tertiary structure, which describes the conformation of the entire protein. Where as secondary structure is stabilized primarily by hydrogen bonds between atoms that form the peptide bonds of the backbone, tertiary structure is stabilized by an array of non-covalent bonds between the diverse side chains of the protein. Secondary structure is limited to a small number of conformations, but tertiary structure is virtually unlimited.

The tertiary structure of the protein is held together exclusively by non-covalent interactions. All of the non-covalent bonds thought to occur between side chains within proteins are hydrogen bonds, ionic bonds and hydrophobic interactions.

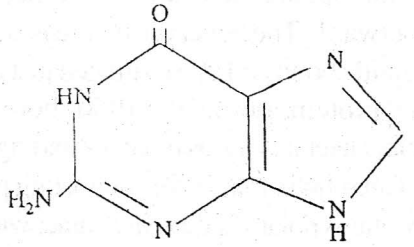
1.1.5.3.4.4 Quarternary structure

Many proteins are made up of more than one chain (or) subunit. The subunits may be linked by convalent disulfide bonds, but more often they are held together by non-covalent bonds as might occur for example, between hydrophobic patches on the complementary surfaces of neighbouring polypeptides. Proteins composed of subunits are said to have quarternary structure. Depending on the protein, the polypeptide chains may be identical or non-identical. A protein composed of two identical subunits is described as a homodimer, while a protein composed of two non-identical subunits is a heterodimer.

1.1.5.4 Nucleic Acids (Figs. 1.31 to 1.37)

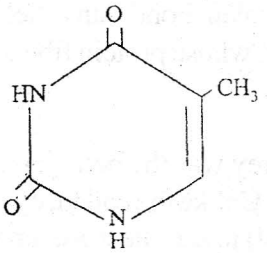


Adenine (A)
(6-aminopurine)

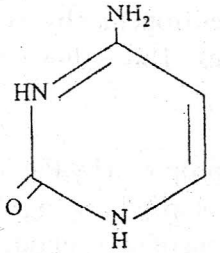


Guanine (G)
(2-amino-6-oxypurine)

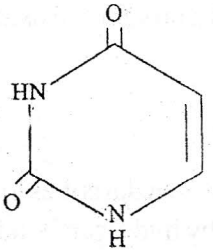
Fig. 1.31



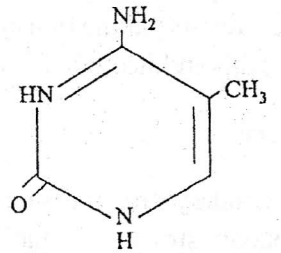
Thymine (T)
(2, 4 dioxy, 5 methyl pyrimidine)



Cytosine (C)
(2-oxy, 4-amino pyrimidine)

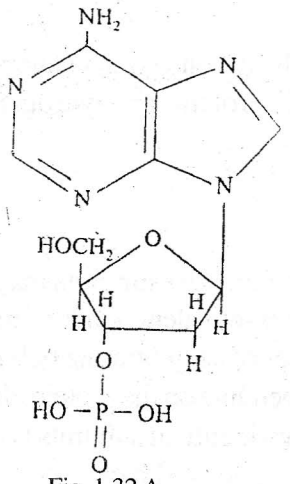


Uracil (U)
(2, 4-dioxypyrimidine)



5-methyl cytosine (mC)

Fig. 1.32



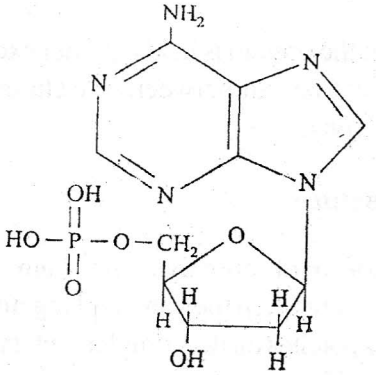
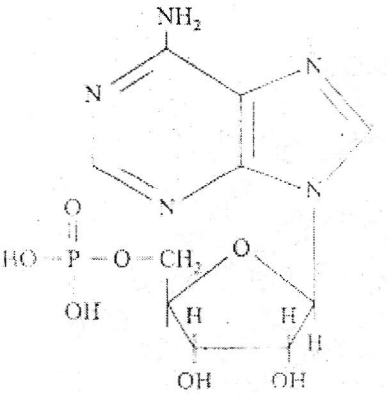
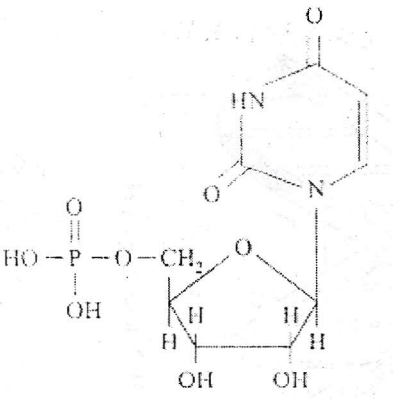


Fig. 1.32 A 3′ and 5′ deoxyribonucleotides



Adenosine 5′—phosphate (AMP)

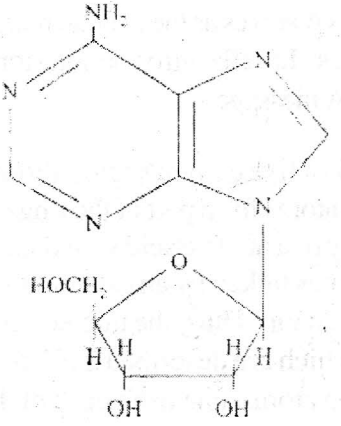


Uridine 5′—phosphate (UMP)

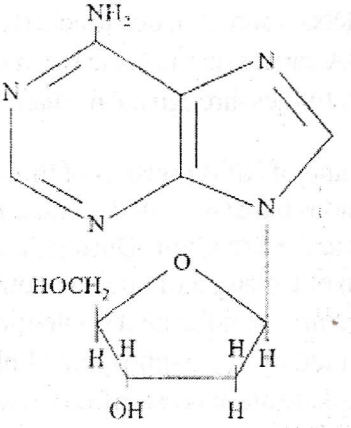
Nomenclature of nucleosides and nucleotides

Nucleoside	*Nucleotide*	*Scientific name*	*Abbreviation*
Adenosine	Adenylic acid	Adenosine-5'-monophosphate	AMP
Guanosine	Guanylic acid	Guanosine-5'-monophosphate	GMP
Cytidine	Cytidylic acid	Cytidine-5'-monophosphate	CMP
*Ribosylthymine	Thymidylic acid	Thymine-5'-monophosphate	TMP
Uridine	Uridylic acid	Uridine-5'-monophosphate	UMP
Deoxyadenosine	Deoxyadenylic acid	Deoxyadenosine-5'-monophosphate	dAMP
Deoxyguanosine	Deoxyguanylic acid	Deoxyguanosine-5'-monophosphate	dGMP
Deoxycytosine	Deoxycytidylic acid	Deoxycytidine-5'-monophosphate	dCMP
*Thymidine	Deoxythymidylic acid	Deoxythymidine-5'-monophosphate	dTMP

Fig. 1.33 *Deoxyribonucleoside thymine is present in DNA, called thymidine. Thymine also occurs in one type of RNA (tRNA), called ribosyl thymine

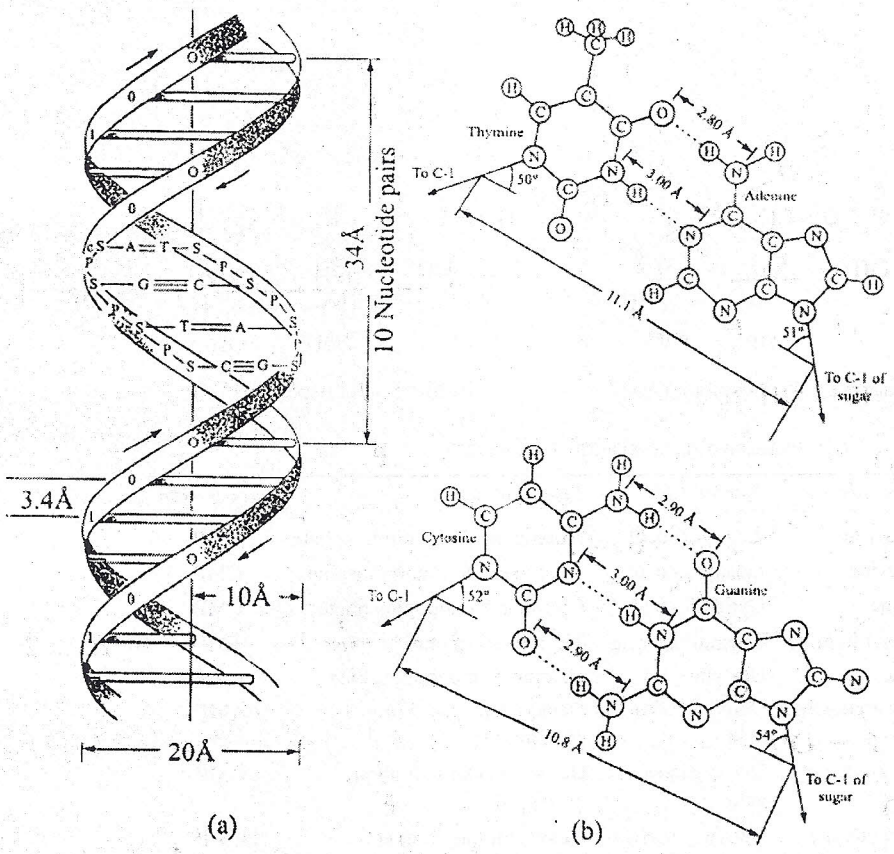


Adenosine
(riboside)



Deoxyriboside

Fig. 1.34



Structure of DNA:
(a) The two polynucleotide chains are twisted to form a double helix
(b) Arrangement of base pairs showing angular and linear dimensions

Nucleic acids are macromolecules constructed as a long chain (strand) of monomers called nucleotides. Nucleic acid function primarily in the storage and transmission of genetic information, but they may also have structural or catalystic roles. There are two types of nucleic acids found in living organisms, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA serves as the genetic material of all cellular organisms, through RNA carries out that role for many viruses. In cells, information stored in the DNA is used to govern cellular activities through the formation of RNA messages.

Each nucleotide in a strand of RNA consists of three parts (1) a five carbon sugar, ribose (2) a phosphate group, and (3) a nitrogenous base (so-called because nitrogen atoms form part of the rings of the molecule). The phosphate is linked to the S¢carbon. During the assembly of a nucleic acid strand, the hydroxyl group attached to the 3¢carbon of the sugar of one nucleotide becomes linked by an ester bond to the phosphate group attached to the S¢carbon of the next nucleotide in the chain. Thus, the nucleotides of an RNA (or DNA) strand are connected by sugar-phosphate linkages, which are described as 3'-5'-phosphodiester bonds because the phosphate atom is esterified to two oxygen atoms, one from each of the two adjoining sugars.

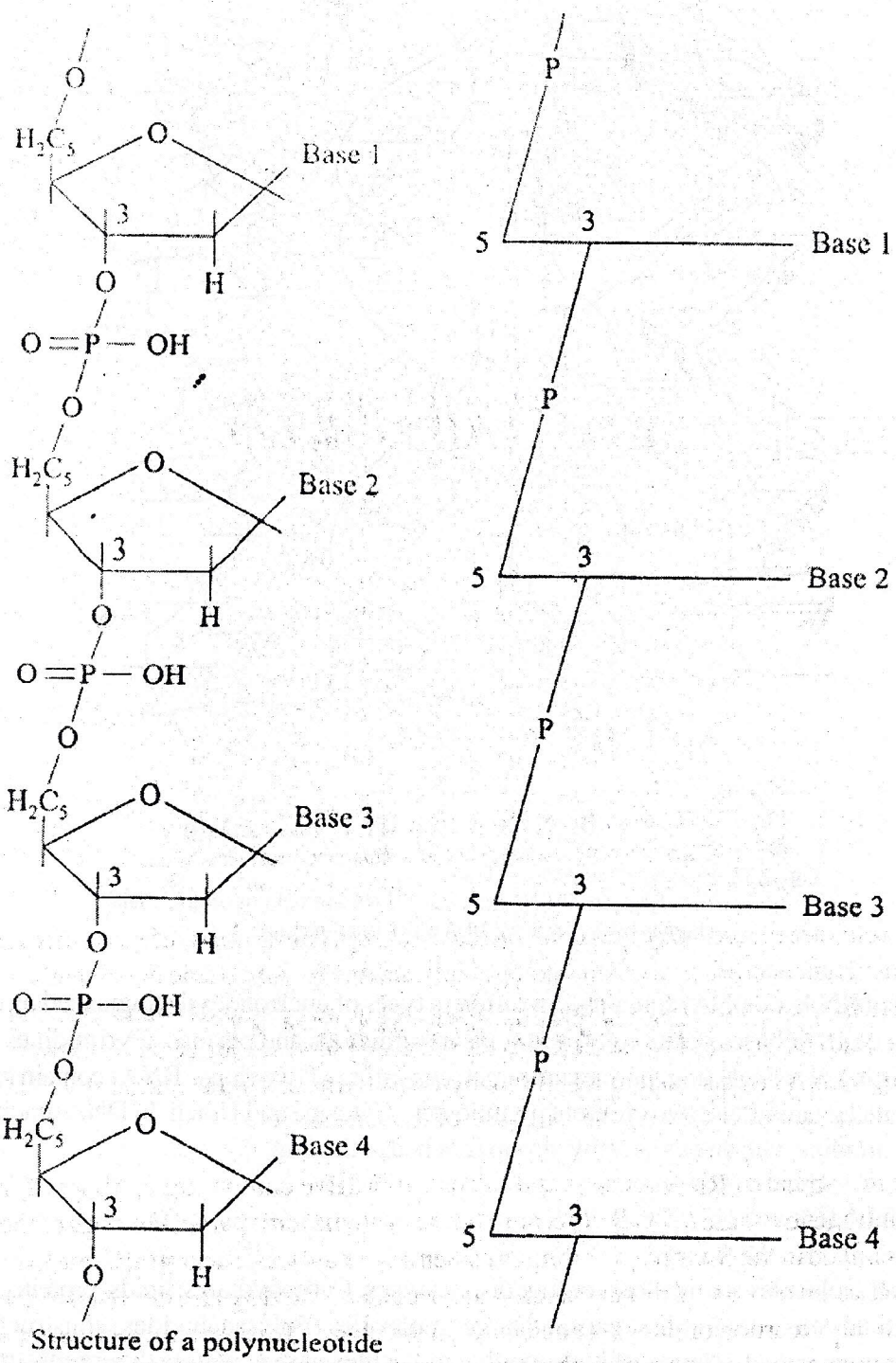


Fig. 1.36 Structure of a polynucleotide showing internucleotide phosphodiester linkages

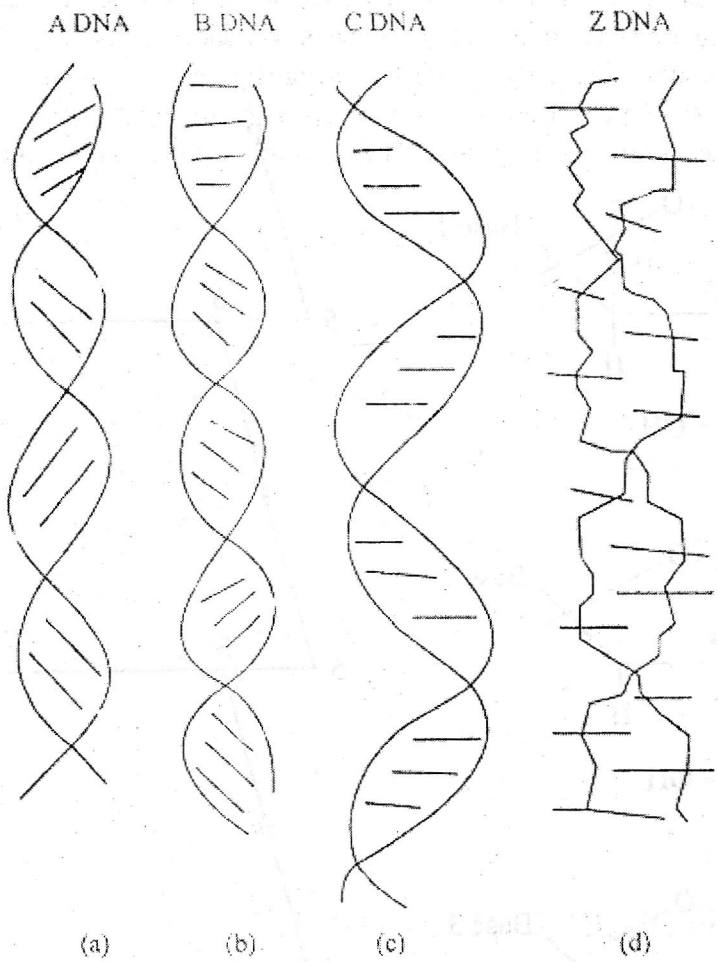


Fig. 1.37 Various conformations of DNA.
(a) A DNA, (b) B DNA, and (C) C DNA are right handed models, whereas the (d) form is Z DNA which is left-handed

A strand of RNA (or DNA) contains four different types of nucleotides distinguished by their nitrogenous base. Two types of bases occur in nucleic acids: pyrimidines and purines. Pyrimidines are smaller molecules, consisting of a single ring; purines are larger, consisting of two rings. RNAs contain two different purines, adenine and guanine, and two different pyrimidines. Cytosine and Uracil. In DNA uracil is replaced by thymine, a pyrimidine with an extra methyl group attached to the ring.

1.1.6 SUMMARY

Biological molecules are members of four distinct types: Carbohydrates, lipids, proteins and nucleic acids. Carbohydrates include simple sugars and larger molecules (Polysaccharides) constructed of sugar monomers. Lipids are a diverse array of hydrophobic molecules having widely divergent structures and functions.

Proteins are macromolecules of diverse function consisting of amino acids linked by peptide bonds into polypeptide chains. The structure of protein can be described at four levels of increasing complexity.

Primary structure is described by the amino acid sequence of a polypeptide; secondary structure by the three dimensional structure (conformation) of sections of the polypeptide backbone; tertiary structure by the conformation of the entire polypeptide; and quarternary structure by the arrangement of the subunits if the protein consists of more than one polypeptide chain. Nucleic acids are primarily informational molecules that consist of strands of nucleotide monomers. Each nucleotide in a strand consists of a sugar, phosphate and nitrogenous base.

1.1.7 TERMINOLOGY

Monosaccharides

Tetroses

Heptoses

Stereoisomers

Glycosidic bonds

Oligosocclaids

Amphipathic

Nucleoproteins

Lipoproteins

Glycoproteins

Disulfide bridge

1.1.8 SELF ASSESSMENT QUESTIONS

1. Describe the structure of nucleotides.
2. Describe the properties of three different types of lipid molecules. What are their respective bi al roles?
3. What are the major properties that distinguish different amino acids from one another? What roles do these differences play in the structure and functions of proteins?
4. Given that proteins act as molecular machines, explain why conformational changes are so important in protein function.

1.1.9 REFERENCE BOOKS

1. **Cell & Molecular Biology** – Gerald Karp.
2. **Cell Biology** – De Robertis & De Robertis.
3. **Biochemistry** – Lehninger.

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UNIT-I

LESSON 1.2

PLASMA MEMBRANE

CONTENTS

1.2.0 Introduction

Although all biomembranes have the same basic phospholipid bilayer structure and certain common functions, each type of cellular membrane also has certain distinctive activities determined largely by the unique set of proteins associated with that membrane. Integral proteins, all or part of which penetrate or span the phospholipid bilayer, and peripheral proteins, do not interact with the hydrophobic core of the bilayer. In this section, we first discuss the basic principles that govern the organization of phospholipids and integral proteins in all biological membranes and then outline the functions of the plasma membrane in prokaryotes and eukaryotes.

The most abundant lipid components in most membranes are phospholipids, which are amphipathic molecules (i.e., they have a hydrophilic and a hydrophobic part). In phosphoglycerides, the principal class of phospholipids, fatty acyl side chains are esterified to two of the three hydroxyl groups in glycerol, and the third hydroxyl group is esterified to phosphate. The phosphate group is also esterified to a hydroxyl group on another hydrophilic compound, such as choline in phosphotidyl choline.

Cholesterol and its derivatives constitute another important class of membrane lipids, the steroids. The basic structure of steroids is the four-ring hydrocarbon cholesterol, the major steroidal constituent on one ring. Although cholesterol is almost entirely hydrocarbon in composition, it is amphipathic because its hy-

droxyl group can interact with water. Cholesterol is especially abundant in the plasma membrane of mammalian cells but is absent from most prokaryotic cells. As much as 30-50% of the lipids in plant plasma membranes consists of cholesterol and certain steroids unique to plants.

Carbohydrates are found in many membranes, covalently bound either to proteins as constituents of glycoproteins or to lipids as constituents of glycolipids. Bound carbohydrates increase the hydrophilic character of lipids and proteins and help to stabilize the conformations of many membrane proteins. The simplest glycolipid glycosyl cerebroside contains a single glucosyl cerebroside, in which a single glucose unit is attached to a ceramide.

1.2.1 Objectives

1) To study the structure and chemistry of plasma membrane
2) The study its role in various biological processes such as transport across the membrane.
3) To study the Properties of the plasma membrane.
4) To study the Chemical composition of plasma membrane.
5) To study the Structure of plasma membrane.
6) To study the Functions of plasma membrane.

1.2.2 Properties

Common features of Biological Membranes:

Membranes are as diverse in structure as they are in function. However, they do have in common a number of important attributes:

1. Membranes are sheet like structures, only a few molecules thick that form closed boundaries between different compartments. The thickness of most membranes is between 60A° (6 um) and 100A° (10 nm).
2. Membranes consist of mainly lipids and proteins. Their mass ratio ranges from 1:4 to 4:1. Membranes also contain carbohydrates that are linked to lipids and proteins.
3. Membrane lipids are relatively small molecules that have both a hydrophilic and a hydrophobic moiety. These lipids spontaneously form closed bimolecular sheets in aqueous media. These lipid bilayers are barriers to the flow of polar molecules.
4. Specific proteins mediate distinctive functions of membranes. Proteins serve as pumps, channels, receptors, energy transducers, and enzymes. Membrane proteins are embedded in lipid bilayers, which create suitable environments for their action.
5. Membranes are non-covalent assemblies. The constituent protein and lipid molecules are held together by many non-covalent interactions, which are cooperative.
6. Membranes are asymmetric. The two faces of biological membranes always differ from each other.

7. Membranes are fluid structures. Lipid molecules diffuse rapidly in the plane of the membrane, as do proteins, unless they are anchored by specific interactions. Membranes can be regarded as two-dimensional solutions of oriented proteins and lipids.
8. Most membranes are electrically polarized with the negative charge inside (typically-60 milli volts). Membrane potential plays a key role in transport, energy conversion and excitability.

Plasma membrane is defined as an elastic delimiting membrane that seperates the cytoplasmic contents from other cells and acts as a selective permeable membrane for the passage of various ions and other substances.

The term cyotplasmic membrane was coined by "Cramel & Nageli". It was named as plasma membrane by "Plaver". Membranes are highly selectively permeable barriers rather than impermeous walls, because they contain specific molecular pumps and gates. Eukaryotic cells also contain intermembranes that form the boundaries of organelles such as mitochondria, chloroplast, lysosomes etc. Some membrane generate signals which can be electrical or chemical. Membranes also contain specific receptors for internal stimuli.

Ex: In the response of target cells to hormones, the membranes play a central role in biological communication.

The two most important energy conversion processes in biological systems are carried out by membrane systems.

1.2.3.1 Photosynthesis

In photosynthesis, light energy is converted to chemical bond energy and it occurs in the inner membranes of chloroplast.

1.2.3.2 Oxidative phosphorylation

In oxidative phosphorylation, ATP is formed by oxidation of fuel molecules in the inner membrane into mitochondria.

1.2.4 Chemical composition of plasma membrane

Membranes are composed of mainly different classes of lipids and proteins which are special non-bounded combinations. Membranes can be regarded as two dimensional solutions of oriented proteins and lipids.

1.2.4.1 Proteins

Membrane contains 3 different classes of proteins based on the function:

1. Structural proteins
2. Enzymes
3. Carrier proteins or permeases

1.2.4.1.1 Structural Proteins

These form the backbone of cellular membrane. The plasma membrane consists of largely structural proteins. Average molecular weight of structural proteins is 3×10^4 daltons. They may not have any catalytic function.

1.2.4.1.2 Enzymes

These form the major component of any membranes and are catalytic in function. Endoplasmic reticulum, mitochondria and plasma membrane contain many enzymes. Important enzymes of Plasma membrane are Na^+ K^+ activated - ATPase, alkaline phosphatase, Mg^{2+} ATPase, RNases. The most important enzymes of plasma membrane is Na^+, K^+ ATPase, Mg^{2+} ATPase. It regulates the transport of different ions across the membranes.

1.2.4.1.3 Carrier Proteins

It transports substances across the membrane against the concentration gradient.

Membranes differ in their protein content. Myelin sheath contain a low content of proteins (18-20%). The remaining is lipid which is well situated for insulation. The plasma membrane contains 50% proteins and other lipids where as Mitochondria and chloroplast membranes are made of 75% protein. The plasma membrane protein fall into 2 categories. They are:

1. Extrinsic or peripheral proteins
2. Intrinsic or integral proteins

The integral proteins are firmly associated with lipid molecule while the extrinsic have weaker association with lipids. It is not easy to remove the intrinsic protein from cellular membrane.

Human erythrocyte glycoprotein consists of 3 distinct regions.

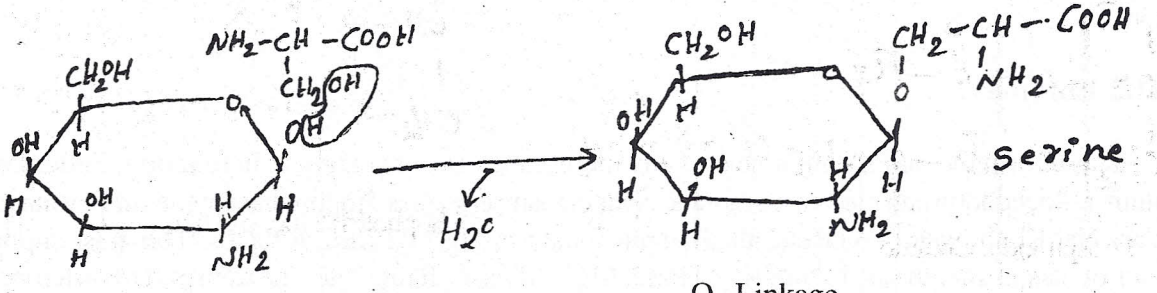
1. N-terminal part:- Which is external to membrane containing mostly CHO moiety.
2. Middle hydrophobic region that is present within the membrane.
3. A hydrophilic C-terminal protein rich in proline but lack in CHO is present in the inner side.

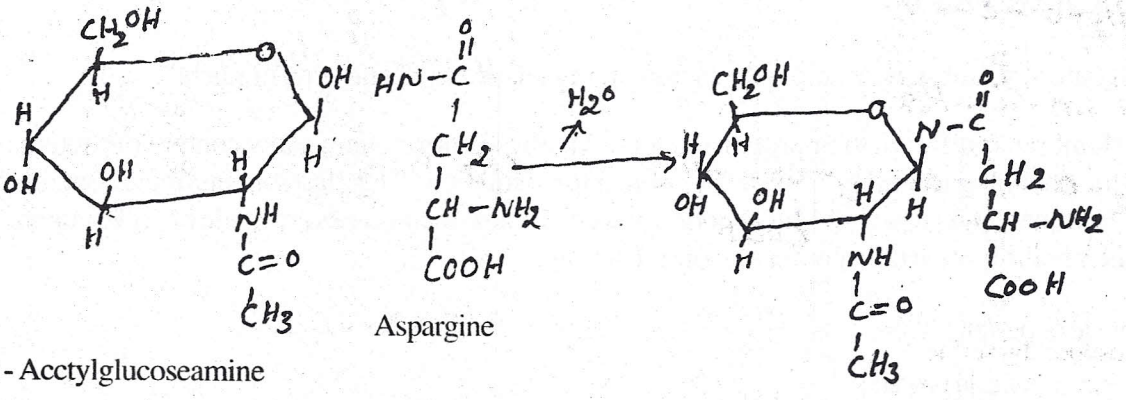
The Glycoprotein of human erythrocyte has a polypeptide back bone which is attached by two types of oligosaccharide units.

1. Straight chain oligosaccharide
2. Branched chain oligosaccharide

Glycoprotein: Membrane glycoprotein of erythrocytes contains 60% of CHO's by weight in the form of 16 oligosaccharide chains, out of which 15 are attached by O-linkages to serine and threonine and one is linked

to Aspargine by an N-linkage. In case of O-linkages the sugar is galactosamine and in N-linkages the sugar is N-acetyl glucosamine.





1.2.4.2 Lipids

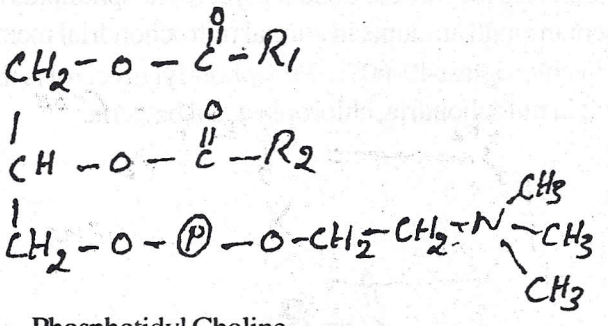
The lipids are esters of fatty acids with glycerol and higher alcohols. The lipids of cellular membrane are polar which contain hydrophilic head and hydrophobic tail. Such molecules are called as amphipathic molecules. The neutral lipids does not exist in plasma membrane but occasionally occurs in cytosol.

The 3 major classes of lipids are:

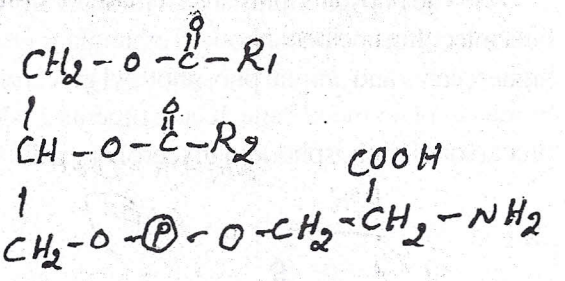
1. Phospholipids
2. Glycolipids
3. Cholesterol

1.2.4.2.1 Phospholipids

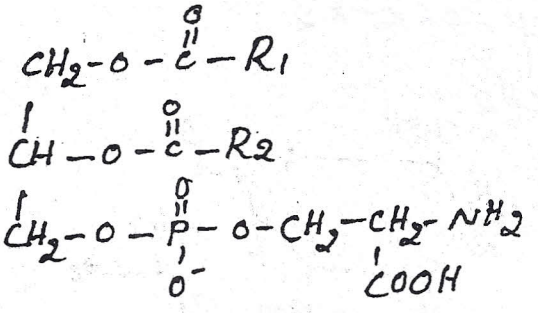
The phospholipids present in plasma membrane are phosphotidyl serine, phosphotidyl choline, phosphotidyl ethanolamine, plasmalogens, cardiomylins, sphingomyelins.



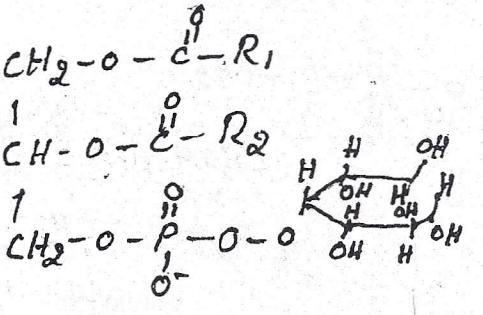
Phosphotidyl Choline



Phophotidylethonolamine

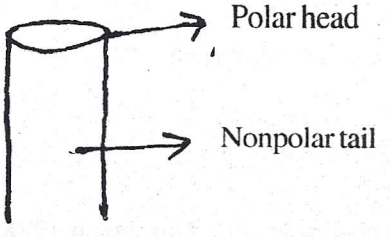


Phosphotidyl serine

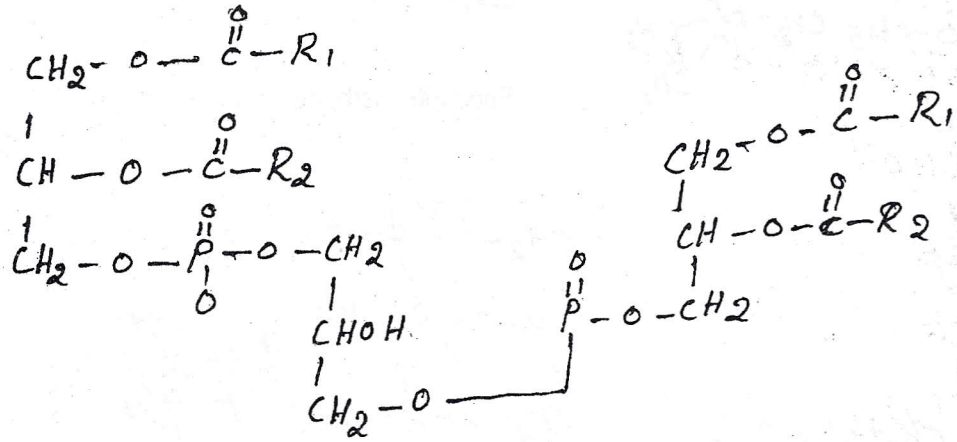


Phophotidyl Inositol

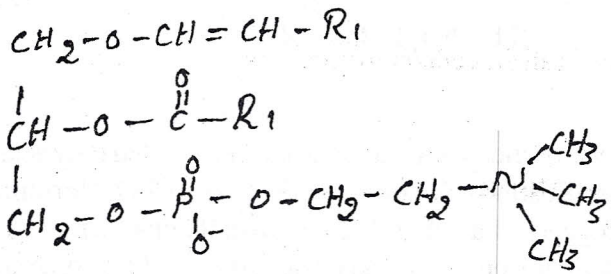
In glycerol, the two hydroxy groups are generally esterified with saturated and unsaturated fatty acids where as the terminal hydroxy groups undergoes esterification with phosphoric acid (H_3PO_4) forming phosphotidic acid. The H_3PO_4 inturn attaches to any nitrogenous base like choline, ethanolamine, serine etc., to form polar head of the lipids. Where as the non-polar tails are long chain fatty acids among which some are saturated and the other unsaturated.



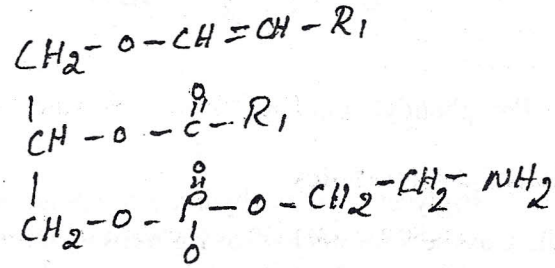
The phospholipids are asymmetrically arranged in human erythrocyte membrane. Phosphotidyl choline or lecithin and phosphotidyl ethanolamine (or) cephalins are the most abundant glycerophospholipids in higher plants and animal phosphotidyl glycerol are present in small amounts in animal mitrochondrial membrane. In plant membrane, it constituents 20-30% and in chloroplast 40-60%. Phosphotidyl glycerol is the precursor of diphosphotidyl glycerol or cardiolipin found in mitochondria, chloroplast and bacteria.



Cardiolipin



Phosphotidyl choline



Phosphotidyl ethanol amine

Plasmalogen:

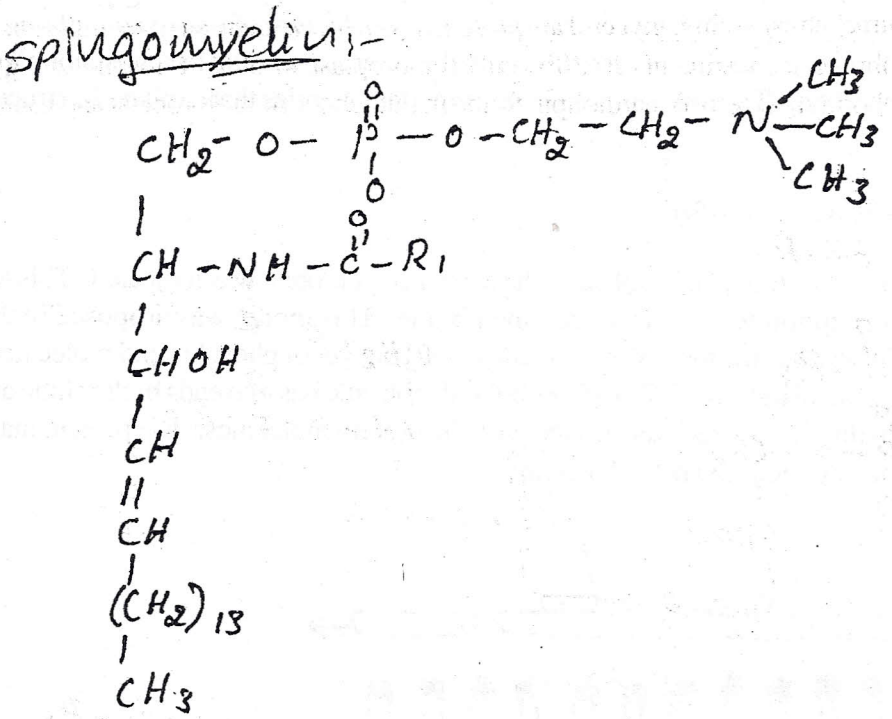
Two types of plasmalogens are present in cellular membrane.

Phosphotidol choline

Phosphotidol ethanolamine

Phosphotidol choline is abundant in heart muscles and absent in myelin sheath. Phosphotidal ethanolamine is abundant in myelin sheath and absent in heart muscles

Spingomyelin:



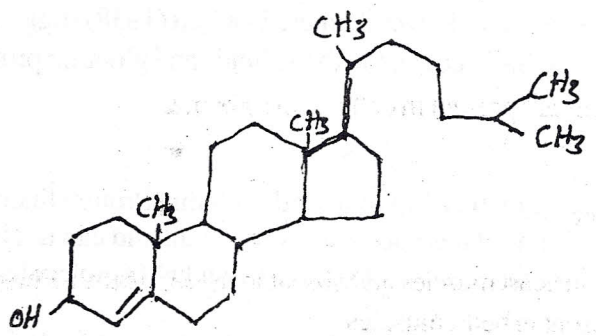
The Spingomyelins are distributed in all the cellular membranes.

1.2.4.2.2 Glycolipids

These are found to be present in brain and nervous tissue.

1.2.4.2.3 Cholesterol

These are present in abundant concentration in eukaryotic cellular membrane where as other subcellular organelles have less cholesterol content.

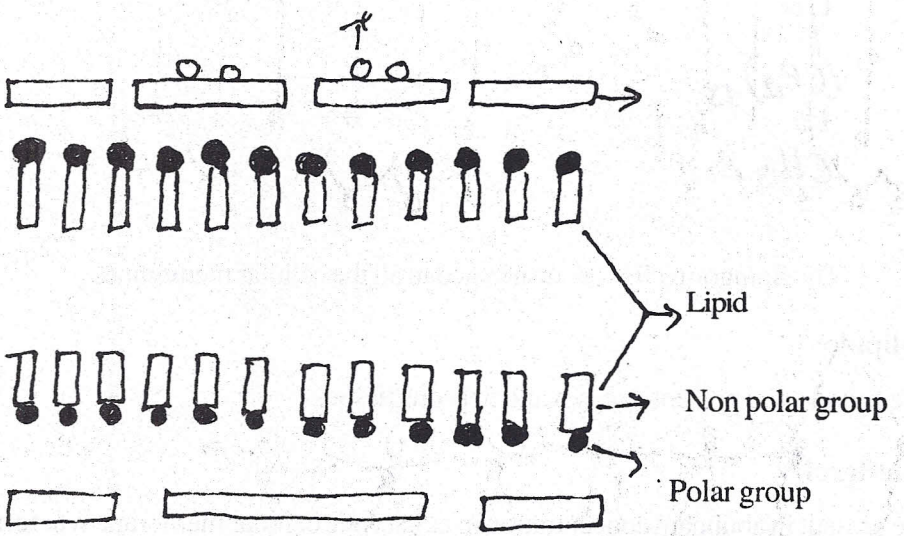


1.2.5 Structures of Plasma membrane (or) Molecular models

Many theories have been put forward regarding the structure of plasma membrane. Most of the theories states that the protein molecules are present on the outer surface and the phospholipidmolecules are sandwitched between them. The following are some important theories that explain the structure of plasma membrane.

1.2.5.1 Danielli – Daveson model

By studying the surface tension of cells, the existence of protein was indicated. This lead Daveson-Danielli to propose a lipoprotein model of cell membrane. This model was proposed in the year 1935. According to this theory, plasma membrane is made up of a bilayer of phospholipid molecules sandwiched between 2 layers of protein molecules. The phospholipid molecule has two ends hydrophilic and hydrophobic ends of which hydrophilic ends is associated with the protein molecules. The protein may arranged as folded protein, globular protein and helical protein.



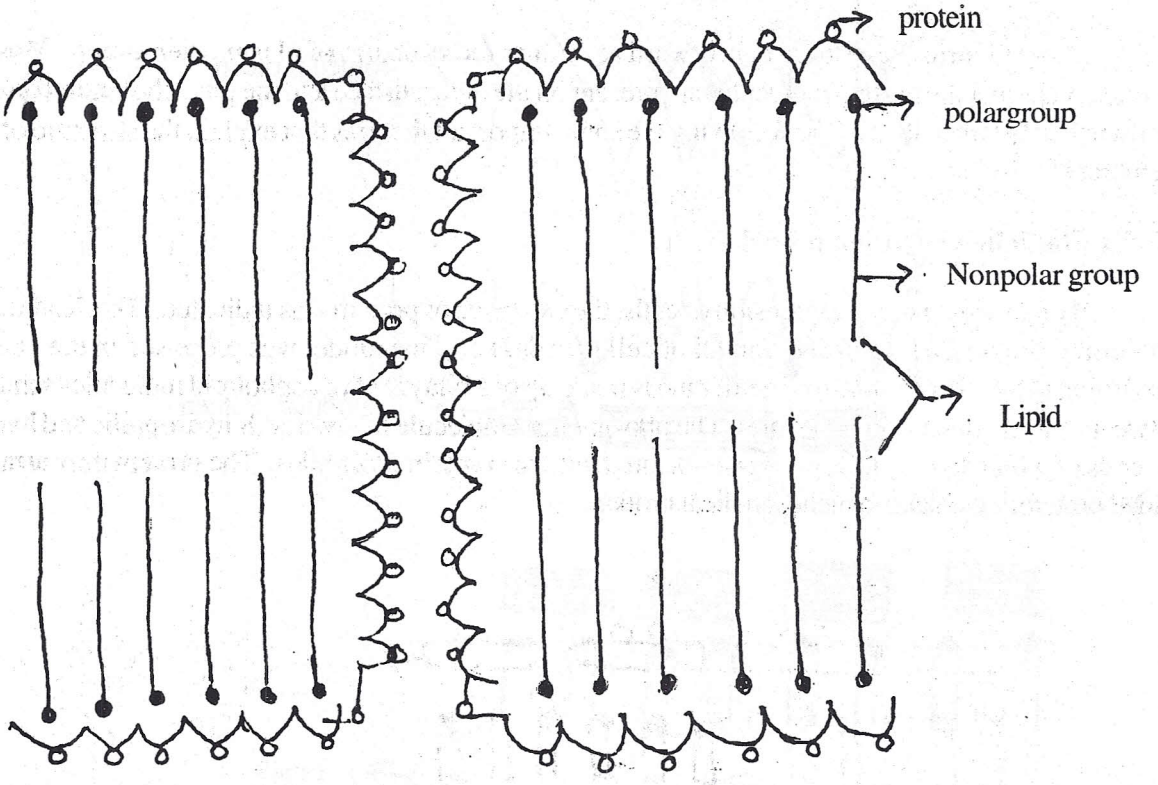
The Danicllli - Daveson Trilamillar sand wich model of cell membrance

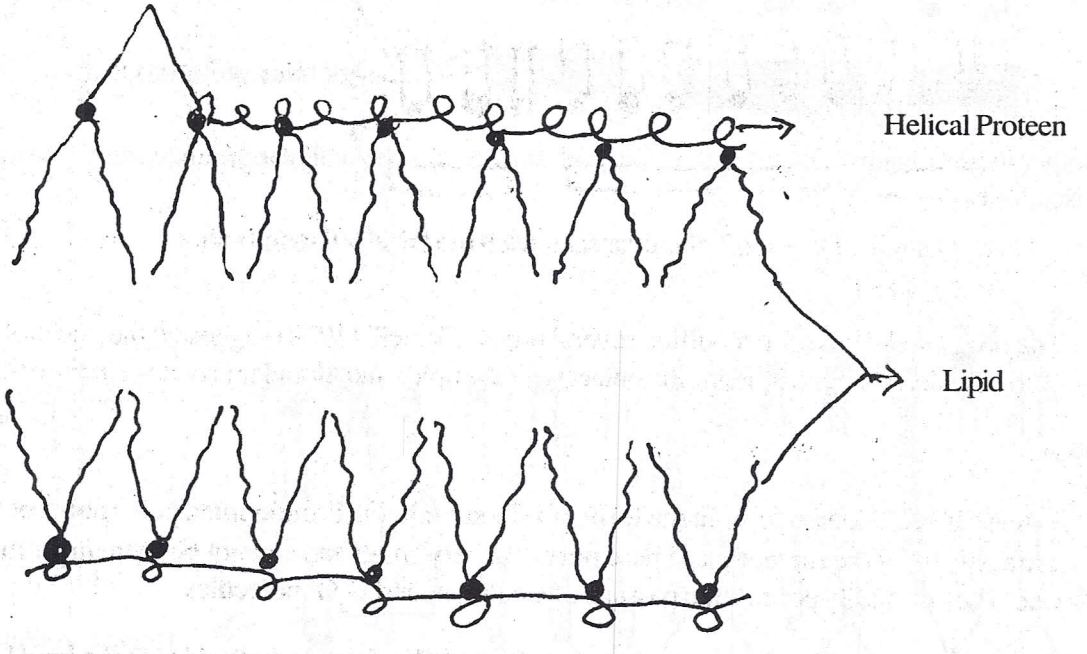
The basic model has been modified several times. Danielli (1938) suggested that the proteins are of 2 types. Tangentially arranged proteins in contact with the lipids and globular proteins, on the outer surface.

Variations:

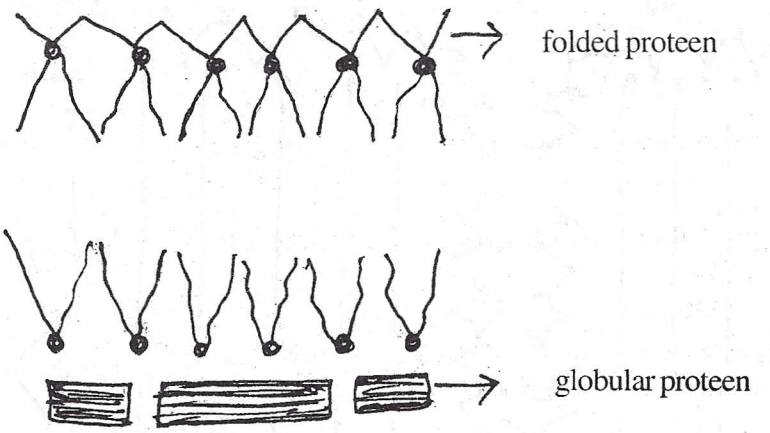
1. The proteins are considered to be in the form of folded b-chain. Protein lined polar pores of about 7A° diameter are present in the membrane. These pores are very small and can not be seen under the electron microscope. They probably permits the passage of small ions and H_2O molecules.

2. In still other variations, the proteins are thought to be coiled a-form on both sides of the lipid layer.

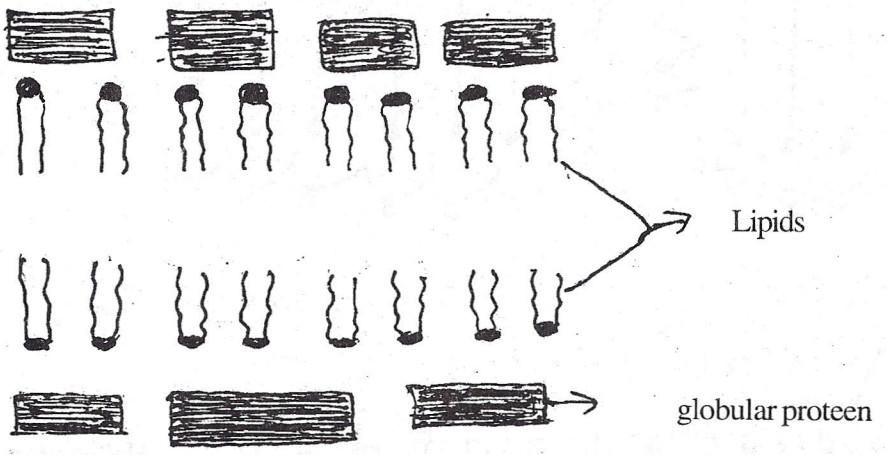

protein
polargroup
Nonpolar group
Lipid


Helical Proteen
Lipid

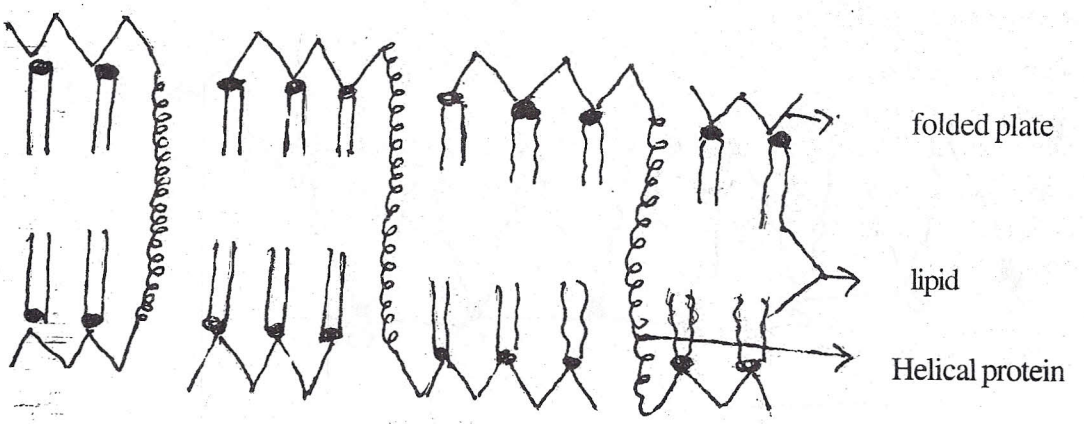
3. It is thought to be asymmetrical with a folded b-chain on one side and globular protein on the other side.



4. Models with globular proteins on both surfaces



5. Another model contains folded proteins on both surfaces and helical proteins extending into the pores are also visualized

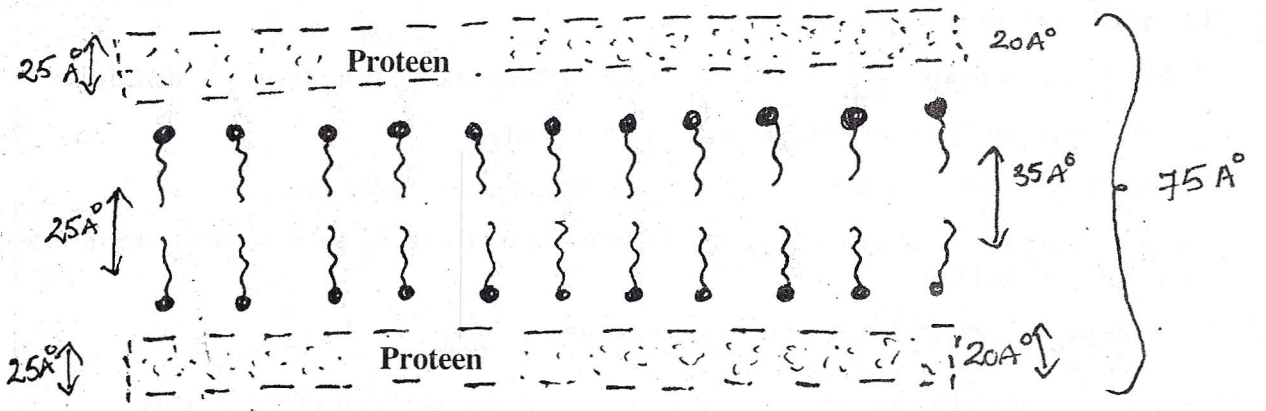


1.2.5.2 Unit membrane model

In 1953, Robertson put forward this unit membrane model. It was considered to be general for a wide variety of plants, animals and also for organelles like mitochondria, chloroplast, lysozymes, golgicomplex etc.

The unit membrane model considered to be trilamellar with a six molecular lipid layer between two protein layers.

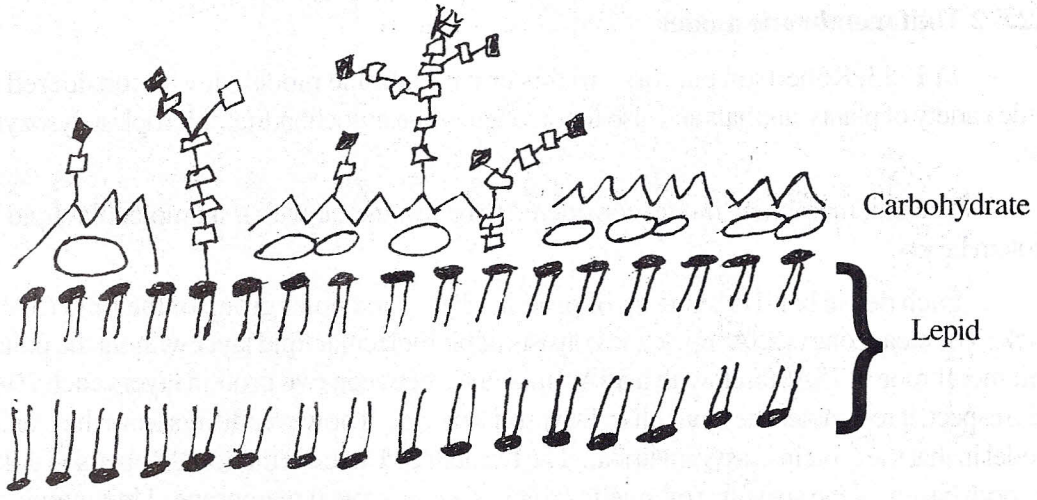
Each dense band is made up of protein (20A°) and polar group of the lipid (5A°) and is thus 25A° thick. The clear zone is 25A° thick and consists of biomolecular lipid layer without the polar groups. Thus the unit membrane is 75A° thick with a 35A° lipid layer between two protein layers each 20A° in thickness. In this respect, it resembles the Daneilli & Daveson's model. It however differs from the Danielli and Daveson's model in that the protein is asymmetrical. The Danielli & Daveson model & Robertson unit membrane model are both based on the structure of myelin which is a non-typical membrane. Unit membranes vary a little in total thickness. The plasmalemma surrounding the cell is thicker at the free surfaces of cells than when it is in contact with other cells. Plasma-lemma is thicker than the unit membrane of endoplasmic reticulum.



The Unit membrane model

1.2.5.3 Greater membrane model

This model resembles the trilaminar model in that a lipid layer is sandwiched between two layers of structural proteins. Robertson however believed that the inner and outer surface of the membrane were different. The internal surface of the membrane is covered with conjugated protein and the outer surface with Glycoprotein which is a superimposed on the structural protein. Attached to glycoprotein are oligosaccharides side chains with negatively charged sialic acid terminals. Thus the membrane is asymmetrical and has structural polarity.



1.2.5.4 Karanu's lipid pillar Model

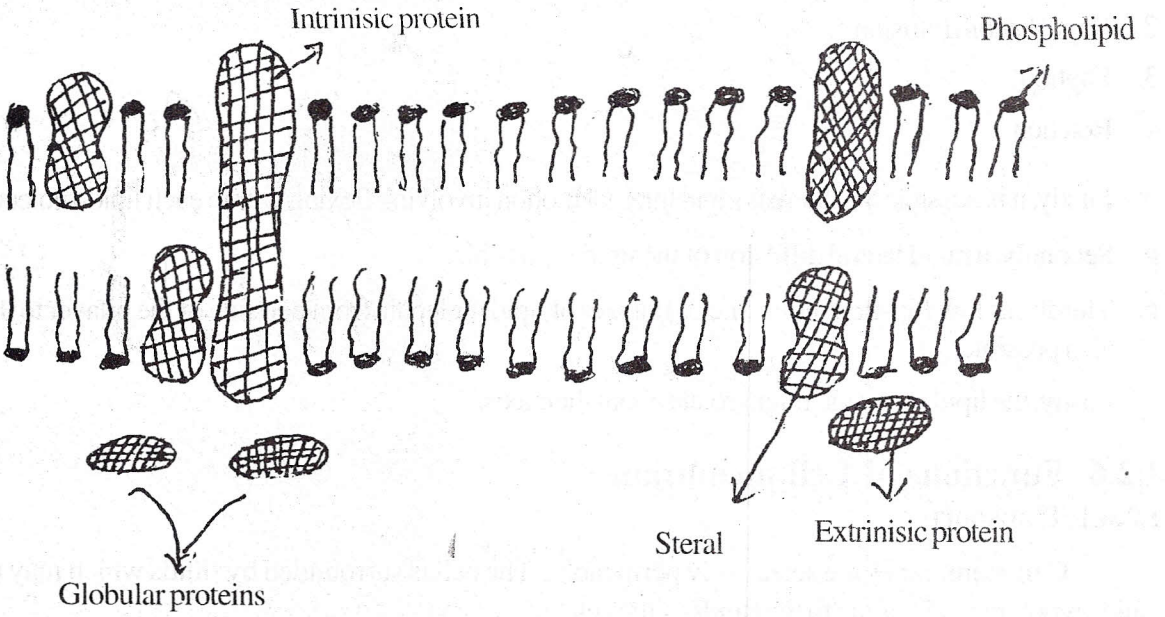
1. This is the modification of the Danielli & Daveson model.
2. This lipid layer exist in two forms.
3. Under certain conditions, it is in form of pillars while in the others, it is in the form of flattened disc.
4. The space between the pillars form pores for the passage of ion.
5. The membrane with pillar structure is thicker than the membrane with disc structure.
6. The interior of each pillar is formed of the non-polar tails of the phospholipids. While the surface is formed of the polar heads.
7. Protein layer are present on both sides of the membrane.
8. The model can not explain active transport (or) the differential permeability of Na^+, K^+ ions.

1.2.5.5 Benson's Model

Benson in 1966 proposed a model on the basis of study of chloroplast membrane.

- The membrane lipids and proteins have a hydrophobic association.
- The lipid tails are bound by hydrophobic regions with complementary hydrophobic regions within the interior of proteins.
- The charged polar heads of the phospholipids lie on the surface of the membrane and are capable of binding ions.
- Flexion of contractive proteins facilities ions transport.

1.2.5.6 The fluid mosaic model of singer & Nicolson



Schematic representation of a section through a eukaryotic fluid-mosaic membrane.

The fluid mosaic model of Singer & Nicolson (1972) is now widely accepted as it best explains the properties of cell membrane. This model tells that there is a continuous bilayer of phospholipid molecules in which globular proteins are embedded. The proteins have been compared to icebergs floating in a sea of the phospholipid bilayer. Thus biological membrane are considered to be quasifluid structure in which lipids and integral proteins are arranged in a mosaic manner. While the Danielli – Daveson model assures hydrophilic bonding between lipids and proteins the Singer & Nicolson model consider the lipid-protein association to be hydrophobic. It should be noted that the phospholipids and many intrinsic proteins are amphipathic molecules i.e., both hydrophilic and hydrophobic groups occur within the same molecule.

The globular proteins of the membrane are considered to be of two different types:

1. Extrinsic (or) peripheral proteins
2. Intrinsic (or) integral proteins

The peripheral proteins are soluble and readily dissociate from the membrane. They are entirely outside the lipid bilayer. The integral proteins are relatively insoluble and dissociate with difficulty. These are amphipathic. These are capable of lateral diffusion in the lipid bilayer. When phospholipids are dispersed in H_2O they form a lipid bilayer. The polar heads of the lipid molecules project into the aqueous phase. The hydrophobic chains aggregate together. Studies with nuclear magnetic resonance (n.m.r) and electron spin resonance (e.s.r.) techniques indicates that the lipid bilayer has many dynamic motional properties.

Motional properties:

1. Flexion
2. Rapid lateral diffusion
3. Flipflop
4. Rotation

- Firstly, it is possible that there is rapid internal motion involving flexing within each lipid-molecule.
- Secondly, a rapid lateral diffusion of the lipid is possible.
- Thirdly, as low flip-flop motion i.e., a transfer of lipid molecule from one side of the bilayer to the other is also possible.
- Lastly, the lipid molecule might rotate about their axis.

1.2.6 Functions of Cell membrane

1.2.6.1 Transport

Cell membranes are selectively permeable. The cell is surrounded by fluids which may be hypertonic, hypotonic or isotonic to the fluids of the cell.

The membrane regulates the passage of certain nutrient molecules into the cell and removal of waste products. All transport of molecules across the membrane may be passive or active. Both these systems involves selectively a carrier molecule.

1.2.6.2 Passive transport

Passage of molecules through the membrane from a high concentration to low concentration energy is not required for this process. It is of 2 types:

1. Simple diffusion: It takes place without the aid of carrier molecule and does not involve any stereo specificity, i.e., L&D isomers may cross at equal rates. It is a slow process.

Ex: Transport of Xylose & Arabinose across the intestinal epithelium.

1.2.6.3 Facilitated diffusion

The process of stereospecificity requires a carrier molecule. It shows starvation kinetics (increase in the concentration of substance to be transported results in an increased rate of transfer upto the asymetric value). The carriers are proteins with relatively low mol. wt. (9000-10,000). The carrier proteins are specific for individual sugars, Ca, Na, K. Proteins move to & fro across the membrane by thermal diffusion.

Ex:- Entry of glucose into erythrocytes. Metabolites bind to carrier proteins at the outer surface to form carrier metabolites complex. This diffuses along the concentration gradient.

1.2.6.4 Active transport

Molecules move from region of low concentration to high concentration i.e., against concentration gradient. It requires energy mainly supplied by ATP.

Primary active transport: It is directly related with the chemical energy (electron flow).

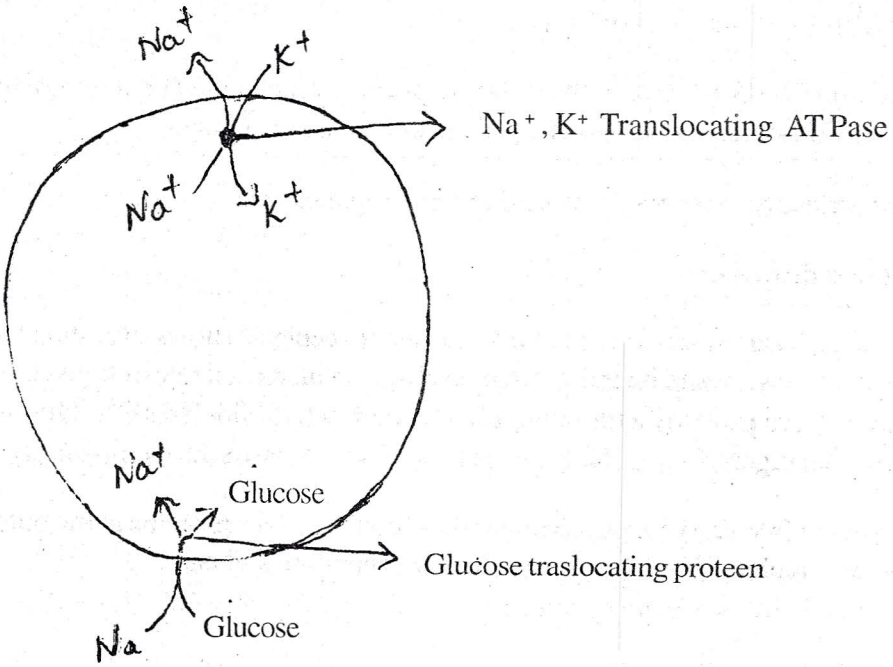
Ex:- Na^+, K^+ translocating ATPase in mammals and protein translocating ATPase in bacteria.

The existence of Na, K pumps has been demonstrated in eukaryotic cells. "Na" is pumped out of the cell by "Na" pump & "K" is pumped into the cell by a coupled process and the two pumps apparently operate simultaneously. In the absence of either Na or K, the movement of both Na & K ceases.

1. Na & ATP approach the inner surface of the ATPase complex.
2. Na & ATP bind to specific sites on ATPase.
3. Phosphorylation of ATPase results in confirmational changes in ATPase and in the binding sites for Na & K.
4. Dephosphorylation results in reversal of confirmation.

It has been suggested that the essential part of the ion transport ATPase includes cyclic phosphorylation and dephosphorylation initiated by Na & K respectively. Na & ATP binds to specific site of ATPase complex. Bound Na is required for phosphorylation of ATPase. This leads to confirmational change resulting in reorientation of enzyme with the membrane causing Na, to pass through membrane. Similarly protein dephosphorylation results in reversion to original confirmation & "K" is transported.

1.2.6.5 Secondary active transport



Ex: Glucose transport system of intestinal epithelium of mammals. The free surface of intestinal epithelium has numerous microvilli which are formed by production of brush border membranes. Primary transport results in Na being pumped out of the cell and "K" into the cell. The electrochemical Na^+ gradient can then utilized for secondary active transport of glucose or amino acid into the cell against the concentration gradient. Thus there is Na-glucose cotransport catalysed by a glucose carrier protein.

The "Na" pump maintains a higher concentration of "Na" outiside the cell than on the inner side. This results in a tendency for "Na" to enter the cell. Thus it does in the form of a carrier sugar or carrier amino acid complex.

1.2.6.6 Group-Translocation

® PEP + Enzyme – (I) ® Enzyme I – (P) + pyruvate

® Enzyme –I- (P) ® Hpr ® Hpr – P + Enzyme-I

® Hpr – P + Sugar $\xrightarrow[\text{Enz-II}]{}$ Sugar – P + Hpr (Heat stable protein)

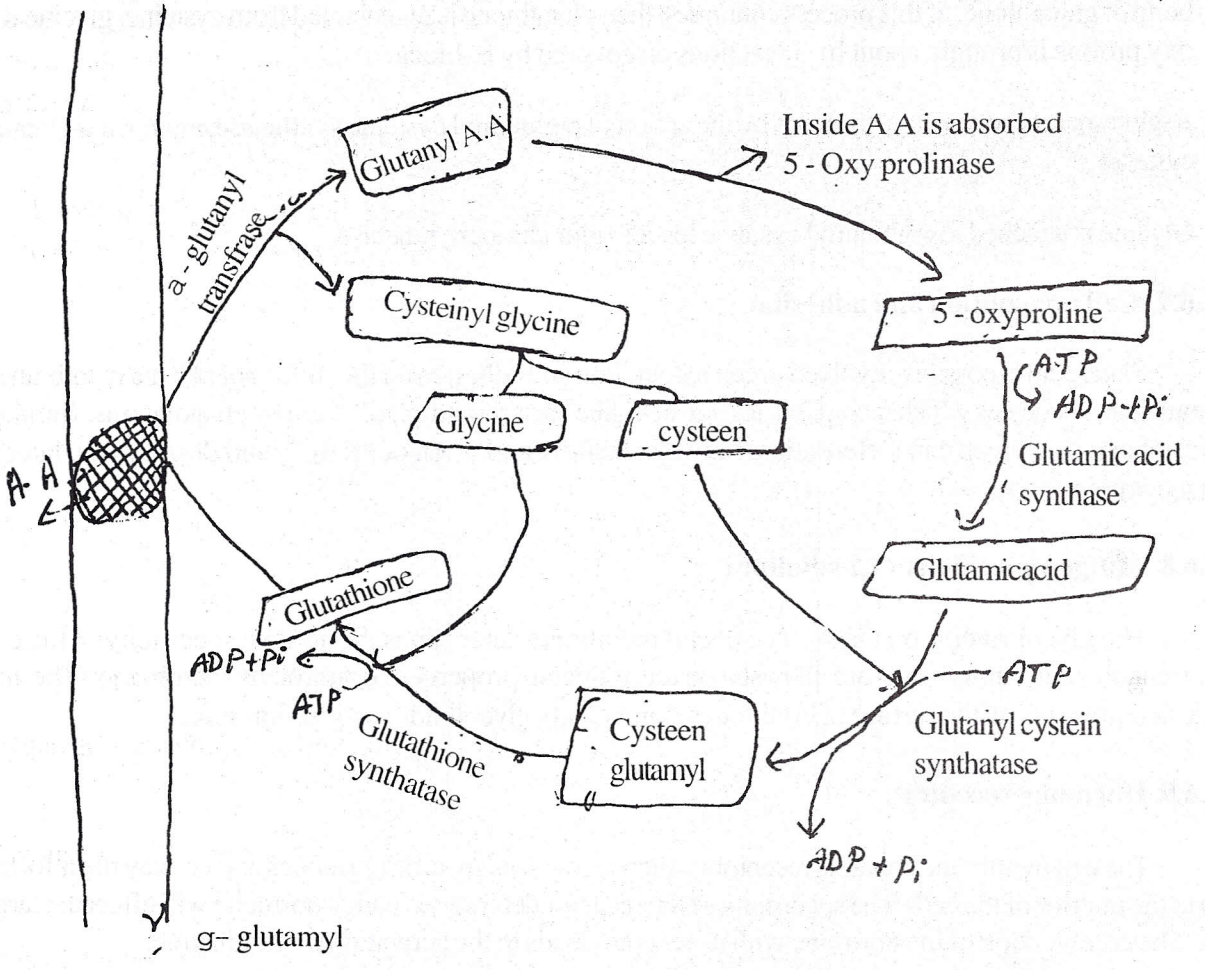
In this process, the substrate is altered by the enzyme that catalyses membrane transport.

Ex:- Transport of fructose, mannitol etc., takes place across the bacterial membranes through the phosphotransferase system (PTS).

PTS always uses PEP as the energy form. The components of PTS or enzyme-I, PTS_2 or enzyme II & Hpr.

PEP donates its phosphorous to enzyme-I forming pyruvate & enzyme-I-(P). This inturn donates to Hpr. Both enzyme and Hpr are general PO^-_4 carriers proteins. Enzyme-II does not participate in phosphorylation but only catalyses the phosphorylation of sugar. The phosphorylated sugar is sent into the cell.

Ex:- Glutamyl cycle:



g-glutamyl cycle was developed by A. Meister and his colleagues for the mechanism of transport of amino acids into the cells of certain animal tissue. It is an example of group translocation in which the substrate transported appears in a different chemical form inside the cell. The g-glutamyl cycle involves a sequence of 6 enzymes, one of which is membrane bound and the remaining are present in the cytosol. g-glutamyl cystein glycine or glutathione are present in higher concentration in animal tissue.

The membrane bound enzyme, g-glutamyl transferase plays a key role in catalysing the reaction which results in the transfer of the glutamyl residue of glutathione to the incoming aminoacid.

amino acid + Glutathione ® g-glutamyl amino acid + cysteinyl glycine

All the aminoacids except proline can accept the g-glutamyl group. The free amino acid comes from outside the cell and glutathione from inside. The g-glutamyl amino acid is discharged in the cytosol as is the cysteinyl glycine. g-glutamyl aminoacid undergoes cleavage to yield the free aminoacid & 5-oxy preproline catalysed by g-glutamyl cyclo-transferase a cytosol enzyme. Cysteinyl glycine undergoes hydrolysis by the action of enzyme peptidase to form cysteine and glycine.

One molecule of aminoacid is transported into the cell at the expense of hydrolysis of the peptide bond of glutathione. If this process continues, then glutathione is regenerated from cysteine, glycine & 5-oxy proline is brought about by 3 reactions discovered by K-block.

- a-glutamate reacts with a-cysteine by the action of g-glutamyl cysteine synthetase and form g-glutamyl cysteine.
- Glycine is attached to g-glutamyl cysteine to yield glutathione synthetase.

1.2.6.7 Cell recognition and adhesion

Sialic acid is possibly involved in cell recognition and adhesion on the surface of plasma membrane of mammalian leucocytes which recognises foreign cells like bacteria and engulf them by phagocytosis. Similarly macrophages of spleen can differentiate between healthy and worn out RBC's and destroys the later by phagocytosis.

1.2.6.8 Antigen specificity - Cardiolipin

The glycoproteins on the surface of cell membrane determines the antigen specificity of the cell. Different blood group systems are all based on the antigenic property of erythrocyte membrane. The antigenic determinants on the surface of erythrocytes are mainly glycolipids and glycoproteins.

1.2.6.9 Hormone receptors

The cell membrane contains receptors which recognises specific hormones and convey the information to the interior of the cell. The specificity of the receptor determines which hormone will effect the target cell. The combination of the hormone with its receptor leads to the activation of the enzyme.

1.2.6.10 Endocytosis

It is the process by which the material is transported into the cell by forming vesicles. It is of two types: Phagocytosis and pinocytosis.

1.2.6.10.1 Phagocytosis (Cell eating)

It involves folding of plasma membrane around the material i.e., being engulfed and subsequent formation of an intracellular vesicles called phagosomes. It fuses with primary lysosome to form secondary lysosome which is having digestive enzymes. In protozoans, it serves for nutritional status of cell. In metazoans it serves as a method of defence against foreign bodies like bacteria. The residual undigested material is ejected out by exocytosis (The reverse process by which the membrane lined material is removed from the cell).

1.2.6.10.2 Pinocytosis

It is intake of fluid material into the cell by the formation of pinocytic vesicles or pinosomes. Both these processes requires the energy.

1.2.7 Summary

Biological membranes are sheath like structures typically 75°A thick, that are composed of proteins and lipids held together by non-covelent interactions. Membranes are highly selective permeability barriers. They create closed compartments. Pumps and gates in the membrane regulate the molecular and ionic compositions of these compartments. Membranes control the flow of information between the cells.

The major classes of membrane lipids are phospholipids, glycolipids and cholesterol. A common feature of these membranes lipids is they are amphipathic molecules. They spontaneously form extensive bimolecular sheets in aqueous solutions because they contain both hydrophilic and hydrophobic moieties. These lipid bilayers – are highly impermeable to ions and for most polar molecules.

The functions of plasma membrane such as transport, communication and energy transduction are mediated by specific proteins. Integral proteins span the lipid layer where as peripheral proteins are bound to membrane surfaces by electrostatic and hydrogen bond interactions. Membranes are dynamic structures in which proteins and lipids diffuse rapidly is the plane of the membrane. The degree of fluidity of a membrane partly depends on the lipids.

1.2.8 Terminology

1. Plasma membrane
2. Lipids
3. Phospholipids
4. glycolipids
5. proteins
6. Integral or Intrinsic proteins
7. Extrinsic or peripheral protein
8. Amphipathic
9. Photosynthesis
10. Phagocytosis
11. Pinocytosis
12. Passive transport
13. Active transport
14. Group translocation
15. Rotation

5)

1.2.9 Self Assessment Questions

1. What are different functions of a plasma membrane and what is the importance of it as a biological system?
2. What are the chemical constituents of a plasma membrane and explain its structure?
3. Explain the different models proposed to explain the structure of plasma membrane.
4. Explain the structure and composition of lipid bilayer of a typical cell.
5. What are the different transport mechanisms for transport of ions and the molecules in and out of the cells.

1.2.10 Reference Books

1. **Genes VII** – Benjamin Lewin, 17th Edition, Oxford University Press.
2. **Biochemistry** - Lubert Stryer, 4th Edition, W.H. Freeman and Company, New York.
3. **Biochemistry** - Lehninger, Nelson and Cox, 3rd edition.
4. **Microbiology** - Ronald M. Atlas, 2nd edition.

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UNIT-I

LESSON-1.3

ENDOPLASMIC RETICULUM AND GOLGI COMPLEX

CONTENTS

1.3.1.0 INTRODUCTION

Endoplasmic Reticulum [ER] forms a continuous system, connected with plasma membrane on one hand and on the other hand with nuclear membrane for performing the metabolic activities of the cell. One expectation is that evagination of the plasma membrane of cell which might result in the formation of a canalicular system, but there is no full evidence that such a process occurs.

During cell division, after karyokinesis, ER forms the nuclear envelope. And hence it was assumed that the ER is formed by the evagination of nuclear membrane.

Fig. 1.3.1 Origin of Endosplamic Reticulum

Endoplasmic Reticulum is the main component of the Endomembrane system, also known as the Cytoplasmic Vacuolar System [made up of Nuclear envelope, Endoplasmic reticulum and Golgi Complex].

ER is the interconnected network of tubular and microsomal vesicular structures in the cytoplasm of the cell. The appearance of ER differ among different eukaryotic cells but it always forms a system of fluid-filled sacs enclosed by the membrane network. So, this ER can be interpreted three-dimensionally as a vast network that subdivides the cytoplasm into two main compartments – one enclosed within the membranes, and the other situated outside and constituting the cytoplasmic matrix (or) cytosol.

1.3.1.1 Objectives

- To study the history of Endoplasmic Reticulum
- To study the occurrence of morphology of ER
- To study the types of ER and Isolation of ER
- To study the functions of ER

1.3.1.2 Definition

KEITH PORTER – defined Endoplasmic Reticulum as – a complex, finely divided vascular system extending from the nucleus through out the cytoplasm to the margins of the cell.

1.3.1.3 History

Garnier (1897) first discovered that certain areas of eukaryotic cytoplasm stain intensely with basic dyes. He named these regions as **ergastoplasm** and these were found to be prominent in cells involved in secretion.

As the resolving power of the light microscope was very low at that time, the studies on ergastoplasm remained in doubt until the discovery of the Electron Microscope in the early 1940s.

In 1945 – Keith Porter, Albert Claude, Ernest Fullman and Thompson reported a soluble walled structure forming a network in the cytoplasm. They found this network more concentrated in the endoplasm of the cell than in the ectoplasm and hence it was given the name Endoplasmic Reticulum by Porter and Kallman in 1959.

So, the existence of ER was discovered in 1945 only after the introduction of electron microscopy applied to cultured cells. The first micrographs showed a lace-like arrangement of tubules that did not reach the periphery of the cell.

The use of high voltage Electron Microscopy on cultured cells rendered a clearer three-dimensional view of the ER. Detailed study of ER became possible with the introduction of thin sectioning end freeze-fracturing of cells. Cell fractionation methods, biochemical analysis and the use of cytochemical techniques for the study of specific components – particularly enzymes, etc., gave much information about ER. Using fluorescent dyes, recently, it has been possible to observe the ER in living as well as in fixed cells (Fig. 1.3.2).

1.3.1.4 Occurrence

It is found in almost all the eukaryotic cells but sometimes absent in eggs and in embryonic or undifferentiated cells ad mature mammalian erythrocytes. Size and number of ER varies from cell to cell.

For ex., in Spermatocytes – only a few vacuoles are present. In Interstitial cells of testis and cells involved in lipid metabolism, such as adipose, brown fat and adrenocortical cells, simple smooth ER is present.

Endoplasmic reticulum is extensively developed in cells active in synthesis, particularly in the synthesis of proteins and hormones, for ex., pancreas and liver cells.

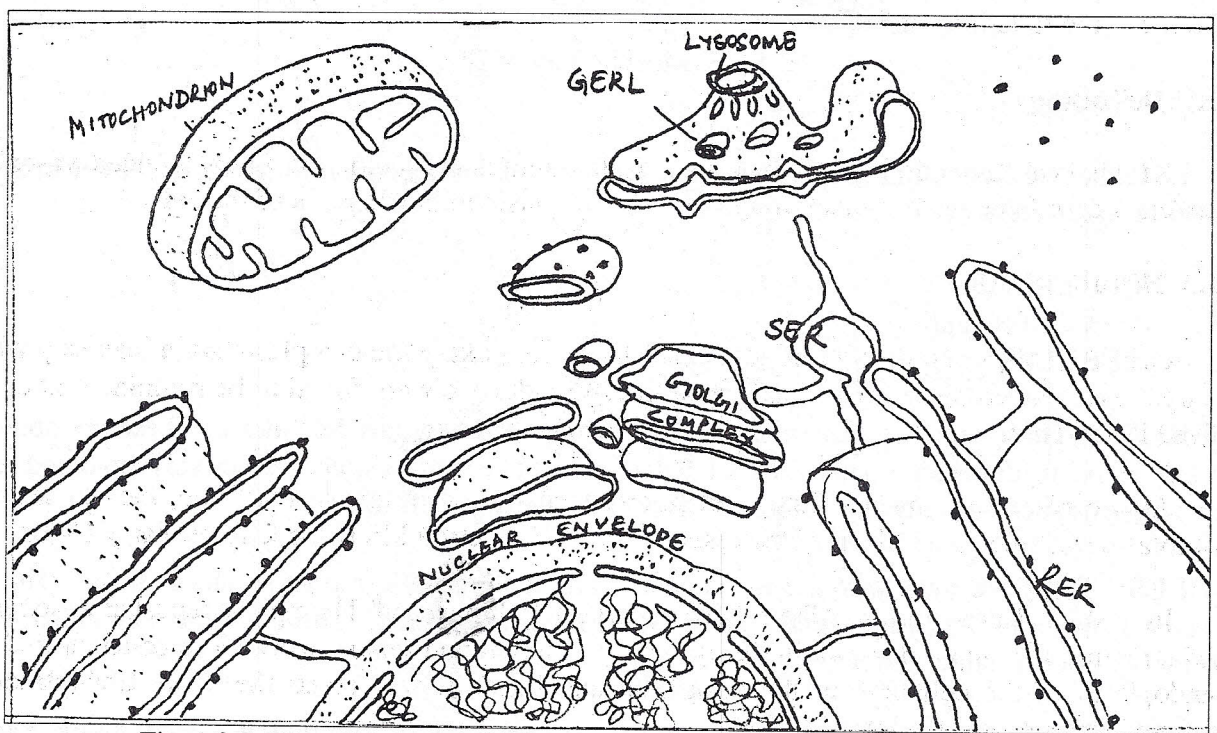


Fig. 1.3.2 Three-Dimensional Diagram of the Endomembrane system of the Cell

In liver, both types of ER are present indicating the different roles performed by liver.

In the Striated Muscles, the endoplasmic reticulum takes a special form and is known as Sarcoplasmic Reticulum.

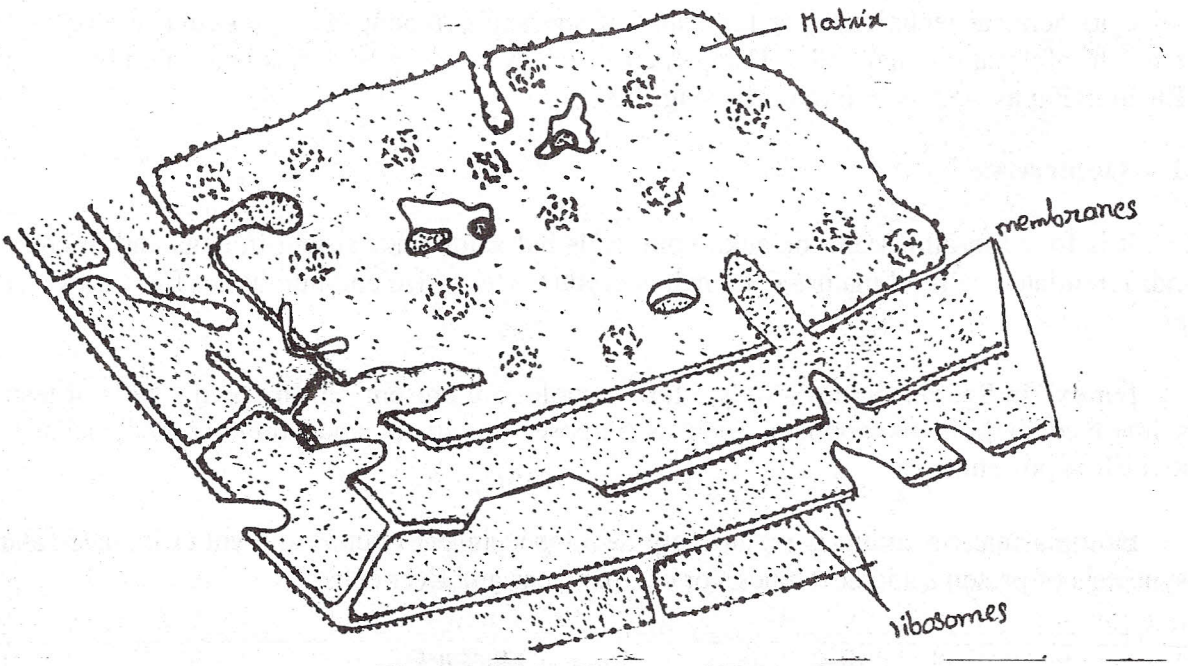


Fig. 1.3.3 Morphology of ER

1.3.1.5 Morphology

Endoplasmic Reticulum [ER] is an extensive network and occurs in three forms either in the same cell or in different cells.

- CISTERNAE
- VESICLES, and
- TUBULES

CISTERNAE: These are long flattened sac-like, unbranched and parallel stacks. These are about 40-50 mμ or A° in diameter. This form of ER is present in cells which are actively involved in protein synthesis such as pancreas, notochord liver cells, plasma cells, etc.

VESICLES: These are oval to round in shape and are about 25-500 mμ or A° in diameter. They often occur isolated in the cytoplasmic matrix. Ribosomes are absent. These are most abundant in SER [Smooth Endoplasmic Reticulum].

TUBULES: Tubules are of different shapes and sometimes they form reticular system along with the cisternae and vesicles. These are present in the non-secreting cells and are about 50-100A° in diameter. These are haphazardly arranged in the cytoplasm of developing spermatides of guinea pig, muscle cells and other non-secretory cells. They are often found in Smooth Endoplasmic Reticulum and are dynamic in nature.

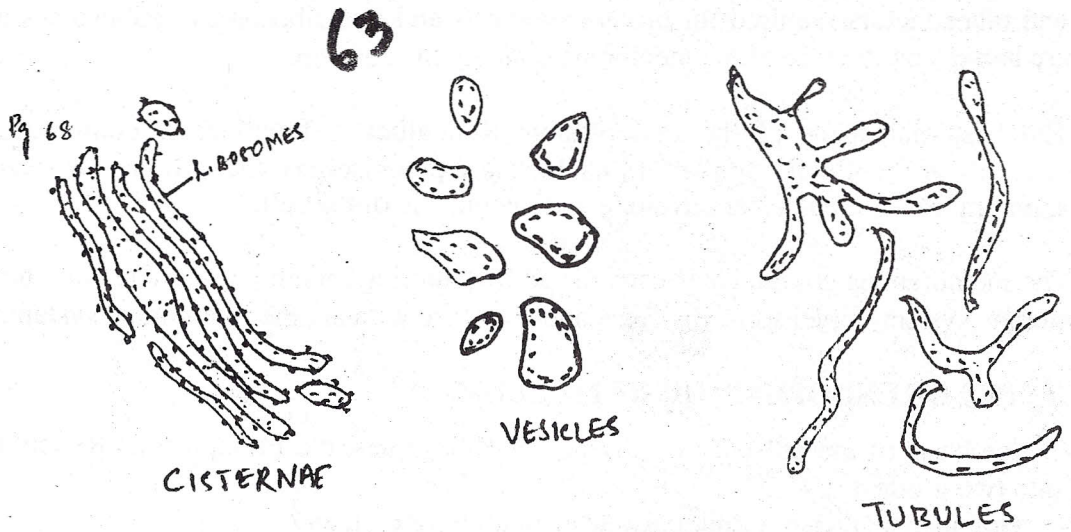


Fig. 1.3.4 CISTERINAE – VESICLES - TUBULES

All these three forms (Fig. 1.3.3) of ER are either present in the same cell or in different cells. Their arrangement also differ in different cells, for ex., as parallel rows in the liver cells of mammals, without any order in pancreatic cells or in the form of a network of tubules in striated muscle cells. Whereas in the notochord cells of Ambystoma larva, the pattern of cisternae arrangement is different from the existing ones.

Like Plasma membrane, ER also exhibits a unit membrane structure. It forms more than half of the total membrane in a cell. The membrane of Endoplasmic reticulum is about 50-60°A thick and is thinner than the plasma membrane. Since it is a double membraned structure, a space or lumen of variable sizes is enclosed within the membranes.

Each membrane is composed of an outer and an inner dense layers of the protein molecules, in between which, two thin and transparent layers of phospholipids are present. The membranes of endoplasmic reticulum contain enzymes which are involved in synthesis of cholesterol, triglycerides and other lipids and hence they play a role in lipid metabolism.

The ER is a continuous system connecting on one side to the Nuclear envelope and on the other side to the cell membrane, thus developing a passage for the transport of secretory and other products. The lumen of endoplasmic reticulum occupies more than 10% of the total cell volume.

Like Golgi complex, it has cytoplasmic face and luminal face (or) extracellular face. The cytoplasmic face is directly opposed to the cytosol whereas the luminal face borders the perinuclear cisternae and corresponds to the interior of the secretory organelles.

The Endoplasmic Reticulum divides the cytoplasm into two compartments – the compartments – the cell sap, and the cisternal space.

The cell sap consists of soluble enzymes which are involved in intermediary metabolism, tRNAs and other factors required for protein synthesis and free ribosomes. Ribosomes attached to the ER are located on the side of the membrane's facing the cell sap.

The Cisternal space of the Endoplasmic Reticulum is functionally continuous with the internal cavities of the Golgi complex, lysosomes and peroxisomes, the perinuclear space between the two membrane's of the nuclear envelope and the outside of the cell.

The membranous continuity is established by shuttling membrane-vesicles that bud off from one membrane system, travel some distance, and then fuse with another membrane system.

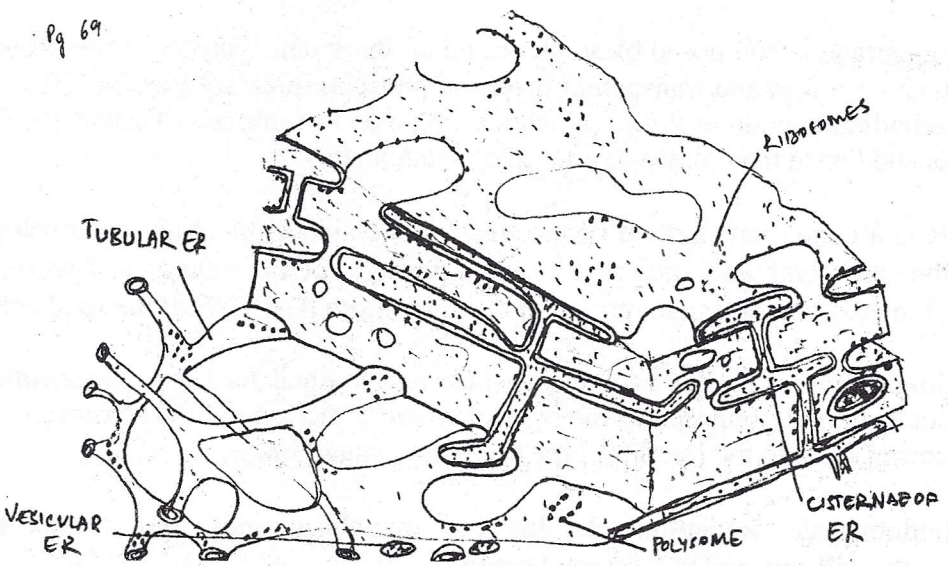
1.3.1.6 TYPES OF ENDOPLASMIC RETICULUM

On the basis of the presence or absence of Ribosomes, the Endoplasmic Reticulum [ER] is divided into two groups:

- Granular (or) Rough Endoplasmic Reticulum [RER], and
- Agranular (or) Smooth Endoplasmic Reticulum [SER]

Granular ER (or) RER

Ribosomes (or) Ribo Nucleo Protein (RNP) particles are attached to the outer surface of Endoplasmic Reticulum [ER] which gives Granular (or) Rough appearance and hence the name Granular or Rough Endoplasmic Reticulum [RER]. These ribosomes are of 100-150A° in diameter. Thus RER provides surface area for the ribosomes in order to carry out protein synthesis. This RER is highly developed in cells which are actively involved in synthesis and secretion of proteins. Only granular ER is found in the cells of pancreatic exocrine gland, whereas in the liver cells, both Smooth Endoplasmic Reticulum [SER] and Rough Endoplasmic Reticulum [RER] are found. It was found that if a cell type has abundant granular ER, it shows little smooth ER and vice-versa (Fig. 1.3.4).



.Fig. 1.3.5 Rough ER

Rough Endoplasmic Reticulum [RER] is studded with ribosomes on the cytoplasmic side of the membrane. RER is organized in stacks of flattened sacs, called cisternae. In pancreatic acinar cells and antibody secreting plasma cells, as many as half of the total ribosomes in the cell are bound to the ER and in the case of protein secreting cells, the ER is often unattractively widened by the protein secreted into it from these ribosomes.

Agranular ER (or) SER

Smooth ER is a small ribosome free region of the rough ER. Such regions are called transitional ER, which represent the specialized region of ER from which the vesicles carrying newly synthesized proteins and lipids bud off for intracelilular transport. It is found in cells which have no active synthesis of proteins, for example, adipose cells, interstitial cells, spermatocytes, leucocytes and epithelial cells of the frog's retina. Smooth ER is well developed in cells which secrete and synthesize steroids.

Smooth ER is sometimes predominant in cells specializing in lipid metabolism. The enzymes that synthesize the lipid component of lipoproteins are located in the membranes of the smooth ER, for example, liver. Muscles cells have a specialized and elaborated smooth ER called the Sarcoplasmic reticulum, that isolate Ca^{2+} from the cytosol. This smooth ER is present in regions rich in glycogen particles (Glycosomes) which are spheroidal in shape, measuring 50 to 200 nm (Fig. 1.3.5).

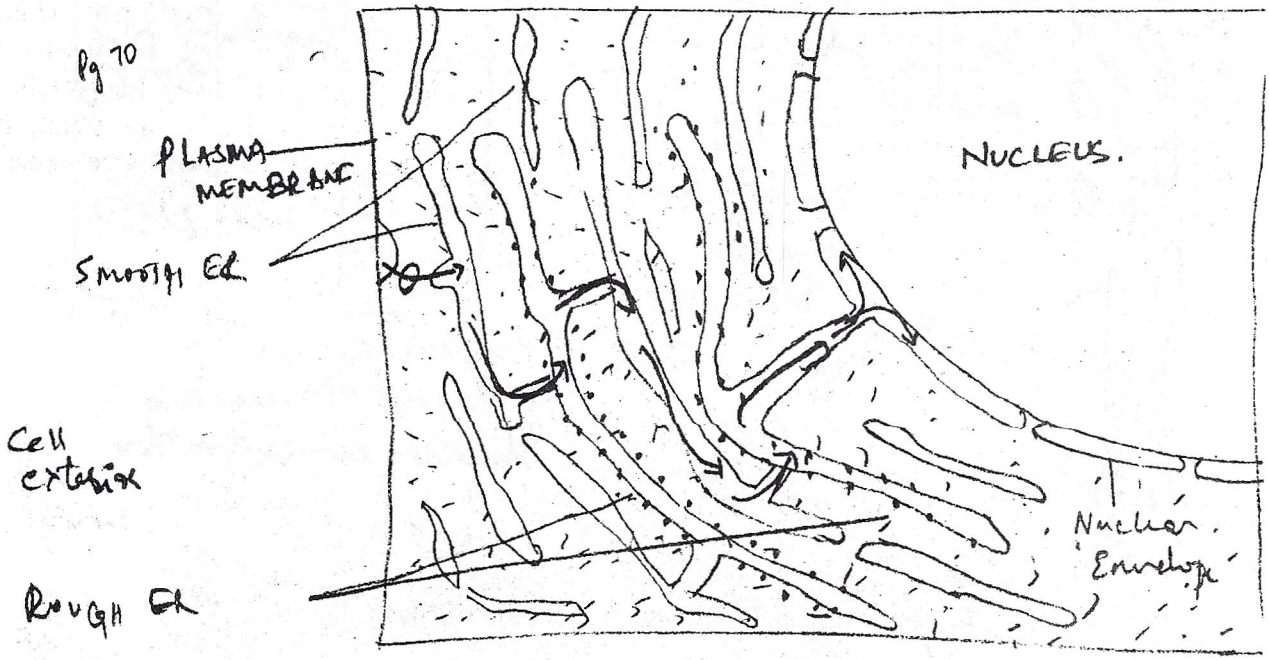


Fig. 1.3.6 Structure of E.R. in continuation with Nucleus

1.3.1.7 Isolation of Endoplasmic Reticulum

The isolation of ER from other components of the cell is made possible with ltracentrifugation.

When tissues or cells are disrupted by homogenization, the ER is fragmented into many smaller closed vesicles called Microsomes. Microsomes derived from rough ER are studded with ribosome and are called Rough Microsomes. Those microsomes which does not contain ribosomes are called smooth Microsomes, which are derived from smooth ER.

Ribosomes, which contain large amounts of RNA, make rough microsomes more dense than smooth microsomes. As a result, the rough and smooth microsomes can be separated from each other by sedimenting the mixture to equilibrium in sucrose density gradients (Fig. 1.3.5).

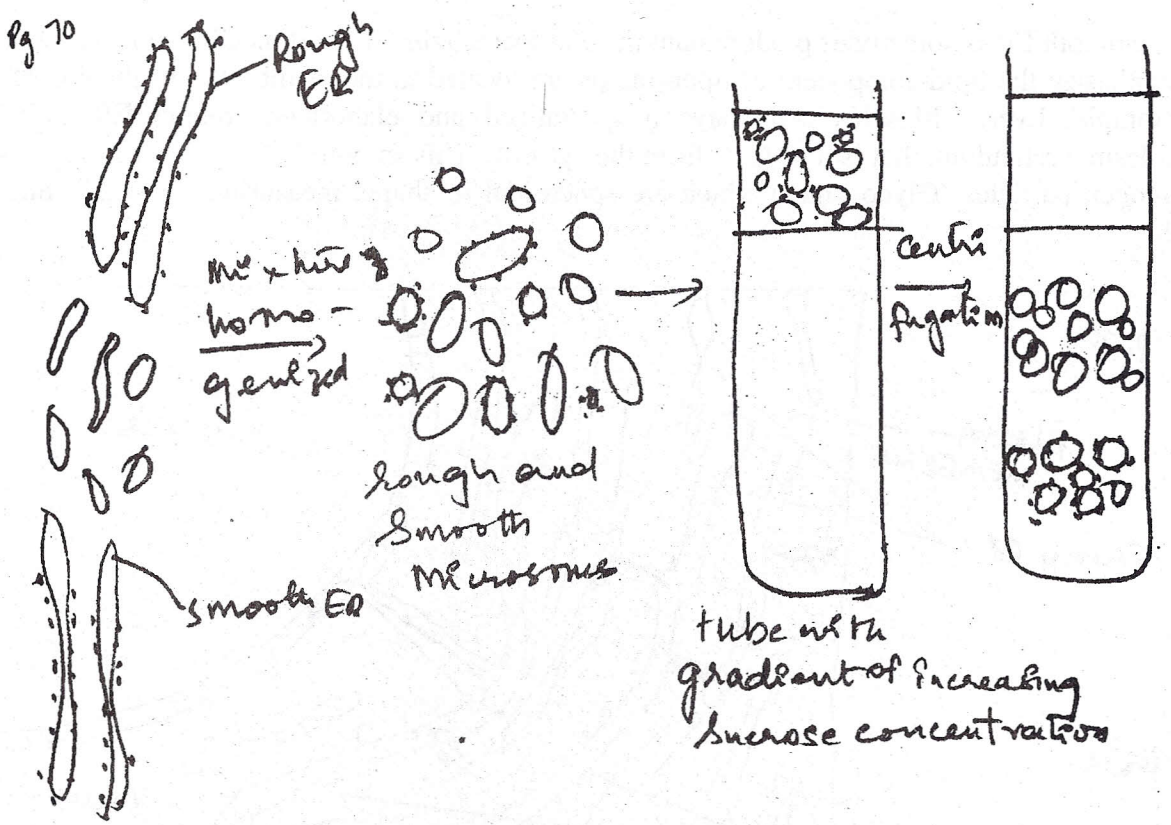


Fig. 1.3.7 Schema of isolation of ER

1.3.1.8 FUNCTIONS

Endoplasmic Reticulum performs a number of functions:

- ER forms a skeletal framework to the cell and thus provides a mechanical support to the cell.
- Since ER is connected with the plasma membrane, it helps in the transport of particles from one place to another either by a process of pinocytosis or endocytosis (cellular drinking from outside to inside) or by exocytosis (from inside to outside).
- The membranes of ER regulate the concentration of certain ions in the cytoplasm.
- **For ex.,** the ER of muscles stores Calcium (Ca^{2+}) ions. These ions are transported into the cytoplasm by a process of active transport, whenever the related muscles have to contract.
- In Muscles, the Smooth ER helps in the conduction of impulses by transmitting impulses from the neuromuscular junction to deep regions of the muscle fibres. It makes the contraction of all muscle fibres smooth.
- ER plays an important role in the transportation of genetic material from muscles to the various organelles in the cytoplasm thereby controlling the synthesis of proteins, fats and carbohydrates.
- Microbodies are small granular bodies filled with the dense materials limited by a single membrane. These formed by dilations of ER. These are rich in the enzymes peroxidase [hence called peroxisomes], catalase, D-amino acid oxidase. In plants, these are filled with enzymes responsible for glyoxylate cycle, hence called Glyoxysomes.
- Plasmodesmata are the cytoplasmic connections developed between the adjoining cells to facilitate the direct exchange of substances between the cells. Electron microscopic studies showed that the endoplasmic reticulum in plants play an important role in the interconnection of cells (plasmodesmata) through the cytoplasmic strands.
- Endoplasmic reticulum helps in the formation of plasma membrane.
- The mitochondria are originated from endoplasmic reticulum by a process of Blebbing.
- Nevikoff described that Golgi complex is formed from Smooth Endoplasmic reticulum.
- ER helps in the formation of Chloroplasts, Lysosomes.
- Proteins are the main building blocks of living cells. Ribosomes are the fine organelles that carry out the synthesis of proteins. ER provides surface to ribosomes to perform the synthesis of proteins. Once the synthesis of protein molecules is over they get discharged from the ribosomes and penetrate into the cavity of ER, where they are stored or exported to the outside.
- Glycosylation is one of the major biosynthetic function of the ER. Most of the proteins isolated in the lumen of the ER before being secreted from the cell or transported to other intracellular

destinations [such as Golgi apparatus, lysosomes or plasma membranes] are glycoproteins. In contrast, the soluble proteins of the cytosol are not glycosylated.

- Phospholipids and cholesterol are the principal building blocks of all lipid bilayers, both of which are synthesized on the membranes of the ER.

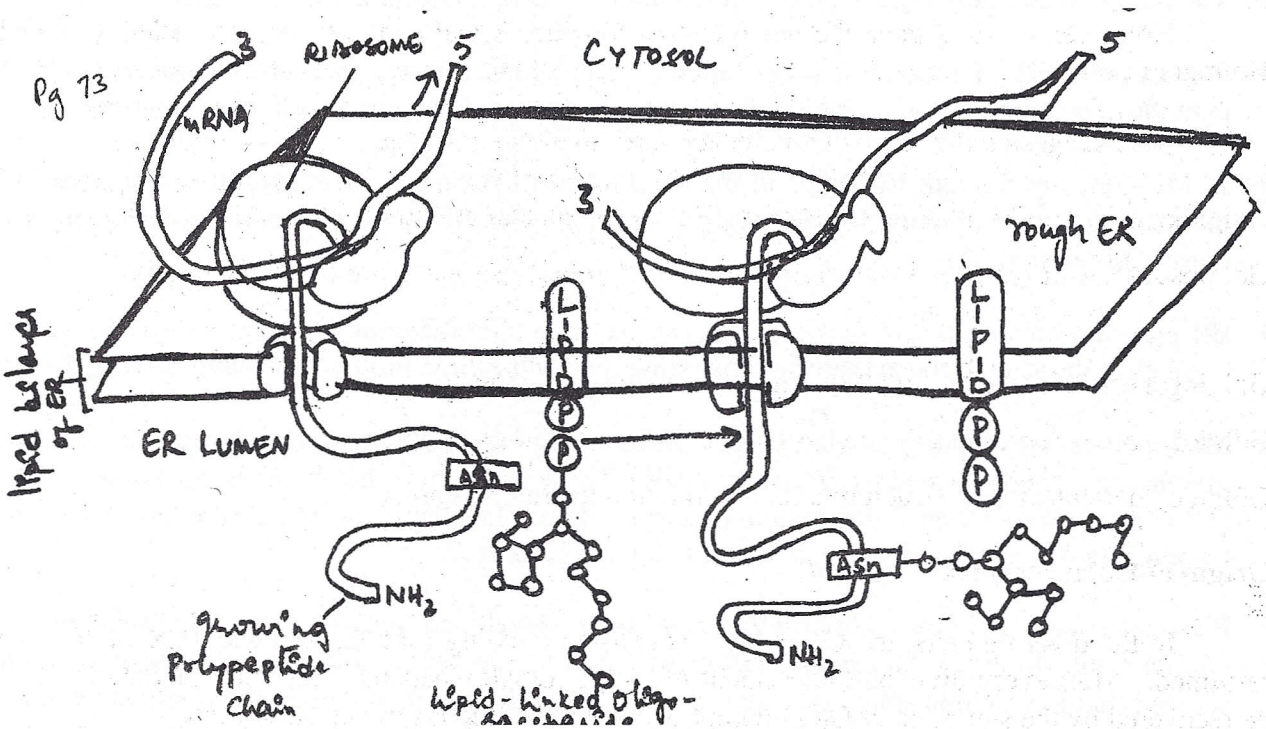


Fig. 1.3.8 Synthesis apparatus of ER

The main phospholipid synthesized in the ER is the phosphatidylcholine [also called lecithin] formed from two fatty acids plus glycerol phosphate and choline.

1.3.2 GOLGI COMPLEX

1.3.2.1 Introduction:

The Golgi complex was first described in 1898 by the 1906 Nobel Laureate, Camillo Golgi. In the past, all thought Golgi complex to be an artifact of various fixation and staining procedures. In 1950, the Golgi complex was first identified in the electron microscope. The Golgi complex was first observed by George in 1867 and later on Camillo Golgi studied this by using the silver salt in the nerve cells of the barn owl and the cat.

Golgi complex was described in terms like Golgi complex, Golgi Body, Golgi Apparatus, Golgisome, Golgi material, Golgi membrane, Dalton Complex, Y Cytomembrane, lipochondria and Dictyosome, etc.

A number of joined Golgi complex system is known as Polysystem.

Holmgren in 1902 gave the name tryptospongium, Cajal supported it and called it as Golgi Holmgren canals.

Sosa suggested the following nomenclature for Golgi complex:

Golgiokinesis - division to the Golgi complex during nuclear division.

Golgiosomes - fragments which are produced by Golgiokinesis and are common among invertebrates.

Golgiogenesis - formation and differentiation of Golgi complex.

Golgiolysis - represents the process of dissolution of Golgi complex.

Golgiocytoarchitecture – structure of cell in relation to Golgi complex

Origin of Golgi complex

In the dividing cells, the Golgi body is constantly being formed, changed, broken down and reformed. After every division, the amount of Golgi complex becomes half and the deficien be recovered by the synthesis of Golgi complex from any of the following structures.

From the Plasmalemma: Danielle in 1964 reproted the formation of Golgi cisternae from the vesicles of plasmalemma in the giant Amoeba, the Pelomyxa. The vesicles may either be for by a process of Pinocytosis (or) Phagocytosis.

From the Nuclear Membrane: Golgi complex generally found near the nuclear membran In 1945, Bouch reported the formation of vesicles from nuclear membrane in brown Algae.

From the Endoplasmic Reticulum: Formation of Golgi complex from ER has been stu in 1962 by Essner and Novikoff. It is Smooth Walled Endoplasmic Reticulum that gives r to Cisternae.

1.3.2.2 OBJECTIVES

- To study the occurrence and Morphology of Golgi complex.
- To study the structure and polarity of Golgi complex.
- To study the chemical nature of Golgi complex.
- To know the different functions of Gol complex.

1.3.2.3 DEFINITION

Camillo Golgi described Golgi complex as the 'internal reticular apparatus' and later on the term Golgi was named after him. Golgi complex is considered as a sorting center that is able to discriminate between the proteins that are to be secreted and those that are to be delivered to the lysosomes.

1.3.2.4 Occurrence

Golgi complex occurs in all the eukaryotic cells except mature sperms and RBC's of animals, certain fungi, sperm cell of bryophytes and pteridophytes and the cells of mature sieve tubes of plants. Golgi complex is totally absent in all the prokaryotic cells like Bacteria and Blue green algae.

1.3.2.5 Morphology

Under favourable conditions in a living cell, the Golgi complex can be stained with Methylene Blue and observed for a limited time. It is also visible by means of Phase Microscopy. It can be isolated from other constituents of the cell by Differential Centrifugation.

Position: The position of the Golgi complex is fixed for each cell type.

For ex:- In the secretory exocrine cells – it is present between the nucleus and the secretory pole.

The position of the Golgi complex is diffused or distributed in the dividing cells. During cell division, it scatters and diffuses throughout the cytoplasm. This is the diffused form of Golgi complex.

The fragments of Golgi complex are called Dictyosomes which are unconnected units of Golgi complex.

After division the Golgi complex localizes at its proper place and occupies a fixed position. This form of Golgi complex is called localized form.

Size:- Varies from one cell type to another depending on the active site of the cell. It is large in the nerve and small in muscle cells. Cells with large-sized Golgi complex shows a considerable decrease in the size when they grow old.

Number: Varies from 3-7 in most animal cells.

In liver cells – there are some 50 dictyosomes/cell.
In plants - varies from 10-20 upto 100 (>25,000 in algal rhizoids)
In Paramoecium – there are two large Golgi complexes.

1.3.2.6 Structure

The Golgi complex consists of the following morphological components:

1. **Flattened sacs (or) Cisternae:** These appear as dense parallel membranes present one above the other. Palay and Palade, described in 1955, that these are the stacks of Smooth Walled Endoplasmic Reticulum. But Sjostrand pointed out that although both can be impregnated with Silver and Osmiumtetraoxide, yet, they differ in thickness of their membranes.

   In Golgi complex, the membrane of flattend sac is about 60-70A° in thickness enclosing a cavity of about 150A° wide whose edges are often dilated. The gap between the adjoining flattened sacs is about 200A° to 300A°.

In few cells, a thin layer of an opaque dense material is seen which at certain regions are prominent and are termed as Nodes by Amos and Grimstone (1968).

Flattened sacs are slightly curved and remain filled with fluid. In animal cells single Golgi complex contains 8-14 upto 25 flattened sacs. Each sac has 2 faces – a forming face (or) immature face which is usually convex and is oriented towards the nucleus; a mature face, which is concave and is oriented toward the cell surface. The sacs possess swelling at either ends giving the characteristic feature.

The flattened sacs on the proximal or concave side are assembled from vesicle arrived from ER, and on the distal or convex side gives rise to other kinds of vesicles, including secretory vesicles that make their way to the plasma membrane. Because of the constant input of new membrane on one side and loss of membrane from the other, the Golgi complex has a short life time. The vesicles from the ER that coalesce to form proximal saccules of the Golgi apparatus are often called **Transition Vesicles**. These are derived from a nearby area of Rough Walled ER. This segment of ER together with the transition vesicles and Golgi flattened sacs is more accurately of as a single unit, the Golgi complex.

2. Large Vacuoles:

These vacuoles about 600A° diameter are present in association with flattened sacs. Sometimes they may contain dense masses or granules. They are also known as condensing vacuoles or secretory vesicle. They represent modified and expanded cisternae in which the two membranes have become widely separated and the vacuolar space enlarged.

3. Cluster of Small Vacuoles:

Small vacuoles present in association with the tubules, give the appearance of clusters. The vesicles range from 20-80A° in diameter. They are intimately associated with the cisternae and may show continuity with them. They arise from sacs by budding or pinching off. They are also known as Transfer vesicles.

There are three types of vesicles:

a. **Smooth Vesicles:** These are the vesicles containing secretory material and hence called secretory vesicles. These bud off from the ends of cisternal tubules within the net.

b. **Coated Vesicles:** There are spherical protuberances with rough surface. They are found at the periphery of the organelles, usually at the ends of single tubules, and are morphologically quite distinct from the smooth vesicles.

c. **Golgian vesicles:** These are large rounded sacs present on the maturing face of Golgi. These are at either by the expanded cisternae or by the fusion of secretory vesicles. These vesicles are filled with some amorphous a granular substances.

1.3.2.7 Polarity of Golgi complex

The Golgi complex exhibits an inherent polarity in that saccules located at opposite ends of the stack differ from each other in size, shape, content, number of associated vesicles and enzymatic activity. The end of the stack receiving newly synthesized proteins from the ER is termed as the Cis region, while the opposite face is termed as the trans region. The Cis face is often termed as convex or outer or immature face, as it is located on the convex, outer surface of the complex, and the trans side is called the inner or concave or nature face.

The saccules located at the inner surface generally contain a greater quantity of granular material and are associated with a large number of vesicles than the saccules of the outer surface.

1.3.2.8 Chemical Nature

Basically the Golgi complex is composed of lipids and proteins just like the unit membrane structure. The Golgi complex isolated from rat liver consists of about 60% protein and 40% lipid. The chemical nature of Golgi complex can be studied under the following heads:

1. Phospholipids: Phospholipids composition of Golgi membrane is intermediate between those of endoplasmic reticulum and plasma membrane. The lipoidal contents of Golgi complex have been described by Baker. According to him, Lecithin and Cephalin are among the most common fatty materials associated with Golgi complex.

2. Proteins and Enzymes: Several different proteins and enzymes have been identified form animal and plant cells. The common enzymes are ADPase, ATPase, CTPase, Glucose-6-phosphatase, NADPH, Cytochrome-C reductase, glycosyl transferase, galactosyl transferase, thiamine pyrophosphate, etc.

3. Carbohydrates: Some carbohydrates have also been identified in both animal and plant cells. These are glucosamine, galactose, glucose, mannose and fructose. Plant Golgi lacks sialic acid, but

it is present in high concentrations in rat liver. Some other carbohydrates like xylase and arabinase are present only in plant cells.

4. Vitamin C: Golgi complex stores vitamin C and liberates it slowly into the cytoplasm in sufficient amounts to prevent oxidation of the cell products. The fraction of Vitamin C stores in the Golgi complex has been shown by Tomitte.

1.3.2.9 FUNCTIONS

1. Role in Secretion: Described by Cajal (1914). The process of secretion in the cells like pancreatic cells and intestinal cells etc., goes on through the following stages:

a. **Ribosome stage:** Ribosomes are present in the cells either on the outer wall of endoplasmic reticulum or lying free in the cytoplasm. During the process, they synthesize the protein whose nature is checked up by the mRNA.

b. **Endoplasmic Reticulum stage:** The protein synthesised is transferred into the lumen of ER where the lumen material is transported to the Smooth Walled ER. The protein, in this stage, is in the form of dilute solution of macro-molecules but in certain cases, it may have small intracisternal granules.

c. **Golgi stage:** In this, the synthesized products are transported from ER to Golgi complex. This process is mediated by transitional vesicles that bud off from the ER more through the cytoplasm, and fuse with the cis face of the Golgi complex.

   The next step involves the sorting, concentrating and modifying the protein products present within the Golgi complex.

d. **Zymogen stage:** As protein destined for secretion pass through the Golgi complex they are concentrated and condensed into a highly compact form suitable for secretion. large vesicles at the Golgi periphery called condensing vacuoles, are the usual site for concentrating protein into granules. The net result is a highly concentrated secretory product contained within the membrane enclosed vesicles emerging on the trans side by the Golgi complex. These small secretory granules in twin fase with one another to form larger particles referred to as Zymogen granules.

   Whenever required the Golgi complex is involved in adding a carbohydrate or fatty acid residues to certain protein synthesized in the rough walled endoplasmic reticulum.

e. **Intraluminar stage:** The secretory granules are released and poured to their respective organs. The discharge of the secretory granules involves its movement toward the apical region and fusion of the membranes. The process is called Exocytosis and requires calcium ions and energy.

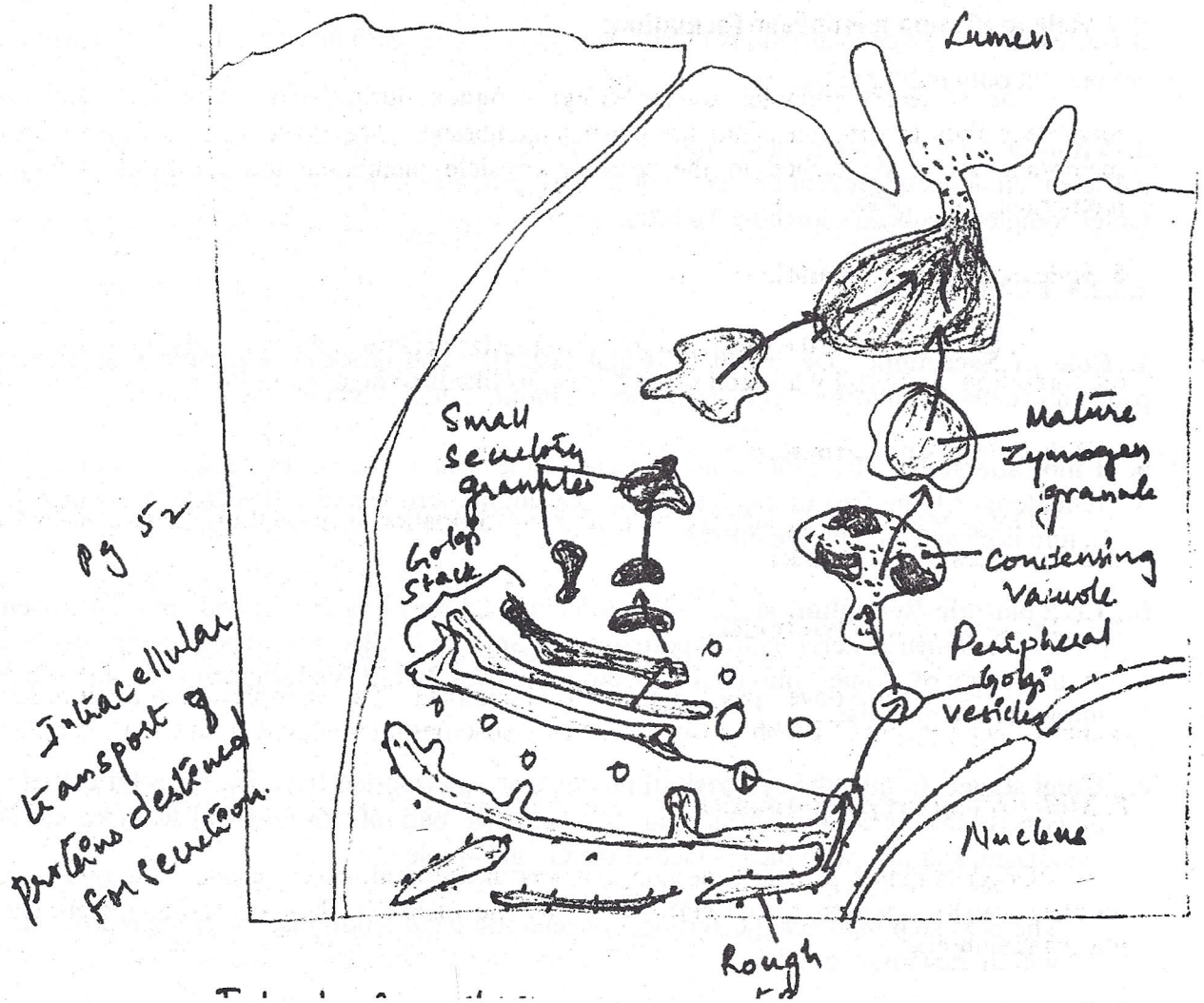


Fig. 1.3.9 Intracellular transport of proteins destined for secretion

2. Role in acrosome formation:

Burgos and Fawcett (1955) found that the Golgi complex plays an important role in the formation of acrosome.

3. Role during Oogenesis:

During Oogenesis, the Golgi complex helps in the formation of yolk

4. Role in Plasma membrane formation:

The secretory granules of the Golgi complex during exocytosis fuse with the plasma membrane thus incorporates into the plasma membrane. The glycoproteins formed on the Golgi membrane remains attached to the secretory vesicle membrane after it fuses with the plasma membrane.

5. Role in Cell wall formation:

In the plant cells, the Golgi complex synthesizes pectin and some carbohydrates necessary for the formation of the cell walls and some secretions like mucilage, gums etc.

6. Role in Pigment formation:

Golgi complex is associated with pigment formation in retinal epithelium of chick embryo and in oocytes of Salamander.

7. Golgi complex and lysosomes:

Lysosomes are developed from the Golgi complex. The endoplasmic reticulum forms Golgi complex by a process of Blebbing and after that lysosomes are released from Golgi complex.

8. Milk protein droplet formation:

Golgi complex produces protein droplets in the mammary gland of lactating mice. These droplets usually open on to the cell surface by the fusion of their enclosing membrane with the plasma membrane.

9. Formation of enamel:

In amyloblasts the Golgi complex plays an important role. During the synthesis of the enamel the Golgi complex reverses its position.

10. Lipid absorption:

Palay and Kartin (1956) reported that the cells of the intestinal wall show higher lipid accumulation in the Golgi complex just after absorption of nutrients.

1.3.3 SUMMARY

Eukaryotic cells contain ER which is an extensive system of membranes that forms numerous tubes and plates in the cytoplasm. ER is the interconnected network of tubular and microsomal vesicular structures in the cytoplasm of the cell. The appearance of the endoplasmic reticulum varies

among different eukaryotic cells but it always forms a system of fluid-filled sacs enclosed by the membrane network. The Endoplasmic reticulum may form a continuum with the outer nuclear membrane and may thus provide a communication network for coordinating the metabolic activities of the cell. The limiting membrane covering the ER is made of bilayered lipids and proteins. The lumen of ER contains a fluid medium called Endoplasmic matrix. The ER shows two distinct morphologies when examined by electron microscopy – Rough ER [RER] and Smooth ER [SER]. RER is the part of ER to which the granular ribosomes are bound to its surface, which give it a rough texture. Its function is, along with the ribosomes, to manufacture proteins. This part is also called the Granular ER. Smooth ER is also called Smooth or Agranular reticulum. Many enzymes are present on the outer surface of this part of the reticulum. Many enzymes are present on the outer surface of this part of the reticulum. Smooth ER is responsible for synthesis of non-protein substances like steroid hormones, sebum, cholesterol, etc. The catabolism of toxic substances like some drugs and carcinogens occurs in smooth ER.

Golgi apparatus (or) Golgi body (or) Golgi complex is present in all the cells except red blood cells. It is situated near the nucleus. The Golgi apparatus consists of a stack (5-8) of flattened membranous sacs known as the cisternae. Individual stacks of membranes of this complex are called Dictyosomes. Golgibodies are the sites of various synthetic activities by which physaccharides and lipid can be added to proteins to form. Lipoproteins, glycoproteins and various polysaccharide derivatives that are essential for the synthesis of various cell parts. The Golgi apparatus receives substances transported from the ER, stores the substances [eg. proteins and lipids] and typically alters their chemical structure. It packages these substances in small segments of membrane called Secretory vesicles. Proteins transported through the rough ER are transferred t the Golgi apparatus by a process of vesicular budding called Blebbing.

1.3.4 TERMINOLOGY

Endoplasmic reticulum
Plasma membrane
Evagination
Canalicular
Karyokinesis
Endomembrane system
Interstilial cells
adipose
adrenocortical cells
Striated muscles
Sarcoplasmic Reticulum
flattened
Sucrose density gradient
Pinocytosis/cellular drinking
Endocytosis
Exocytosis
Active transport
Nemomuscular junction

Cytoplasmic vacuolar system
Microsomal
Vesicular
Interpreted
Cytoplasmic Matrix (or) Cytosol
Ergastoplasm
Resolving power
Ectoplasm
Endoplasm
Micrographs
Lace-like arrangement
Freeze-Fracturing
Cell fractionation
Cytochemical techniques
Fluorescent dyes
Embryonic
Undifferentiated cells
Mature mammalian erythrocytes
Spermatocytes
sac-like
Reticular system
Listernae
Vesicles
Tubules
hapazardly
Spermatides
Cell sap
Cisternal space
Perinuclear space
Granular ER
Agranular ER
Ribo nucleo proteins
acinar cells
Transitional ER
Glycosomes
Ultracentrifugation
homogenization
Microsomes
Microbodies
Peroxidase
Glyoxylate cycle
Glyoxysomes
Plasmodesmata
Cytoplasmic strands
Blebbing
Lysosomes
Glycosylation
Golgi complex
Polysystem
Tryptospongium
Golgi Holmgren canals
Golgiokinesis
Golgiosomes
Golgio genesis
Golgiolysis
Golgiocytoarchitecture
Internal reticular apparatus
Phase

1.3.5 SELF ASSESSMENT QUESTIONS

1. Write a note on the history of Endoplasmic Reticulum?
2. Describe the occurrence, morphology and structure of ER?
3. What are the types of ER. Add a note on them.
4. Describe the various functions of ER.
5. Write a short note on the history, occurrence, and Morphology of Golgi complex.
6. Write down the chemical nature of Golgi complex.
7. Describe the structure of Golgi complex and write down the functions.

1.3.6 REFERENCE BOOKS

1. **Cell and Molecular Biology** – Gerald Karp
2. **Cell and Molecular Biology** – De Robertis & De Robertis
3. **Cell Biology, Genetics, Molecular Biology, Evolution and Ecology** - P.S. Verma and V.K. Agarwal.
4. **The Cell** - Bruce Alberts.

UNIT-I

LESSON 1.4

MITOCHONDRIA

1.4.0 INTRODUCTION

Mitochondria (Greek: Mito=thread, chondrion=granule) are filamentous or granular cytoplasmic organelles of all aerobic cells of higher animals and plants and also of certain micro-organisms like algae, protozoa and fungi. These mitochondria are generally absent in bacteria. The mitochondria contain specific DNA named as mt DNA which is useful for cytoplasmic inheritance and it also contains ribosomes for the protein synthesis.

Mitochondria are generally called "power house of the cell" because they produce high energy phosphates in the form of ATP by utilising the food we take and this energy is used for various purposes. **e.g.:** contraction of muscle, movements of a sperm etc. Mitochondria are also prominent in many plant cells. These are the primary suppliers of ATP in non-photosynthetic tissues, as well as being a source of ATP in photosynthetic leaf cells during dark periods.

Mitochondria are osmotically active, this means, when these organelles are kept in hypotonic medium i.e., when the concentration of the surrounding medium was less than the internal mitochondria, then these organelles will swell. When these are placed in hypertonic medium, i.e., concentration of the surrounding medium was higher than the internal environment, then these organelles will shrink. This property of mitochondria suggest that these organelles were bounded by a semi-permeable membrane.

1.4.1 Objectives

- To study the structure, properties and functions of mitochondria.
- To study the compartmentalization of mitochondria.
- To study the enzymes localized in mitochondria.
- To study how to isolate, observe and characterize the mitochondria.
- To study various functions of mitochondria.
- To study biogenesis of mitochondria.

1.4.2 History

Mitochondria were first observed by Kolliker in 1850 as granular structures in the striated muscles. Flemming in 1882 named them as "Fila". Richard Altmann in 1890 named them as "Bioplasts" and he was the person who found the relationship between mitochondria and cellular oxidation. The term "mitochondria" which is presently used today was coined by Benda in 1897.

Sir Hans Adolph Krebs in 1937 worked out various reactions of the citric acid cycle. Kennedy and Lehninger in 1950 showed that the citric acid cycle, oxidative phosphorylation and fatty acid oxidation took place in mitochondria. Mitchell in 1961 proposed "chemiosmotic-coupling hypothesis" for the production of ATP in the mitochondria. Palade in 1954 described the ultrastructure of cristae.

Previously, mitochondria have been known by various names such as fuchsinophilic granules, parabasal bodies, plasmosomes, plastosomes, fila, vermicules, bioplasts and chondriosomes.

1.4.3 Morphology

We can observe mitochondria by staining with a dye called Janus green. This dye gives greenish blue colour to them. This is due to the action of cytochrome oxidase system present in the mitochondria. It maintains the dye in its oxidised (colored) form. But in the surrounding cytoplasm, this dye is reduced to a colorless leukobase. Some fluorescent dyes which are more sensitive have also been used to observe mitochondria even in intact cultured cells and are suitable for metabolic studies.

1.4.3.1 Number: The number of mitochondria in a cell depends mainly on the type and functional state of the cell. The number may vary from cell to cell and from species to species. An average mammaliar liver cell contains about 1500 mitochondria, which will represent approximately 15 to 20 per cent of the cell's volume. These mitochondria are present in higher number in muscle cells which require large amounts of ATP for their contraction. Amoeba contains 50,000; Sea urchin eggs contains 1,40,000 to 1,50,000; oocytes of amphibians contain 3,00,000 mitochondria.

1.4.3.2 Size: Typical mitochondrion is approximately 0.2 to 1.0 μm in cross-sectional diameter and 1 to 4 μm in length. As mitochondria and bacteria are having approximately similar sizes, this feature suggests that mitochondria have evolved from bacteria that lived symbiotically within other cells.

1.4.3.3 Shape: The typical mitochondrion is sausage-shaped. But the shape varies depending upon the physiological conditions of the cell. They may be filamentous or granular or may be club shaped or racket shaped or vesicular or ring or round-shaped.

1.4.4 Distribution

The distribution of mitochondria within the cytoplasm depends upon the cell's function as they are energy suppliers of the cell. In some cells, these organelles move freely and supply ATP wherever needed. But in other type of cells, these organelles are permanently localised near the region of the cell where more energy is needed. **eg:** in muscle cells, mitochondria are grouped like ring around I-band of myofibril. It was also proved that large number of mitochondria are present at the membranes which need continuous supply of energy for the active transport of water and solutes.

1.4.4.1 Orientation: Mitochondria have definite orientation. If we see a cylindrical cell, these organelles are generally oriented in basal-apical direction and lie parallel to the main axis. If we see a leucocyte, these organellus were found radially with respect to centrioles.

1.4.5 Structure

A mitochondrion consists of two membranes and two compartments (Fig. 1). the two membranes are:

(1) Outer membrane
(2) Inner membrane

1.4.5.1 Outer membrane: Outer membrane is the limiting membrane which completely surround the mitochondrion. It is 6 nm in thickness and smooth in nature. It contains a number of transport proteins called porins. So, this membrane is permeable to all molecules of molecular weight 10,000 daltons or less.

1.4.5.2 Inner membrane: Inner membrane in contrast to outer membrane is not smooth in nature and is impermeable. Although part of the inner membrane may lie just inside the outer membrane, much of it occurs as deep folds or invaginations called cristae. In a mammalian liver, these cristae occur as wide sheets or lamellar in shape cutting across the entire diameter of the mitochondrion. These cristae greatly increase the amount of surface available to house, the cell machinery needed for aerobic respiration. Inner membrane has an outer cytosol or 'C' face toward perimitochondrial space and an inner matrix or M-face toward matrix.

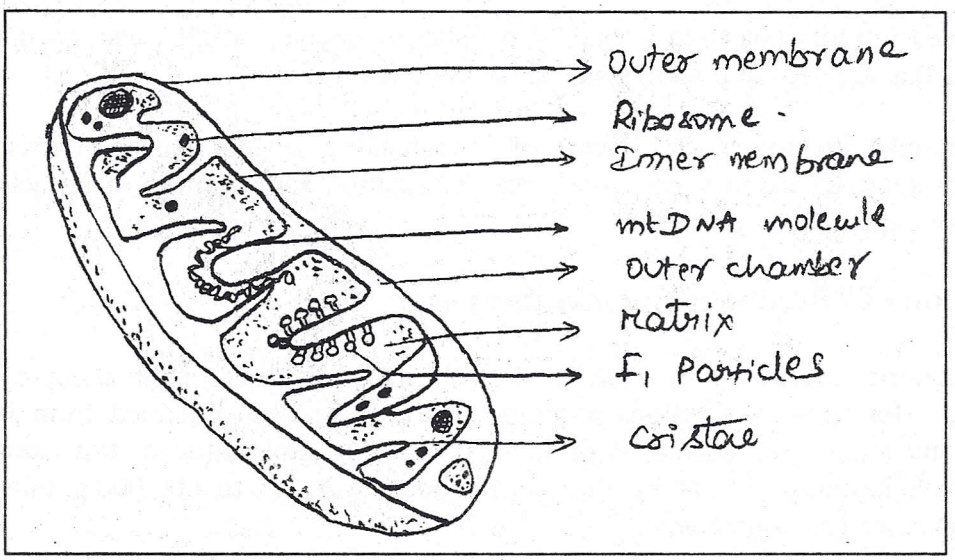


Fig. 1. A longitudinally cut mitochondrion showing its internal structure

1.4.5.3 F_1 particles: F_1 particles are located on the M side of the inner mitochondrial membrane. If mitochondria were allowed to swell and break in a hypotonic solution and when immersed in phosphotongstate, then the cristae appears covered by particles of 8.5 nm, that have a stem linking each with the membrane. These are called "Elementary" or "F_1-Particles". These are regularly spaced at intervals of 10 nm on the inner surface of inner membrane. Approximately, 10^4 to 10^5 elementary particles are present per mitochondrion. These particles correspond to a special ATPase involved in the coupling of oxidation and phosphorylation.

The two compartments are:

(1) Outer compartment
(2) Inner compartment

1.4.5.4 Outer compartment: It is also called peri-mitochondrial space. This is 6-8 nm space which is present in between the outer membrane and inner membrane. This space is continuous into the core of the cristae.

1.4.5.5 Inner compartment: It is also called inner chamber or matrix. Matrix is homogenous in nature containing a number of enzymes, ribosomes (smaller than those fand in the cytosol), lipids, proteins and certain granules. These granules are important in some cells concerned with the transport of ions and water, including kidney tubule cells, epithelial cells of small intestine and osteoblasts of bone forming cells.

In addition to these, matrix contains double stranded circular DNA. This genetic material is important because it encodes a small number of mitochondrial polypeptides (13 in humans) that are

tightly integrated into the inner mitochondrial membrane. Human mtDNA also encodes 2 rRNA's and 22 tRNA's that are used in protein synthesis within the organelle.

This double structure (solid-liquid) of mitochondria is important in providing a good explanation for some mechanical properties like deformation and swelling of mitochondria under physiological or experimental conditions.

1.4.6 Isolation of Mitochondrial membranes

Mitochondria can be easily isolated by cell fractionation brought about by differential centrifugation. Homogenous fractions of mitochondria have been obtained from liver, skeletal muscle, heart and some other tissues. In differential centrifugation, mitochondria sediment at 5,000 to 24,000 g. while in living cells, at the ultracentrifugation of 20,000 to 4,00,000 g, mitochondria are deposited intact at the centrifugal pole.

Because of the difference in the composition of outer membrane and inner membrane, the two membranes can be easily separated. The outer membrane is composed of approximately 50 per cent lipid by weight, and contains more cholesterol and is higher in phosphatidyl inositol and lower in cardiolipin. It also contains a mixture of enzymes involved in diverse activities like degradation of tryptophan, oxidatin of epinephrine, elongation of fatty acids.

Inner membrane contains more than 100 different polypetides and has a very high protein to lipid ratio, which is approximately in 3:1 ratio by weight. The inner membrane is devoid of cholesterol and is rich in unusual phospholipid, cardiolipin. Both these features are also characteristic of bacterial plasma membrane, from which the inner membrane is presumably derived by evolution.

The outer mitochondrial membrane is thought to be derived from an outer membrane of bacteria. Both contain porins, integral proteins of 29,000 daltons, which are resistant to trypsin digestion. These porins form an integral channel of diameter 2-3 nm. As long as porin channels are opened, it can allow upto 5,000 daltons. Thus, ATP, NAD, co-enzyme A which are having mass less than 1,000 daltons can pass freely. In contrast, the inner membrane is highly impermeable and it requires special transporters for the entry of molecules into the matrix.

The outer membrane can be separated by placing the mitochondria in a hypotonic solution so that it will swell and breakage followed by contraction of inner membrane and matrix. Some detergents like digitonin and lubrol are often used for this purpose. Since outer membrane is lighter and much stronger, density gradient centrifugation can separate the two membranes. When the outer membrane is removed, structure formed is called 'Mitoplast' (Fig. 2). The mitoplast contains inner membrane with unfolded cristae and the matrix. All the enzymes in inner membrane and matrix are intact. That is why this mitoplast can carry oxidative phosphorylation smoothly.

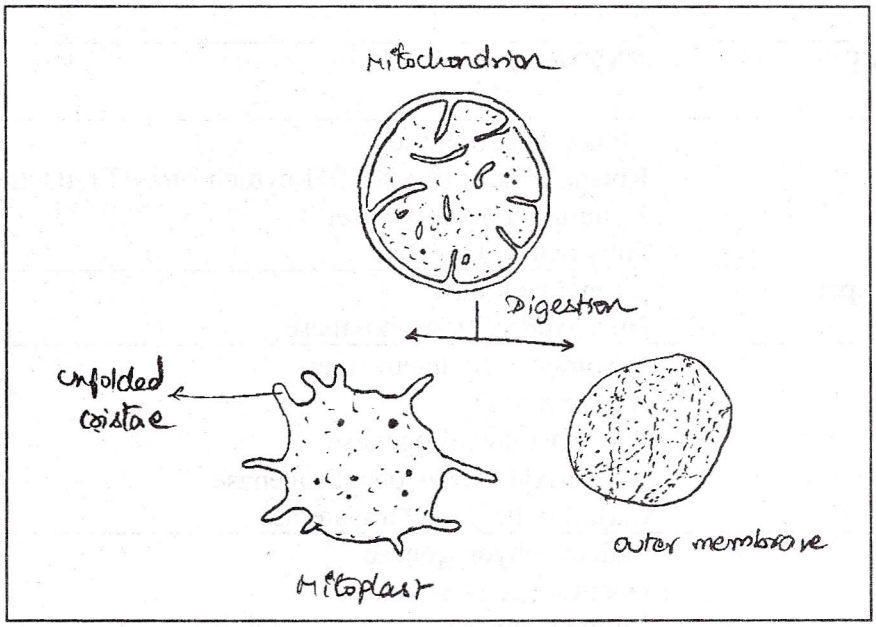


Fig. 2. Action of digitonin on liver mitochondria to produce mitoplast and "folded-bag" of outer membrane.

The remaining component i.e., outer membrane gives the appearance of a "folded bag". Isolation of two membranes helps us in localization of various enzymes in mitochondria.

1.4.7 Mitochondrial Enzymes

Many enzymes are localized in the various compartments of the mitochondria and are listed in Table 1.

The outer membrane contains NADH-cytochrome-C-reductase system that consists of a flavoprotein and cytochrome b5. The most specific enzyme system of the outer membrane is monoamine oxidase, which may serve as an enzyme marker. This membrane also contains kynurenine hydroxylase, fatty acid coenzyme-A ligase, a phospholipase and various enzymes of the phospholipid metabolism. Monoamine oxidase acts as an enzyme marker.

Perimitochondrial space contains adenylate kinase, nucleoside diphosphokinase, DNase I and 5'-endonuclease. Adenylate kinase is the enzyme marker for peri mitochondrial space.

Table 1. Enzyme distribution in mitochondria

COMPARTMENT	ENZYME
Outer membrane	Monoamine oxidase. Rotenone-sensitive NADH-cytochrome-C-reductase. Kynurenine hydroxylase. Fatty acid CoA ligase.
Perimitochondrial space	Adenylate kinase Nucleoside diphosphokinase
Inner membrane	Respiratory chain enzymes ATP synthase Succinate dehydrogenase β-hydroxybutyrate dehydrogenase Carnitine fatty acyl transferase
Matrix	Malate dehydrogenase Isocitrate dehydrogenase Citrate synthase α-keto acid dehydrogenase β-oxidation enzymes.

The inner membrane contains proteins with various functions **e.g.:** those that carry out oxidative reactions of respiratory chain, an enzyme complex called ATP synthase and some specific transport proteins that can regulate passage of metabolites into and out of the matrix. The significant enzymes of inner membrane are – enzymes of electron transport system, NAD, FAD, diphosphopyridine nucleotide dehydrogenase (DPN), four cytochromes (cyt b, cyt c, cyt c_1, cyt a, cyt a_3), ubiquinone or co-enzyme Q_{10}, non-heme copper and iron, ATP synthase, succinate dehydrogenase, hydroxybutyrate dehydrogenase, carnitine fatty acid acyl transferase. Cytochrome oxidase is the marker enzyme of inner membrane.

Mitochondrial matrix contains a highly concentrated mixture of hundreds of enzymes, including those required for oxidation of pyruvate and fatty acids and for the citric acid cycle. Matrix also contains several identical copies of the mtDNA, special 55S mitochondrical ribosomes, tRNA's and various enzymes required for expression of mitochondrial genes. Thus the matrix contains malate dehydrogenase, isocitrate dehydrogenase, fumarase, aconitase, citrate synthase, α-keto acid dehydrogenase, β-oxidation enzymes. Matrix contains different nucleotides, nucleotide co-enzymes and inorganic electrolytes like K^+, HPO_4^-, Mg^{+2}, Cl^-, SO_4^{2-}.

1.4.8 Functions

Mitochondria perform most important functions such as oxidation, dehydrogenation, oxidative phosphorylation and carry respiratory chain of the cell. They are the actual respiratory organs of the cell, where food stuffs are oxidised. During this oxidation process, large amount of energy is released which is stored in the form of ATP. Because mitochondria can synthesise large amount of energy. It is called 'power house' of the cell. In animal cells, 95% of the energy is

contributed by mitochondria and remaining 5% is obtained by anaerobic respiration outside the mitochondria.

1.4.8.1 Oxidation of carbohydrates: The carbohydrates that we take through the diet are first broken down to 3-carbon compound, pyruvic acid by a series of chemical reactions. Later, pyruvic acid enters mitochondria and there it is completely oxidised to CO_2 and water, the electrons that are produced in these reactions pass through different electron carriers and production of high energy compounds will be seen. All these reactions can be grouped under –

a) Glycolysis
b) Oxidative decarboxylation
c) Kreb's cycle or Tricarboxylic acid cycle or citric acid cycle
d) Respiratory chain & oxidative phosphorylation

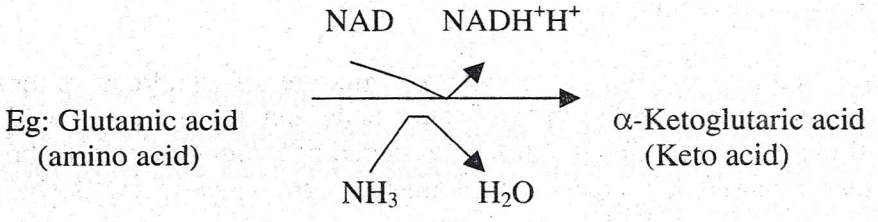
1.4.8.2 β-oxidation of fatty acids:

Enzymes of outer and inner membranes mediate the movement of free fatty acids into mitochondrial matrix. In the matrix, each fatty acid molecule is in the form of 'active fatty acid' or 'fatty acyl coA' and by β-oxidation, one molecule of acetyl CoA is released in each turn of cycle and they are directed to Kreb's cycle.

1.4.8.3 Oxidation of Proteins:

Peptide bonds in the proteins were cleaved with the help of protease enzymes or by hydrolytic cleavage. Later, nitrogen molecules is removed from aminoacids by either deamination or transmination reactions.

In the oxidative deamination, amino group is split off from rest of the molecule forming ammonia. The remainder of the amino acid then enters the main stream of metabolism as keto acid.



In transamination reaction, amino group is shifted from one molecule to another in exchange for an oxygen.

Eg: Glutamic acid + Oxaloacetic acid → α-Ketyglutaric acid (Keto acid) + Aspartic acid (amino acid)

In this case of transamination, amino group is not lost completely but is transferred to one of the substrates in the reaction.

1.4.8.4 Thermogenesis:

Some energy produced during oxidation of glucose is captured in the form of ATP and the rest is either lost as heat or used to regulate body temperature of warm blooded animals.

In young animals and hibernating species, a specialized tissue called brown fat is present which is important for temperature regulation. This tissue produces large quantities of body heat necessary for arousal of hibernation. The color of brown fat comes from its high concentration of mitochondria, which are spars in ordinary fat cells. The mitochondria appear to catalyze electron transport in the usual way but are much less efficient at producing ATP. Hence, a higher than usual fraction of the oxidatively released energy is converted directly to heat.

1.4.8.5 Biosynthetic activities:

Mitochondria contain DNA and the machinery needed for protein synthesis and can make less than a dozen different proteins which include subunits of ATPase, 3 out of 7 subunits in cytochrome oxidase. Synthesis of haeme by a mitochondrial enzyme, δ-amino levulinic acid synthetase by early steps in the conversion of cholesterol to steroid hormones in adrenal cortex are also catalyzed by mitochondrial enzymes.

1.4.9 Biogenesis of mitochondrion

Mitochondrial and chloroplast biogenesis became of great interest with the demonstration that these organelles contain DNA and ribosomes and are able to synthesize proteins. They are called 'Semi autonomous organelles' because their biogeneis is the result of the cooperation of two genetic systems (i.e., mitochondrial and nuclear). A mitochondrion has one or more circular DNA molecules about 5.5 μm long localized in the matrix and is probably attached to the inner membrane at the point where DNA duplication starts. This duplication is under nuclear control and the enzymes used are imported from the cytosol. This DNA is able to code for ribosomal RNA, transfer RNA by a few proteins of the inner membrane.

Mitochondrial DNA from human cells is a circular DNA molecule of about 15,000 base pairs. A significant proportion of this DNA is devoted to coding for the protein synthesizing machinery. MtDNA codes for the mitochondrial ribosomal RNAs (12S and 16S), for about 19 tRNAs and for the mRNAs of about 12 proteins.

The two genetic systems, Nuclear component and mitochondrial component cooperate to make the mitochondrial proteins. About 90% of the proteins are coded by nuclear genes and synthesised in the cytosol. Then they are transferred into the mitochondrion by a post-transcriptional mechanism.

1.4.9.1 Symbiont Hypothesis: Mitochondria and chloroplasts might be intracellular parasites that have established a symbiotic relationship with the eukaryotic cell. Bacteria were thought to have originated the mitochondria, and blue-green algae originated, the chloroplasts. With the recognition

that these organelles have certain degree of autonomy, symbiont hypothesis has been reframed. According to the revised theory, the original host cell is conceived of as an anaerobic organism deriving its energy from glycolysis and the parasite contains reactants of Kreb's cycle by respiratory chain and is able to carry on respiration and oxidative phosphorylation. This hypothesis is based on many similarities between mitochondria, chloroplast by prokaryotes, which when considered from an evolutionary view point, may be more circumstantial. Some similarities include –

a) Localization of respiratory chain
b) Membranous projections extending from plasma membrane called mesosomes comparable to mitochondrial crests.
c) Mitochondrial DNA is circular, as it frequently is in chromosomes of prokaryotes.
d) Protein synthesis in mitochondria and in bacteria is inhibited by chloramphenicol where extra mitochondrial protein synthesis of higher cell is not affected.

1.4.10 Summary

Mitochondria are filamentous or granular cytoplasmic organelles found in eukaryotes and are absent in prokaryotes. These are called 'power house of the cell' as they produce large amount of high energy phosphates. These are osmotically very active. They can be observed by staining them with janus green. Number of mitochondria in a cell may vary depending upon functional state and from species to species. An average mammalian liver cell contains 1500 mitochondria, Amoeba contains 50,000; sea urchin eggs contains 1,40,000 to 1,50,000, oocytes of amphibians contain 3,00,000 mitochondria. Each mitochondria is approximately 0.2 to 1.0 μm in diameter by 1 to 4 μm in length. The shape may vary from sausage to filamentous, granular or club shaped. These are generally localised near the region of the cell where energy is needed more. The structure of mitochondrion consists of two membranes and two compartments. They are outer membrane and inner membrane and perimitochondrial space and matrix respectively. Different enzymes were localised in these 4 fractions, each having specific marker enzymes. Because of the difference in the composition between outer and inner membranes, these membranes can be easily separated with the help of enzymes like digitonin. The component which is free without outer membrane is called mitoplast. Mitochondria have a number of functions like oxidation of carbohydrates, fatty acids, proteins etc. Mitochondria are semiautonomous organelles because their evolution is a result of cooperation of two genetic systems. According to symbiont hypothesis, mitochondria might be intracellular parasites, which have many similarities with prokaryotes.

1.4.11 Terminology

Mitochondria, cytoplasmic organelles, mtDNA, power house of cell, osmotically active, hypotonic, hypertonic, semipermeable membrane, compartmentalization, outer mitochondrial membrane, inner mitochondrial membrane, perimitochondrial membrane, mitochondrial matrix, F_1

particles, cristae, mitoplast, porin, mitochondrial enzymes marker enzymes, thermogenesis, biogenesis, symbiont hypothesis.

1.4.12 Self-Assessment Questions

1. Why are mitochondria termed as 'power houses of cells'?
2. What are the characteristic features of mitochondria?
3. Compare the properties of inner and outer mitcohondrial membranes, intermembrane space and the matrix.
4. What are the different functions of mitochondria?
5. Describe the biogenesis of mitochondria.
6. Explain why mitochondria are considered as semiautonomous organelles.

1.4.13 Reference Books

1. **Cell and Molecular Biololgy** by Gerald Karp.
2. **Cell Biology, Genetics, Molecular Biology, Evolution and Ecology** by P.S. Verma and V.K. Agarwal.
3. **Cell and Molecular Biology** by De Robertis & De Robertis.
4. **The Cell** by Bruce Alberts.

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UNIT-II

LESSON - 2.1

DNA AS GENETIC MATERIAL

CONTENTS

2.1.0 Introduction

The nature of a cellís phenotype is controlled by protein synthesis within that cell. The genetic material must determine the presence and effective amounts of the enzymes in a cell.

An enzyme is a protein that acts as a catalyst for a specific metabolic process without itself being altered by the reaction. Enzymes allow these reactions to occur within the cell by lowering the free energy of activation [DG^{á}] of a particular reaction.

An average protein is 300 to 500 amino acids long; only 20 naturally occurring aminoacids are used in constructing these proteins. The sequence of aminoacids determine the final structure of an enzyme. The genetic material determines the sequence of the aminoacids. In most metabolic processes, such as the biosynthesis (or) degradation of bio-molecules different enzymes facilitates each step in the pathway.

In 1868, Johann Friedrich Miescher, a young Swiss Medical Student, became fascinated with an acidic substance that he isolated from pus cells obtained from bandages used to dress human wounds. He named this acidic substance as "**nuclein**".

2.1.1 Objectives

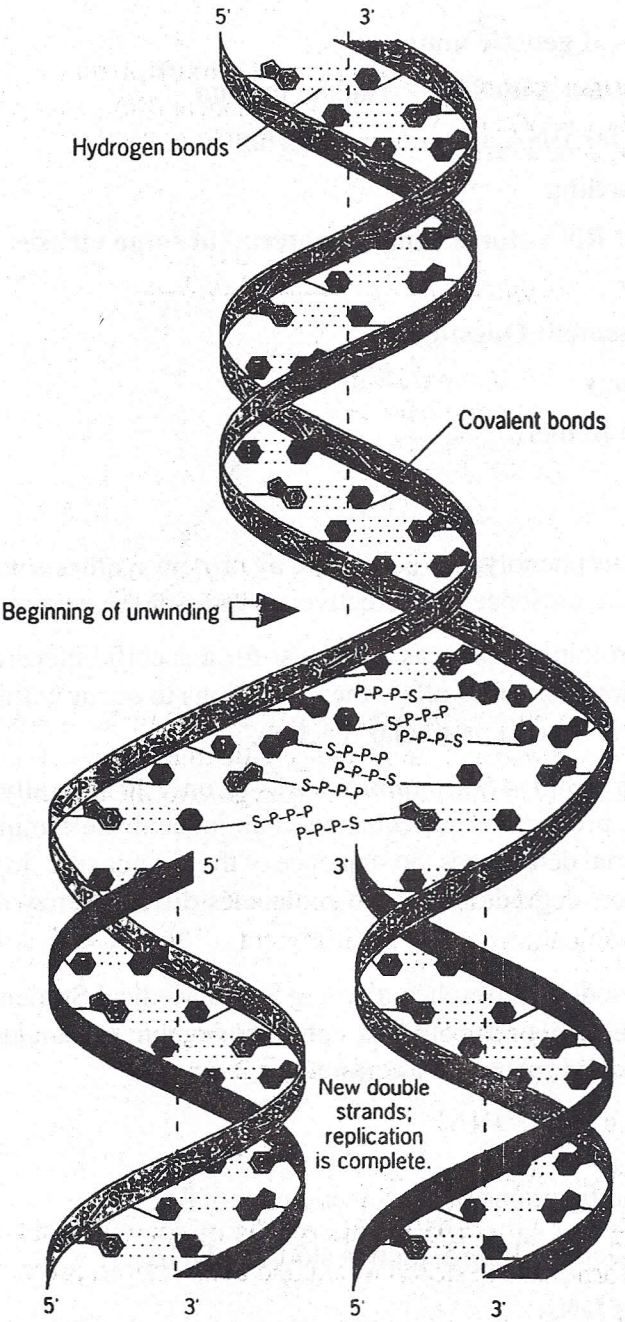
* To study the functions of the genetic material
* Proof that Genetic Information is stored in DNA
* Structure of DNA

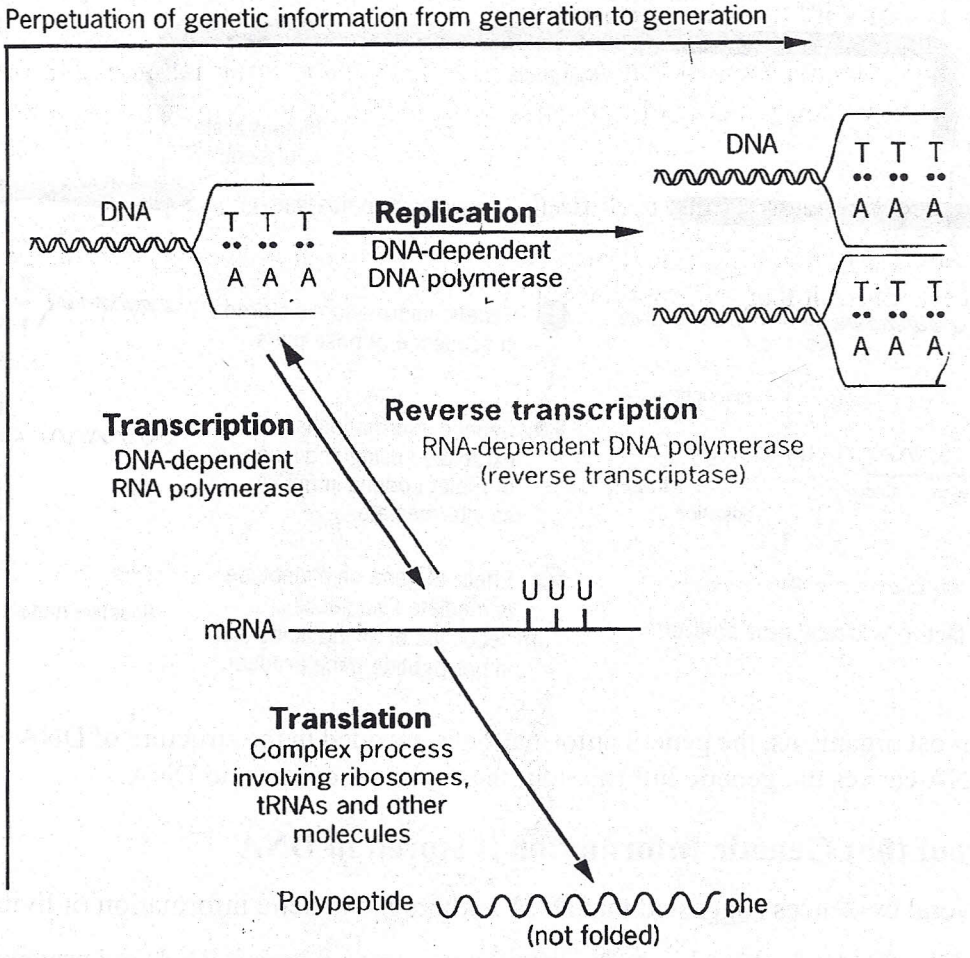
2.1.2 Functions of the genetic material

In 1865, Mendel showed that genes transmitted genetic information and in first few decades of the 20th century, their patterns of transmission from generation to generation were studied exclusively.

The genetic material must perform three essential functions:

⇒ The genotypic function, **Replication**. The genetic material must store genetic information and accurately transmit that information from parents to offspring, generation after generation.





⇒ The phenotypic function, **Gene Expression**. The genetic material must control the development of the phenotype of the organism. That is, the genetic material must dictate the growth and differentiation of the organism from the single-celled zygote to the mature adult.

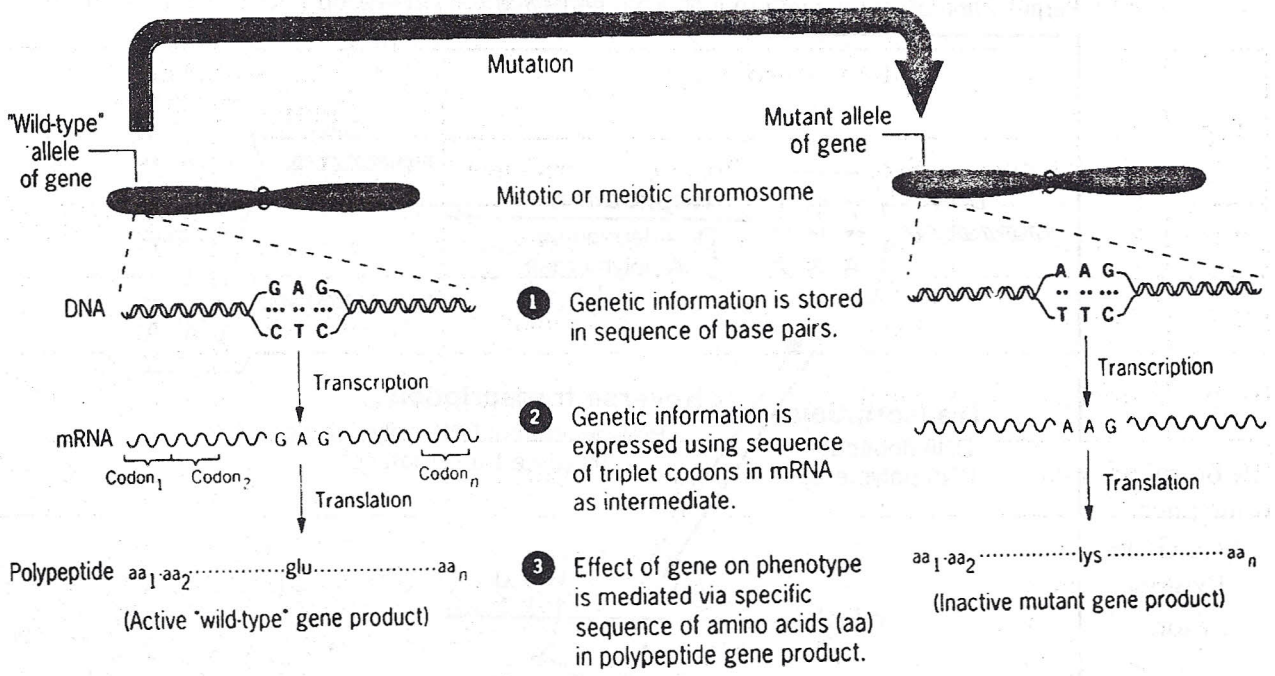
⇒ The evolutionary function, **Mutation**. The genetic material must undergo change so that organisms can adapt to modifications in the environment. Without such changes, evolution could not occur.

Chromosomes are composed of 2 types of large organic molecules [macromolecules] called proteins and nucleic acids. These nucleic acids are of 2 types:

Deoxyribo Nucleic Acid [DNA]

Ribo Nucleic Acid [RNA]

During the 1940ís and early 1950ís, the results of some elegant experiments clearly established that the genetic information is stored in nucleic acids, not in proteins.



In most organisms, the genetic information is encoded in the structure of DNA. In some small viruses, RNA carries the genetic information; these viruses contain no DNA.

2.1.3 Proof that Genetic Information is stored in DNA

Several evidences suggested that DNA harbors the genetic information of living organisms.

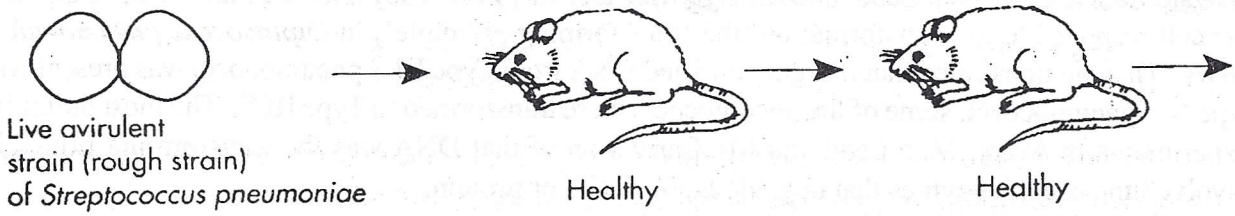
- Most of the DNA of cells is located in the chromosomes, whereas RNA and proteins are abundant in the cytoplasm.
- A precise correlation exists between the amount of DNA per cell and the number of sets of chromosomes per cell. For **eg.** most somatic cells of diploid organisms contain twice the amount of DNA as the haploid germ cells [gametes] of the same species.
- The molecular composition of the DNA is the same [with rare exceptions] in all the different cells of an organism, whereas the composition of both RNA and protein is highly variable from one cell type to another.
- DNA is more stable than RNA or proteins, which are synthesized and degraded quite rapidly in living organisms. Since the genetic material must store and transmit information from parents to offspring, we might expect it to be stable, like DNA.

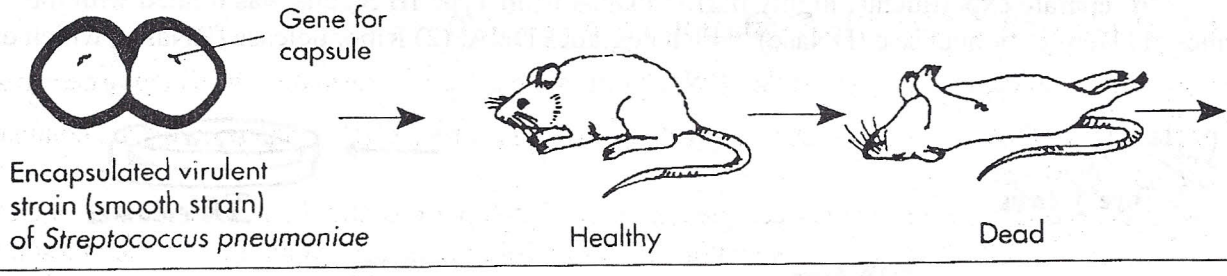
2.1.4 Evidence for DNA as the genetic material

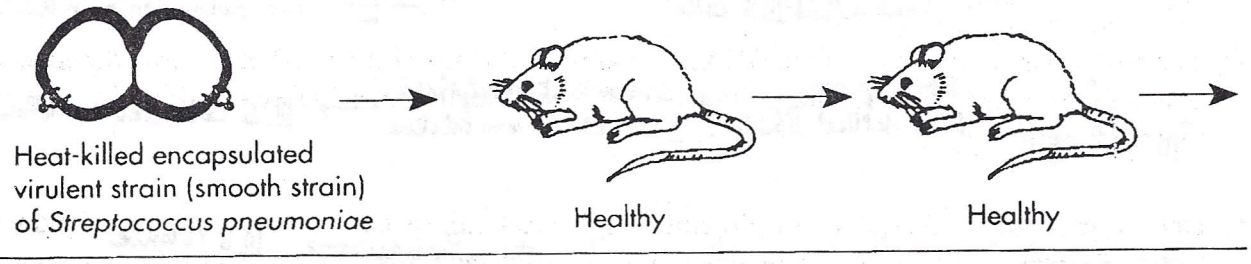
In 1928, E. Frederick Griffith reported that heat-killed bacteria of one type could 'transform' living bacteria of a different type. Griffith demonstrated this transformation using two strains of the bacterium *Streptococcus pneumoniae* [previously called *Diplococcus pneumoniae*].

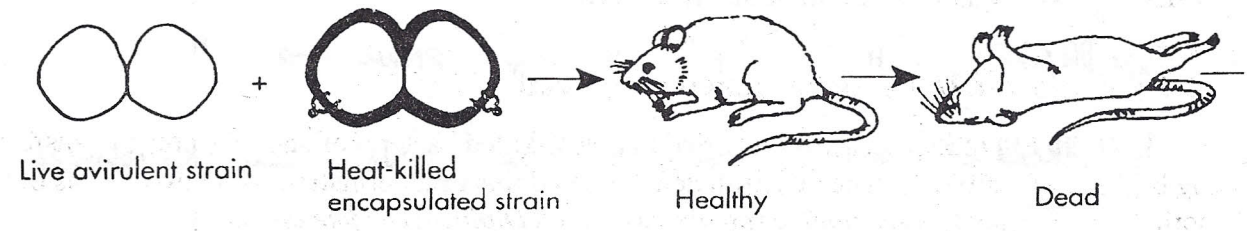
Characteristics of *Diplococcus pneumoniae* strains when grown on Blood Agar Medium.

Type	Colony Morphology				Reaction with Antiserum prepared against	
	Appearance	Size	Capsule	Virulence	Type II S	Type III S
II R	Rough	Small	Absent	Avirulent	None	None
II S	Smooth	Large	Present	Virulent	Aggluti-nation	None
III R	Rough	Small	Absent	Avirulent	None	None
III S	Smooth	Large	Present	Virulent	None	Aggluti-nation









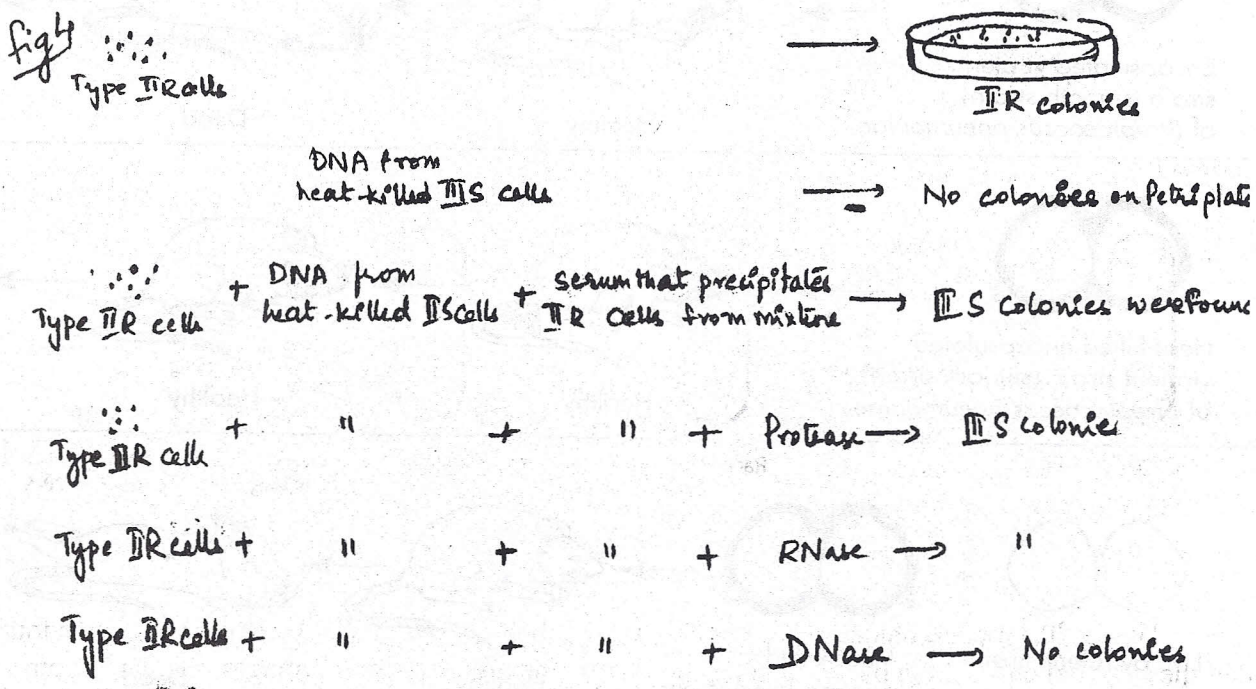
One strain (S) produced smooth colonies on media in a petriplate becaue the cells had polysaccharide capsules. It caused a fatal bacteremia [Bacterial infection] in mice. Another strain (R), which lacked polysaccharide capsules, produced rough colonies on petriplate. It did not have a pathological effect on mice. Bacteria of the rough strain are engulfed by white blood cells of the mice; bacteria of the virulent smooth strain survive because they are protected by their polysaccharide coating.

Griffith found that neither heat-killed S-type nor live R-type cells, by themselves, caused bacteremia in mice. However, if a mixture of life R-type and heat-killed S-type cells was injected into mice, the mice developed a bacteremia identical to that caused by injection of living S-type cells. Thus, something in the heat-killed S-cells transformed the R-type bacteria into S-type cells.

2.1.6 Proof that DNA mediates transformation

The first proof that the genetic material is DNA rather than protein or RNA was published by Oswald Avery, Colin MacLeod, and Maclyn McCarty in 1944. They showed that the component of the cell responsible for transformation [the 'transforming principle'] in *Diplococcus pneumoniae* is DNA. They demonstrated that if highly purified DNA from Type III S pneumococci was present with type II R pneumococci, some of the pneumococci were transformed to Type III S. The most definitive experiments in Avery, Mac Leod and Mc Carty's proof that DNA was the transforming principle, involved the use of enzymes that degrade DNA, RNA or protein.

In separate experiments, highly purified DNA from Type III S cells was treated with the enzymes (1) Deoxyribonuclease (DNase), which degrades DNA, (2) Ribonuclease (RNase), which de-



grades RNA, or (3) Proteases, which degrade proteins; the DNA was then tested for its ability to transform Type II R cells to type III S. Only DNase treatment had effect on the transforming activity of the DNA preparation.

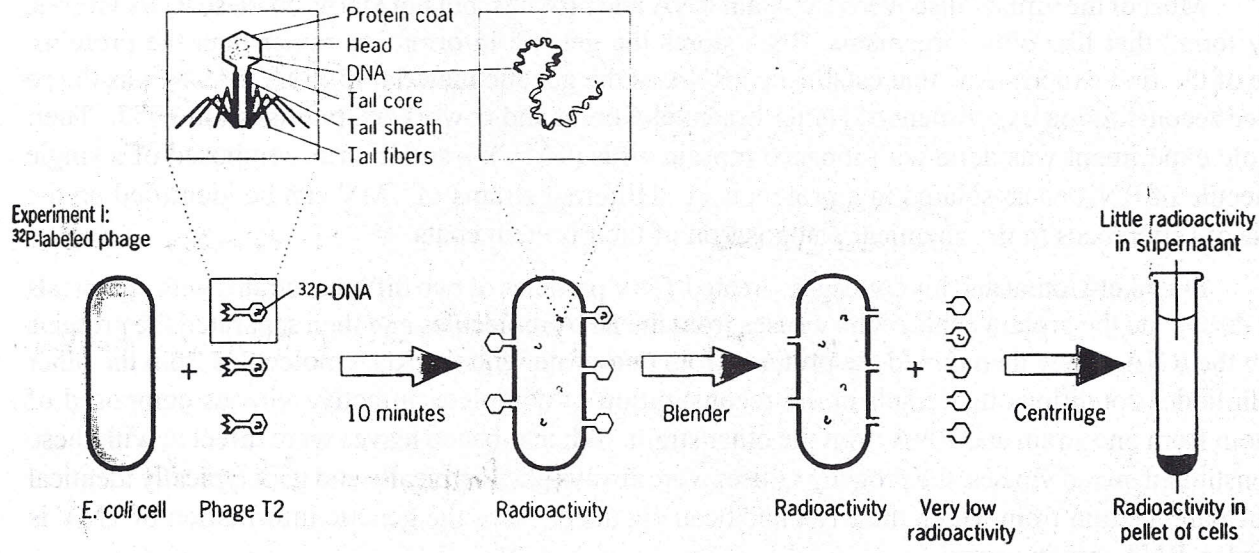
This study proved the first experimental evidences that DNA was the genetic material; DNA transformed R-type bacteria into S-type bacteria.

2.1.5 Phage Labeling

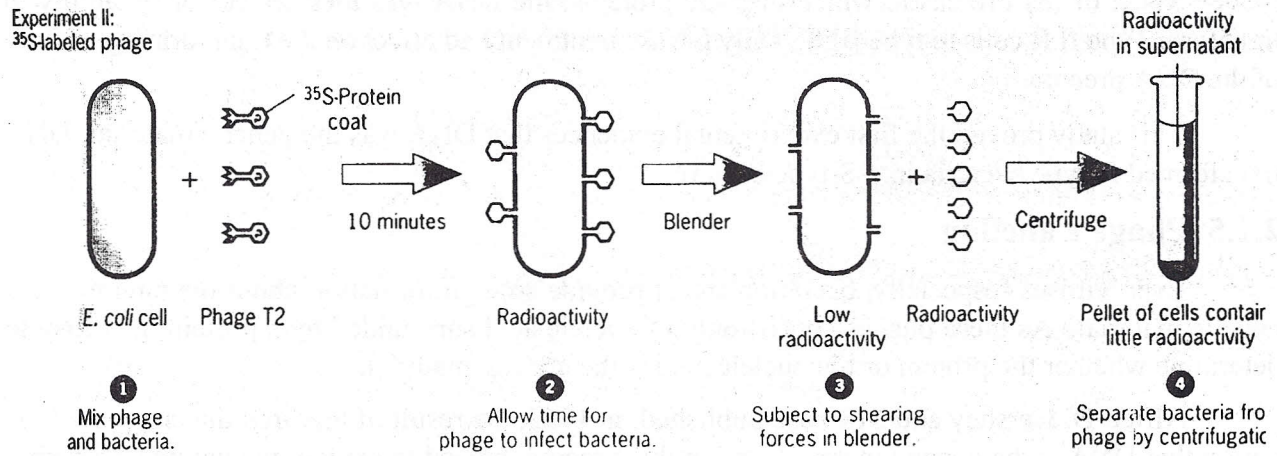
Even viruses [especially, bacteriophages] provide some information about the nature of the genetic material. As these phages consist only of nucleic acid surrounded by a protein, it is easy to determine whether the protein or the nucleic acid is the genetic material.

Alfred D. Hershey and M Chase published, in 1952, the result of research that supported the notion that DNA is the genetic material, and in this process, helped to explain the nature of the viral infection process. Both of them designed an experiment using radioactive isotopes of sulphur and phosphorus to keep the track separately of the viral proteins and nucleic acids during the infection process, as all the nucleic acids contain ëPhosphorusí but not ëSulphurí and most of the protein ëSulphurí [ex: Cysteine and Methionine] but not ëPhosphorusí.

They used the **bacteriophage T_2** and the bacterium *Escherichia coli*. The phages which were labelled were made to infect bacteria growing in culture medium containing the radioactive isotopes ^{35}S or ^{32}P. Hershey and Chase then proceeded to identify the material injected into the cell by phages attached to the bacterial wall.



When ^{32}P, labelled phages were mixed with unlabeled *E. coli* cells, Hershey and Chase found that the ^{32}P label entered the bacterial cells and that the next generation of phages that burst from the infected cells carried a significant amount of the ^{32}P label.



When ^{35}S-labeled phages were mixed with unlabelled *E. coli*, the researchers found that the ^{35}S label stayed on the outside of the bacteria for the most part. Hershey and Chase thus demonstrated that the outer protein coat of a phage does not enter the bacterium it infects, whereas the Phageís inner material, consisting of DNA, does enter the bacterial cell.

Since the DNA is responsible for the production of the new phages during the infection process, the DNA, not the protein, was proved to be the genetic material.

2.1.7 Proof that RNA stores the Genetic Material in some viruses

Most of the viruses discovered, contain RNA and proteins, but not DNA. In these RNA viruses, they found that like other organisms, RNA stores the genetic information rather than the proteins. One of the first experiments that established RNA as the genetic material in RNA viruses was the so called reconstitution experiment of Heinz-Fraenkel-Conrat and coworkers, published in 1957. Their simple experiment was done with tobacco mosaic virus (TMV), a small virus composed of a single molecule of RNA encapsulated in a protein coat. Different strains of TMV can be identified on the basis of differences in the chemical composition of their protein coats.

Fraenkel-Conrat and his colleagues treated TMV particles of two different strains with chemicals that dissociate the protein coats of the viruses from the RNA molecules and then separated the proteins from the RNA. They then mixed the proteins from one strain with the RNA molecules from the other strain under conditions that result in the reconstitution of complete, infective viruses composed of protein from one strain and RNA from the other strain. When tobacco leaves were infected with these reconstituted mixed viruses, the progeny viruses were always phenotypically and genotypically identical to the parent strain from which the RNA had been obtained. Thus the genetic information of TMV is stored in RNA, not in protein.

2.1.7 Summary

A genetic material must be able to control the phenotype of a cell or organism (i.e., to direct protein synthesis), it must be able to replicate, and it must be located in the chromosomes. Avery and

his colleagues demonstrated that DNA was the genetic material when they showed that the transforming agent was DNA. Griffith had originally demonstrated the phenomenon of transformation of Streptococcus bacteria in mice. Hershey and Chase demonstrated that it was the DNA of bacteriophage T2 that entered the bacterial cell. Fraenkel-Conrat demonstrated that in viruses without DNA [RNA viruses], such as tobacco mosaic virus, the RNA acted as the genetic material. Thus by 1953, the evidence was strongly supportive of nucleic acids (DNA or in its absence, RNA) as the genetic material.

2.1.8 Self Assessment Questions

1. Give different functions of genetic material.
2. Illustrate how DNA is acting as a genetic material?
3. Demonstrate Avery, Mac Leod and Mc Cartyís experiment.
4. What is meant by phage labeling. Demonstrate in *E. coli*.
5. Prove how RNA act as genetic material in viruses.

2.1.9 Terminology

Genetic material

DNA

RNA

Gene expression

Diplococcus pneumoniae

Transfoormation

DNase

RNase

Bacteriophage T_2

Escherichia coli

^{35}S-labeled cells

^{32}P-labeled cells

Reconstitution experiment

Tobacco Mosaic virus

2.1.10 References

1. **Principles of Biochemistry** - Nelson, Cox and Lehninger
2. **Biochemistry** - Lehninger
3. **Molecular Biology** - Frieffelder
4. **Cell and Molecular Biology** - Lodisch and Baltimore

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LESSON-2.2

DNA AND THE STRUCTURE OF THE GENETIC MATERIAL

CONTENTS

2.2.0 Introduction

Deoxyribonucleic acid (DNA) is a chain like macromolecule that function in the storage and transfer of genetic components. Major components of the cells are DNA covering around 5 to 15% of their dry weight. Although nucleic acids are so named because DNA was isolated from cell nuclei, both DNA and RNA also occur in other parts of the cells.

Just as the aminoacids are the building blocks or monomeric units of polypeptides, the nucleotides are the monomeric units of nucleic acids. Just as one type of protein molecule is distinguished from another by the sequence of the characterized side chains or R groups of the amino acid monomers, each type of nucleic acids is different and distinguished by the sequence of the characteristic heterocyclic bases of its nucleotide monemers.

In this chapter, we examine first, the structure and properties of nucleotides, which serve not only as building blocks of nucleic acids, but also have important functions in intermediary metabolism.

DNA and the structure of the Genetic material

2.2.1 Objectives

- To study the general structure of the nucleotides
- To study the pyrimidines and purines
- To study the nucleosides
- To study DNA
- To study the Structure of DNA double helix.

2.2.2 General structure of the Nucleotides in DNA

The monomeric units of DNA are called deoxyribonuleotides. Each nucleotide contains three characteristic components: (1) a nitrogenous heterocyclic base, which is a derivative of either pyrimidine or purine; (2) a pentoses, (3) a molecule of phosphoric acid.

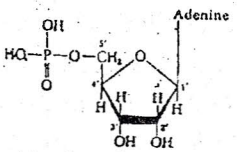
Four different deoxyribonucleotides serve as the major components of DNAs. They differ from each other only in their nitrogenous base components after which they are named. The four bases characteristic of the deoxyribonucleotide units of DNA are the purine derivatives adenine and guanine and the pyrimidine derivatives cytosine and thymine. Thymine is characteristically present in DNA. It is 5-methyl uracil. Deoxyribonucleotides contain 2-deoxy-D-ribose as their pentose component. This sugar occurs in furanose form in nucleotides. The pentose is joined to the base by a β-N-glycosyl bond between carbon atom 1 of the pentose and Nitrogen atom 9 of purine bases or Nitrogen atom 1 of pyrimidine bases. The phosphate group of nucleotides is in ester linkage with carbon atom 5 of the pentose.

When the phosphate group of a nucleotide is removed by hydrolysis, the structure remaining is called a nucleoside. Thus nucleotides are the 5'-phosphates of the corresponding nucleosides. The cells also contain the 5'-diphosphates and 5'-triptrosphates of the common nucleosides.

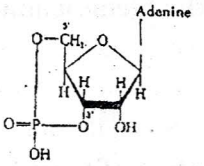
2.2.3 The Pyrimidines and Purines

The parent compounds of the two classes of nitrogenous bases found in nucleotides are the heterocyclic compounds, pyrimidine and purine which have pronounced aromatic character. Purine may itself be regarded as a derivative of pyrimidine. It consists of a pyrimidine ring and an imidazole ring fused together. Three pyrimidine derivatives - uracil, thymine and cytosine and two purine derivatives. Adenine and Guanine, constitute the major nitrogenous bases found in nucleotides. Uracil is absent in DNA.

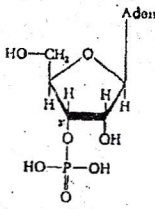
Free pyrimidine and purine bases are relatively insoluble in water. They are weakly basic compounds that may exist in two or more tautomeric forms depending upon the pH. They are responsible for the observed hydrogen bonding between bases in native DNA molecules.



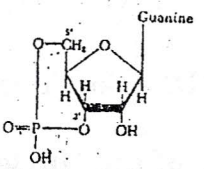
Adenosine 5'-phosphoric acid



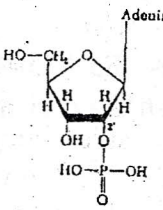
Adenosine 3',5'-cyclic phosphoric acid
(cyclic AMP; cAMP)



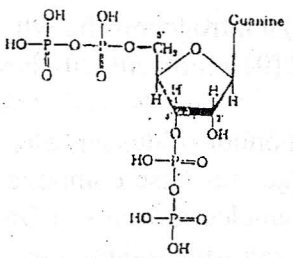
Adenosine 3'-phosphoric acid



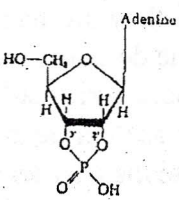
Guanosine 3',5'-cyclic phosphoric acid
(cyclic GMP; cGMP)



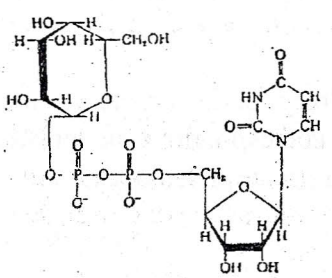
Adenosine 2'-phosphoric acid

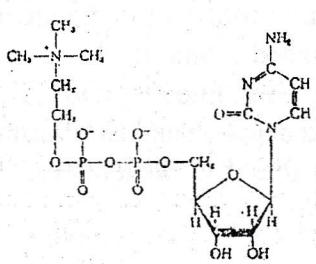


Guanosine 5'-diphosphate 3'-diphosphate
(also called guanosine tetraphosphate)

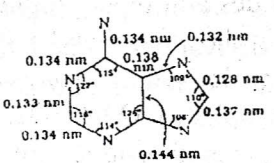


Adenosine 2',3'-cyclic phosphoric acid


Uridine diphosphate glucose
(UDPG)


Cytidine diphosphate choline

Dimensions of the adenine molecule.



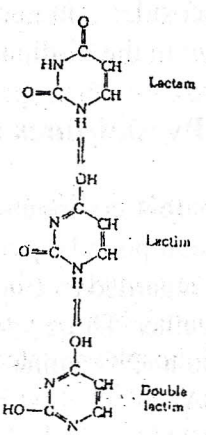


Fig. 2.2.1 Structure of Nucleotide bases

A large number of other purine and pyrimidine derivatives, called the rare or minor basis, occur is small amounts in some nucleic acids. Among the rare pyrimidines are 5-methyl cytosine and 5-hydroxymethyl cytosine. The minor purines include 6-methyl adenine and 2-methyl guanine. Most of them are methyl derivatives. Some contain acetyl, isopentenyl or hydroxymethyl groups. The purines and pyrimidine bases of nucleic acids strongly absorb U-V light in the region 250-280 nm. This property is very useful in the detection and quantitative analysis not only of the free bases, but also of nucleotides andnucleosides. Free purine and pyrimidine bases are easily separated by chromatographic or electrophoretic methods.

2.2.4 Nucleosides

Deoxyribonucleosides contain 2-deoxy-D-ribose in DNA. Free nucleosides occur only in trace amounts in most cells and these are the products of chemical or enzymatic hydrolysis of nueleotides. Nucleosides are much more soluble in water than their corresponding free bases. They are readily separated and identified by chromatographic methods. Like the glycosides, nucleosides are relatively stable in alkali. The purine nucleosides are rather easily hydrolysed by acid to yield the free base and the pentose. However, the pyrimidine nucleosides are resistant to acid hydrolysis.

2.2.5 Nucleotides

Deoxyribonucleotides occur in the free form in cells in significant amounts. Phosphoric groups are relatively strong acids at pH – 7.0. The free nucleotides thus exist primarily in the form N-O-PO_3^{2-}, where N is nucleoside group. Due to the presence of a pyrimidine or purine base, all the nucleotides show strong ultraviolet absorption in the region 250-280 nm. Nucleotides are easily separated and quantitated by ion-exchange chromatography.

Nucleosides and nucleotides contain two nearly planar rings, that of the base and that of the ribofuranose. In the most stable conformation of nucleotides, the rings are not coplanar but are almost at right angles to each others, placing the 2' hydrogen or hydroxyl of the ribofuranose ring in close proximity to nitrogen atom 3 of the purines or oxygen atom-2 of the pyrimidines.

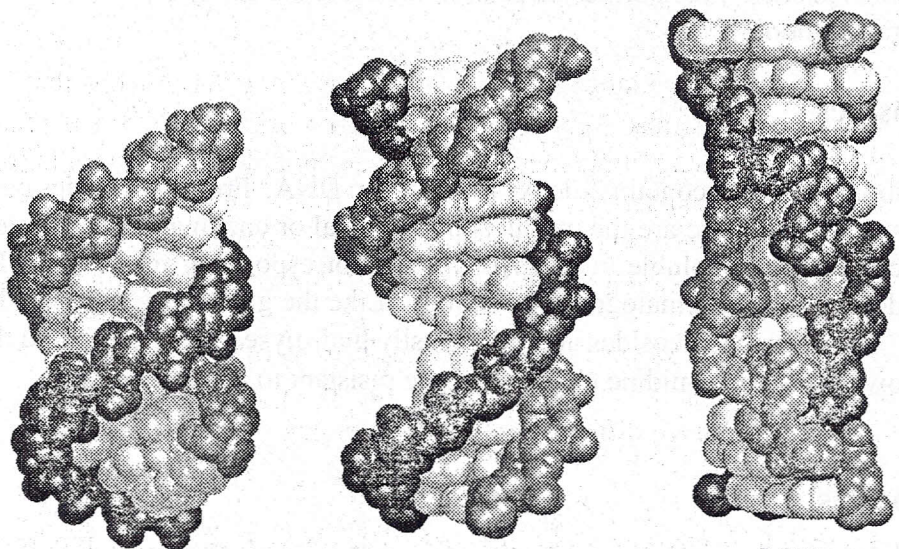
2.2.6 Nucleoside 5'-Diphosphates and 5'-Triphosphates

All the common ribonucleosides and deoxyribonucleosides occur in cells not only as the 5'-monophosphates, but also as the 5'-diphosphates and 5'-triphosphates, i.e., as the 5'-pyrophosphoric and the 5'-triphosphoric esters of the nucleosides. We have three series of 5'-phosphorylated nucleosides – For Adenine: Adeosine-5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP) and adenosine-5'-triphosphate (ATP). The phosphoric acid residues are designated as α, β, γ respectively.

The nucleoside 5'-diphosphoric acids and 5'-triphosphoric acids, generally designated as NDPs and NTPs and are relatively strong acids.

2.2.7 DNA

DNA was first isolated (from pus cells and salmon sperm) and intensively studied by Friedrich Miescher, a Swidish scientist, in a series of remarkable investigations beginning in 1869. He named it "nuclein" from its occurrence in cell nuclei. Over 70 years of research were required to completely identify the major building block units and the backbone structure of nucleic acids.



DNA molecules from different cells and viruses vary in the ratio of the four major types of nucleotide monomers in their nucleotide sequence and in their molecular weight. Besides the four major bases (adenine, guanine, thymine and cytosine) found in all DNAs, small amounts of methylated derivatives of these bases are present in some DNA molecules, particularly those from viruses. The DNAs isolated from different organisms and viruses normally have two strands in complementary double-helical arrangement. In most cells, the DNA molecules are so large that they are not easily isolated in intact form. In prokaryotic cells which contain only a single chromosome, essentially all the DNA is present as a single double-helical closed circular DNA. It is a macromolecule exceeding $2x10^8$ in molecular weight.

In eukaryotic cells, which contain several or many chromosomes, there are correspondingly several or many DNA molecules. In bacteria, the DNA molecule, which makes up about 1% of the cell weight, is found in the nuclear zone. It is usually attached, apparently in a single point, to an infolding of the cell membrane called a mesome. In bacteria no protein is associated with the DNA. Sometimes small molecules of extra chromosomal DNA occur in the cytoplasm of bacteria; such DNA molecules, which carry only a few genes, are called either plasmids or episomes, depending on their genetic relationship to the chromosomal DNA.

In diploid eukaryotic cells, nearly all the DNA molecules are present in the cell nucleus, where they are combined in ionic linkage with basic proteins called histones. In addition to the nuclear DNA,

diploid eukaryotic cells also contain very small amounts of DNA in the mitochondria, it differs in its bar composition and molecular weight from nuclear DNA. Mitochondrial DNA (designated as mtDNA) has a molecular weight of about 10 million; it accounts for 0.1 to 0.2 per cent of the total cellular DNA. Chloroplasts also contain a distinctive type of small DNA molecule. Many viruses contain DNA, which may range in molecular weight from about 2 million to over 100 million, depending upon the viral species. The structure and biological function of various types of DNA are also important.

2.2.8 Base Equivalences in DNA

One of the most important biochemical evidence supporting the concept, that DNA is the bearer of genetic information was, the discovery that the base composition of DNA is related to species of origin. Before reliable chromatographic methods became available, it was thought that the four major bases found in DNA – adenine, guanine, cytosine and thymine – occur in equimolar ratio in all DNA molecules. Chargaff *et al.*, applied quantitative chromatographic methods to the separation and quantitative analysis of four basis in hydrolysates of DNA specimens isolated from different organisms.

1. The base composition of DNA varies from one species to another.

2. DNA specimens isolated from different tissues of the same species have the same base composition.

3. The base composition of DNA in a given species does not change with age, nutritional state, or changes in environment.

4. In nearly all DNAs examined, the number of adenine residues is always equal to the number of thymine residues, that is A=T, and the number of guanine residues is always equal to the number of cytosine residues (G=C). As a corollary, it is clear that the sum of the purine residues equals the score of the pyrimidine residues that is **A+G=C+T**.

5. The DNAs extracted from closely related species have similar base composition, whereas those of widely different species are likely to have widely different base composition. The base composition of their DNAs can in fact be used to classify organisms.

2.2.9 The Watson-Crick Model of DNA structure

The base equivalences observed in DNA from different species raised the intriguing possibility that there is a level of structural organization of DNA which is compatible with certain base equivalences and incompatible with others. In fact, it had already been suspected that DNA has a specific three-dimensional conformation. Since solutions of native DNA are highly viscous, it appeared that DNA molecules are long and rigid rather than compact and folded. Heating freshly isolated DNA produces significant changes in its viscosity and other physical properties, without breaking the covalent bonds in the DNA backbone. Because of such observations and the illuminating discovery of the α-helical arrangement of the polypeptide chains in some fibrous proteins by L. pouling and his col-

leagues by x-ray diffraction studies. By the early 1950s it was inevitable that the powerful x-ray method was to be applied to the problem of DNA structure. In fact, fibres of DNA had already been subjected to x-ray diffraction analysis as early as 1938; when W. Astbury and F.O. Bell first observed that fibrous DNA specimens show reflections corresponding to a regular spacing of 0.34 nm along the fiber axis, but the significance of this observation was not clear because the DNA was known to be impure. In the period 1950-1953, more refined x-ray data were obtained on fibres from highly purified DNA, particularly through the work of R. Franklin and M.H.F. Wilkins. DNA can be obtained in two forms, A and B, which differ in degree of hydration; the B form is biologically important one. The B form of DNA was found to possess two periodicities, a major one of 0.34 nm and a secondary one of 3.4 nm, reminiscent of the major and minor periodicities observed in α-keratins.

In 1953, J.D. Watson and F.H. C. Crick postulated a precise three-dimensional model of DNA structure based on the x-ray data of Franklin and William and the base equivalences observed by Chargaff. This model not only accounted for many of the observations on the chemical and physical properties of DNA, but also suggested a mechanism by which genetic information can be accurately replicated.

The Watson-Crick model of DNA structure proposed that two right handed polynucleotides chains are coiled in helical fashion around the same axis, thus forming a double helix. The two chains or strands are antiparallel; i.e., their 3′,5′-internucleotide phosphodiester bridges run in opposite directions. The coiling of the two chains is such that they cannot be separated except by unwinding the coils; such coiling is termed plectoromic. The purine and pyrimidine bases of each strand are stacked on the inside of the double helix, with their planes parallel to each other and perpendicular to the long axis of the double helix. The bases of one strand are paired in the same planes with the bases of the other strand. The pairing of the bases contributed by the two strands is such that only certain base pairs fit inside this structure in such a manner that they can hydrogen-bond to each other.

The allowed pairs are A-T and G-C, which are precisely the base pairs showing equivalence in DNA. The disallowed purine pair A and G is rather large to fit inside a helix having a thickness of 2.0 nm and the disallowed pyrimidine pair C and T would appear to be too far apart within a 2.0 nm helix to form stable hydrogen bonds with each other. The allowed pairs A-T and G-C are only about the same size. They are also more strongly hydrogen-bonded than A-G and C-T pairs. Thus, the Watson-Crick double helix involves the maximum possible pairs giving maximum fit and stability.

To account for the 0.34 nm periodicity, observed by x-ray methods, Watson and Crick postulated that the bases are stacked at a center-to-center distance of 0.34 nm from each other. There are exactly 10 nucleotide residues in each complete turn of the double helix to account for the secondary repeat distance of 3.4 nm. DNA can theoretically exist in other helical forms, but they would not have the 0.34 nm repeat distance along the long axis observed in native DNA. The Watson and Crick double helix, which is about 2.0 nm in diameter, has one shallow and one deep groove. The relatively hydrophobic bases are within the double helix, shielded from water. The polar sugar residues and negatively charged phosphate groups are located on the periphery, exposed to water. The double helix is stabilized not only by the hydrogen bonding of complementary base pairs, but also by electronic interactions between the stacked bases as well as by hydrophobic interactions.

The two antiparallel chains of double-helical DNA are not identical in either base sequence or composition. Instead, the two chains are complementary to each other. Wherever adenine appears in one chain thymine is found in the other and vice-versa, and wherever guanine is found, in one chain, cytosine is found in the other and vice-versa. The double-helical structure of DNA leads to the second clear cut of the Watson Crick hypothesis namely a mechanism by which genetic information can be accurately replicated. Since the two strands of double-helical DNA are structurally complementary to each other and thus contain complementary information, the replication of DNA during cell division was postulated to occur by replication of the two strands, so that each parent strand serves as the template specifying the base sequence of the new complementary strand. The end result of such a process is the formation of two daughter double-helical molecules of DNA, each identical to that of the parent DNA and each containing one strand from the parent. Proof emerged for the antiparallel polarity of the two strands of DNA and for the "semi-conservative" nature of the replication process described above. The Watson-Crick hypothesis had an immediate and profound impact on the nature of experimentation designed to unravel the molecular nature of genetic information transfer. The double-helical or duplex structure of DNA quickly gained support from many other kinds of evidence. The length of homogenous native DNA molecules of known weights, such as relatively small DNAs of certain bacterial viruses, can be measured accurately and directly by electron microscopy.

2.2.10 Summary

That DNA bears genetic information was first shown by experiments in which addition of DNA isolated from one strain of a bacterium transformed another strain in a heritable manner to the strain from which the added DNA was derived. The amount of DNA per cell increases with the cell's position on the evolutionary scale. All somatic (diploid) cells of a given species of higher organism contain same amount of DNA, which is not modified by diet or environmental circumstances. The base composition of DNA specimens varies characteristically from one species to another. In double stranded DNAs, the number of adenine residues equals the number of thymine residues; similarly, the number of guanine residues equals that of the cytosine residues.

From x-ray analysis of DNA fibres and from the base equivalences in DNA, Watson and Crick postulated that native DNA consists of two antiparallel chains in a double-helical arrangement with the complementary basis.

A-T and G-C are paired by hydrogen bonding within the helix and the covalent backbone on the outside. The base pairs are closely stacked perpendicular to the long axis; 0.34 nm apart. The double helix is about 2.0 nm in diameter. This structure provides an explanation for accurate replication of the two strands of DNA.

2.2.11 Terminology

DNA
Purines
Pyrimidines
Watson and Crick
Double helix

2.2.12 Self Assessment Questions

1. Explain the DNA double helical structure.
2. Explain the Watson and Crick model of DNA
3. Write short notes on:
   Nucleotides
   Nucleosides
   Nucleic acids

2.2.13 Reference Books

1. Biochemistry – Nelson, Cox, Lehninger.
2. Biochemistry – Lehninger
3. Textbook of Biochemistry – A.V.S.S. Rama Rao
4. Textbook of Biochemistry – A.C.Deb.

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Lesson - 2.3.

TYPES OF RNA (mRNA, rRNA, tRNA)

2.3.0. Introduction

The central dogma of Molecular Biology defines: Genes are perpetuated as sequence of Nucleic acids by function by being expressed in the form of proteins. Three types of processes are responsible for the inheritance of genetic information and for its conversion from one form to another.

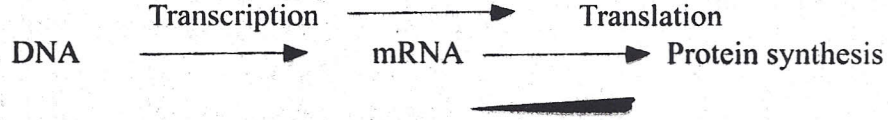
Information is perpetuated by replication where a double-stranded nucleic acid which is DNA is duplicated to give identical copies.

Information is expressed by a two stage process.

Transcription generates a single stranded RNA identical in sequence with one of the strands of the duplex DNA. Several different types of RNA are generated by transcription. The three principal classes involved in the synthesis of proteins are

1. messenger RNA (mRNA)
2. transfer RNA (tRNA)
3. ribosomal RNA (rRNA).

Translation converts the nucleotide sequence of RNA into the sequence of amino acids comprising a protein. An mRNA is translated into a protein sequence; tRNA and rRNA provide other components of the apparatus for protein synthesis.



Because the genetic material in the nucleus is physically separated from the site of protein synthesis which takes place in the cytoplasm of eukaryotic cells, it was clear that the DNA could not itself be translated into protein, so to carry the information from one compartment to another an intermediatory must be needed.

Messenger RNA, the name reflects its role. RNA is synthesized from DNA by process called Transcription in eukaryotic cell nucleus and is transported to the cytoplasm; the site where protein synthesis takes place. It is assumed that eukaryotic cell keeps its master set of sequence in the nucleus, while a "Working set" consists of cytoplasmic mRNA copies of the DNA sequences that are to be expressed. Transcription in eukaryotic cells takes place in the nucleus while translation would take place in the cytoplasm. But incase of prokaryotic cells transcription and translation would be taking place in the same compartment and is called coupled transcription and translation and it takes place in the cytoplasm.

One strand of DNA act as a template on which the synthesis of mRNA takes place is called Transcription. The strand of the DNA ascting as a template for the synthesis of RNA is called as template strand or antisense strand. The template strand of DNA and RNA which is synthesized on it are complementary to one another. The other DNA strand which is not a template has a sequence similar to the sequence present in mRNA is called the coding strand or sense strand.

Most of the cellular genetic material is DNA but some viruses contain RNA as their genetic material and the replication of viral RNA takes place in the infected cells.

The mRNA is the intermediate in carrying the genetic information from one or few genes in DNA to the ribosomes where this organelle provides the environment for the synthesis of the protein. rRNA are the structural components of the ribosomes. Transfer RNAs (tRNA) are the adaptor molecules that faithfully translate the information in mRNA into a specific sequence of amino acids.

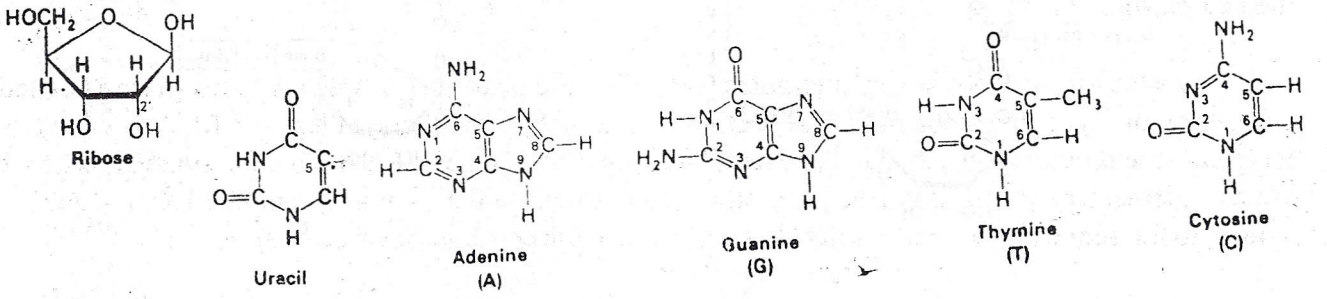
All forms of RNAs are synthesized by an enzyme called RNA polymerase that takes the instructions from the DNA template called as transcription and followed by translation, the synthesis of proteins according to the instructions given by the mRNA templates. In case of Eukaryotic cells three different kinds of RNA polymerases called RNA polymerase I, RNA polymerase II, and RNA polymerase III are responsible for transcription but in Prokaryotes only one kind of RNA polymerase is involved in the synthesis of RNA.

The components of the organelle ribosome is rRNAs and ribosomal proteins and the rRNAs play a structural and catalytic roles and for each of the 20 amino acids atleast there is one tRNA molecule.

Eukaryotic cells contain additional small RNA molecules called Small Nuclear RNA (SnRNA) molecules; which participate in the splicing of RNA exons. A small RNA in cytosol called Small cytosolic RNA (ScRNA) are also present.

2.3.2. Chemistry and Structure of RNA

RNA like DNA is a long unbranched polymer consisting of ribonucleotides joined by 3'‡ 5' phosphodiester bonds. The covalent structure of RNA differs from that of DNA in two respects : as indicated by its name the sugar units in RNA are ribose rather than deoxy ribose. Ribose contains a 2'-hydroxyl group not present in deoxyribose. Each ribonucleotide i.e., the precursor in the RNA is composed of a sugar ribose and phosphate and a nitrogenous base. Each of the nitrogenous base contains a heterocyclic ring which is the parent component of purine and pyramidine. The different purines present in the RNA are Adenine (A) and Guanine (G) and the two pyrimidines present are Cytosine (C) and Uracil (U). The pyrimidine Thymine (T) is absent in RNA.



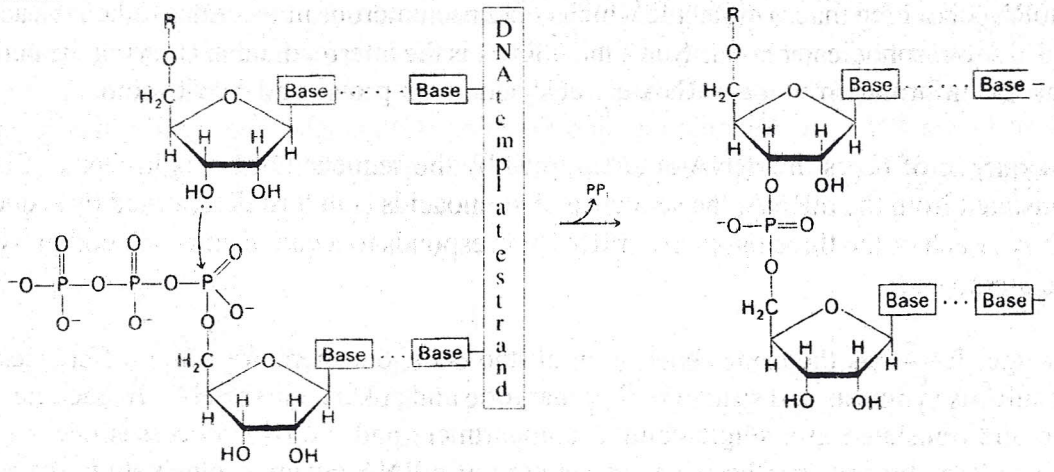
Uracil like Thymine can form a base pair with Adenine but lacks a methyl group present in Thymine RNA molecules are always designated from 5' – 3' direction. The 5' end of an RNA contains a tri phosphate and 3' end of RNA contains an hydroxyl group. Each of the ribonucleotide is linked by a phosphodiester bond between the 3' OH group of one nucleotide and the 5' phosphate group of the next nucleotide. RNA molecules can be of single stranded or double stranded. RNA cannot form a double helix of the B type DNA because of steic interference by the 2'-hydroxyl groups of its ribose units but RNA can adopt a modified double-helical form in which the base pairs are tilted by 20 degrees away from the perpendicular to the helix axis, a structure like A-DNA. Since RNA is usually a single stranded. Consequently, an RNA molecule need not have complementary ratios in fact proportion of Adenin differs from that of Uracil and the proportionofGuanine differsfrom that of Cytosine in most of the RNA molecules. The RNA molecules do contain regions of double-helical structure that are produced by the formation of hair pin loops (Fig.). In these regions the Adenine pairs with uraciland guanine pairs with cytosine. The base pairing of the bases in the RNA is often imperfect. Guanine can also base pair with uracil but is less strong than guanine and cytosine base pair. Some of the opposing bases may not be complementary at all, and one or more bases along a single strand may be looped out to facilitate the pairing of the others.

2.3.3. Synthesis of Cellular RNAs:

Jerard Hurwitz and Samuel Weiss (1960) independently discovered as enzyme that synthesizes RNA according to instructions given by a DNA template and the enzyme was named as RNA polymerase. This enzyme from E. coli requires the following components for the synthesis of RNA:

1. Template: The preferred template is double-stranded DNA. Single-stranded RNA can also serve as template.

2. Activateds precursors: All four ribonucleoside triphosphates ATP, GTP, CTP and UTP are required.



3. Divalent Metal ions: Mg^{2+} or Mn^{2+} are effective. Mg^{2+} meet there quirement invivo.

RNA polymerase catalyzes the initiation and elongation of RNA chains. The reaction catalyzed by enzyme is

$(RNA)_n$ residues + ribonucleoside triphosphate = $(RNA)_{n+1}$ residue + PPi

The synthesis of RNA is similar to that of DNA in several respects. First, the direction of synthesis is 5′ ‡ 3′. Second the mechanism of elongation is similar. The 3′OH group at the terminus of the growing chain makes a nucleophic attack on inner most phosphate of the incoming nucleoside triphosphate. Third the synthesis is driven forward by the hydrolysis of pyrophosphate.

2.3.4. Different types of RNA:

The three different types of cellular RNAs are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA).

2.3.4.1 Messenger RNA (mRNA)

The concept of mRNA was formulated by Francois Jacob and Jacques Monod. They summarised that the messenger RNA must be a very short-lived intermediate with the following properties

i. The messenger RNA should be a polynucleotide

ii. The base composition of the messenger RNA should reflect the base composition of the DNA that specifies it.

iii. The messenger RNA should be very heterogeneous in size because genes or groups of genes vary in length.

iv. The messenger RNA should be transiently associated with the ribosomes, the site of protein synthesis.

v. The messenger RNA should be synthesized and degraded very rapidly.

The mRNA is a long macro molecule which contains numerous nucleotides linked to each other by means of 3′ - 5′ phosphodiester bond. Since the mRNA is the intermediate in carrying the hereditary informationas, the information in the mRNA is translated into a protein by the ribosome.

The sequence of bases in mRNA is determined by the sequence of bases in DNA Since the protein is translated from the mRNA, the sequence of aminoacids is in turn determined by sequence of bases in a DNA. Each of the three bases in a mRNA corresponds to a codon and each codon would be coding for an aminoacid.

Messenger RNA has the same function in all the cells, but there are some differences in the details of the mRNA synthesis and structure of prokaryotic and eukaryotic mRNA. In bacteria mRNA is transcribed and translated in a single cellular compartment and the two processes occur simultaneously but in eukaryotic cells synthesis and maturation of mRNA occurs exclusively in the nucleus. Only after completion of the events the mRNA exported to the cytoplasm where it is translated by ribosomes.

Bacterial mRNAs vary greatly in the number of proteins for which they code. Some mRNA represent only one gene and code for only one protein, they are monocistronic. Other mRNAs carry sequences coding for several proteins, they are polycistronic. In these cases, a single mRNA is transcribed from a group of adjacent genes (Operon).

All mRNAs contain two types of regions. The coding region consists of a series of codons representing the amino acid sequence of a protein, starting usually with AUG and ending with terminating codon. But the mRNA is always longer than the coding region. In a monocistronic mRNA, extra regions are present at both the ends. An additional sequence at the 5'end preceding to the start codon is described as the leader. An additional sequence following the termination signal; forming the 3'end is called trailer. The leader and trailer sequences are a part of mRNA and they are not used to code for proteins.

The polycistronic mRNA contains more than one coding regions (cistrons). The region between two cistrons (coding regions) is called inter cistronic region or nontranslated spacer. This intercistronic region contains as many as 30 nucleotides in bacterial mRNA but they can also be as short as 1 to 2 bases. Some times the last base of one cistron becomes the first base of next cistron.

The bacterial mRNAs usually is unstable and is therefore translated into protein for only short period of time (typically a few minutes) but eukaryotic mRNA is more stable and is usually translated for few hours. The degradation of mRNA in bacteria closely follow the translation.

The mRNAs in eukaryotic cells are modified at the 5′ ends and 3′ ends. The 5′ end of an mRNA is capped with a methylated guanine ribonucleotide. This addition is catalyzed by an enzyme called guanyl transferase. The new guanine linked to the terminal nucleotide of the mRNA forming 5′ - 3′ triphosphate bond. This structure is called a Cap and the cap is methylated at N^7 of Guanine it is called cap O.

The 3'end of an mRNA has a polyadenylate tail. The Adenine residues are added to the 3'end of an mRNA by an enzyme called poly A polymerase. The presence of the methyl cap at the 5'end and a polyadenylate tail towards the 3'end protects the mRNA from degradation.

2.3.4.2. The transfer RNA (Adaptor RNA)

Since the bases in mRNA code for the aminoacids, but no amino acids are in direct contact of mRNA, Crick suggested that translation might be mediated by an adoptor molecule. This adoptor is a transfer RNA (tRNA), a small molecule whose polynucleotide chain is only 75 – 85 bases long.

A tRNA has two crucial properties

i. It represents a single amino acid to which it is covalently linked.

ii. It contains a trinucleotide sequence, the anticodon which is complementary to the codon representing its amino acid. The anticodon enables the tRNA to recognize the codon via., complementary base pairing.

The tRNA have some unusual features including its secondary and tertiary structures. They have unusual bases that are generated by modification of the four stranded bases. The tRNA forms a secondary structure. It is represented as a clover leaf which has stem for single stranded loops.

The clover leaf of tRNA has the following arms:

i. The acceptor arm consists of a base-paired stem that ends in an unpaired sequence whose free 2'- or 3'-OH group can be linked to an amino acid.

ii. The T ψ C arm is named for the presence of this triple sequence (ψ stands for pseudouridine, a modified base)

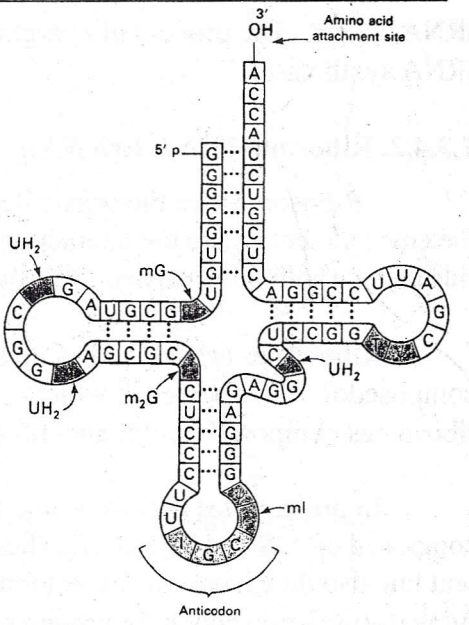
iii. The anticodon are always contains the anticodon triplet in the center of the loop.

iv. The D-arm is named for its content of the base dihydrouridine (another of the modified bases in tRNA).

The most variable feature of RNA is the so called extra arm which lies between the T ψ C arm and the anticodon arm. Based on the extra arm tRNAs can be divided into two classes Class 1. tRNAs have a small extra arm consisting of only 3 – 5 bases. They represent 75% of all tRNAs. Class 2. tRNAs have a large extra arm, it may be having 13 – 21 bases.

The base pairing that maintains the secondary structure is invariant most of base pairing to maintain the secondary sstructure is pairing between A-U and G-C but occasional G-U and G-G and A-A pairs are found, which are unusual.

tRNA has a copmplex tertiary structure:



The three dimensional structure of a tRNA molecule was first solved in 1974. It includes the following features.

a. The molecule is L shaped.

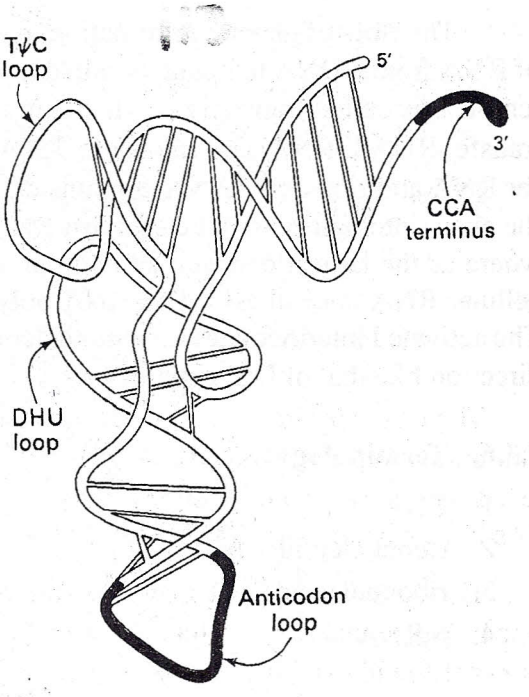
b. There are two segments of double helix. They are like A-DNA as expected for an RNA duplex. Each of these helixes contain about 10 base pairs which correspond to one turn of helix, the helical segments are perpendicular to each other giving the molecule its L shape.

c. Most of the bases in the non helical regions participate in unusual hydrogen-bonding interactions. These tertiary interactions are between the bases that are not usually complementary (eg., G.G, A.A and A.C).

Moreover, the ribose phosphate back bone interacts with some bases and even with another region of the back bone itself. The 2'OH groups of the ribose units act as hydrogen bond donors or acceptors in many of the interactions. In addition most of these bases are stacked. The hydrophobic interactions of aromatic rings play a role in stabilizing the architecture of the molecule.

d. The CCA terminus containing the amino acid attachment site is at one end of the L. The other end of the L is the anticodon loop and the DHU and TWC loops form the corner of L.

The tertiary structure is created by hydrogen bonding mostly involving bases that are unpaired in the secondary structure. The bonds of the clover leaf are described as secondary H-bonds; the additional bonds of the tertiary structure are called tertiary H-bonds.



When a t RNA is charged with amino acid corresponding to its anti codon, it is called amino acyl tRNA. The amino acid is linked by an ester bond, from its carboxyl group to the 2' or 3' hydroxyl group of the ribose of the 3' terminal base of the tRNA.

There is atleast one tRNA for each aminoacid. A tRNA is named by using three letter abbreviation for the amino acid as a super script. For example $tRNA^{Ala}$ is tRNA for Alanine. If more than two tRNAs for 1 amino acid the super script numerals are used. Example $tRNA_1^{Tyr}$,

$tRNA_2^{Tyr}$, etc. The process of charging an amino acid to tRNA is catalysed by the enzyme amino acyl tRNA synthetase.

2.3.4.2. Ribosomal RNA LrRNA):

Ribosomes are the organelles which plays an important role in protein synthesis. It provides the environment where the interaction between the mRNA containing the codons coding for a polypeptide and the anticodon carried by the adaptor tRNA which carries the amino acid would be taking place.

Ribosomes are organelles present in prokaryotic and eukaryotic cells. In prokaryotic cells it is composedof 70S ribosomes which is made up of 2 subunits 30S and 50S type. In eukaryotic 80S ribosomes composed of 40S and60S sub unit.

In prokaryotes 30S ribosome is composd of 16S rRNA and 21 proteins and the 50S subunit is composed of 23S and 5S rRNA. These rRNA not only contribute to the structure of the ribosomal sub unit but also they have various catalytic roles. The 16S rRNA of 30S subunit is responsible for identifying the initiation codon for protein synthesis in mRNA. The sequence in 16S rRNA close to the 3'end is complementary to the sequence called Shine Dalgarno Sequence located upstream of initiating codon, AUG. The 23S ribosomal RNA has the catalytic activity of catalyzing the peptide bond formation between amino acids of a polypeptide chain Lpeptidyl transferase) and 5S rRNA which add to the structural stability. The 16S rRNA in 30S subunit is associated with 21 proteins designated as S1, S2, S3, to S21 and 23S and 5S rRNAs of 50S subunit are associated with 33 proteins designated as L1, L2, — L33, etc. The eukaryotic cells contain 18SrRNA in its 40S subunit and 28S, 5S and 5.8S rRNAs in its 60S ribosomal subunit associated with proteins.

2.3.5. Summary

The flow of genetic information in normal cells is from DNA to RNA to protein. The synthesis of RNA from a DNA template is called transcription, where as the synthesis of a protein from an RNA template is called translation. There are three kinds of RNA molecules, messenger RNA (LmRNA, transfer RNA LtRNA, and ribosomal RNA LrNA). These RNA molecules are single stranded. Transfer RNA and ribosomal RNA contains extensive double-helical regions that arise from the folding of the chain into hair pins. The smallest RNA molecules are tRNAs which contain about 75 nucleotides where as the largest ones are among the mRNAs which may have more than 5000 nucleotides. All cellular RNA is synthesized by RNA polymerase according to instructions given by DNA template. The activated intermediates are ribonucleotide triphosphates. The direction of RNA synthesis is 5' ‡ 3' direction like that of DNA synthesis.-

2.3.6. Terminology:

1. RNA
2. ribonucleotide
3. ribonucleoside
4. mRNA

5. tRNA
6. rRNA
7. purines
8. pyrimidines
9. uracil
10. methyl cap
11. poly adenylate tail
12. anti codon arm
13. clover leaf structure
14. acceptor arm
15. Shine Dalgarno Sequence

2.3.7. Self assessment questions:

1. Define transcription and explain the chemistry and structure of RNA?
2. What is mRNA and what role itsplays in the cell. Explain its structure?
3. Describe the structure and function of tRNA?
4. What are rRNAs and explain the role played by them and what are the different rRNAs?
5. What are the different RNAs present in the cell and explain the role played by them?

2.3.8. Reference Books:

1. Genes VII ——Benjamin Lewin———3rd edition
2. Biochemistry —— Lubert Stryer ——— 3rd edition
3. Molecular cell Biology—Lodish , David Baltimore
4. Biochemistry ———Lehninger , Nelson and Cox.

UNIT-II

LESSON - 2.4

PROTEIN SYNTHESIS

2.4.0 Introduction

The central dogma of gene expression (molecular biology) defines genes as organised sequenced of bases in Nucleic acids, but function by being expressed in the form of proteins. The link between the DNA and the enzymes is RNA. DNA directs the transcription of its sequence into RNA by the enzyme, RNA polymerase. The sequence of bases in the RNA is then translated into corresponding sequence of aminoacids to form a protein.

The RNA that corresponds tot he protein coding gene is called messenger RNA (or) mRNA. The mRNA is translated into a protein by an organelle called ribosome. At the ribosome each set of three nucleotides in the mRNA pairs with three complementary nacleotides in a small RNA molecule

ñ a transfer RNA or tRNA which in turn would be carrying an aminoacid. The cracking of genetic code by Nirenberg and his coworkers showed that enetic information is stored in the form of nucleotide triplets (codons). Each of the codon would be representing an amino acid.

GENETIC CODE

FIRST POSITION	SECOND POSITION U	C	A	G	THIRD POSITION
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Iie	Thr	Arn	Ser	U
	Iie	Thr	Arn	Ser	C
	IIe	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

2.4.1 Objectives

- To study the concept of genetic code
- To explain the prokaryotic translation process
- To study the steps involved in eukaryotic translation
- Protein targetting fundamental study
- To study protein degradation
- To study prions

2.4.2 Features of the Genetic code

1. The genetic code is comma less, i.e., it does not have any penetration signaling the end of one codon and the beginning of the next. However, the genetic code contains special signal sequences, for initiation and termination of the polypeptide chains.

2. The initiating amino acid is N-formyl melthionine in prokaryotes and methionine in eukaryotes. Both are encoded by the same initiation codon AUG.
3. Genetic code is triplet. There are 4 bases in a DNA but the number of aminoacids are 20. So four bases cannot code for 20 aminoacids. If the bases are in combination of two, $4^2 = 16$. So 16 bases as double + cannot code for 20 amino acids. If the bases are in combinations of three, $4^3 = 64$. It would be coding for 20 aminoacids. So three bases together code for single aminoacid.
4. Three of the 64 codons, (UAA, UAG, UGA) do not code for any amino acid. They terminate the polypepticle chain. These are referred to as stop codons, termination codons or nonsense codons.
5. Although the genetic code is commaless, it starts with the initiation codon AUG and ends with any one of the termination codons. The region extending from the initiation codon to the termination codon is known as the cistron encoding a protein product. If the protein product is not known, this is known as an open reading frame (ORF).
6. The code is non ambiguous. Each codon specifies a single amino acid.
7. The genetic code is degenerate. More than one codon codes for a single amino acid. A maximum of 6 codon can code for 1 amino acid and a minimum of 1 codon codes for one amino acid.
8. **Wobble hypothesis:** The number of codons coding for 20 amino acids are 61, but the number of tRNAís carrying the amino acids to the site are less than 61 but greater than 20. So more than one tRNA have to complementary base pair with more than one codon since codon is degenerate.

   The base pairing between the codon of mRNA and the anticodon of tRNA reads to be mentioned. The first two bases in a codon of mRNA base pairs with the last two bases of the aticodon of tRNA according to strict Watson and Crick base pairing but the pairing between first base of anticodon and last base of codon in mRNA do not pair according to strict Watson and Crick base pairing. There is a little Wobble played between these two bases. Depending on the base present at the first position of anticodon, we can say how many bases it can pair within a codon.

Anticodon 1st base	Last base of a codon
C	G
A	U
U	A/G
G	C/U
I	U/A/C

9. The features described above are similar in most organisms. However, mitochondria and some unicellular organisms show slight deviations from the "universal" genetic code. In mitochondria, the codon for methionine is AUA and not AUG, UGA which is a stop codon codes for Tryotophan and AGA signals termination instead of encoding arginine.

2.4.3 Translation

Translation or protein biosynthesis takes place in the cytosol by the concised action of all the three RNAs ñ rRNA, tRNA and mRNA. The ribosome containing the rRNA moves along the mRNA while tRNA brings the amino acids. The polypeptide is synthesized from the N-terminal to the C-terminal end corresponding to the 5' → 3' direction of the codons in mRNA.

2.4.3.1 Translation in prokaryotes: Protein synthesis takes place in three stages ñ initiation, elongation and termination. Activation of amino acids, the building blocks of proteins occurs before initiation of polypeptide chain synthesis.

2.4.3.1.1 Activation of amino acids: The 20 amino acids are esterified to their corresponding tRNAs by the action of amino acyl - tRNA synthetase in a two step process. In the first step, the amino acid reacts with ATP, to form an aminoacyl adchylate intermediate. In the next stage, the amino acyl group is transferred from the enzyme-bound intermediate to the terminal adenine residue in the 3' CCA terminus of tRNA. The overall reaction is as follows:

$$\text{Aminoacid} + \text{tRNA} + \text{ATP} \rightarrow \text{Aminoacyl - tRNA} + \text{AMP} + \text{pPi}$$

The aminoacyl ñ tRNA synthetases are specific for the corresponding tRNAs as well as the amino acids. These enzymes are capable of proof reading and correcting errors.

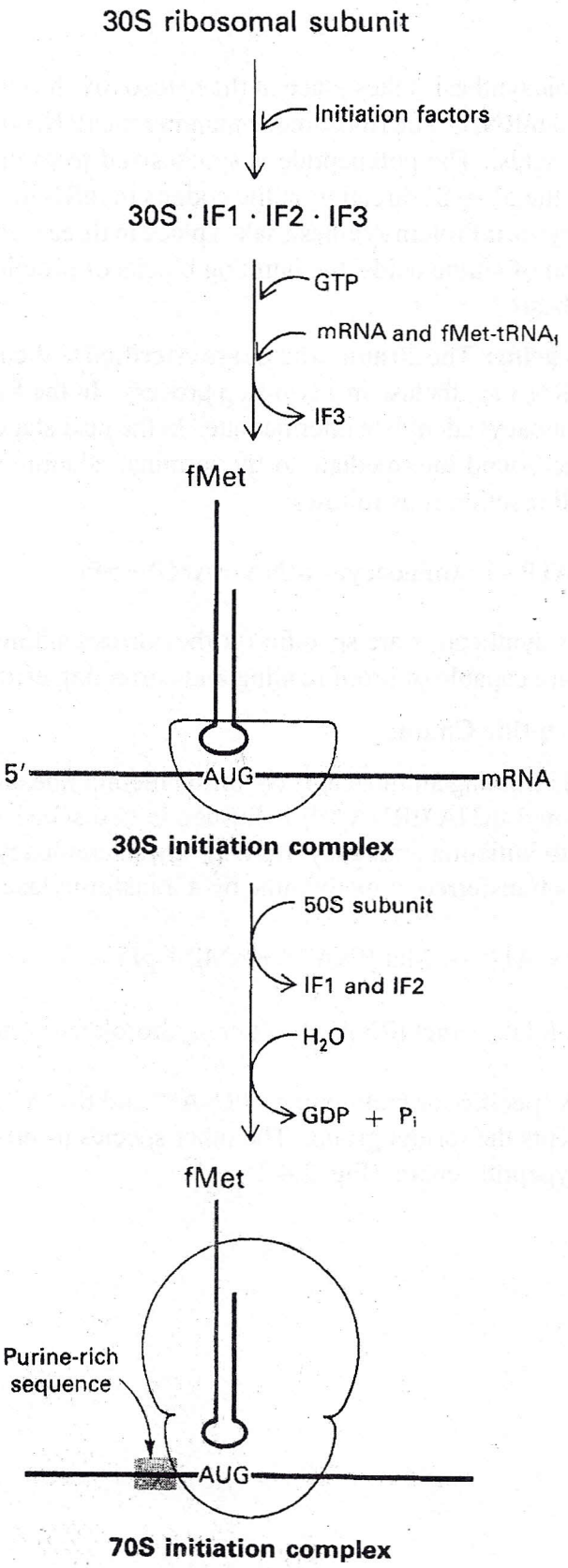
2.4.3.1.2. Initiation of Polypeptide Chain:

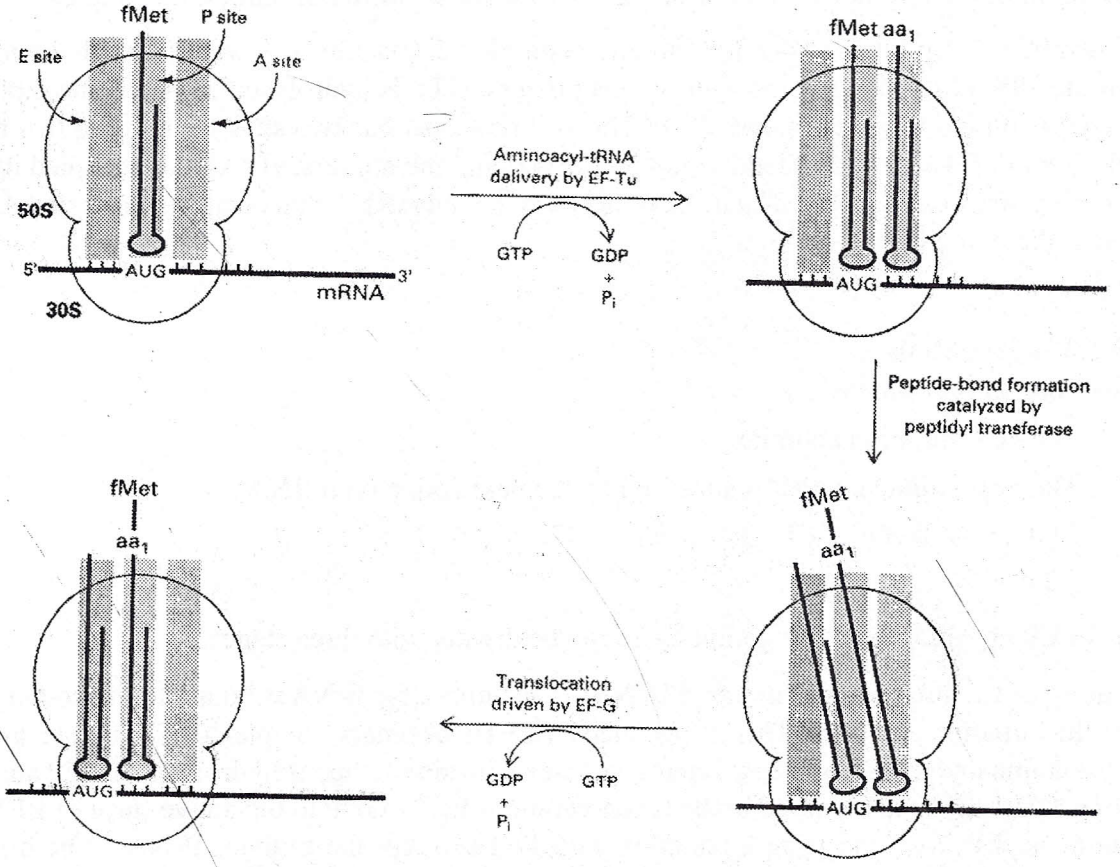
Initiation: In prokaryotes, the initiating aminoacid is N-formyl methionine encoded by the initiation codon AUG. N-formyl methionyl tRNA ($tRNA_f^{fmet}$) is formed in two successive stages. In the first stage, methionine is esterified to initiating aminoacyl $tRNA^{fmet}$ by an aminoacyl tRNA synthetase. In the next step, a formyl group is transferred to methionine by a transformylase to form $tRNA^{fmet}$.

$$\text{Methionine} + tRNA^{fmet} + \text{ATP} \rightarrow \text{Met } RNA^{fmet} + \text{AMP} + \text{pPi}$$

$$N^{10} \text{ ñ formyl tetrahydrofolate} + \text{met } tRNA^{fmet} \rightarrow \text{tetrahydrofolate} + \text{fmet } tRNA^{fmet}$$

There are two species of tRNA specific for methionine - $tRNA^{met}$ and tRNA. Although, both accept methionine, only $tRNA^{fmet}$ accepts the formyl group. The other species inserts methionine residues in internal positions of the polypeptide chain. (Fig. 2.4.2)


30S ribosomal subunit
Initiation factors
30S · IF1 · IF2 · IF3
GTP
mRNA and fMet-tRNA$_i$
IF3
fMet
5′
AUG
mRNA
30S initiation complex
50S subunit
IF1 and IF2
H_2O
GDP + P_i
fMet
Purine-rich sequence
AUG
70S initiation complex



Requirements for initiation

(i) The 30S and the 50S subunits.
(ii) mRNA encoding aminoacid sequence of the polypeptide chain
(iii) Initiating N-formyl methionyl $tRNA^{fmet}$
(iv) Initiation factors IF-1, IF-2, and IF-3
(v) GTP

Steps in initiation

The initiation complex is formed in a stepwise process.

1. The three initaition factors (IF-1, IF-2 and IF-3) together with GTP bound to the 30S subunit.
2. The mRNA binds to the 30S subunit. Approximately 7 bases upstream of the AUG codon is a polypurine stretch termed as the Shine-Dalgarno (SD) sequence, 5í AGGAGGU3í. The SD sequence is recognised by the basepairs with a pyrimidine sequence in the 16S rRNA of the 30S subunit. This interaction positions the mRNA for initiation of translation.
3. IF-2 complexed with GTP assists the initiator tRNA to bind to the 30S subunit. The initiator tRNA is correctly positioned with its anticodon base pairing with the initiation codon AUG on mRNA.

4. Binding of the initiator tRNA releases IF-3 from 30S to form 30S initiation complex.
5. The 50S subunit binds to the 30S initiation complex displacing IF-1 and IF-2 with the formation of the 70S initiation complex. During this process, GTP is hydrolysed to guanosine diphosphate (GDP) and inorganic phosphate (Pi). The 70S ribosome has two sites for binding two tRNAs ñ the peptidyl (P) site occupied by peptidyl tRNA and the aminoacyl (A) site occupied by the incoming aminoacyl tRNA. The initiator tRNA is the only tRNA that occupies the P site. All others enter the A site in the ribosome.

2.4.3.1.3. Elongation

Requirements for elongation

(i) The 70S initiation complex.

(ii) The next aminoacyl tRNA specified by the next codon on mRNA.

(iii) Elongation factors EFTu, EFTs, and EFG.

(iv) GTP.

Steps in Elongation: The elongation cycle can be divided into three stages.

1.Binding of the incoming aminoacyl tRNA: The aminoacyl tRNA is bound to the codon in the A site of the initiation complex. This is mediated by EFTu. A binary complex of EFTu-GTP associates with the aminoacyl tRNA to form a ternary complex with simultaneous hydrolysis of GTP and release of EFTu-GDP. EFTs is involved in the regeneration of EFTu-GDP to the active form of EFTu-GTP. All aminoacyl tRNAs can form a complex with EFTu except the initiator tRNA. The binding of amino acyl t-RNA to the A site is blocked by antibiotics such as tetracycline.

2. Peptide bond formation: A peptide bond is formed between t-RNA^{fmet} in the P site and the newly bound amino acyl tRNA in the A site. The reaction is catalyzed by peptidyl transferase present in the 50S subunit. This product is dipeptidyl tRNA bound to the A site. This leaves an uncharged initiator tRNA on the P site. Peptide bond formation is inhibited by puromycin.

3. Translocation: The ribosome moves to the next codon on the mRNA. This results in shifting of the dipeptidyl tRNA from the A site to the P site with simultaneous removal of the uncharged tRNA from the P site. The A site is now vacant for entry of the next aminoacyl tRNA. Translocation is catalysed by EF-G (translocase) and GTP. After translocation, EFG dissociates from the ribosome and GTP is hydrolysed to GDP and Pi.

All three steps are repeated until a termination codon is encountered.

2.4.3.1.4 Termination: The polypeptide chain is terminated after the last amino acid is added. Termination is signalled by any one of three termination codons ñ UAG (amber), UAA (ochre) and UGA (opal). In *E. coli*, three release factors RF-1, RF-2 and RF-3 catalyse termination. RF-1 recognises UAA and UAG, wehreas RF-2 recognises UAA and UGA. RF-3 in association with GTP helps RF-1 and RF-2 in promoting termination. Termination involves release of the completed polypeptide chain, ejectioning the tRNAs from the ribosome and dissociation of mRNA from the ribosome. EF-G and a ribosome releasing factors are required for the complete dissociation of the subunits.

Energetics: The synthesis of each peptide bond requires 4 high-energy phosphate bonds of which 2 ATP molecules are required for activation of amino acids; 1 GTP for binding of amino acyl tRNA to the A site and 1 GTP during translocation.

2.4.3.2 Translation in Eukaryotes: The basic mechanism of translation in eukaryotes is the same as in prokaryotes. However, there are differences in the number of components and some of the steps. These are described below:

1. Ribosomes:- The composition of the eukaryotic ribosome is different from that of prokaryotes. The sedimentation coefficient of the eukaryotic ribosome is 80S with a larger 60S and smaller 40S subunits.

2. mRNA:- The eukaryotic mRNA has a 5í cap which has a role in initiation of translation. The eukaryotic mRNAs are monocistronic, i.e., they encode a single polypeptide chain unlike the prokaryotic mRNA which is polycistromic (encoding several polypeptide chains).

3. Initiation: There are a number of differences between eukaryotic and prokaryotic initiation.

(i) The initiating animo acid is methionine. The initiator tRNA is $tRNA^{fmet}$ which is distinct from $tRNA_m$ recognised by internal non-initiator methionine codons.

(ii) There is no SD sequence to direct initiation to the initiation codon AUG. A scanning hypothesis has been proposed to explain the mechanism of selecting the start codon. According to this hypothesis, the 40S subunit containing the initiator tRNA attaches to the 5í end of the mRNA and scans along the mRNA until it finds the appropriate initiation codon AUG within the sequence. 5í-CCRCCAUGG-3í, where R=purine. This sequence is referred to as the Korak consensus sequence.

(iii) Initiation factors: More proteins are involved in initiation in eukaryotes compared to prokaryotes. Nine initiation facators (labelled eIF) as well as cap binding protein (CBP) have been characterised. The eIFs are classified according to their functions. Some of these are analogous to the three prokaryotic IFs.

(iv) Initiation Complex Assembly: The order of assembly of the initiation complex is different in eukaryotes. The initiator tRNA is bound to the small subunit before mRNA binds. Bonding of CBP to the 5′-cap may initiate mRNA binding and promote binding of eIF-4A and eIF-4B to mRNA. ATP is hydrolysed and bound by eIF-4A leading to unwinding of secondary structure in the 5′-untranslated region of the mRNA.

Formation of the 80S initiation complex requires the action of eIF-4C and eIF-5. eIF-4C assists in binding of 60S subunit with 40S, eIF-5 displaces eIF-2 and eIF-3. The released eIF-2-GDP is converted to eIF-2-GTP by eIF-2B. Viral infection and interferon production promote phosphorylation of eIF-2 and subunit protein synthesis.

4. Elongation: The elongation cycle in eukaryotes is similar to that in prokaryotes. Three elongation factors, eEE1α, eEF1$\beta\gamma$ and eEF2 are analogous to prokaryotic EFTu, EFTs and EFG respectively in their functions.

5. Termination: A single release factor eRF is involved in termination of protein synthesis in eukaryotes. It recognises all the three stop codons and requires GTP for activity.

6. **Inhibitors of protein synthesis:** Protein biosynthesis is inhibited by antibiotics, drugs, and toxins in both prokaryotes and eukaryotes. The mechanism of action of some of these agents are described below:

Anti-bacterial drugs: Many antibiotics inhibit protein synthesis in prokaryotes and are effective as antibacterial agents.

1. **Tetracycline:** A broad spectrum antibiotic blocks the A site on the ribosome and inhibits binding of amino actyl-tRNA.
2. **Streptomycin:** An aminoglycosidic used to treat heart infections, binds to the S112 protein of the 30S subunit. It causes:

(i) destabilization of the initiation complex

(ii) misreading of the genetic code

(iii) impaired dissociation of ribosomes into subunits.

(iv) membrane damage

3. Erythromycin binds to the 50S subunit and inhibits translocation.

4. Chloramphenicol inhibits peptidyl transferase present on 50S subunit and blocks peptide bond formation.

Other Inhibitors: Some antibiotics inhibit protein biosynthesis but are not useful as therapeutic agents.

1. Cycloheximide binds to the 60S subunit of eukaryotes and inhibits the peptidyl transferase activity.
2. Puromycin synthesised by the mold streptomyces alboniger is a structural analogue of the 3í-end of aminoacyl tRNA. It is treated by the ribosome as an incoming amino acyl tRNA and bound to the A site. This is followed by transfer of the polypeptide from the P site to puromycin to form peptidyl puromycin on the A site. As a result, new aminoacid residues are not bound at the A site leading to termination of protein synthesis.

2.4.3.3 Toxins:

1. Diphtheria Toxins: They are toxic proteins secreted by corye bacterium diphtheriae which infect the nasopharynx and inhibit eukaryotic protein synthesis. The toxin causes transfer of ADP ribose from nicotinamide adinine dinucleotide (NAD^+) to eEF-2 (ADP ribosylation). This inactivates eEF-2 resulting in inhibition of translocation.

Ricin produced by castor beans produces N-glycosidase, a toxin that removes an adenine residue from 28S rRNA. The depurination inhibits protein synthesis in eukaryotes. Prolonged use of castor oil which contains ricin causes diarrhoea, loss of intestinal function and eventually death.

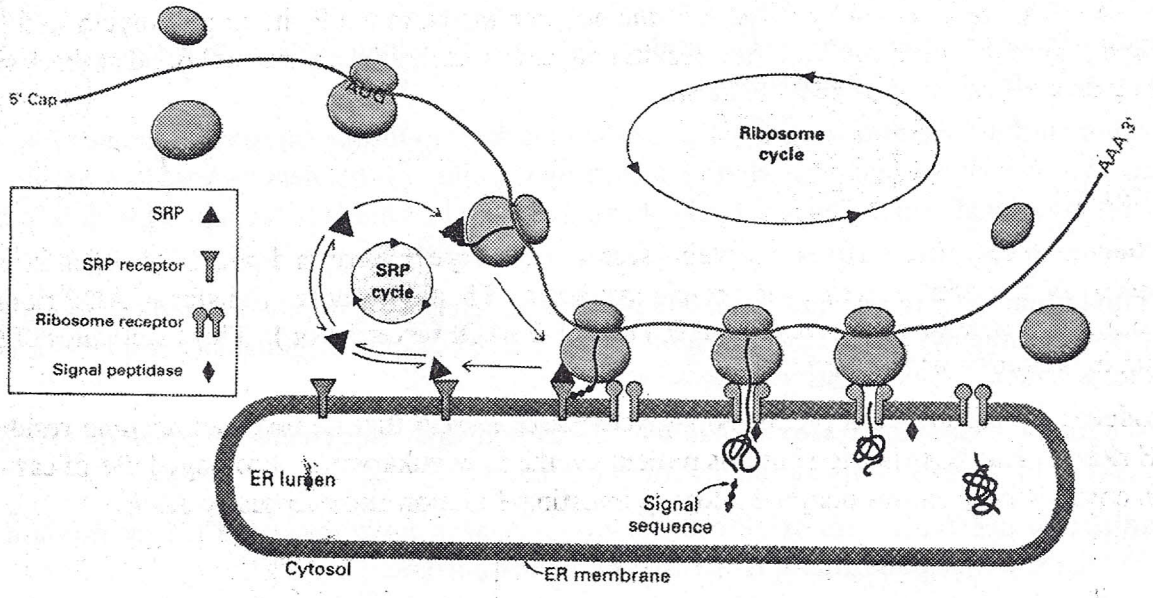
2.4.4 Protein Targetting

Protein synthesised by ribosomes have several destinations while some are secreted into the surrounding milieu, others are sequestered into intracellular organelles or delivered to the cell membrane.

Secretory Proteins: These proteins contain specific polypeptide ëleadersí on their amino terminal ends termed as signal sequences (signal peptides). These are found in both prokaryotes and eukaryotes. The signal peptides have 13-36 aminoacids with a stretch of hydrophobic amino acids. In eukaryotes, a signal recognition particle (SRP), a ribo nucleo protein, recognises the arrested SRP-ribosome complex binds to a docking protein or SRP receptor on the cytosolic side of the Endoplasmic Reticulum (ER). Ribosome-receptor proteins on the ER membrane tightly bind the ribosome releasing the SRP and allow translation to continue. The nascent polypeptide enters the lumen of the ER through a pore created by the protein translocator. A signal peptidase present in the luminal surface of the ER cleaves the signal peptide from the rest of the protein. The protein is then transported to the Golgi where it is modified by glycosylation. The final localization of the protein depends on the different patterns of glycosylation.

Plasma Membrane Poteins: Unlike seretory proteins, plasma membrane proteins are not released into the lumen of the ER. They are inserted into the ER membrane, transported into the Golgi and then to the cell surface.

Proteins of the endoplasmic reticulum: Many proteins in the ER assist nascent proteins to fold correctly into their native conformation. Some of these proteins are termed as turned chaperones. The proteins of the ER are synthesised on the rough endoplasmic reticulum and pass into the lumen like secretory proteins. They are then transported to the Golgi by vesicles. These proteins have a C-terminal retention signal with the amino acid sequence Lys-Asp-Glu-Lur (KDEL) using the one letter amino acid symbols. Receptors in the Golgi bind to the KDEL sequence and return the protein to the ER via vesicles.



Lysosomal proteins: These are targeted to their destination by the addition of a mannose-6-phosphate signal in the cis compartment of the Golgi. This signal is recognised and bound by receptor proteins in the trans compartment of the Golgi which packs the protein in transport vesicles to the appropriate destination.

Nuclear proteins: These proteins have a nuclear localization signal of 4-8 amino acids present internally in the protein. Protein uptake takes place via unclear pores and requires ATP.

Mitochondria and Chloroplast proteins: These are synthesized by free cytosolic ribosomes. Uptake of proteins into the mitochondrial matrix requires a matrix targeting sequence, the heat shock proteins sp 60 and sp 70, and energy from electro chemical gradient and ATP.

2.4.5 Post-Translational Modifications

A protein becomes functionally active only when it is folded in its native conformation. In addition, most proteins undergo processing or covalent modifications in order to attain biological activity. These changes are called post-translational modifications, because they occur after the protein is synthesized. The various post-translational modifications are described below:

1. **Modification of N-terminal and C-terminal amino acids:** The formyl group of the initiating aminoacid is removed in prokaryotes. In eukaryotes, the initiating, methionine residue and one or more succeeding residues may be enzymatically removed. The N-terminal residue may be acetylated in some proteins whereas in others, the C-terminal residue may be modified.
2. **Removal of signaling sequences:** These are removed by specific peptidases.
3. **Phosphorylation:** The hydroxyl groups of scr, thr, and tyr residues of some proteins are phosphorylated by ATP to yield phosphoserine, phospho threonine and phosphotyrosine respectively which become negatively charged. The milk protein causes contains several phosphoserine groups which binds Ca^{2+}. Phosphorylation of serine residues may activate enzymes such as glycogen phosphorylase. During malignant transformation, specific tyrosine residues of some proteins become phosphorylated.
4. **Hydroxylation:** Prolene and lysine residues in collagen are hydroxylated to hydroxyproline and hydroxylysine only after translation.
5. **Carboxylation:** Addition of COOH groups to glutamate to form γ-carboxyglutamate (Gla) residues in prothrombsin and other clotting factors by vitamin K-dependent carboxylases occurs as a post-translational event. The negatively charged Gla residues bind Ca^{2+} required for blood coagulation.
6. **Methylation:** Lysere residues in muscle proteins are enzymatically methylated to monomethyl lysine and dimethyl lysine. Methylation of the COOH groups of glutamate residues in some proteins removes their negative charges.
7. **Attachment of carbohydrate side chains:** The carbohydrate side chains are covalently linked to asn, ser or thr resiues of glycoproteins after translation.
8. **Addition of prosthetic groups:** Prosthetic groups such as biotin to acetyl CoA carboxylase, are added to the polypeptide after it is released from the ribosome.

9. **Formation of disulphide boads:** The interchain and intrachaian disulphide bridges between cysteine residues are formed enzymatically in several proteins.

2.3.6 Protein Degradation

The stability of a protein depends on its N-terminal amino acid residues. The N-terminal amino acids differ in their half-lives. While some amino acids (ala, cys, gly, met, pro, ser, thr and val) with a half life more than 20 h have high stability, others (arg, his, ile, leu, lys, phe, trp, tyr) have a short half-life of 20-30 minutes. The remaining amino acids (asn, asp, gln, glu) are destabilizing in nature. A protein which is damaged, modified or contains a destabilizing N-terminal amino acid, undergoes ubiquitinylation. This involves covalent linkage of ubiquitin to lysine residues in the protein. The ubiquitinylated protein is then digested by an ATP-dependent reaction by a 26S protease complex (proteasome). Later the intact ubiquitin is released for reuse.

2.4.7 Prions

Prions are small, proteinaceous infectious particles devoid of nucleic acids. These are resistant to inactivation by UV radiation, formalin, heat, nucleus and extremes pH. Prion proteins are found in the brains of all mammals and they protect against dementia and other neurodegradative diseases.

The prion diseases in animals include scrapie, a neurological disease of sheep and Boxine spongiform encophelopa the (BSE, mad cow disease) among several others. In humans, Kuru and Cruetzfeld ñ Jakob disease (CJD) are two of the well known prion diseases. Kuru, confined to foretribes of Papua New Guinea, is associated with cannibalism. CJD is a very rare-but well characterized, severe, progressive dementia sees in parts of North Africa and Slovakia. It is transmitted during corneal or during transplantation and by contaminated neurosurgical devices or growth hormone properations.

Prion diseases show the following features:

1. Long incubation period
2. Protracted, severe, progressive, almost fatal course.
3. Degeneration of CNS.
4. Absence of inflamatory reaction and immune response.

2.4.8 Summary

1. The genetic code is read in codons, each codon representing one amino acid. Of the 64 codons, 61 code for the 20 aminoacids, while the remaining three signal termination of protein synthesis. Although the genetic code is commaless, it starts with the initiation codon AUG and ends with any one of the termination codons. The degeneracy of genetic code usually involves the third base in the codon called the wobble base. Mitochondria and some unicellular organisms show slight deviations from the universal genetic code.

2. Protein synthesis takes place in three stages ñ initiation, elongation and termination. Activation of amino acids, occurs before initiation of polypeptide chain synthesis. The initiation amino acid is N-formyl methionine in prokaryotes, and methionine in eukaryotes. Initiation requires mRNA, initiator tRNA, initiation factors and GTP. The elongation cycle is divided into three stages : binding of incoming aminoacyl tRNA, peptide bond formation and translocation. Termination is signalled by any one of the three termination codons and assisted by release factors. Protein biosynthesis is inhibited by antibiotics, drugs and toxins.
3. Proteins synthesised by ribosomes may be secreted into the surrounding milieu, sequestered into intracellular organelles and delivered to the cell membrane. The protein is transported from the site of synthesis to the Golgi where it is modified by glycosylation. The final location of the protein depends on the different patterns of glycosylation.
4. Most proteins undergo post-translational modifications to attain biological activity. These include modifications of N-terminal and C-terminal amino acids, removal of signaling, sequences, phosphorylation, hydroxylation, carboxylation and formation of disulphide bonds.
5. A protein which is damaged, modified or contains a destabilizing N-terminal amino acid undergoes ubiquitinylation. The ubiquitinylated protein is then digested by a proteasome.
6. Prions are proteinaceous infectious particles devoid of nucleic acids. They protect against dementia and other neuro degenerative diseases. The prion diseases in animals are scrappie BSE etc., and in humans, Kuru and CJD. The development of prion diseases is associated with conversion of the normal PrP^{c} to infectious PrP^{sc}.

2.4.9 Self Assessment Questions

1. Describe the salient features of the genetic code. How does mitochondrial genetic code differ from the universal genetic code.
2. Explain the role of GTP in protein synthesis.
3. Describe the mechanism of initiation of protein synthesis in prokaryotes. How does this differ from the mechanism operating in eukaryotes?
4. What are Shine-Dalgarno and Kozack sequences?
5. Discuss the steps involved in elongation of polypetide chain in eukaryotes and prokaryotes.
6. How is polypeptide chain synthesis terminated.
7. What is the reaction catalysed by amino actyl tRNA synthetase.
8. Give an account of the various post-translational modifications.
9. Explain how newly synthesized proteins are delivered to their correct destinations.
10. What are prions?
11. Describe the process of protein degradation.
12. Discuss the mechanism of action of various inhibitors of protein synthesis.

2.4.10 Terminology

Translation

Prokaryotes

Eukaryotes

Initiation

Elongation

Termination

Toxins

Protein Targetting

Post translational modification

Protein degradation

Prions

Shine-Dalgarno sequences

2.4.11 Reference Books

Nelson, Cox, Lehninger ñ *A Text of Biochemistry*.

Nagini, S. - *Textbook of Biochemistry.*

Rama Rao, A.V.S.S. ñ *A Text book of Biochemistry*.

Voet & Voet - Fundamentals of Biochemistry.

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UNIT-III

LESSON-3.1

CHROMOSOME STRUCTURE AND GIANT CHROMOSOMES

CONTENTS

3.1.1 INTRODUCTION

The chromosomes are the nuclear components of special organisation, individually and functionally. They are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species. The role of chromosomes is unquestionably made clear in the heredity of the organisms whether plant or animals, as the carriers of genes and are faithfully replicated at each cell generation in the cell lineage of the organism. The morphology of chromo-

somes enable us to predict the genetic and cytogenetic consequences depending upon the length, shape, size, number and the distribution of the euchromatin and heterochromatin inside the chromosome of a particular species.

Karl Nagli (1842) observed rod-like chromosomes in the nuclei of plant cells. The name chromosome was coined by W. Waldeyer (1888), Geitter (1940) and Kaupmann (1948) have described the morphology of chromosomes. R.D. Korenberg (1974) proposed the nucleosome model of the basic chromatin material.

3.1.2 OBJECTIVES

- ⇒ To study the size and shape of chromosomes
- ⇒ To study the material and chemical composition of chromosomes
- ⇒ To study different models of chromosome structure
- ⇒ To study the structure of giant chromosomes
- ⇒ To study the deformation of giant chromosomes

3.1.3 MORPHOLOGY

3.1.3.1 Size : The size of chromosome is normally measured at mitotic metaphase and may be as short as 0.25 μm in fungi and birds, or as long as 30 μm in some plants such as Trillium. However, most metaphase chromosomes fall within a range of 3 μm in fruitfly (Drosophia), to 5 μm in man and 8 μm to 12 μm in maize. The organisms with less number of chromosomes contain comparatively large-sized chromosomes than the chromosomes of the organisms having many chromosomes.

The monocotyledon plants contain large-sized chromosomes than the dicotyledon plants. The plants in general have large-sized chromosomes in comparison to the animals. Further, the chromosomes in a cell are never alike in size, some may be exceptionally large and others may be too small. The largest chromosomes are lampbrush chromosomes of certain vertebrate oocytes and polytene chromosomes of certain dipteran insects.

3.1.3.2 Shape : The shape of the chromosomes is changeable from phase to phase in the continuous process of the cell growth and cell division. In the resting phase or interphase stage of the cell, the chromosomes occur in the form of thin, coiled, elastic and contractile, thread like stainable structures, the chromatin thread. In the metaphase and the anaphase, the chromosomes become thick and filamentous. Each chromosome contains a clear zone, known as centromere or kinetocone, along their length. The centromere divides the chromosomes into two parts, each part is called chromosome arm. The position of centromere varies from chromosome to chromosome and it provides different shapes to the chromosome.

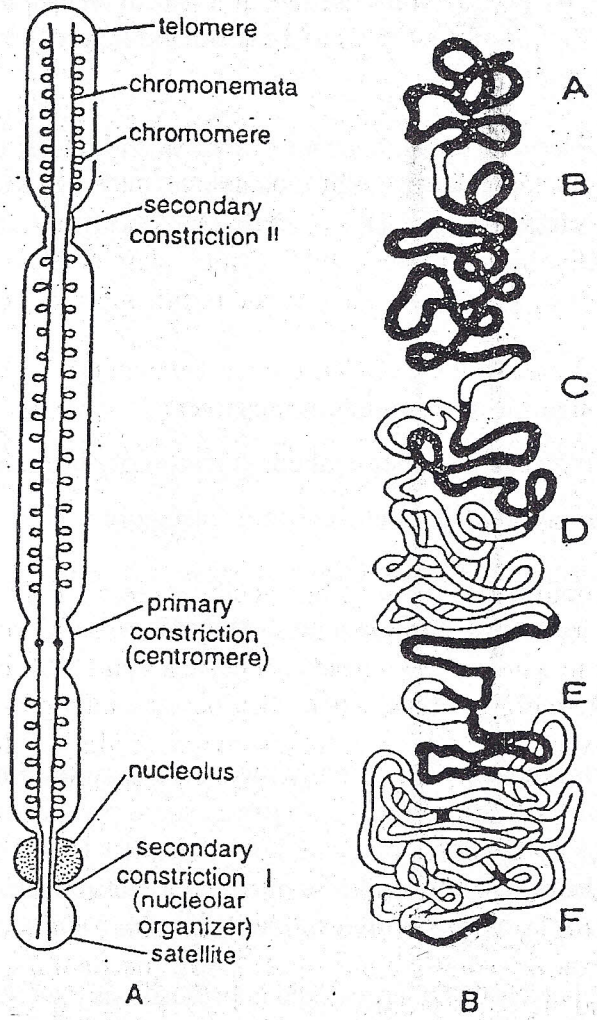

Fig. 3.1.1 A-Structure of a typical chromosome; B-Model of constitutive heterochromatin in a mammalian metaphase chromosome, A-Constitutive heterochromatin; B-Secondary construction; 1 or nucleolar organizer; C-Primary constriction or centromere, D-Euchromatin; E-Secondary construction, II-possible site of 5S rRNA cistrons; F-Telomere (after De Robertis *et al.*, 1975).

1. **Teleocentric:** The rod-like chromosomes which have the centromere on the proximal end are known as the telocentric chromosomes.

2. **Acrocentric:** The acrocentric chromosomes are also rod-like in shape but these have the centromere at one end and thus giving a very short arm and an exceptionally long arm. The locusts (Acrididae) have the acrocentric chromosomes.

3. **Submetacentric:** The submetacentric chromosomes are J-or L-shaped. In these, the centromere occurs near the centre or at medium portion of the chromosome and thus forming two unequal arms.

4. **Metacentric:** The metacentric chromosomes are V-shaped and in these chromosomes the centromere occurs in the centre and forming two equal arms. The amphibians have metacentric chromosomes.

3.1.3.3 Structure

1. **Chromatid:** At mititoc metaphase each chromosome consists of two symmetrical structures called chromatids. Each chromatid contains a single DNA molecule. Both chromatids are attached to each other only by the centromere and become separated at the beginning of anaphase, when the sister chromatids of a chromosome migrate to the opposite poles.

2. **Chromonema:** During mitotic prophase, the chromosomal material becomes visible as very thin filaments, called chromonema. A chromonema represents a chromatid in the early stages of condensation. Therefore chromatid and chromonema are two names for the same structure: a single linear DNA molecule with its associated proteins. The chromonemata form the gene bearing portions of the chromosomes.

3. **Chromomeres:** The chromomeres are bead-like accumulations of chromatin material that are sometimes visible along interphase chromosomes. The chromosome-bearing chromatin has an appearance of a necklace in which several beads occur on a string. Chromomeres become especially clear in the polytene chromosomes, where they become aligned side by side, constituting the chromosome beads. At meataphase the chromosomes are tightly coiled and the chromomeres are no longer visible. They are believed to correspond to the units of genetic function in the chromosomes.

4. **Centromere and Kinetochore:** Centromere is the region of the chromosome to which are attached the fibres of mitotic spindle. The centromere lies within a thinner segment of chromosome, the primary constriction. The regions of chromosome flanking the centromere contain highly repetitive DNA and may stain more intensely with the basic dyes. Centromeres are found to contain specific DNA sequences with special proteins bound to them, forming a disc sh structure called kinetochore. Under Electron Microscope, the kinetochore appears as a plate - or cup-like disc, 0.20 to 0.25 nm, in diameter situated upon the primary constriction or centromere

   The chromosomes of most organisms contain only one centromere and are kno as monocentric chromosomes. Some species have diffuse centromeres, with microtubules attached along the length of the chromosome, which are called holocentric chromosomes.

5. **Telomere:** Each extremity of the chromosome has a polarity and therefore, it prevents other chromosomal segments to be fused with it. The chromosomal ends are known as the telomeres. If a chromosome breaks, the broken ends can fuse with each other due to lack of telomeres.

6. **Secondary constriction:** The chromosomes besides having the primary constriction of the centromere possess secondary cosntriction at any point of the chromosome. Secondary constriction can be distinguished from primary cor riction or centromere, because chromosome bends only at the position of centromere during ana nase.

7. **Nuclear organizers:** These areas are certain secondary constrictions that contain the genes coding for 5.8S, 18S and 28S ribosomal RNA and that induce the formation of nucleoli. The secondary constriction may arise because the rRNA genes are transcribed very actively and thus interfering with chromosomal condensation. In human beings, the nucleolar organiers are located in the secondary constrictions of chromosomes 13, 14, 15, 21 and 22 all of which are acrocentric and have satellite.

8. **Satellite:** Sometimes the chromosomes bear round elongated or knob like appendages known as satellites. The satellite remains connected with the rest of the chromosome by a thin chromatin filament. The chromosomes with the satellite are designated as the sat chromosomes. The shape and size of the satellite remain constant.

3.1.4 MATERIAL OF THE CHROMOSOMES

The material of the chromosomes is the chromatin. Depending on their staining properties, the following two types of chromatin may be distinguished in the interphase nucleus.

1. **Euchromatin:** Portions of chromosomes that stain lightly are only partially condensed; this chromatin is termed euchromatin. It represents most of the chromatin that disperse after mitosis has completed. Euchromatin contains structural genes which replicate and transcribe during G and S phase of interphase. The euchromatin is considered genetically active chromatin, since it has a role in the phenotype expression of the genes. In euchromatin, DNA is found packed in 3 to 8 nm fibre.

2. **Heterochromatin:** In the dark-staining regions, the chromatin remains in the condensed state and is called heterochromatin. In 1928, Heitz defined heterochromatin as those regions of the chromosome that remain condensed during interphase and early prophase and form the so-called chromocentre. Heterochromatin is characterised by its especially high content of repetitive DNA sequences and contains very few, if any, structural genes. It is late replicating and is not transcribed. It is thought that in heterochromatin the DNA is tightly packed in the 30 nm fibre.

Types of heterochromatin: In an interphase nucleus, usually there is some condensed chromatin around the nucleolus, called perinucleolar chromatin and some inside the nucleolus, called intra-nucleolar chromatin. Both types of this heterochromatin appear to be connected and together, they are referred to as nucleolar chromatin.

Dense clumps of deeply staining chromatin often occur in close contact with the inner membrane of the nucleolar enuclone and is called condensed peripheral chromatin. Between the peripheral heterochromatin and the nucleolar heterochromatin are regions of lightly staining chromatin, called disposed chromatin. In the condensed chromosomes, the heterochromatic regions can be visualised as regions that stain more strongly or more weakly than the euchromatin regions, showing the so-called positive or negative heteropyknosis of the chromosomes i.e.

hetero = different + pyknosis ñ staining.

Heterochromatin has further been classified into the following types

1. Constitutive heterochromatin:

In such a heterochromatin the DNA is permanently inactive and remains in the condensed state through out the cell cycle. This most common type of heterochromatin occurs around the centromere, in the telomeres and in the C-bands of the chromosomes. In Drosophila virilis, constitute heterochromatin exists around the centromeres and such pericentromeric heterochromatin occupies 40 per cent of the chromosomes.

2. Facultative heterochromatin: Such type of heterochromatin is not permanently maintained in the condensed state; instead it undergoes periodic dispersal and during these times is transcriptionally active. Frequently, in facultative heterochromatin, one chromosome of the pair becomes either totally or partially heterochromatic. The best known case is that of the X-chromosomes in the mammalian female, one of which is active and remains euchromatic, where as the other is inactive and forms at interphase, the sex chromatin or barr body.

3.1.5 CHEMICAL COMPOSITION

Chromatin which has been isolated from rat liver contains DNA, RNA and Protein. The protein of chromatin is of two types. The histones and the non-histone. Rat liver chromatin has been used as a model for chromatin. It possess a histone to DNA ratio near 1:1, a non-histone protein to DNA ratio of 0.6 : 1 and a RNA/DNA ratio of 0.1 : 1.

1. DNA: DNA is the most important chemical component of chromatin, since it plays the central role of controlling heredity.

2. Histones: Histones are very basic proteins, basic because they are enriched in the amino acids arginine and lysine to a level of about 24 mole per cent. Arginine and lysine at physiological pH are cationic and can interact electrostatically with anionic nucleic acids. Thus being basic, histones bind tightly to DNA which is an acid. There are five histones in eukaryotic chromosomes, namely H_1, H_2A, H_2B, H_3 & H_4.

Histone H_1 is the least rigidly conserved histone protein. It contains 210 to 220 amino acids and may be represented by a variety of formis even within a single tissue. H1 is present only once per 200 base pairs of DNA and is rather loosely associated with DNA.

3. Non-Histones: In contrast to the modest population of histones in chromatin, non-histone proteins display more diversity. In various organisms, number of non-histones can vary from 12 to 20. Heterogeneity of these proteins is not conserved in evolution as the histones. These non-histones differ even between different tissues of the same organism suggesting that they regulate the activity of specific genes.

About 50 per cent non-histones of chromatin have been found to be structural proteins and include such proteins as actin and α and β tubulins and myosin. Many of the remaining 50 per cent of non-histones include all the enzymes and factors that are involved in DNA replication, in transcription and in the regulation of transcription.

3.1.6 ULTRASTRUCTURE

The field of ultrastructure of the chromatin is still the area where electron microscope had failed to provide us a clear picture of the organisation of DNA in the chromatin. For the study of chromosomes with the help of electron microscope, whole chromosome mounts as well as sections of chromosomes were studied. Such studies had demonstrated that chromosomes have very fine fibrils having a thickness of 2 nm ñ 4 nm. Since DNA is 2 nm wide, there is a possibility that a single fibril corresponds to a single DNA molecule.

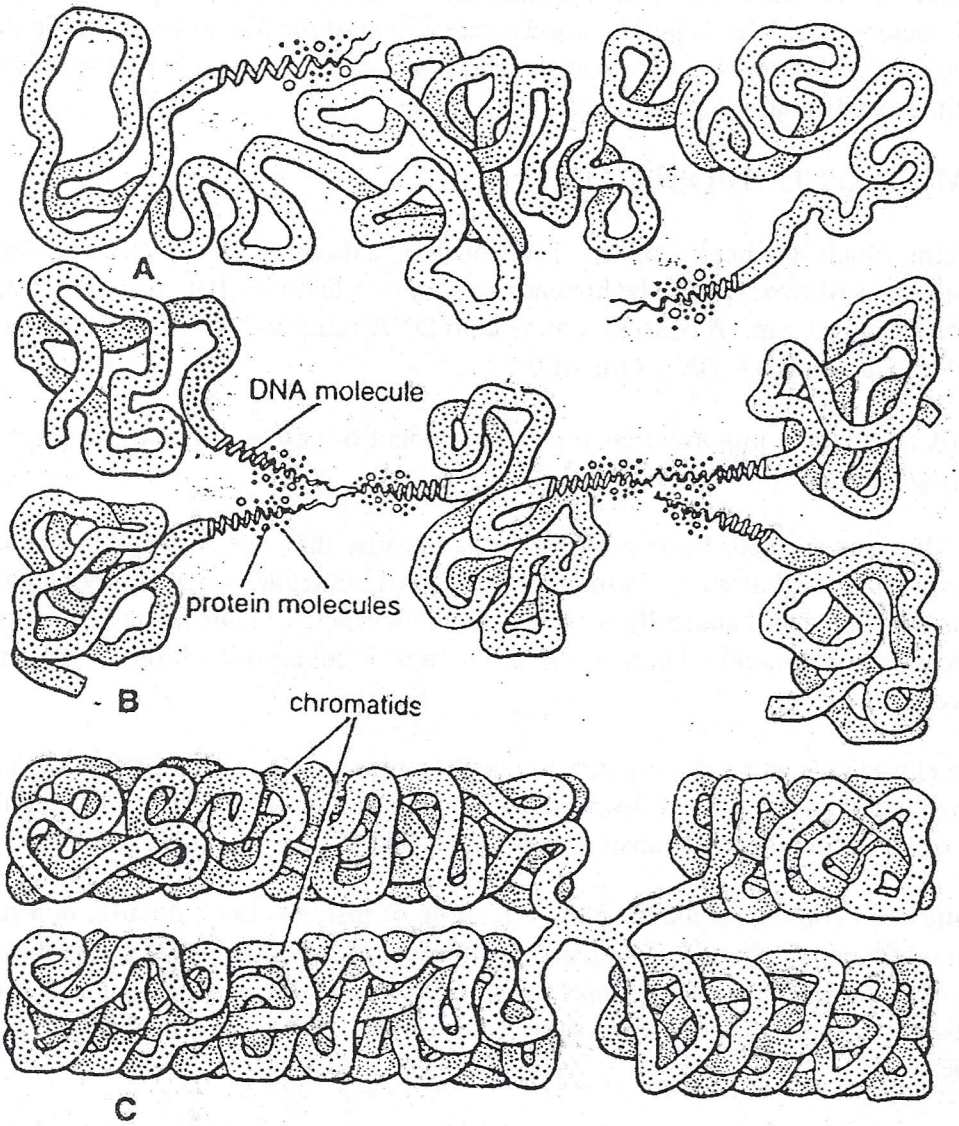

Fig. 3.1.2 Duprawís folded fibre model of chromatin in interphase (A and B) and in metaphase (C).

3.1.6.1 Single-stranded and Multi-stranded Hypothesis

When chromosomes are compared in related species which differ widely in DNA content, such differences may be attributed to one of two causes: (1) lateral multiplication of chromonemata leading to multiple or multi-strandedness (2) tandem duplication of DNA or chromonemata where lengthwise duplication is responsible for chromatin differences. This latter condition will retain the single stranded feature of chromosomes.

Although multiple strandedness has been demonsurated in several cases of plants such as Vicia faba and animals such as dipteran salivary gland chromosomes, there are evidences against such hypothesis to become a generalization. In all these cases, however, tandem duplication of chromonemata (or DNA) evidently takes place. Indeed, there are many evidences to support the idea of single-stranded nature of chromatin. This was confirmed by the technique of pulsed gel electrophoresis that in yeast Saccharomyces cerevisae, each chromosome is formed from a single linear DNA molecules.

3.1.6.2 Folded-fibre Model and Nucleosome Concept

If we presume that a single long DNA molecule, we have no choice but to believe that DNA should be present in a coiled or folded manner. The manner of coiling and folding of DNA was a matter of debate and dozens of models were available for this purpose; of them only two stand out and are important. A popular model was the folded ñ fibre model, proposed by E.J. Duprow in 1965. According to it, the bulk of the chromosome is visualized to be composed of a tightly folded fibre which has a rather homogenous diameter of 200 to 300 A°. This folded fibre is supposed to contain the DNA histone helix (of 30 A° diameter) in a super coiled condition. Another model is most significant and universally accepted one and is called nucleosome model which was proposed by R.D. Kornberg (1974) and confirmed by P. Outdet *et al.* (1975). Thus, while in the folded-fibre model, it was proposed that the histones were bound on the outside of the DNA core surrounded by histones was incorrect (Berns, 1983). In fact, from a genetic perspective, a significant feature of packing mechanism through the nucleosomes lies in its topology; at no point is the DNA buried; instead, it is freely exposed along the entire surface of the ìspoolî, available for genetic expression. Nucleosomes seem to be universal device for compacting the long DNA molecules of embryonic cells.

3.1.6.3 Nucleosomes and Solenoid Model of Chromatin

In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeating array of DNA-protein particles, called nucleosomes. If it was stretched out, the DNA double-helix in each human chromosome would span the cell nucleus thousands of time. Histones play a crucial role in packing this very long DNA molecule in an orderly way (i.e. nucleosome) into nucleus only a few micrometres in diameter. Thus, nucleosomes are the fundamental packing unit particles of the chromatin and give chromatin a ìbeads-on-a-stringî appearance in electron micrographs taken after treatments that unfold higher-order packing (Olins and Olins, 1974).

The nucleosome ëbeadsí can be removed from long DNA ìStringî by digestion with enzymes that degrade DNA, such as bacterial enzyme, micrococcal nuclease. After digestion for a short period

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with micrococcal nuclease, only the DNA between the nucleosome beads is degraded. The rest is protected from digestion and remains as double-stranded DNA fragments 146 nucleotide pairs long bound to a specific complex of 8 nucleosome histones (the histone octomer). The nucleosome beads obtained in this way have been crystallized and analyzed by x-ray diffraction.

Each nucleosome is a disc-shaped particle with a diameter of about 11 nm and 5.7 nm in height containing 2 copies of each 45 nucleosome histones ñH_2A, H_2B, H_3 and H_4. This histone octamer forms a protein core [(i.e., a core of histone tetramer (H_3, H_4) and the apolar regions of 2 (H_2A and H_2B)] around which the double-stranded DNA helix is wound 1æ time containing 146 base pairs. In undigested chromatin the DNA extends as a continuous thread from nucleosome to nucleosome. Each nucleosome bead is separated from the next by a region of linker DNA which is generally 54 base pair long and contains single H_1 histone protein molecule. Generally, DNA makes two complete turns around the histone octomers and these two turns (200 bp long) are sealed off by H_1 molecules to 242 base pairs. Thus on an average, nucleosomes repeat at intervals of about 200 nucleotides or base pairs. For example, an eukaryotic gene of 10,000 nucleotide pairs will be associated with 50 nucleosomes and each human cell with 6×10^9 DNA nucleotide pairs contains 3×10^7 nucleosomes.

3.1.7 GIANT CHROMOSOMES

Some cells at certain particular stages contain large nuclei with giant or large-sized chromosomes. The giant chromosomes are the polytene and lampbrush chromosomes.

3.1.7.1 Polytene Chromosome (Salivary Gland chromosomes)

An Italian cytologist E.G. Balbian (1881) had observed peculiar structure sin the nuclei of certain secretary cells (eg. of salivary glands) of midge, Chironomus (Diptera). These structures were long and sausage-shaped and marked by swellings and cross striations (transverse bands),. Unfortunately, he did not recognize them as chromosomes, and his report remained buried in the literature. It was not until 1933 that Theophilus Painter, Ernst Heitz and H. Bauer rediscovered them in Drosophila and recognized them as the chromosomes. Since these chromosomes were discovered in the salivary gland cells, they were called salivary gland chromosomes. The present name polytene chromosomes was suggested by Koller due to the occurrence of many chromonemata (DNA) in them.

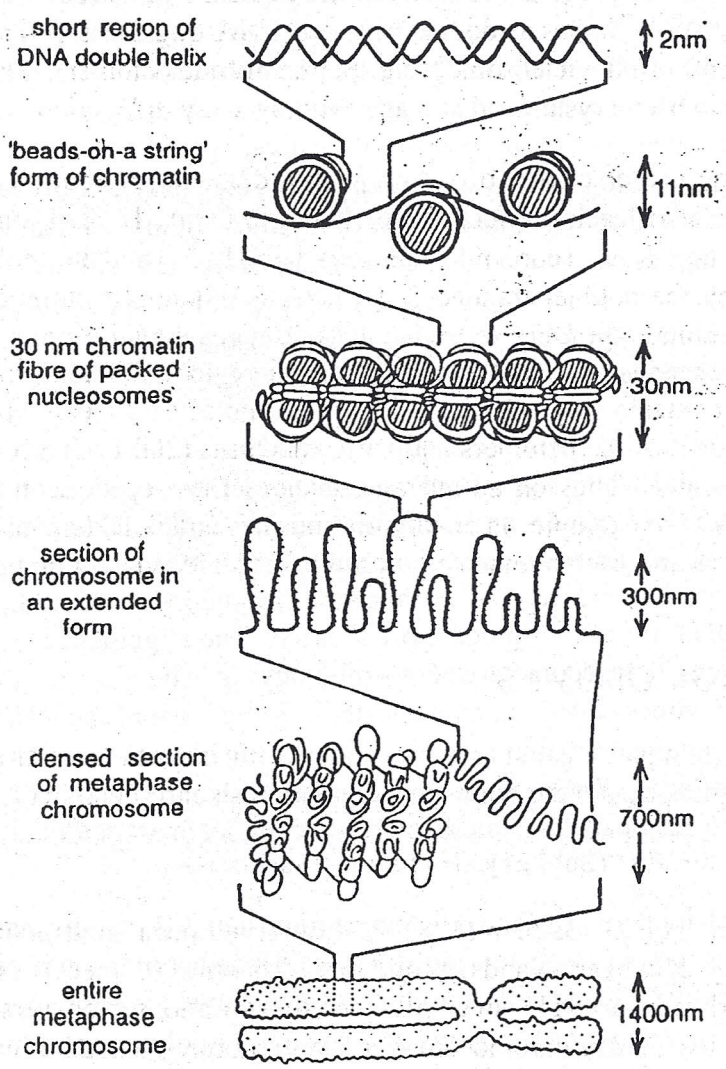

Fig. 3.1.3 Schematic diagram of some of many orders of chromatin packing which may give the highly condensed metaphase chromosome (after Alberts *et al.* 1989).

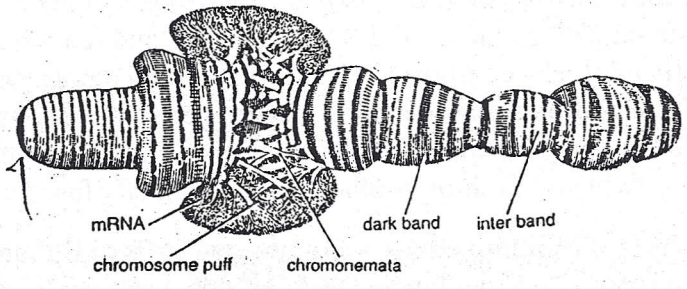

Fig. 3.1.4 Polytene chromosome of an insect, showing bands and interbands and a puff or Balbian ring.

Thus, some cells of the larvae of the dipteran insects such as flies (eg. Drosophila), mosquitoes and midges (chromosomal) become very large having high DNA content. These cells are unable to undergo mitosis and are destined to die during metamorphosis (those cells of larva which are destined to produce the adult structures after metamorphosis, i.e. imaginal discs remain diploid). Such polytenic cells are located most prominently in the salivary gland, but also occur in Malphighian tubules, rectum, gut, foot pads, fat bodies, ovarian nurse cells etc., polyteny of giant chromosomes is achieved by replication of the chromosomal DNA several times without nuclear division (endomitosis); and the resulting daughter chromatids do not separate but remain aligned side by side. In the process of endomitosis the nuclear envelope does not rupture and no spindle formation takes place. In fact, polyteny differs from polyploidy, in which there is also an excess DNA per nucleus, but in which the new chromosomes are separate from each other.

A polytene chromosome of Drosophila salivary gland has about 1000 DNA molecules which are arranged side by side and which arise from 10 rounds of DNA replication (2^{10}=1024). Other dipteran species have more DNA, for example, chironarmus has 16000 DNA molecules in their each polytene chromosomes. Further, the polytene chromosomes are visible during interphase and prophase of mitosis. In them, the chromomere (regions in which the chromatin is more tightly coiled) alternate with regions where the DNA fibres are folded more loosely. The alignment of many chromomeres give polytene chromosomes their characteristic morphology, in which a series of dark transverse bands alternates with clear zones called interbands. About 85 per cent of the DNA in polytene chromosome is a constant characteristic within a species and helps in chromosome mapping during cytogenetic studies. For example, in Drosophila melanogaster there are about 5000 bands and 5000 interbands per genome and each band and interband represent a set of 1024 identical DNA sequences arranged in file.

Another peculiar characteristic of the polytene chromosomes is that the maternal and paternal homologous chromosomes remain associated side by side. This phenomenon is called somatic pairing. Consequently in the salivary gland cells, the chromosome number always appear to be half of the normal somatic cells, e.g., *Drosophila melonogaster*, has only 4 polytene chromsomes. In Drosophila, pericentromeric heterochromatin of all polytene chromosome also coalesces in a chromocentre.

The preparation of a slide of the polytene chromosomes of dipterans for light microscopy is rather easy. The larvae are taken at the third instar stage and the salivary glands are dissected out and squashed in aceto-carmine. In such preparations, these chromosomes in aggregate reach a length of as much as 2000 μm in *Drosophila melanogaster*. In female Drosophila, the polytene chromosomes are found in the form of five long and one short strands radiating from a single more or less amorphous chromocentric one long strand corresponds to the X-chromosome and remaining far long strands are the left and right arms of II and III chromosomes. The shortest strand represents the small dot-like IV chromosome. Each of these chromosomes contains maternal and paternal homologues in somatic pairing which lacks in the sex chromosomes of male fruit flies. Thus, in male Drosophilla, X-chromosome remains single and twin and Y-chromosome exists indistinctly fused with the chromocentre.

Chromosome puffs (or) Balbiani rings: Chromosome puffs or Balbiani rings are the swellings of bands of the polytene chromosomes where DNA unfolds into open loops as a consequence of intense gene transcription.

3.1.7.2 Lampbrush Chromosomes

The lampbrush chromosomes were first observed in Salamander (amphibian) oocytes in 1882. He coined the name because the chromosomes look like the brushes which were used for cleaning the glass chimneys of old-fashioned paraffin or kerosene. They were described in detail in shark oocytes by R.Ruckert in 1892. Thorpe (1984) and Burns and Bottino (1989) preferred the term est tube brush chromosomes for them. The lampbrush chromosomes occur at the diplotene stage of meiotic prophase in the primary oocytes of all animal species, both vertebrates and invertebrates. Thus they have been described in Saigtta (Chaetogratha), Sepia (Mollusca), Echinaster (Echinodermata) and in several species of insects, shark, amphibians, reptiles, birds and mammals (humans). Lambrush-chromosomes are also found in spermatocytes of several species, gaint nucleus of Acetabularia and even in plants. Generally, they are smaller and hairy in invertebrates than in vertebrates. Lampbrush chromosomes are best visualized in salamander oocytes because they have a high DNA content. For example, the largest chromosome having a length upto 1mm have been observed in urodele amphibian. Thus, lampbrush chromosomes are much larger (longer) than the polytene chromosomes of insects.

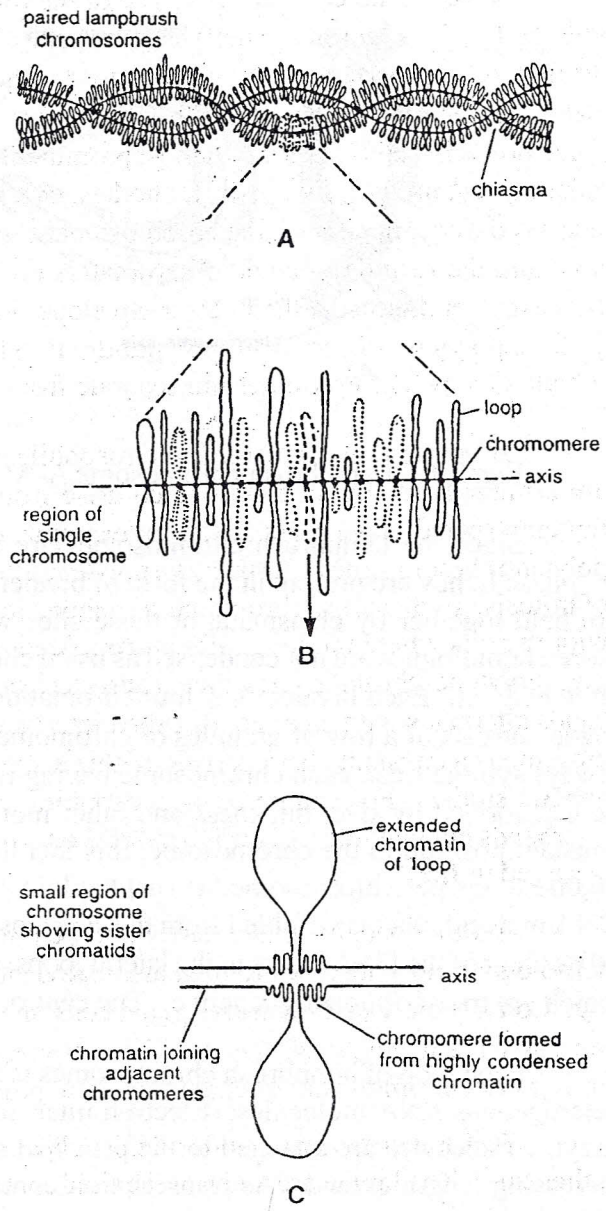

Fig. 3.1.5 Lampbrush chromosome structure. A-Bivalent or paired homologous chromosomes in pairing showing chiasmata, B-A part of one homologue showing paired loops given out by two chromatids; C-Single pair of loop (after Alberts *et al.*, 1989).

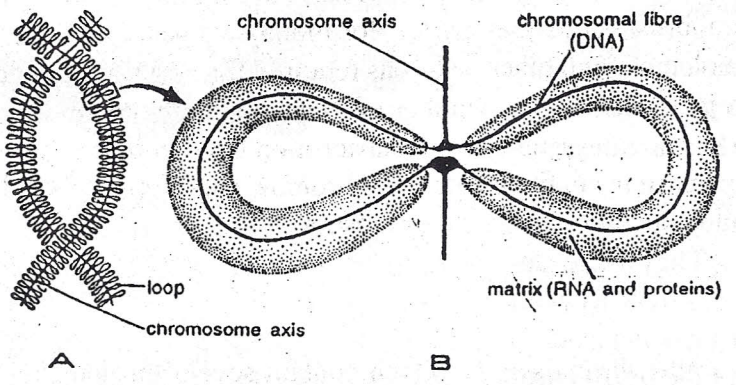

Fig. 3.1.6 Lampbrush chromosome A-At low magnification; B-Loop magnified

Since the lampbrush chromosomes are found in the prolonged diplotene stage of meiotic prophase I, they are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata, at those sites where crossing over has previously occurred. The paired homologues are not condensed as usual chromosomes would be instead, they are very long and stretched out. Each bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or chromomeres from which lateral lopps extend. The loops are always symmetrical, each chromosome having two of them, one for each chromatid. The loops can be categorized by size, thickness and other morphological characteristics. Each loop appears at a constant position in the chromosome, this fact helps in the chromosome mapping. There are about 10,000 loops per chromosome set (or) haploid set. Each loop has an axis which is made of single DNA molecule that is unfolded from the chromosome for the intense RNA synthesis. Thus about 5 to 10 per cent of the DNA exists in the lateral loops, the rest being tightly condensed in the chromomeres which are transcriptionally inactive. The centromeres of the chromosomes bear no loops.

Each loop of lampbrush chromosomes is found to perform intense transcription of hnRNA or heterogenous RNA molecules. Electron microscopy of the loops has shown that RNA polymerase enzyme molecules are attached to the principal axis (DNA) of the loop from which RNA fibrils of increasing length extend. As transcription continues along the DNA strand of loop, the fibrils of RNA (i.e., hn RNA) lengthen. Proteins get associated with these RNA fibrils as they are formed and ultimately ribonucleoprotein product is released.

Thus, each lateral loop is covered by a matrix that consists of RNA transcripts with hnRNA ñ binding proteins attached to them. Generally this matrix is asymmetrical, being thicker at one end of the loop than at the other. RNA synthesis starts at the thinner end and progresses toward the end of the loop than at the other. RNA synthesis starts at the thinner end and progresses toward the thicker end. Preparations spread for electron microscopy exhibit the typical ëChristmas treeí images with nascent ribonucleoprotein chains attached perpendicularly to the DNA axis. Many of the loops correspond to a single transcriptional unit and they are transcribed continuously from end to end; they form a continuous thin-thick matrix. However, other loops contain several units of transcription such loops include an extended section of chromatin that is not transcribed at all.

Further, the number of pairs of loops gradually increases during meiosis till it reaches maximum in diplotene. Such a lampbrush stage may persist for months or years as the primary oocyte builds up a supply of mRNA molecules and other materials required for its ultimate development into a new individual. As meiosis proceeds further, number of loops gradually decreases and the loops ultimately disappear either due to disintegration or by reabsorption back into the chromosome. For example, the addition of histone proteins to the lampbrush chromosomes stops the synthesis of RNA on the loops and causes the loops to retract into the chromosomes.

3.1.8 SUMMARY

The DNA is compacted into chromosomes, which contain several proteins which are thick enough to be visible by light microscopy during the mitotic phase of the cell cycle. The DNA-protein complex of eukaryotic chromosomes is called chromatin. The protein component of chromatin consists primary of five distinct proteins: histones H_1, H_2A, H_3 & H_4. The last four histones aggregate to form an octomeric protein that contains two molecules of each. DNA is wrapped around the histone octamer, forming a particle called a nucleosome. Centromeres and telomeres are regions of eukaryotic chromosomes specialized for spindle fiber attachment and stabilization of the tips, respectively.

Polytene chromosome are found in certain organs in insects. These gigantic chromosomes consist of about 1000 molecules of partly folded chromatin aligned side by side. Polytene chromosomes do not replicate further, and cells that contain them do not divide. They are useful to geneticists primarily as morphological markers for particular genes and chromosome segments.

3.1.9 TERMINOLOGY

Chromatin

Chromocenter

Diffuse centromere

euchromatin

folded chromosome

H1 histone

H2B hisone

H3 histone

H4 histone

heterochromatin

highly repetitive sequence

histone

holocentric chromosome

nucleoid

nucleosome

Satellite DNA

telomere

3.10 SELF ASSESSMENT QUESTIONS

1. Distinguish between the members of each pair:
   (i) diploid-haploid, (ii) Chromatid-chromosome, (iii) euchromatin-heterochromatin
2. Describe the basic structure of chromatin. What is the role of histones in this structure.
3. Why the study of chromosomes has become very significant in the field of biology?

3.11 REFERENCE BOOKS

1. **Cell Biology, Genetics, Molecular Biology, Evolution & Ecology** - P.S. Verma & V.K. Agarwal.
2. **Genetics** - P.K. Gupta
3. **Genetics** - Strickberger.

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LESSON-3.2

CHROMOSOME VARIATION IN NUMBER

3.2.0 INTRODUCTION

In our development of many of the principles considered upto now, we have assumed the constancy of genetic material during the period of observation. This assumption has made it easier to derive the various genetic laws without having to be concerned about unexplained changes that may occur in the midst of an experiment.

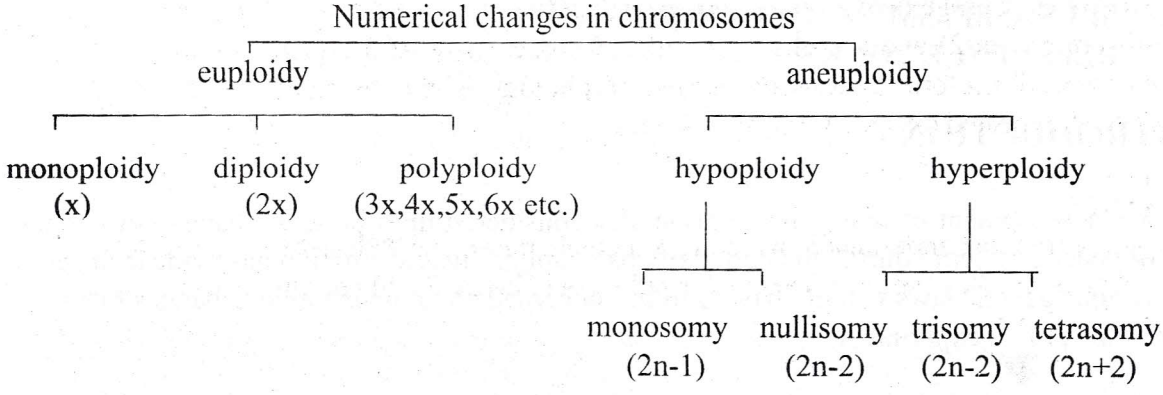
In general, genetic changes, when they arise, have been called mutations. This term usually refers to any change in the amount, organization, or content of genetic material, other than an exchange resulting from recombination between homologous chromosomes. For ease of classification mutations may be divided into modifications that are cytologically visible in the nucleus as chromo-

some changes, and into cytologically invisible "gene" or "point" mutations, which nevertheless have an observable developmental effect on the phenotype of an organism. By convention, the term mutation is commonly used for gene changes, and the more obvious chromosomal changes are known as chromosomal variations or aberrations.

Among chromosomal variations, the easiest to observe are usually those that involve changes in number. These may be of two types: euploid variations, which involve changes in the number of entire sets of chromosomes; and aneuploid variations, which involve changes in the number of only single chromosomes within a set. Let us imagine that 7 is the basic chromosome number (x) in a particular class of individuals where diploid number (2n) is 14. In this case, chromosome numbers 2n=15 and 2n=13 would be aneuploids, while those having 2n=7, 21, 28, 35 (or) 42 would be euploids.

3.2.1 OBJECTIVES

- To study the variations in chromosome number.
- To study the type of variations.
- To study different aneuploid conditions, i.e., addition of one or more chromosomes like monosomy, nullisomy, trisomy, tetrasomy.
- To study different monosomic conditions like double monosomics as triple monosomics.
- To study trisomy condition in humans which causes several morphological abnormalities in human beings like down syndrome, Edwards Syndrome etc.
- To study different euploid conditions like monoploid, polyploid conditions.
- To study different methods followed to produce monoploids.
- To study different interspecific crosses that produce monoploids.
- To study different polyploid conditions like autopolyploids & allopolyploids.
- To study the chromosomal constitution of allopolyploids by developing synthesised allopolyploids.
- To study segmental allopolyploids.



Different kinds of numerical changes in chromosomes
x = basic chromosome number; 2n = somatic chromosome number

3.2.2 ANEUPLOIDY

Aneuploidy can be either due to loss of one or more chromosomes (hypoploidy) or due to addition of one or more chromosomes to complete chromosome complement (hyperploidy). Hypoploidy is mainly due to loss of a single chromosome – monosomy (2n-1),or due to loss of one pair of chromosomes – nullisomy (2n-2). Similarly, hyperploidy may involve addition of either a single chromosome trisomy (2n+1) or a pair of chromosome – tetrasomy (2n+2). In representing chromosome number of aneuploids, here we are using 2n as the euploid chromosome number, even though 2n actually represents the somatic chromosome number of any organism, whether euploid or aneuploid.

3.2.2.1 Monosomy

Since monosomics lack one complete chromosome, such aberrations create major imbalance and cannot be tolerated in diploids. These could be easily produced in polyploids. A polyploid has several chromosomes of same type and therefore this loss can be easily tolerated. The number of possible monosomics in an organism will be equal to haploid chromosome number in common wheat, since 21 pairs of chromosomes are present 21 possible monosomics are known. These 21 monosomics in wheat were produced by E.R. Sears in the variety chinese spring and are being used for genetic studies all over the world. Monosomics were also isolated in cotton (2n=52) by J.E. Endrizz; and his co-workers, and in tobacco (2n=48) by E.R. Clausen and D.R. Cameron.

As indicated above, monosomics are normally found in polyploids and diploids cannot tolerate them. Nevertheless, in tomato (2n=24), which is a diploid, rarely monosomics could be produced. During the lost decade surprisingly a complete set of monosomics has also been produced in maize which is a diploid crop. Double monosomics (2n-1-1) or triple monosomics (2n-1-1-1) could also be produced in polyploids like wheat. Double monosomics mean that the chromosome number is 2n-2, like that in a nullisomic, but the missing chromosomes are non-homologoous.

Monosomic condition for a particular chromosome may be associated with a characteristic morphology. Moreover, in progeny of a monosomic we will get a mixture of disomics (2n), monosomics (2n-1) and nullisomics (2n-2) and a nullisomic will not possess any of the genes located on this specific chromosome. Therefore, by looking on the morphology of monosomics and that of their progeny, genes can be located on specific chromosomes.

3.2.2.2 Nullisomy

Nullisomics are those individuals, which lack a single pair of homologous chromosomes, so that the chromosomes. So that the chromosome formula would be 2n-2 and not 2n-1-1, which would mean a double monosonic.

3.2.2.3 Trisomy

Trisomics are those organisms, which have an extra chromosome (2n+1). Since the extra chromosome may belong to any one of the different chromosomes of a haploid complement, the number of possible trisomics in an organism will be equal to its haploid chromosome number. For

instance, we know that haploid chromosome number in barley is n=7, consequently, seven trisomics are possible. Trisomics, where extra chromosome is identical to two homologous are called primary trisomics. Besides these, there are secondary and tertiary trisomics. While a secondary trisomic means that extra chromosome should be an isochrome, a tertiary trisomic would mean that extra chromosome should be the product of a translocation. Trisomics were obtained for the first time in Datura Stramonism by A.F. Blakeslee and his co-workers. Since haploid chromosome number in this species is n=12, 12 primary trisomics, 24 secondary trisomic and a large number of tertiary trisomics are possible. Most of the trisomics were identified by size, shape and other morphological features of the fruit.

One of the most extensively studied trisomic series is that produced and studied by T. Tsachiya in barley. Trisomics are also known in Homo Sapiens. Trisomy for certain chromosomes causes definite morphological abnormalities in human beings. Mongolism (Down's Syndrome) is one such feature which is common in children and is characterised by mental retardation, a short body, swollen tongue and eyelid folds resembling those of Mongolian races.

3.2.2.3.1 Trisomy in humans: In human beings, the following three syndromes have been studied:

A. Down's Syndrome (DS) or Trisomy-21:

Downs syndrome is named after the physician J. Langdon Down who first described this genetic defect in 1866 and it was formally called mongolism or mongolian idiocy. It is usually assocaited with a trisomic condition for one of the smallest human autosomes (i.e. chromosome 21). It is the most common chromosomal abnormality in live births ($^{1}/_{650}$ births). There are about 50 physical characteristics shown by down syndrome infants soon after birth. These include mild or moderate mental retardation; eyes that slant up and out with internal epicanthal folds; a tongue that is large, swollen and protruding, small and under developed ears; a single palmar crease; short stature; stubby fingers, an enlarged liver and spleen. Women over 45 years of age are about twenty times more likely to give birth to a child with down syndrome than women aged 20. Non-disjunction of chromosome pair 21 during oogenesis is the main cause of occurrence of trisomy-21.

B. Edward's Syndrome or Trisomy-18

First described in 1960 by John H. Edwards and his colleagues, trisomy-18 is found to contain an incidence of about 0.3 per 1000 births. It is characterized by multiple malformations, primarily low-set ears, small receding lower jaw, flexed and clenched fingers cardiae malformations. Horelip and Cleft plate often occurs. Death takes place around 3 to 4 months of age.

C. Patau syndrome or Trisomy-13

This syndrome was described in 1960 by Klaus Patau and co-workers. Its incidence is about 0.2 per 1000 births. Individuals with patau syndrome appear to be markedly mentally retarded. Tri-

somy in non-humans. Trisomy-22 has been reported in chimpanzees (*McClure et al. 1969*), this shows Downs syndrome-like phenotypic features Trisomy-21 has been reported in the gorilla.

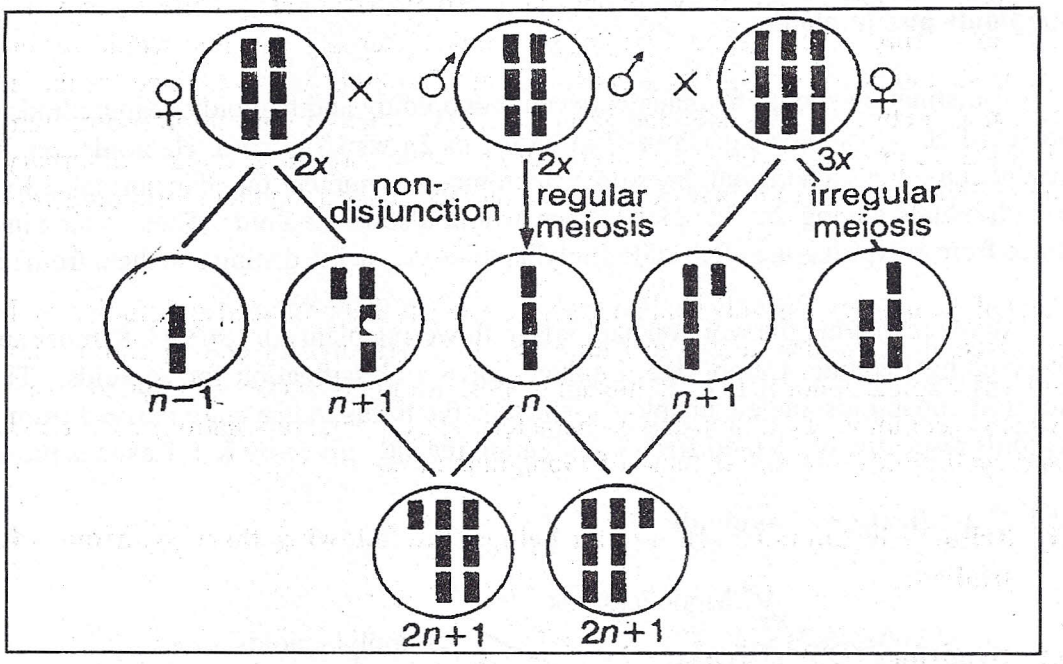


Fig. Production of trisomics due to formation of n+1 type of gametes in diploid (2x) and triploid (3x) individuals

3.2.2.3.2 Production of trisomics: Trisomics may originate spontaneously due to production of n+1 type of gametes due to rare non-disjunction of a bivalent. However, more often trisomics are produced artificially either by selfing triploids (produced by crossing diploids and autotetradploids) or by crossing these triploids as females with diploids as male (3x X 2x). In either case, trisomics are obtained in large number and can be identified through phenotypic effects of individual chromosomes.

3.2.2.3.3 Cytology of trisomics: A trisomic has an extra chromosome which is homologous to one of the chromosomes of the complement. Therefore, it forms a trivalent. This trivalent may take a variety of shapes in primary and secondary trisomics. In a tertiary trisomic a characteristic pentavalent is observed.

3.2.2.4 Tetrasomy

Tetrasomics have a particular chromosome represented in four doses. Therefore, general chromosome formula for tetrasomics is 2n+2 rather than 2n+1+1, the later being a double trisomic. All 21 possible tetrasomics are available in wheat. Besides these tetrasomics, E.R. Sears was also able to synthesize a complete set of compensating nullisomic tetrasomics (2n-2+2), where addition of a pair of homologous chromosomes would compensate for the loss of another pair of homologous chromosomes. Such non-homologous chromosomes, which are able to compensate for each other, are considered to be genetically related and are called homologous chromosomes.

3.2.3 Euploidy

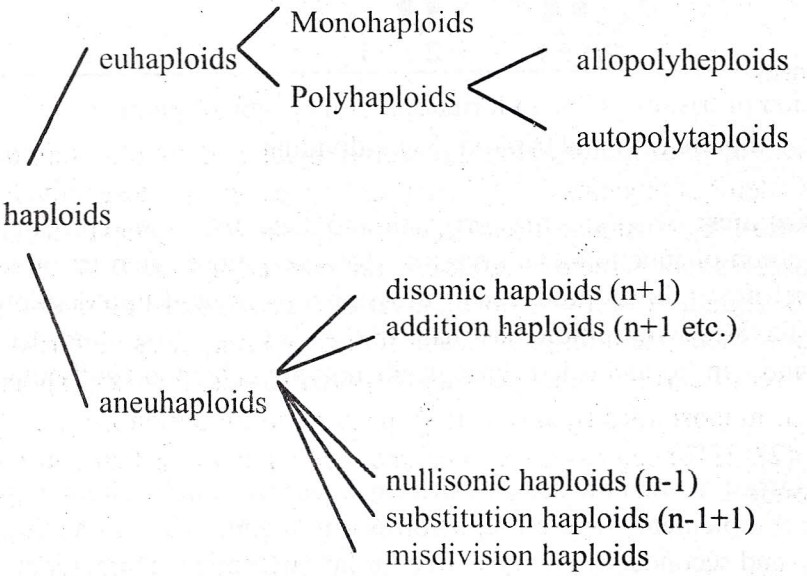
Euploids can be monoploids, diploids or polyploids.

Monoploidy and haploids

A distinction should be made between monoploidy and haploidy. Monoploids have a single basic set of chromosomes e.g. 2n=x=7 in barley or 2n=x=10 in corn. Haploids, on the other hand represent individuals having half the somatic chromosome number found in normal individual. Therefore, individuals having 2n=3x=21 in wheat would also be haploids. These later kind of haploids obtained from polyploids are often called polyhaploids in order to distinguish them from monohaploids.

While reviewing the work on haploids in flowering plants in 1963, G. Kimber and R. Riley of plant breeding Institute, Cambridge, England, gave a classification for haploids. They classified haploids in euhaploids and aneuhaploids which as the terms indicate are derived from euploids and aneuploids respectively. A modified classification recently given by K.J. Kasha is presented.

3.2.3.1 Classification of haploids



3.2.3.2 Origin and production haploids: Haploids in some cases as in male insects (Hymenoptera) are found as a routine and are produced due to parthenogenesis. In these insects, queen and drones are diploid females. Haplois may also originate spontaneously due to parthenogenic development of egg in flowering plants. Such rare haploids have actually been obtained in tomatoes and cotton under cultivation. Rarely haploids may originate from pollen tube rather than from egg, synergies or antipodals of embryo sac. These haploids will be called androgenic haploids. Haploids can be artificially produced by any one of the following methods: (i) X-rays treatment, (ii) delayed pollination, (iii) temperature shabs, (iv) colchicine treatment, (v) distant interspecific intergeneic hybridization, (vi) anther or pollen culture. Among these techniques, the most important ones are distant hybridization and anther culture, distant hybridization.

Interspecific crosses in genera of Solaraceae (**eg.** Solanum and Nicotiana) have been employed for the production of both parthenogenetic and androgenic monoploids. By this technique monoploids have been obtained in large number in potato. K.J. Kosha from University of Guelph, Canada evolved an excellent technique for producing haploids in large number in barley. He discovered that if the cross *Hardeum vulgare* x *H. bulbosum* is made, chromosomes of *H. bulbosum* are eliminated in early zygotic divisions, so that a few days after pollination, embryos can be cultured to get haploids. This technique is being extensively utilized now all over the world.

(b) The production of haploids by anther and pollen culture was demonstrated for the first time in the laboratory of S.C. Maheswari of Delhi University. Subsequently, haploids by this technique could be produced in other plant genera **e.g.** Oryza (rice) and Nicotiana (tobacco).

Morphology of haploids: Haploid plants have reduced size of all vegetative and floral parts. In haploid Nicotiana Kostoff reported that the leaves, flowers and overall plant size were smaller. The size of seed and stomata as well as diameter of pollen were found smaller in haploids than in the diploids. Even the size of nucleus (or the nuclear volume) of a haploid often was found to be just half than the nucleus of the diploid ell.

3.2.3.3 Cytology of haploids

Sine in a haploid set, the chromosomes are non-homologous and have no homologous pair with, they are found as univalents at metaphase I of meiosis. Consequently, these univalents distribute at random during anaphase-I. For instance, a haploid in maize (2n=20) will have 10 chromosomes and the number of chromosomes in a gamete of haploid plant can range from 0-10. Consequently, considerable sterility will be found. Moreover, since univalents are scattered all over the cell, they may constitute a restitution nucleus including all chromosomes and may thus give rise to gametes having a complete haploid set of chromosomes. Haploids (polyhaploids; 2n=3x=21) were used by E.R. Sears for production of monosomics by pollinating the haploid by pollen from a diploid individual (hexaploid; 2n=6x=42). If the egg has a chromosome number, one less than the complete set, this will result into monosomic.

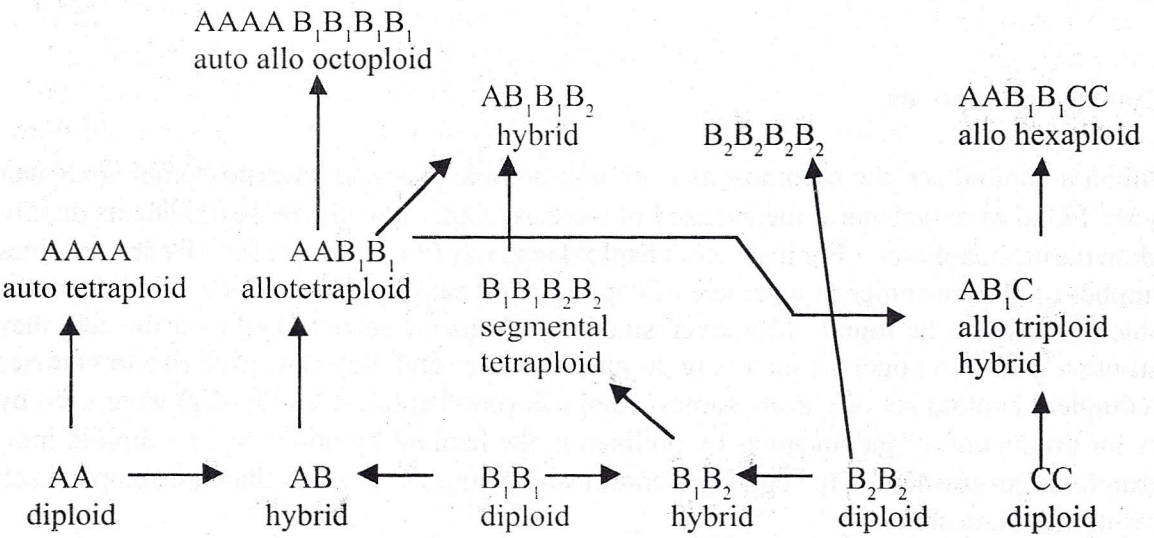
3.2.3.4 Uses of haploids

In a haploid, since there is only one copy of each chromosome and only one allele of each gene, so in it each gene is expressed whether it is dominant or recessive. This facilitates genetic experiments and this is the reason why micro-organisms have been helpful in genetic studies. For the same reason, scientists are trying hard to develop haploid strains of flowering plants. Success has been achieved in developing haploid strains of Nicotiana, Datura and Triticum. From these haploid strains have also been developed pure breeding strains which are resistant for the insecticides and also for toxic compounds normally produced by the parasites of these plants.

3.2.3.5 Polyploidy

Any organism with more than two genomes (2x) is called a polyploid. Many plant genera include species whose chromosome numbers constitute a euploid series. For example, the rose genus Rosa includes species with the somatic numbers 14, 21, 28, 35, 42 & 56. These numbers are multiples of 7. Therefore, this is a euploid series of the basic haploid number 7 which gives diploid, triploid, tetraploid, pentaploid, hexaploid and octaploid species. Except diploids rest of these belong to polyploid category. Ploidy levels higher than tetraploid are not commonly encountered in natural populations, but our most important crops and ornamental flowers are polyploids **e.g.**, wheat (Hexaploid 6x), straw berries (Octaploid, 8x), many commercial fruits and ornamental plants. Generally, polyploidy is common in plants (more common in monocots) but rare in animals.

Type of Polyploidy: There are following three different kinds of polyploids: (i) autopolyploids, (ii) allopolyploids, (iii) segmental allopolyploids. Suppose that there are four different haploid sets of chromosomes A, B_1 B_2 B_C in which B_1 and B_2 genomes are related. By using these genomes, all three types of polyploids can be derived as



Mode of formation of different kinds of polyploid

3.2.3.5.1 Autopolyploids

The autopolyploids are those polyploids, which consist of same basic set of chromosomes multiplied. For example, if a diploid species has two similar sets of chromosomes or genomes (AA), an autotriploid will have three similar genomes (AAA), and an autotetraploid, will have four such genomes (AAAA).

(i) Origin and Production of autopolyploids

The autopolyploids may occur in nature or may be produced artificially. When they are found in nature, their autopolyploidy nature is deduced by their meiotic behaviour. One of the common

example of natural and autopolyploid is 'doob' grows (cynodon dactylon) which is quite commonly cultivated in Uttar Pradesh and Bihar. its autotriploid status was established from its meiotic behaviour by Prof. P.K. Gupta, an eminent cytogeneticists of Northern India, working in Department of Agriculture Botany of Meerut University. Polyploids may arise naturally by following means: (i) in natural populations polyploidy may arise as a result of interference with cytokinesis, once chromosome replication has occurred.

(ii) It may occur either in somatic tissues which give rise to tetraploid branches or during meiosis which produces unreduced gametes. All these natural induction of polyploidy may occur due to chilling.

Some of common examples of autotriploid crop plants, which are mainly produced by artificial methods are seedless varieties of watermelons, sugarbeet, tomato, grapes and banana. Similarly, many important crop plants include autotetraploids such as rye (*Sccale cereale*), corn (*Zea mays*), red clover (*Trifolion pratense*), berseen (*Trifolium alexandrium*), marigolds (Tagetus), Snap dragons (Antirrbinum), phlox, greepes, apples, *Oenothera lamorkiana*.

3.2.3.5.1.1 Induced autopolyploidy

The autopolyploidy have been induced in many plant and animal cells by artificial means such as chemical (**eg.** chloralhydrate, colchine, sulphanilamide, mercury chloride, hexachlorcyclo-hexane etc.), radio active substances (**eg.** radium and x-ray) and temperature shocks. These inducers usually disturb the mitotic (or) meiotic spindle and cause non-segregation of already duplicated chromosomes, during cell division.

Colchicine is a drug (i.e. an alkaloid obtained from the corms of plants – *Colchicum autmunale* and *C. luteum*) and its aqueous solution is found to prevent the formation and organisation of spindle fibres, so the metaphase chromosomes of the affected cells (called C-mataphase or colchicine metaphase) do not move to a metaphase plate and remain scattered in the cytoplasm. Even the process of cytokinesis is prevented by colchicine and with duplications of chromosomes, the number goes on increasing. As colchicine interferes with spindle formation, its effects are limited to dividing and meristomatic cells.

3.2.3.5.1.2 Effects of autopolyploidy

Autopolyploid results in gigantism of plant cells i.e. leaves, flowers and fruits of an autopolyploid are larger in size than a diploid plant. For example, the size of flower epidermis of leaf of a tetraploid sarifraga Pensylvanica was found greater than the diploids. Some of significant effects of autopolyploidy are as follows: (1) With the increase in cell size, the water content increases which leads to a decrease in osmatic pressure. This results into loss of resistance against frost, etc. (2) Due to slower rate of cell division, the plants growth rate decreases. This leads to a decrease in auxin supply and a decrease in respiration. (3) Due to slow growth rate, the time of blooming of an autopolyploid is delayed. (4) At higher ploidy level, such as auto octoploids, the adverse effects become highly pronounced and lead to the death of the plants.

Polyploid varieties with an even number of genomes (**eg.** tetraploids) are often fully fertile, whereas those with an odd number (**eg.** triploids) are highly sterile.

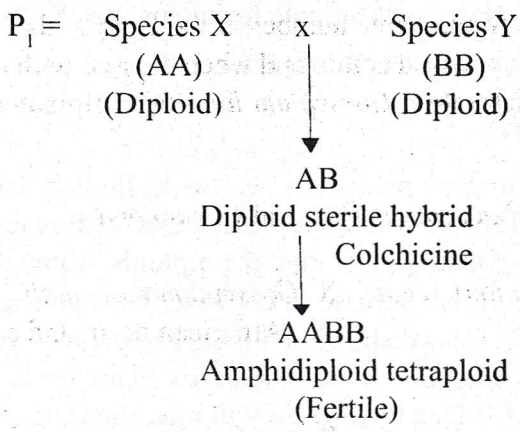
3.2.3.5.1.3 Uses of induced Polyploidy

Since in the induced polyploids, the fertility level and seed set are low, so seedless fruits can be produced by using triploids as in case of seedless watermelons which were produced by a Japanese scientist, Dr. Hitoshi Kihara. These triploids are obtained from seeds raised by a cross of tetraploid and diploid plants. The tetraploids have been produced from the diploids by colchicine treatment. By adopting these methods a variety of triploids such as sugarbeet, tomato and grapes and tetraploids such as rye, barley, corn, apple, grapes, marigolds, grapdragons, lily, phlax etc., have been obtained. Among the forage crops, tetraploid barseem isavery popular crop in Nothern India.

3.2.3.5.2 Allopolyploids

When the polyploidy results due to doubling of chromosome number in a F_1 hybrid which is derived from two distinctly different species, then, it is called allopolyploidy and the resultant species is called an allopolyploid. Let A represent a set of chromosomes (genome) in species X, and let B represent another genome in a species Y. The F_1 hybrids of these species then would have one A genome and another B genome. The doubling of chromosomes in the F_1 hybrids will give rise to allotetraploids with two A and two B genomes.

Rephanobrassica is a classical example of allopolyploidy or amphipolyploidy. In 1927, a Russian geneticist G.D. Karpechenko performed across between radish (Raphanus section 2n=18) and cabbage (*Brassica oleracea*, 2n=18) and in F_1 got sterile (diploid) hybrids. Among these sterile F_1 hybrids, he found certain fertile plants which were found to contain 36 chromosomes. These fertile tetraploids were called Raphanobassica.



Formation of amphidiploid tetraploid

3.2.3.5.2.1 Synthesized Allopolyploids

To find out the origin of naturally occurring allopolyploids some cytogeneticists produced certain allopolyploids in laboratory by employing artificial means. Common hexaploid wheat and tetraploid cotton furnish two such examples.

(i) *Triticum spelta* is a hexaploid wheat which was artificially synthesized in 1946 by E.S. McFadden and E.R. Sears and also by H. Kihara. They crossed an emmer wheat, Triticum dicoccoides, (tetradploid : 2n=28) with goat grass, Aegilops squorrosa (diploid; 2n=14) and doubled the chromosome number in the F_1 hybrid. The artificially synthesized hexaploid wheat was found to be similar to the primitive wheat *T. spelta*.

P_1 Triticum dicoccoides (Tetraploid emmer wheat) AABB (2n = 28; 14 bivalents) x Aegilops squarreled (Diploid goat grass) DD (2n = 14; 7 bivalents)

↓

ABD
Triploid hybrid
(2n=21; 21 univalents)

↓ Colchicine

AA BB DD
Synthesized hexaploid wheat
(*Triticum spelta*)
(2n=42; 21 bivalents)

Artificial synthesis of hexaploid wheat

3.2.3.5.2 *Gossypium hirsutum*: The new world cotton plant, is another interesting example of allopolyploidy. Old world cotton, *Gossypium herbaceum*, has 13 pairs of chromosomes, while American (a) "upland cotton" also contains 13 pairs of chromosomes J.O. Beasley crossed the old world and American cottons and doubled the chromosome number in the F_1 hybrids. The allopolyploids thus produced resembled the cultivated new world cotton and when crossed with it gave fertile F_1 hybrids. These results, thus, suggested that tetraploid *Gossypium hirsutum* originated from two diploid species, namely:

G. herbaceum (2n=26) and *G. raimondii*

Gossypium herbaceum (Old world cotton) X *Gossypium raimondii* (American or upland cotton)

↓

F_1 hybrid

(2n=26; 26 univalents)

↓ Colchicine

New world cotton
(*Gossypium hirsutum*)
(2n=52; 26 bivalents)

Artificial synthesis of new world cotton

3.2.3.5.3 Segmental allopolyploids: Different genomes of some allopolyploids are not quite different from each other. Consequently in these polyploids chromosomes belonging to different genomes do pair together to some extent. This indicates that segments of chromosomes and not the whole chromosomes are homologous. Therefore such allopolyploids are called segmental allopolyploids. The segmental allopolyploids are intermediate between autopolyploids and can be identified by their peculiar meiotic behaviour.

Triticum aestivum X *Sesale cereale*
(Hexaploid wheat 2n=26) (Diploid rye; 2n=14)

F_1 hybrid (Sterile)
(Tetraploid; 2n=28)

↓ (Chromosome doubling)

Octoploid triticate
(2n=56)

Artificial synthesis of a octoploid triticale

It is generally believed that most naturally occurring polyploids are segmental allopolyploids. Our common hexaploid bread wheat too is found to be segmental hexaploid, because the three diploid genomes (A, B and D) are related (homoeologous) to each other.

3.2.4 SUMMARY

Polyploidy involves the presence of extra sets of chromosomes. Many polyploids are sterile because their multiple sets of chromosomes segregate irregularly in meiosis. However, polyploids produced by chromosome doubling in interspecific hybrids may be fertile if their constituent genomes segregate independently. In some polyploid tissues, sister chromatids remain together, forming a large, polytene chromosome.

Aneuploidy involves the under-or over-representation of a chromosome or chromosome segment. *In a trisomy, such as down syndrome* in human beings, a chromosome is over represented, in a monosomy, such as Turner syndrome, it is unrepresented. Deletions and duplications of particular chromosome segments also cause aneuploidy – hypoploidy in the case of deletion and hyperploidy in the case of duplication.

3.2.5 TERMINOLOGY

Heteroploidy
aneuploidy
euploidy
Monosomy
Nullisomy
Trisomy
Tetrasomy
Double monosomics
Triple monosomics
Primary Trizomics
Secondary trizomics
Tertiary trisomics
Trisomic analysis
Homoeologous chromosomes
Monoploids
Euhaploids
Aneuhaploids
Androgenic haploids
Autotriploid
Autotetraploid
Anti colchicine
Auto octoploids
Amphidiploid

3.2.6 SELF ASSESSMENT QUESTIONS

1. How will you distinguish cytologically? (i) between a double monosonic and a nullisomic; (ii) between a primary trisomic and a secondary trisomic.
2. What are the different kinds of polyploids? How will you distinguish between autopolyploids and allopolyploids?

3. Discuss the role of polyploidy in evolution using specific examples of some natural polyploids, whose evolutionary history is known.
4. How can triploidy lead to seedlessness?
5. In common wheat (n=21), nullisomics are known for each of the 21 chromosomes. No nullisomics are known in barley (n=7) why?
6. How can you distinguish between the terms haploidy and monoploidy? How can haploids be produced and utilized in plant breedings.

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UNIT-III

LESSON-3.3

ABNORMALITIES OF CHROMOSOMES STRUCTURAL CHANGES IN CHROMOSOMES

STRUCTURAL CHANGES IN CHROMOSOMES

3.3.0 INTRODUCTION

The presence of arrangement of many genes on a single chromosome permit a change in genetic information to occur through a change in chromosome structure. In these cases, the number of chromosomes usually remains the same, but their genetic material becomes altered through the loss, gain or rearrangement of particular sections.

In origin, such structural changes are now known to be caused by breaks in the chromosome, or in its cell-division subunit, the chromatid. Each break produces two ends which may then follow three different paths:

1. They may remain un-united, thereby leading to the eventual loss of that chromosomal segment which does not contain the centromere.

2. Immediate reunion or restitution of the same broken ends may occur, leading to reconstitution of the original chromosome structure.

3. One or both ends of one particular break may join those produced by a different break, causing an exchange, or non-restitutional union.

Depending upon the number of banks, their locations, and the pattern in which broken ends join together, a wide variety of structural changes are possible. Structural changes can be of following types:

(i) deficiency, which involves loss of a part of chromosome,

(ii) duplication, which involves addition of a part of chromosome,

(iii) inversion, which involves a reverse order of genes in a part of chromosome

(iv) translocation, which involves exchange of segments between non-homologous chromosomes.

3.3.1 OBJECTIVES

- To study the structural changes in chromosomes.
- To study the different types of structural changes.
- To study the deficiency (or) deletion of a part of chromosome.
- To study the chromosomal pairing in a deficiency heterozygote.
- To study different inversion methods.
- To study translocation, which involves exchange of segments between non-homologous chromosomes.

Structural abnormalities may be found in both the homologous chromosomes of a pair, or in only one of them. When both homologous chromosomes are involved, these are called structural homozygotes e.g., deficiency homozygote, duplication homozygote etc. If only one chromosome is involved, this will be called a structural heterozygote. The constitutions of a translocation homozygote and translocation homozygote are represented in the figure.

3.3.2 DEFICIENCIES

Deficiency is due to loss of a part of chromosome. Smaller deficiencies, present in heterozygotes condition (only on one of the two homologous chromosmes), can be tolerated by an organism. Such individuals at meiosos will form a loop in a bivalent that can be observed at pachytene stage. Loops can also be observed in salivary gland chromosomes of Drosophila which are found in a permanent state of pairing, so that even small deficiencies could be detected in these chromosomes. Deficiencies have an effect on inheritance also. In presence of a deficiency, a recessive allele will behave like a dominant allele (pseudo-dominance). This principle of pseudo-dominance exhibited by deficiency heterozygotes has been utilized for location of genes on specific chromosomes in Drosophilla, maize and other organisms. L.J. Stadler, who was a pioneer in radiation work in plants devised a method where a homozygous recessive stock was pollinated by irradiated pollen from dominant stock, so that if irradiation induced a deletion, recessive allele will express due to pseudo-dominance.

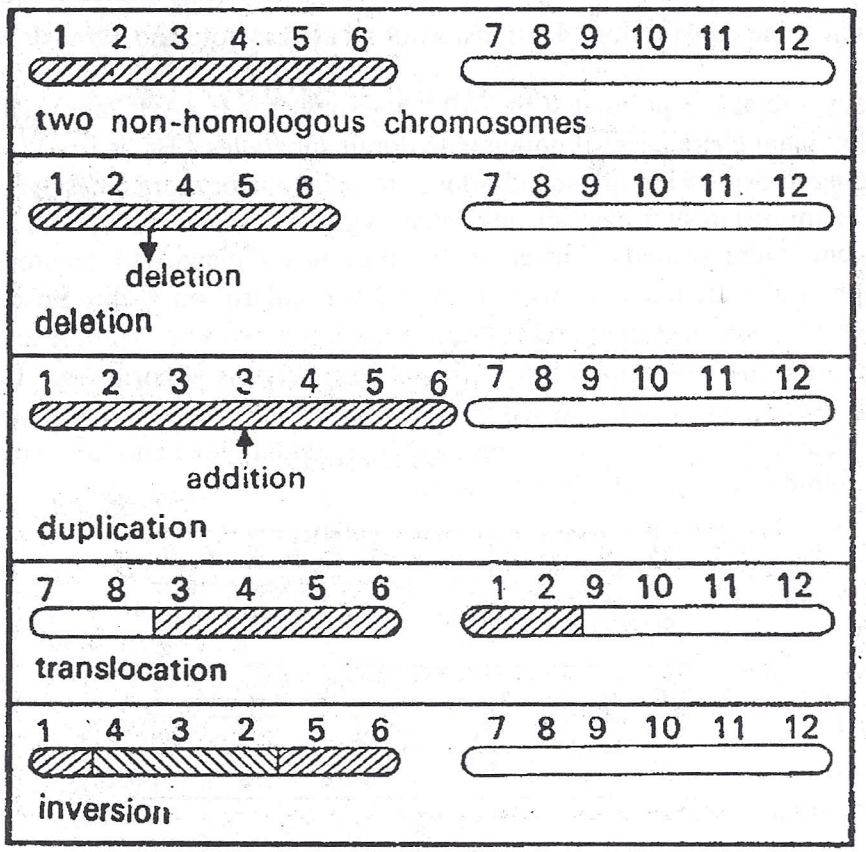


Fig. 3.3.1. Different kinds of changes in chromosome structure

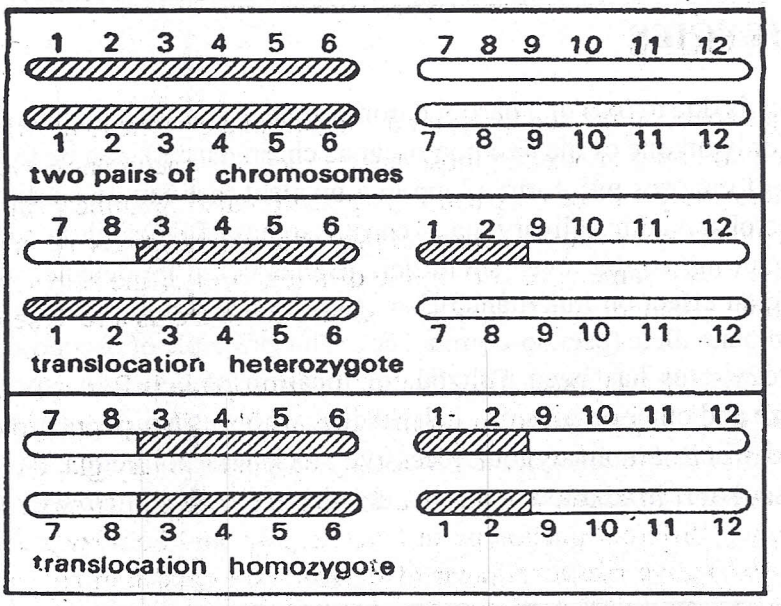


Fig. 3.3.2 Chromosome constitution of a translocation heterozygote and a translocation homozygote

If homozygote abc is pollinated by ABC, heterozygous F_1 (ABC/abc) will be produced expressing only dominant characters. If pollen with dominant alleles ABC is irradiated, a deletion may be induced leading to expression of psedudo-dominance by one or more recessive alleles. If meiosis at pachytene is examined in such a deficiency heterozygote, presence of loop will indicate location of gene. Several genes were located on different chromosomes of maize and tomato, utilizing deficiencies. In drosophila also deficiencies were recorded particularly on x-chromosomes in regions of genes W (white eye), fa (facet eye) and v (vermilion coloured eye). Deficiencies have also been recorded in Waltzing mice in region of gene w inducing nervous abnormality. In human beings, a deficiency was discovered, which was associated with cat like-cry so that the child carrying this deficiency had a cat like cry and also had microcephally (small head and low mental faculty). This deficiency was found in a segment of chromosome 5.

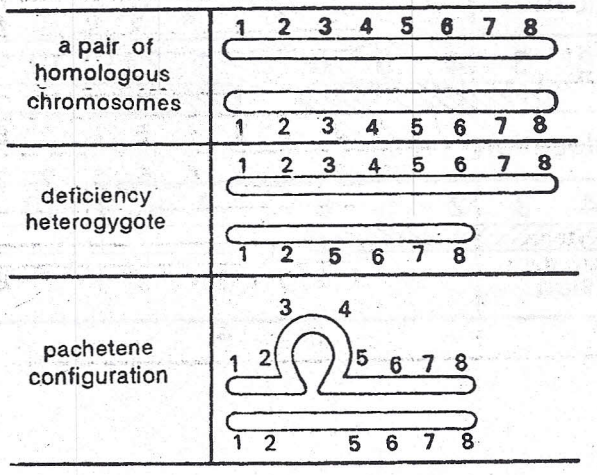


Fig. 3.3.3 Chromosome pairing in a deficiency heterozygote

3.3.3 DUPLICATIONS

Duplications are obtained due to addition of a part of a chromosome. If duplication is present only on one of the two homologous chromosomes, at meiosis (pachytene), cytological observations characteristic of deficiency will be obtained in duplication also. Duplication f a chromosome segment, may be brought about by addition at any of the following positions. (i) in adjacent region, (ii) at a displaced position of the same arm, (iii) on the different arm of the same chromosome, (iv) on a different chromosome. Sometimes, the duplication may be found as a reverse repeat.

One of the classical examples of duplication in Drosophila is Bar eye. Bar eye is a character, where eyes are narrower as compared to normal eye shape. This phenotypic character is due to duplication for a part of a chromosome. By the study of gaint salivary gland chromosomes, it could be demonstrated that ëBarí character was due to a duplication in region 16A of x-chromosome. Barred eyes will have slightly different phenotype in heterozygous and homozygous individuals. Barred individuals (16A, 16A) gave rise to ultrabar (16A 16A 16A) and normal wild type (16A) due to unequal crossing over.

Some other duplications konwn in Drosophila lead to following phenotypic effects :

(i) a reverse repeat in chromosome 4 causes eyeless dominant (ly)

(ii) a tandem duplication in chromosome 3 causes confluens (co) resulting in thickened veins.

(iii) another duplication causes hairy wing (Hw).

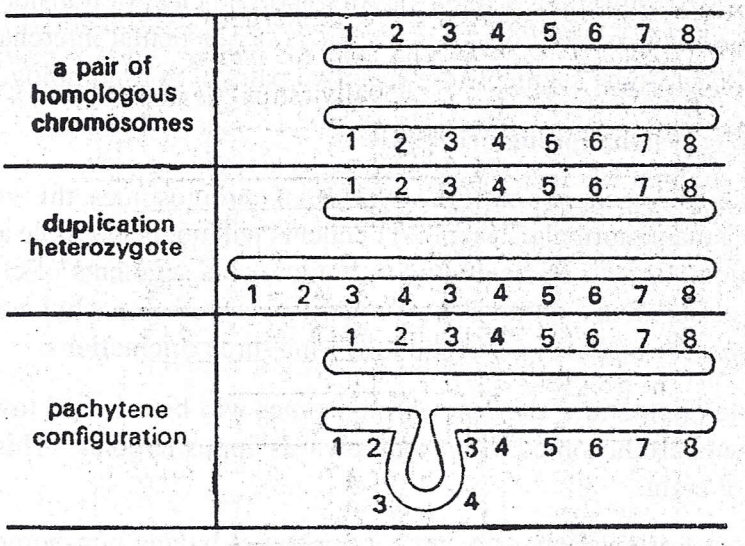
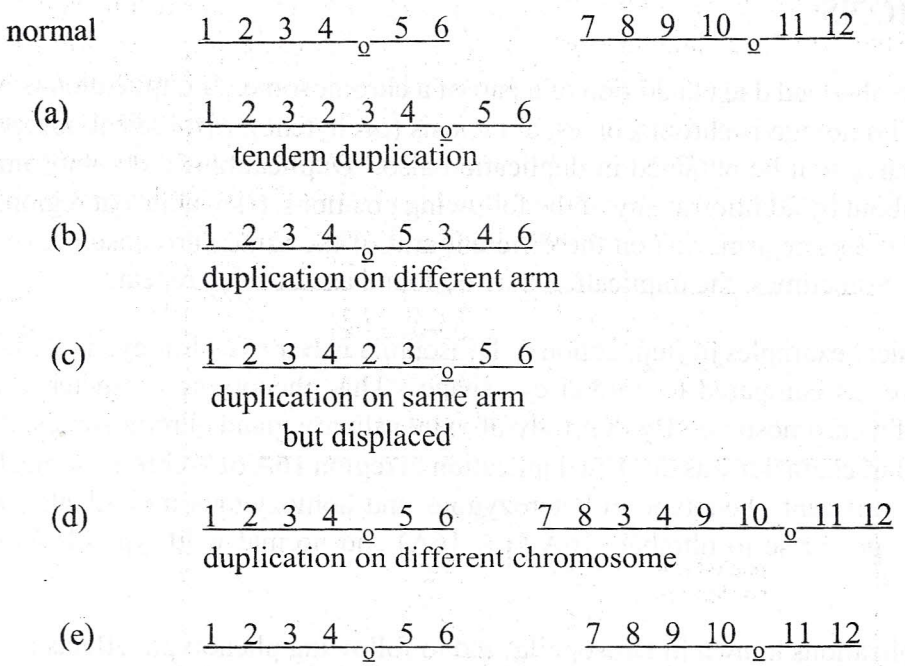


Fig. 3.3.4 Chromosome pairing in a duplication heterozygote



Different kinds of duplication in chromosomes

3.3.4 TRANSLOCATIONS

Translocations is a broad term including all types of unilateral or bilateral transfer of chromosome segments from one chromosome to another. An important class of translocations having evolutionary significance is known as reciprocal translocations or segmental interchanges, which involve mutual exchange of chromosome segments between two pairs of non-homologous chromosomes.

3.3.4.1 Cytology of a translocation heterozygote

If a translocation is present in one of the two sets of chromosomes, this will be a translocation heterozygote. In such a plant, normal pairing into bivalents will not be possible among chromosomes involved in translocation. Due to pairing between homologous segments of chromosomes, a cross shaped (x) figure involving four chromosomes will be observed at pachytene. This ring of four chromosomes at metaphase can have one of the following three orientations.

Alternate: In alternate orientation, alternate chromosomes will be oriented towards the same pole. In other words, adjacent chromosomes will orient towards opposite poles. This will be possible by formation of a figure of eight.

Adjacent I: In adjacent I orientation, adjacent chromosomes having non-homologous centromeres will orient towards the same pole. In other words, chromosomes having homologous centromeres will orient towards opposite poles. A ring of four chromosomes will be observed.

Adjacent II: In adjacent II orientation, adjacent chromosomes having homologous centromeres will orient towards the same pole. A ring of four chromosomes will be observed.

Alternate disjunctions will give functional gametes. Adjacent I and adjacent II disjunctions will form gametes, which would carry duplications or deficiencies and as a result would be non-functional or sterile. Therefore, in a plant having a translocation in heterozygous condition, there will be considerable pollen sterility. A ring of four chromosomes, as described above, is found under conditions when a single interchange is found. If two interchanges are involving three non-homologous chromosomes, a ring of six chromosomes is found, and he size of ring can increase with additional interchanges. More than one ring can also be found if two or more interchanges are independently found, each involving two different non-homologous chromosomes.

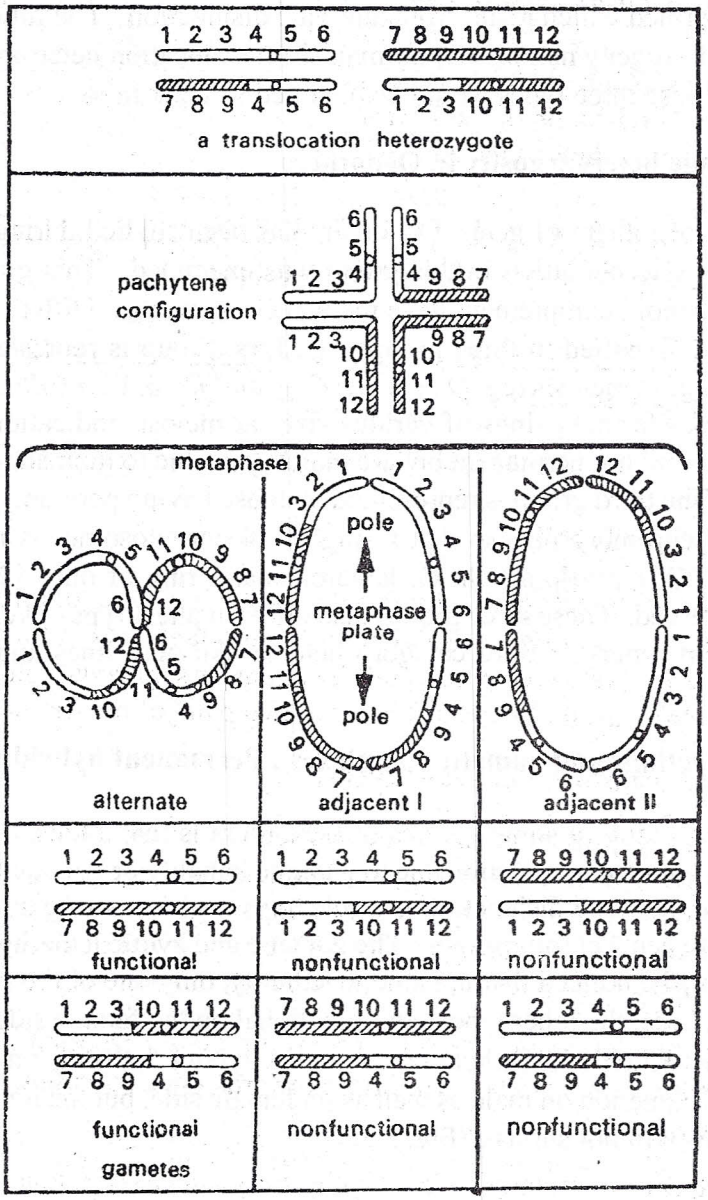


Fig. 3.3.6 Chromosomal pairing and different kinds of gametes formed in a translocation heterozygote

The first case of translocation was found in Denothera, which was originally described as a mutation by de vries while working for his Mutation Theory. Oenothera, Tradescantia and Rhoeo are such cases, where translocations in heterozygous condition are frequently found in nature. In many crop plants they have been artificially induced.

3.3.4.2 Breeding behaviour of a translocation heterozygote

Presence of translocation heterozygosity can be detected by presence of semi-sterility and low seed set. This can then be confirmed at meiosis by quadrivalent formation. Only two types of functional gametes are formed which result from alternate disjunction. The functional gametes will give rise to three kinds of progeny namely: (i) normal, (ii) translocation heterozygote, and (iii) translocation homozygote. These three types would be obtained in 1:2:1 ratio.

3.3.4.2.1 Interchange heterozygosity in Oenothera

Subgenus Euoenothera of genus Oenothera has been studied during 1920-1930 and cytogenetic structure leading to evolution in this group was examined. This group has 2n=14 and all 7 chromosomes of a haploid complement have median centromeres. Different speies in the subgenus Euoenothera, can be classified in three groups : (i) First group is represented by species showing bivalents or small rings at meiosis (eg. *O hookeri, O. grandiflora, O. argillicola).* (ii) Second group is represented by species forming rings of various sizes at meiosis indicationg the presence of interchanges. These rings are not permanent but are maintained due to their superiority in adaptive value (eg. O irrigua). (iii) The third group is represented by those having permanent translocation heterozygosity involving all chromosomes, so that a ring of 14 chromosomes is regularly formed (e.g. O biennis, O. strigosa, O. parviflora). In O. lamarckiana, a ring of only 12 instead of a ring of 14 chromosomes is observed. These three groups also differ in phenotypes like flower size, etc., and can be identified. The members of third category behave like pure lines and are actually permanent heterozygotes.

3.3.4.2.2 Balanced lethals and gametic complexes : Permanent hybridity in Oenothera

Permanent hybridity in some species of Oenothera is maintained due to operation of a balanced lethal system, which may function due to gametic lethality or zygotic lethality. Since complete rings are formed and alternate disjunction is a rule, only two types of gametes are formed showing complete linkage between 7 chromosomes. The gametic and zyotic lethality leads to survival of only heterozygotes. It may be noticed that in gametic lethality, only one of the two types of gametes will function on the male side, the other type being functional on the female side, thus giving rise to only one type of progeny, which will be heterozygous. In zygotic lethality on the other hand, both the types of gametes will function on male as well as on female side, but the homozygote progeny due to recessive lethal genes will not survive (Fig. 3.3.7).

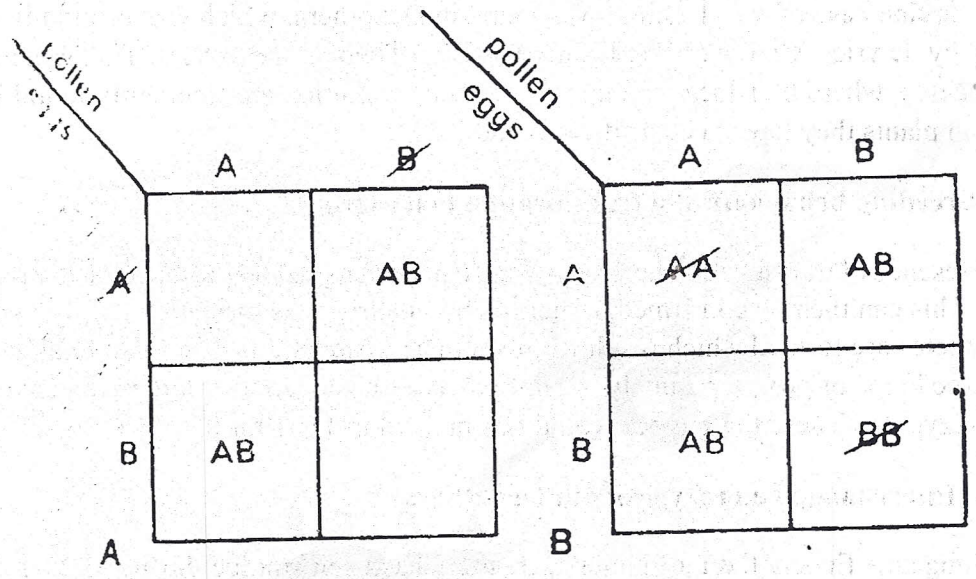


Fig. 3.3.7 Gametic lethality (A) and zygotic lethality (B) showing balanced lethal systems

3.3.5 INVERSIONS

An inversion is produced when there are two breaks in a chromosome and the intercalary segment reunites in reverse order i.e. the segment rotates at 180°. Let us imagine that a chromosome 1-2-3-4-5-6-7-8 gives rise to another chromosomes having the order 1-2-7-6-5-4-3-8. The segment 3-4-5-6-7 has rotated here at 180° giving an inverted order of genes 7-6-5-4-3. A similar hypothetical example using a chromosome ABCDEF has been shown, where due to coiling, breaks occur between B and C as well as between D and E. Reunion at broken ends may lead to inversion of the segment CD into DC.

The inversion can be of two types:

(i) Paracentric inversion
(ii) Pericentric inversion

One of the possible mechanisms, which may give rise to chromosomal inversions

Paracentric inversions are those inversions, where inverted segment does not include centromere. On the other hand, in a pericentric inversion, inverted segment includes centromere. In order to remember these terms and their meaning, one should bear in mind that pericentric means surrounding the cendromere or on the periphery of centromere.

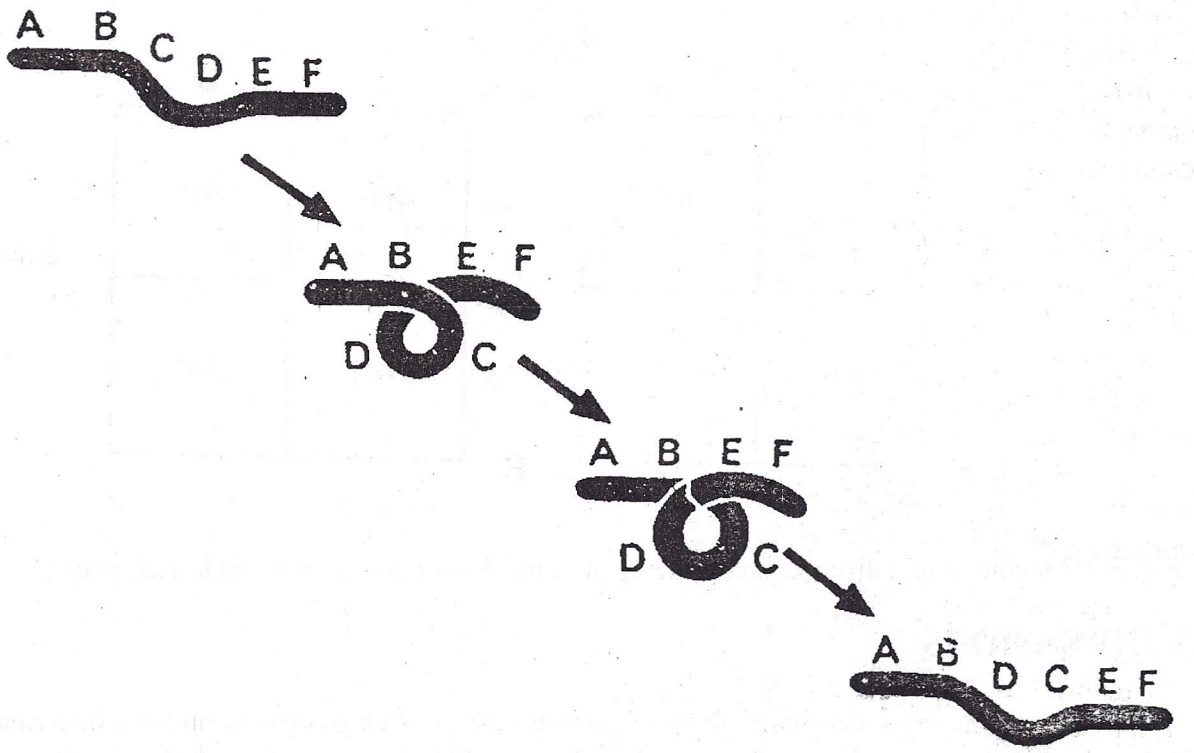


Fig. 3.3.8 One of the possible mechanisms, which may give rise to chromosomal inversions

3.3.5.1 Cytology of inversions

Due to an inverted segment in one of the two homologous chromosomes, the normal kind of pairing is not possible in an inversion heterozygote. In order to enable pairing of homologous segments, a shape of loop is formed by each of two chromosomes. This kind of configuration will be observed both in paracentric as well as in pericentric inversion. As will be observed, the products of crossing over and the subsequent stages of meiosis will differ in these two kinds of inversions.

3.3.5.2 Paracentric inversion

A single crossing over or an odd number of cross overs in inverted region will result into formation of a dicentric chromosome (having two centromeres) and an acentric chromosome (with no centromere). Of the remaining two chromatids, one will be normal and the other will carry the inversion. The dicentric chromatid and acentric chromatid will be observed at anaphase I in the form of a bridge and a fragment. Double cross overs and cross overs within and outside inversion will give various kinds of deficiencies and duplications. These will also rise to a variety of characteristic configurations at anaphase I and anaphase II.

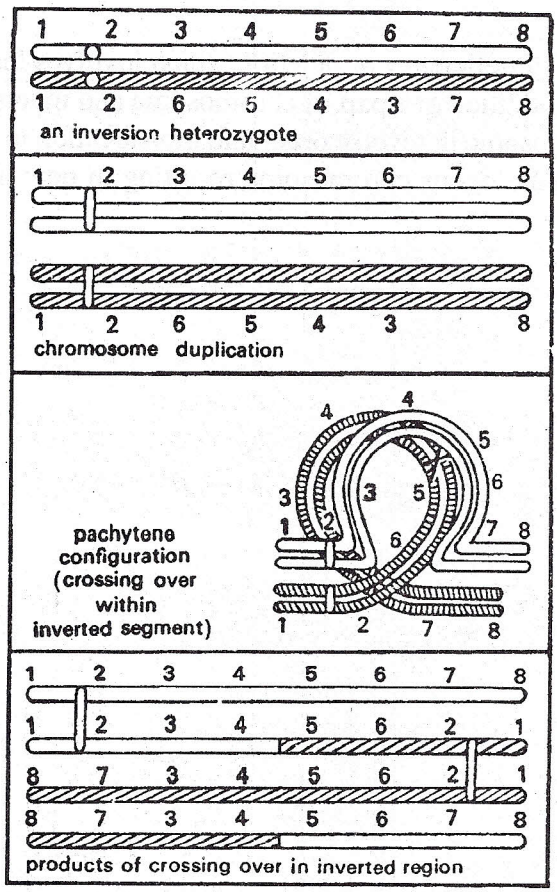


Fig. 3.3.9 (a) Chromosome pairing and products of crossing over in a paracentric inversion heterozygote

3.3.5.3 Pericentric inversion

In a pericentric inversion (where centromere is present within the inverted segment), the pachytene configuration observed is similar to the one described above for paracentric inversion. However, the products of crossing over and configurations at subsequent stages of meiosis differ. In this case, two of the four chromatids resulting after meiosis will have deficiencies and duplications. However, unlike paracentric inversion, no dicentric bridge or acentric fragment will be observed. Consequently at anaphase I, no bridge or fragment will be seen.

However in percentric inversion, if two breaks are not situated equidistant from the centromere, this will result in a change in shape of the chromosome. For instance, a metacentric chromosome may be submetacentric and vice versa.

3.3.5.4 Overlapping inversions

Sometimes a second inversion is induced in a chromosome which already has one inversion. This results in an overlapping inversion, if the segments involved in first and second inversions contain a common region.

3.3.6 SUMMARY

Structural changes in chromosome may be of deficiency or deletion which involves loss of a broken part of a chromosome duplication involves addition of a part of chromosome and inversion in which broken segment reattached to original chromosome in reverse order and translocation in which the broken segment becomes attached to a non-homologous chromosome resulting in new linkage relation.

3.3.7 TERMINOLOGY

Deficiency
Deletion
Duplication
Inversion
Translocation
Structural homozygotes
Structural heterozygotes
Terminal deletion
Deficiency heterozygote
Reciprocal translocations
Paracentric inversion
Dicentric chromatid
Acentric chromatid

3.3.8 SELF ASSESSMENT QUESTIONS

(a) Salivary gland chromosomes of Drosophila are commonly used for study of structural changes in chromosomes why?

(b) Semi-sterility in plants may be due to different factors. How would you establish in a particular case, whether or not it is due to translocation heterozygosity.

(c) It is often said that an inversion suppressses crossing over. What does it mean?

(d) How will you distinguish cytologically.

(i) between a paracentric inversion and a pericentric inversion.

(ii) between a translocation homozygote and a translocation heterozygote.

3.3.9 REFERENCE BOOKS

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3. *Genetics* - Strickberger.

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UNIT-III

LESSON - 3.4

CELL DIVISIONS : MITOSIS AND MEIOSIS

3.4.0 INTRODUCTION

A primary problem, undoubtedly faced in the very dawn of life, concerned with the physical growth of primitive organisms. Without cell division, growth occurs through an increase in volume and an enlargement of the outer surface membrane. However, as the surface of a spherical organism increases by the square of its radius (r), its volume increases proportionately greater by the cube of this number i.e., an increase in size (r) produces a relatively smaller increase in surface area (r^2) than

in volume (r^3). Quite rapidly, therefore, the inner constituents of such an expanding organism have proportionately less surface from which to obtain food, oxygen and the various metabolic wastes and products. In the absence of division, death would quickly ensue, both as a result of these causes. And because of the many physical stress and accidents that could rupture such an unwidely membrane. Some form of cell division must, therefore have been a primary necessity for the maintainance of life.

3.4.1 OBJECTIVES

- To study the physical growth of primitive organisms
- To study the distribution of genetic material to the daughter cells
- To study the equal distribution or division of genetic material in somatic cell division or Mitosis.
- To study the duplication of genetic material to be propagated to next generation.
- To study the haploid cell formation from an diploid organism in the meiotic division.
- To study the segregation of genetic material in meiotic division.
- To study the crossing over mechanism carried out in meiosis.
- To study the genetic variation i.e., through crossing over in the pachytene stage of meiosis.

3.4.2 MITOSIS

Cell division, however, is not a simple answer to these problems unless assurance is provided that the essential cell constituents are properly distributed to the daughter cells. It is easy to see that an incomplete distribution to cells and their descendants of something as vital as the genetic material, the carrier of biological information, would seriously endanger their future. One successful answer to this problem was the evolution of a mitotic mechanism that produces an even cellular division of essential hereditary components.

In prokaryotes such as bacteria, partitioning of the nuclear material ñ that is, the bacterial chromosome occurs by utilizing the attachment of the chromosome and its newly formed replicate to a section of the bacterial cell membrane. As the bacterium elongates during cell division, one portion of the membrane carrying one chromosome separates from another portion carrying its replicate. When sufficient seperating between the two chromosomes has been achieved, the membrane between them invaginates and cleaves, so that each of the chromosomes is now in a separate daughter cell. Prokaryotic replication mechanismís are carried out in the process.

In eukaryotes cell division is more complex because more than one chromosome is usually present. Manipulation of eukaryotic chromosomes occurs through microtubules, which are structurally similar to the fibers of cilia and flagella. Most often these microtubular fibers are organized into spindle-shaped bodies that appear to be generated by the centrioles, which are inturn, similar in many respects to the basal bodies involved in flagella formation. The eukaryotic modes of cell division undoubtedly evolved early in the history of these organisms, since these processes are shared by most eukaryotes. The remainder of this chapter will concern itself with the eukaryotic processes of mitosis & meiosis. In general, although individual variations of the mitotic process and structures exist, the usual stages of mitosis can be described as follows:

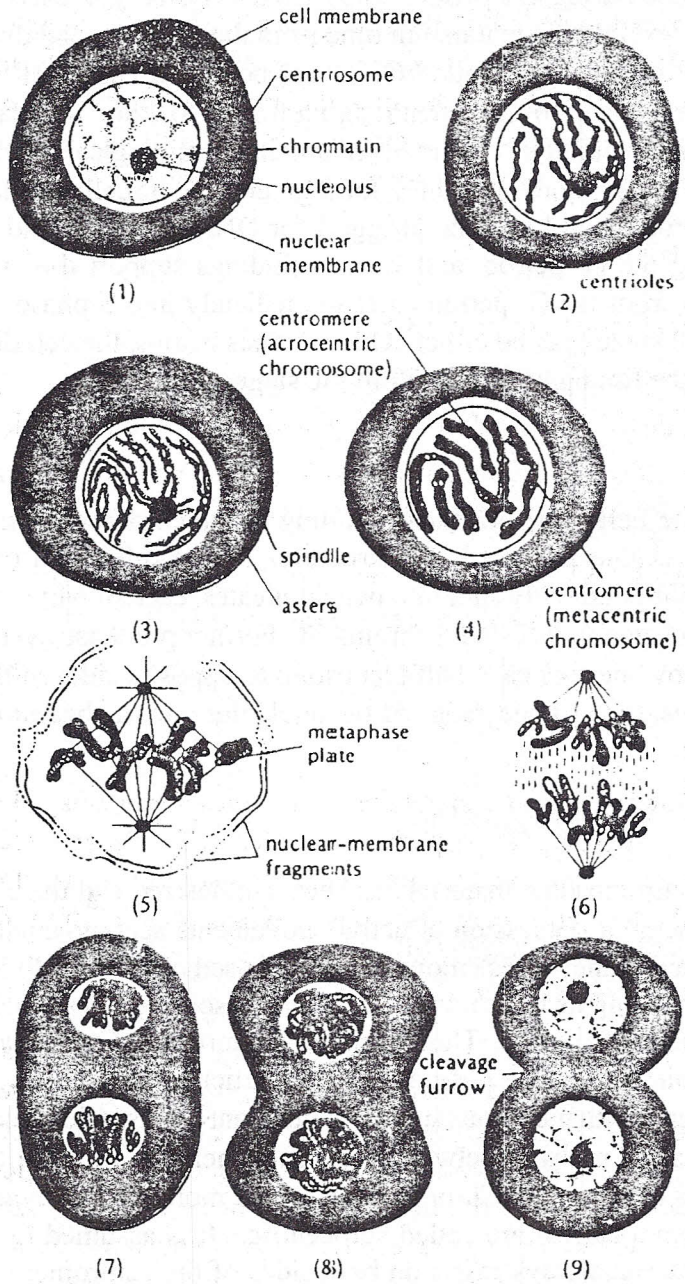

Fig. 3.4.1 Various stages of Mitosis in a somatic cell

3.4.2.1 Interphase

The interphase period between successive cell divisions consists of processes associated with growth and preparation for mitosis. In many cases, the specific chemicals that constitute the newly synthesized chromosomes (DNA and histones), as well as the proteins that will soon give rise to the

mitotic spindle are found during this period. The period of DNA synthesis during interphase is called the ëSí (Synthetic) period and is separated in time from the previous cell division by a gap called G_1. After DNA synthesis a further gap called G_2 occurs before the next cell division begins. The G_1 period shows considerable variability, often ranging from 3 to 4 hours to days, weeks or months and appears to depend upon the kind of cell involved and its physiological condition. As is true of the S period, the G_2 period shows more constancy for a given type of cell; G_2 usually ranges from 2 to 5 hours, and S lasts about 7 to 8 hrs. The ëtriggerí for DNA synthesis and subsequent cell division seems to occur during the G_1 period, and various findings support this: specially prepared nuclei exposed to cytoplasm from the G_1 period enter immediately into S phase, whereas exposure to the cytoplasm of other cell stages has no effect. Once mitosis begins, the cell division process is usually quite rapid, although the timing of different mitotic stages may vary.

3.4.2.2 Prophase

In prophase, the first stage of mitosis, coiling of the chromosomes, or their ëcondensationí, begins, making them visible as thread like structures. As a rule, each of these mitotic prophase chromosomes appear longitudinally split into two duplicates, each of which is called a ëchromatidí as long as it remains connected to its ësisterí chromatid. Further prophase events are the splitting of the centrosome and the movement of each half (centriole) to opposite sides of the nucleus, the synthesis of the mitotic apparatus, the disappearance of the nucleolus and the beginning of the break down of the nuclear membrane.

3.4.2.3 Metaphase

Once the new chromosome material has been synthesized and the chromosomes have coiled and condensed, they begin a succession of active movements accompanied by the complete break-down of the nuclear membrane. These movements are based on the attachment of each chromosome through a specific point along its length, the centromere (also called ëkinetochoreí), to a double-poled spindle-shaped structure, the spindle. The centromere in most species is characterized by a localized constriction that occupies a constant position for any particular chromosome, but some species pos-sess ëdiffuseí centromeres with numerous spindle attachment points. Chromosomes whose centromere is localized approximately midway between each end, there by forming two equal chromosomal ëarmsí are described as metacentric. Chromosomes with a more terminally placed centromere, form-ing unequal chromosomal ëarmsí are called acrocentric. It is assumed by some investigators that some chromosome material always exists on both sides of the centromere, even in those instances when the centromere appears to be at the very tip of the chromosome (telocentric).

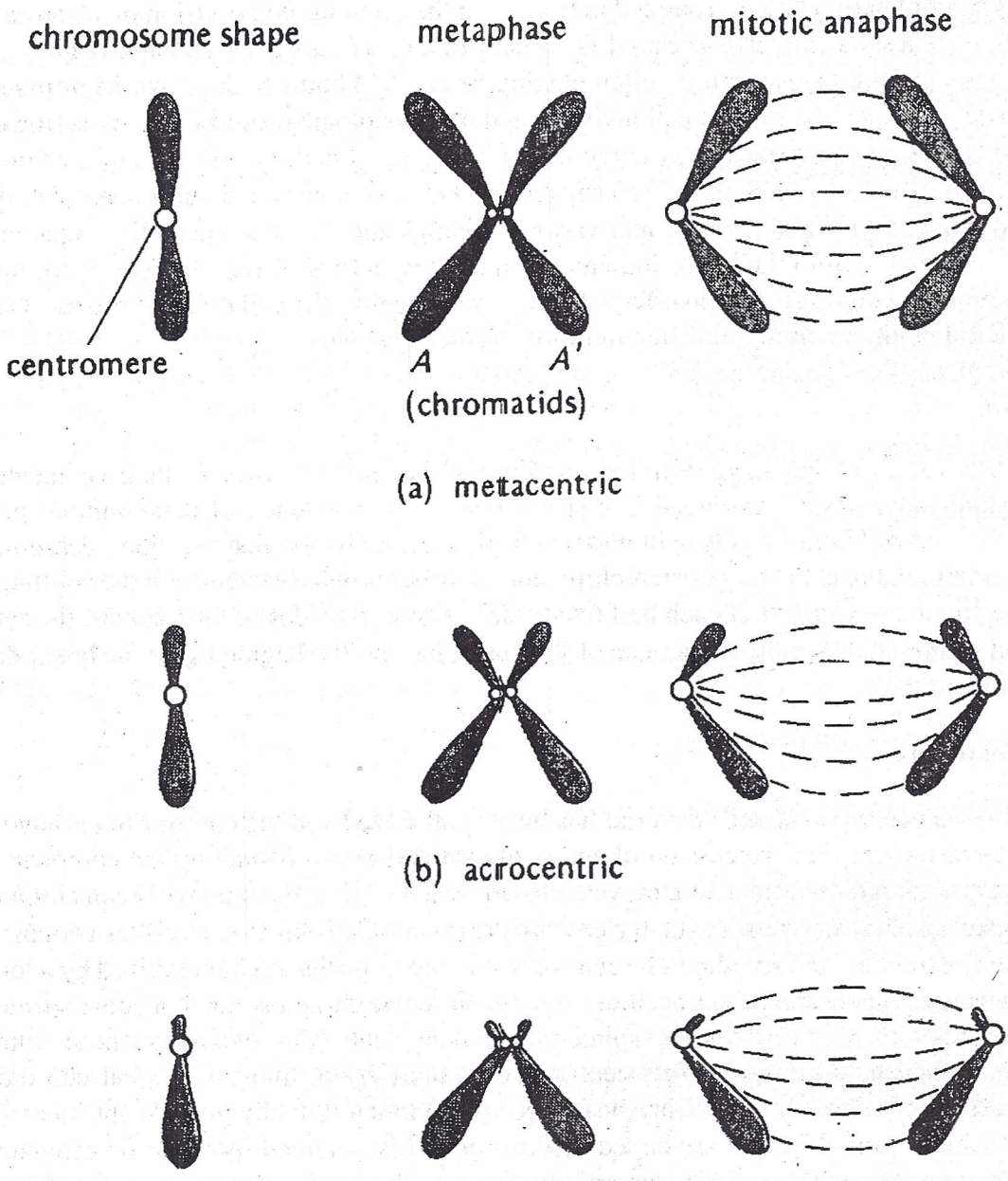

Fig. 3.4.2 Diagrammatic representation of three types of chromosomes based on centromere position

In animal cells, the spindle to which the chromosome centromeres attach is usually formed between the two centrioles that were formerly together on one side of the nucleus. As these centrioles separate and move to new positions on opposite sides of the nucleus, they appear to radiate distinctive lines (astral rays) forming a network between them a continuous microtubular spindle fibers. In many plants, the centrosomes and their accompanying centrioles are missing, although spindles are nevertheless present. Whether plant or animal, each chromosome is now in duplicated condition as a

result of the previous S-period synthesis, and the two sister chromatids remain attached to each other in regions immediately adjacent to their centromeres. One of the most distinctive events in cell division now occurs ñ the movement and arrangement of all chromosomes on a flat plane (metaphase plate) mid-way between the two poles of the spindle. These movements and those that follow in the stages have been precisely described by Bajer, Mole-Bajer, Nicklas and others, but the kinetic forces responsible for them are nít yet known.

3.4.2.4 Anaphase

The shortest of the mitotic phases, anaphase, occurs when the mutual attachment between the two sister chromatids ceases. At that point, the centromere of each chromatid on the metaphase plate separates from its sister centromere and the products of this division, which may now be called ëdaughter chromosomesí move toward opposite poles of the spindle. The appearance of this event is that of an active repulsion between former sister centromeres, each digging its chromosome along with it.

3.4.2.5 Telophase

During the telophase period each of the two polar groups of daughter chromosomes, undergoes a reversion to the more extended interphase state. The nuclear membrane is re-established and the nucleoli reformed. Division of the cytoplasmic portion of the cell (cytokinesis) is also completed during this period, giving rise to a cell plate in plant cells and the intended cell furrow in animals.

3.4.3 Meiosis

The exact replication and splitting of each chromosome into two identical parts and their subsequent separation into two cells would not ordinarily lead to any change in chromosome number between the parent and daughter. Cells with a similar number. In organisms whose cells are always formed by sexual means (absence of conjugation between sex cell nuclei), the number of chromosomes should therefore remain constant between generations. In sexually reproducing organisms, however, where a zygote (embryonic cell) is formed by fertilization between male and female gametes or sex cells i.e. sperm and egg or pollen and ova, the embryonic cells would have double the chromosomes of each parent if no reduction in number occurred during sex cell formation. The finding that, barring very unusual incidents, the chromosome number remains constant between the generations of a species, indicates that such continuous doubling does not occur. Indeed several organisms with continuous doubling of chromosome number would rapidly achieve large unbalanced cells with tremendously unwieldy nuclei and insufficient cytoplasm.

At some unknown point in the history of life. Sexually reproducing organisms evolved a mechanism that enabled them to regularly reduce the number of chromosomes in each gamete to half the usual number. If, for example, four were the regular diploid chromosome number in somatic cells, then the reduced or haploid number in the sex cells would be two. Meiosis is simply the process by which the chromosomes are separated during the formation of sex cells and their numbers reduced from the diploid to the haploid condition. Fertilization then marks the event in which two haploid nuclei join to reform a diploid cell. A large number of animal species, as well as higher plants, are

ordinarily composed of diploid cells except for their gametes. Others, such as the mold Neurospora, are haploid for most of their life cycle but then, through fertilization of two haploid sex cells, produce a diploid zygote that undergoes meiosis to form again a haploid stage. The number, as well as the size and shape of the chromosomes of a species, is usually constant and is called its karyotype.

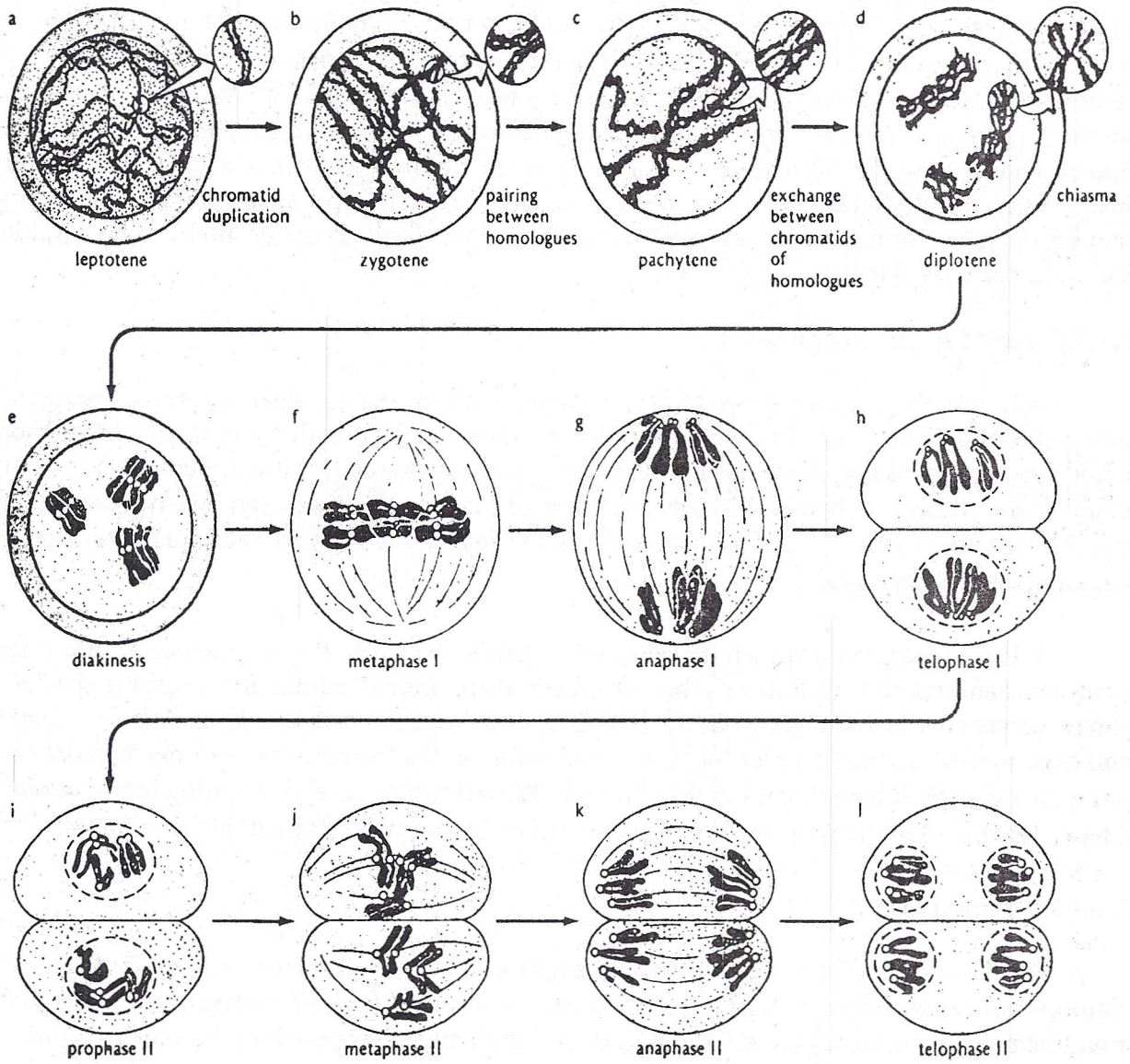

Fig. 3.4.3. Principle Stages of Meiosis

In diploid cells prior to meiosis each individual chromosome usually has a pairing mate, or homologue, so that a parental karyotype of four chromosomes (diploid number) would consist of two homologous pairs e.g. AA^1 and BB^1. Meiosis in such an organism would then produce haploid gametes each one containing two individual chromosomes, one from each pair (A or A^1, together with B

or B^1, e.g., AB, A^1B, AB^1 or A^1B^1). Upon fertilization by another gamete, a diploid zygote is formed with two homologous chromosome pairs (two A^1s & tow B^1s) which in many higher organisms, now divides mitotically to produce the diploid tissue of the adult. In this fashion, each parent contributes one member of a homologous pair of chromosomes to an off spring.

The advantage of homologous pairing derives from differences in biological function between chromosomes. For example, if each pair, AA^1 and BB^1, affects somewhat different characteristics of the individual, then it will be of advantage for an organism to have a member of each of the two pairs represented. Meiosis ensures the presence of one A as well as one B. Chromosome in a gamete by dividing the two chromosomes of each homologous pair into separate gametes. If there were no homologous pairing nor subsequent separation between the two members of such a pair, it is easy to imagine that the chromosomes would undergo a random reduction producing gametes with varying number of chromosomes occurred in each such gamete, many zygotes might still be formed lacking at least one essential chromosome.

3.4.3.1 First Meiotic Prophase

As a rule, the meiotic divisions follow a standard scheme during which two successive divisions of the chromosomes occur. The first division represents a reduction division in which members of homologous pairs of chromosomes are separated into daughter cells without duplication, i.e., their numbers are reduced to half. Although variations of this scheme have been discovered, one of the significant and common features of meiosis is the initial pairing and subsequent separation of homologous chromosomes.

It is not yet known at which stage, the initial pairing between homologues occurs, nor what the exact mechanisms of attraction may be. However, there are indications from various species that chromosomes are often attached to the inner surface of the nuclear membrane by their tips, or telomeres, and there may be a specific order for those attachments so that chromosomes of one haploid set can pair easily with their homologues in the other set. Whatever the cause, this pairing becomes obvious during the fairly lengthy first meiotic prophase, which has been subdivided into five stages.

3.4.3.1.1 Leptotene

The leptotene is the first of the meiotic stages which differs from the previous interphase. The chromosomes first appear as long, slender threads with many bead like structures (chromomeres) along their length. In some plants, the chromosomes are dumped to one side of the nucleus (Synizesis). In some animals (many insects), they appear polarized with their ends drawn together toward that portion of the nuclear membrane close to the centriole. Although considerable biochemical evidence indicates the replication of chromosome material has occurred, it is usually difficult to observe any morphological duplication at this stage. Meiotic prophase chromosomes generally appear as single and individual structures until the pachytene stage.

3.4.3.1.2 Zygotene

During the zygotene stage homologous chromosomes appear to attract each other and enter into a very close zipper like pairing (Synapsis). This pairing is highly specific and occurs between all homologous chromosome sections even if present on different non-homologous chromosomes. For example, if a piece of chromosome material, **e.g., a**, has been shifted from one of the A chromosomes to one of the B pair (translocation), the **a** part of that particular **B** chromosome will be attracted to an **A** chromosome still containing the **a** material. In almost all cases, pairing or synapsis is confined to two homologous chromosome areas at a time. In triploid organisms, having three members for each homologous group rather than two, pairing nevertheless occurs only between two chromosomes in any one region, although there are reports to triple pairing of homologous chromosomes in triploid chickens.

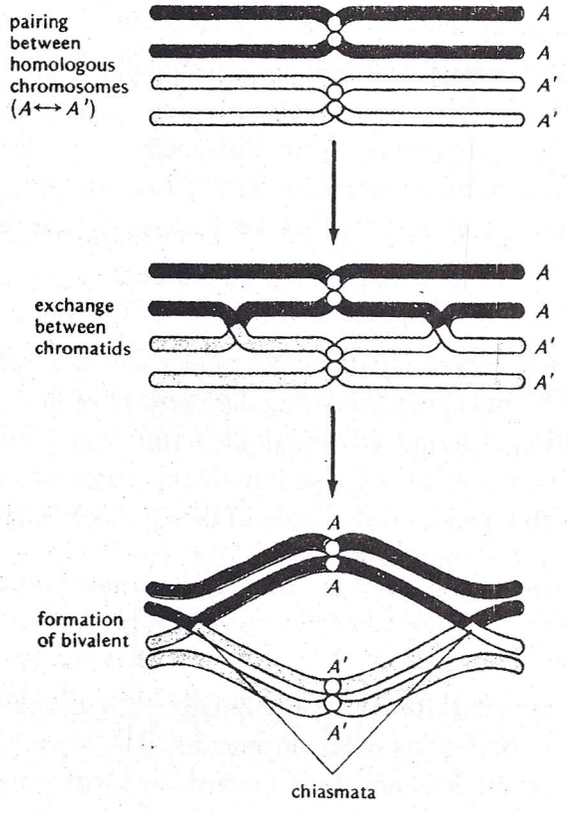

Fig. 3.4.4 Pairing between two metacentric homologous chromosomes, 'A & A' & subsequent exchange of chromosome material in each arm leading to the formation of a bivalent with two chiasmata.

synaptonemal complex can be observed between synapsed chromosomes through electron microscopy. It appears as a ribbon like group of three longitudinal components organized in two dense lateral elements and a thin central element composed primarily of proteins. The synaptonemal complex may function to pull chromosomes together helping them to pair more precisely and efficiently. Moses and others have suggested that this structure may be correlated with the occurrence of genetic crossing over. Under the light microscope, a somewhat different but easily observable physical counterpart to genetic crossing over the chiasmata can be noted during the next stages.

3.4.3.1.4 Diplotene

At the diplotene stage each chromosome now acts as though it were repulsing its closely pairing homologue, especially near the centromere. Distinctly visible separations then occur between homologous chromosomes expect for specific regions where an actual physical crossing over appears to have taken place between homologous chromatids. These crossed areas or chiasmata are X-shaped attachments between the chromosomes and seem to be the only remaining force holding each bivalent together until metaphase. In many organisms their position and number seem to be constant for a particular chromosome.

3.4.3.1.5 Diakinesis

At diakinesis, coiling and contraction of the chromosomes continue until they are thick heavy-staining bodies. In this process, the bivalents usually migrate close to the nuclear membrane and become evenly distributed. The nucleolus either disappears or detaches from its associated chromosome. During the later part of this stage, or the early part of metaphase, the nuclear membrane dissolves and the bivalents attach themselves by their centromeres to the rapidly formed spindle.

3.4.3.2 First Metaphase

In metaphase, the chromosomes reach their most condensed state and appear relatively smooth in outline. The chiasmata that had first appeared during diplotene have now moved toward the ends of each chromosome (terminalization), leaving only the single terminal attachment between the formerly paired arms of homologous chromosomes. These remaining chiasmata prevent the separation of homologous chromosomes which now lie on each side of the equitorial plate of the spindle stretched by their respective centromere toward opposite poles.

3.4.3.3 First Anaphase

Although previously duplicated along its entire length, each chromosome still maintains only a single functional centromere for both of its sister chromatids. The separation or 'disjunction' of one homologous chromosome from another in anaphase towards opposite poles therefore results in this single centromere dragging both chromatids (dyad) along with it. The chiasmata slip off the ends of the chromosome as they are pulled apart, and the chromatids moving towards the pole are now bound together at only one point, the centromere. Since two chromatids compose each dyad, their appearance depends on the position of the centromere a double V if the chromosome is either metacentric or acrocentric and a single V if telocentric.

Our previous view of the first meiotic division as a reduction division that separates two homologous chromosomes into daughter cells must now be modified in accordance with the exchange of chromosomal material manifested by chiasmata. That is, if no exchange occurs between homologous chromosomes in meiosis, their separation is purely reductional because a chromosome in one daughter cell doesn't contain any material from its homologue in the other cell. However, if genetic exchange

occurs, each separating dyad in anaphase carries part of its homologue, resulting in an equal (equational) division of the exchanged chromosome material to both daughter cells. If we consider that each of a homologous pair of chromosomes is contributed by a different parent, chiasma exchange in meiosis will redistribute chromosome material from both parents to the daughter cells. Gametes formed from such daughter cells may, therefore, carry a wide mixture of chromosome material, part from one parent and part from another.

In addition, since it is a matter of chance which of the parental homologous chromosomes are separated to each daughter cell, the more pairs of chromosomes an organism has, the greater the chances that a gamete will contain material from both parents and the less the chances that all its chromosomes will be from one parent. For example, an organism with two homologous pairs may have received chromosomes A and B from one parent and their homologues A^1 and B^1 from the other. This individual, having the constitution AA^1 BB^1 can form four different haploid gametes in equal proportions. AB, AB^1, A^1B, A^1B^1 of which two are parental. On the other hand, the meiotic division of cells with four pairs of chromosomes will yield, on the average, only one out of eight gametes with all chromosomes from either of the two parents.

3.4.3.4 Telophase and Interphase

These stages vary considerably between organisms. Generally, once the dyads reach one of the spindle poles, a nuclear membrane is formed around them, and the chromosomes pass into a short interphase before the second meiotic division begins. In the plant Trillium, the anaphase group of dyads enters immediately into the second meiotic division (Prophase II), skipping telophase and interphase. In most cases, the sequence of events is so rapid that the meiotic interphase chromosomes are not as physically extended as in mitosis, nor is there sufficient time to form a single large nucleolus. Mechanical division of the cell (cytokinesis) may occur during this stage (in corn) or may be postponed until simultaneous formation of four daughter cells at the end of the second meiotic division.

3.4.3.2 Second Meiotic Division

The chromosomes enter the prophase of the second meiotic division as dyads or two sister chromatids connected in their centromere region. As soon as these connected centromeres divide, each chromatid (monad) separates from its sister and moves to the opposite pole in the second anaphase. The second telophase and cytokinesis follow rapidly, giving rise to four haploid cells from each initial diploid cell that entered meiosis. In a cell consisting of a diploid number of four chromosomes, or two pairs of homologous, the meiotic events leading to a reduction in chromosome number can be summarized as follows:

First meiotic division

Metaphase-I: Two bivalents or tetrads (four chromosomes or eight chromatids) on the metaphase spindle.

Anaphase-I: Two dyads (two chromosomes of four chromatids) pass to each pole.

Second meiotic division

Metaphase-II: Two dyads on the spindle in each daughter cell.

Anaphase-II: Two monads (two chromosomes) pass to each pole.

3.4.4 TERMINOLOGY

Microtubules
Basal bodies
Mitotic
Chromatid
Centromere
Spindle fibers
Metacentric
Acrocentric
Telocentric
Cytokinesis
Fertilization
Diploid
Haploid
Telomeres
Synapsis
Chiasmata

3.4.5 SUMMARY

Mitosis, a mode of nuclear and cytoplasmic division, provides for the production of two daughter nuclei that contain identical chromosome sets and are genetically identical to each other and to the parent cell from which they arose. The division is divided into five phases: interphase, prophase, metaphase, anaphase and telophase. Cytokinesis, the division of the cytoplasm, follows telophase.

Meiosis, a fundamental process of cell division in sexually reproducing eukaryotes, involves three main events, the pairing of homologous chromosomes, the exchange of genetic material by crossing over; and the segregation of the members of a homologous pair of chromosome into different daughter nuclei. Meiosis involves one round of DNA replication followed by two separate cell divisions. The result of meiosis in a diploid cell is four haploid cells.

3.4.6 SELF ASSESSMENT QUESTIONS

1. The cells of a particular male contain one pair of homologous chromosomes, **eg.** AA^1 and one additional chromosome without pairing mate, **eg. B**. What is the chromosome constitution of each of the four gametes produced by one complete meiotic division?
2. An organism has two homologous pairs of chromosomes, one pair metacentric, and the other pair telocentric, (a) draw the metaphase plate of the first meiotic division, (b) draw the metaphase plate of a second meiotic division.
3. Explain why you would expect genetic differences between cells to arise from meiosis and not from mitosis.
4. By what mode of cell division would you expect sperm cells to be formed in the haploid male bee?
5. Would heredity through cytoplasmic particles affect the degree of resemblance between an off-spring and a particular parent?

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UNIT-IV

LESSON 4.1

MENDELIAN GENETICS

CONTENTS

4.1.0 Introduction

Gregor Johann Mendel (1822-1884) is called father of Genetics. With the help of his experiments on garden pea, he was able to formulate laws, which explain the manner of inheritance of characters. Mendel's laws have been tested and proved. Although Mendel described his results in 1866, his work was recognised only in 1900, when his work was rediscovered simultaneously by Hugo de Vries, a Dutch biologist, Carl Correns, a German botanist and Erich Von Tschermak, an Australian botanist.

4.1.1 Objectives

⇒ To study Mendel's experiments
⇒ To study Pre-Mendelian experiments
⇒ To study different criteria of experimental plant selection
⇒ To study Mendel's plant material

⇒ To study phenomenon of dominance
⇒ To study principal of segregation
⇒ To study principle of Independent assortment
⇒ To study Monohybrid and Dihybrid crosses
⇒ To study Back cross and Test cross

4.1.2 Pre-Mendelian experiments

Mendel was not the first to conduct hybridization experiments, but he was the first to consider one trait at one time and this was his secret of success. His experiments were extensions and development of hybridization experiments on pea conducted by Knight (1799) and Goss (1824).

J. Kolreuter was the scientist, who performed hybridization experiments before Mendel. He conducted his experiments in tobacco and compared the hybrids with their parents to demonstrate that the hybrids may resemble one or the other parent or may be intermediate between them. He also showed that both the parents make equal contribution to the hybrids. This was demonstrated by reciprocal crosses i.e., A x B and A x B . He also found that hybrids themselves may be fairly uniform, but their offsprings exhibit considerable diversity. Kolreuter selected tall and dwarf varieties of tobacco and hybridized. He found that all F_1 hybrids were intermediate in size. These in next generation gave plants varying in size from tall to dwarf. But he could not explain his results. Likewise, Gartner, Naudin and Darwin could not explain their results in an expectable way.

4.1.3 Mendel's Selection of experimental plant

For hybridization experiments, Mendel's considerations about the expeimental material were –

a) **Variation:** The organism should have a number of detectable differences, and at a time only single detectable character should be considered.

b) **Reproduction:** The organism should be sexually reproducing because, then only the offspring will be able to receive different characters from both the male and female parents.

c) **Controlled mating:** It is useful to maintain pure parents. Along with this, maintainance of records of offsprings of many generations is beneficial.

d) **Short life cycle:** The organism should have very short life cycle, so as to study their life history completely.

e) **Large number of offsprings:** This will help in deducing correct conclusions.

f) **Convenience in handling:** The organism that can be raised and maintained conveniently and inexpensively in the laboratory was selected.

4.1.4 Mendel's Material

Mendel chose garden pea (*Pisum sativum*) as his plant material since it had the following advantages:

(i) well defined characters
(ii) bisexual flowers
(iii) predominantly self-fertilization
(iv) easy hybridization

Besides these, garden pea being self-fertilized, had pure lines due to natural self-fertilization for a number of years. Therefore, any variety used was pure for the characters it carried. Previous workers considered the individual as a whole complex of characters, but Mendel's success was mainly based on the fact that he considered a single character at one time. Seven pairs of contrasting characters are shown in Table 1.

Table 1. Seven pairs of contrasting characters in Pea

Characters	Dominant	Recessive
Seed characters		
Shape	round	Wrinkled
Cotyledon colour	yellow	green
Coat colour	grey	white
Pod characters		
Shape	Inflated	Constricted
Colour	green	yellow
Stem characters		
Position of pod	axial	terminal
Plant height	tall	dwarf

4.1.5 Crossing Technique

Since garden pea is self-fertilizing, the anthers have to be removed before maturity. This operation of removal of anthers is called emasculation. The stigma is protected against any foreign pollen with the help of a bag. The pollen, then at the dehiscence stage, is brought from the plant to be used as a male parent and is dusted on the feathery stigma of the emasculated flower. For each of seven pairs of characters, plants with one alternative trait were used as female, and those with the other alternative as male. Reciprocal crosses were also made. The population obtained as a result of crossing plants showing contrasting characters is called F_1 generation. The progeny of F_1 plants was obtained by self-fertilization and it forms F_2 generation. Similarly, F_3, F_4 etc., generations can also be obtained.

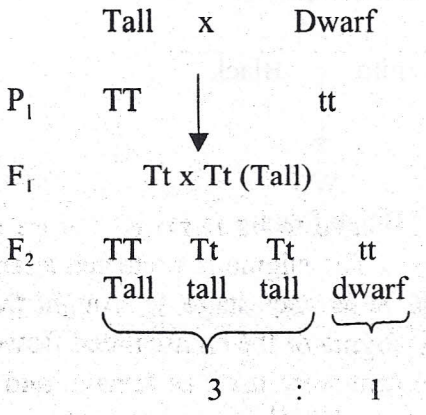
Mendel adopted self-fertilization technique for obtaining genetically pure varieties of pea plants for a single character. He presented his experimental results in "Experiments in Plant Hybridization". Mendel's results can be explained using a specific example. When tall plants were crossed with dwarf plants, all plants in the F_1 generation were tall. When F_1 plants were self-fertilized, both tall and dwarf plants were obtained in the F_2 generation. The tall and dwarf plants were obtained in a ratio close to 3:1. On the basis of his results, he recognized:

(1) phenomenon of dominance
(2) Law of segregation
(3) Law of independent assortment

4.1.6 Monohybrid crosses

Cross between two plants differing in single pair of contrasting characters is known as monohybrid cross.

Mendel cross-fertilized tall and dwarf pea plants to investigate their pattern of inheritance. Mendel obtained tall plants in two reciprocal crosses. He significantly noted that dwarf character seemed to have disappeared in the F_1 progeny, and all hybrids were tall. To explore the hereditary make up of these tall hybrids, he allowed them to undergo self-fertilization. In the F_2 progeny, he found that both tall and dwarf plants were in the ratio of approximately 3:1. He noted the reappearance of dwarf character in the F_2 generation and he inferred that these hybrids carried a latent genetic factor for dwarfness, one that was masked by the expression of another factor for tallness. He said that the latent factor was recessive and that the expressed factor was dominant.



Mendel performed similar experiments to study inheritance of six other traits like seed texture, seed colour, pod shape, pod color, flower color, flower position.

4.1.7 Phenomenon of Dominance

In a cross between pure (homozygous) organisms for contrasting characters of a pair, only one character of the pair appears in the first filial generation.

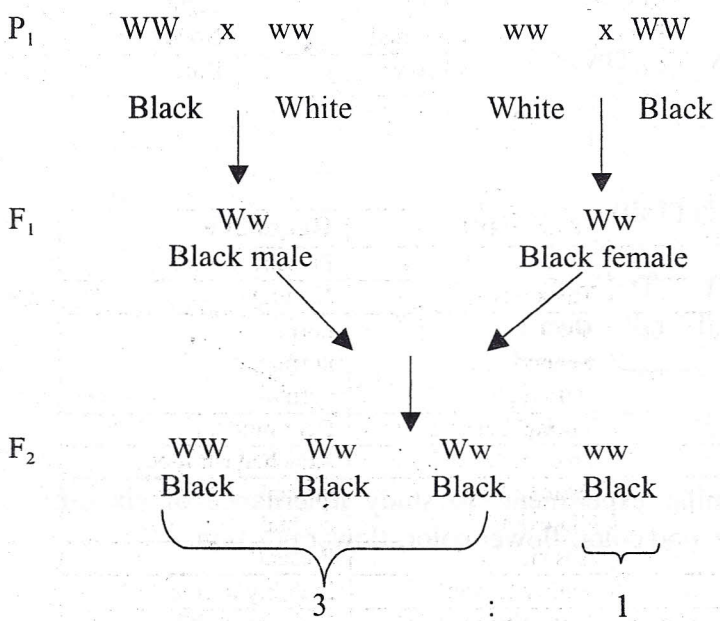
Examples of phenomenon of Dominance.

1. Phenomenon of Dominance in plants:

Name of the Plant	Dominant	Recessive
1. Nettle	Serrated leaves	Smooth marginal leaves
2. Sunflower	Branched habit	Unbranched habit
3. Cotton	Coloured lint	White lint
4. Maize	Round Starchy Kernel	Wrinkled sugary kernel
5. Snapdragon	Red flower	non-red flower
6. Barley	Beardlessness	Beardness
7. Wheat	Susceptibility to rust	Immunity to rust
8. Tomato	Two celled fruit	many celled fruit

2. Application of Phenomenon of Dominance in Animals:

a) When homozygous black guinea pig is crossed with homozygous white guinea pig, F_1 generation were found to be black. When F_1 are interbred, then black and white offsprings were in the ratio of 3:1 which shows that black coat colour dominates over white coat colour.



b) Other examples of animals include:

Name of the animal	Body character	Dominant	Recessive
1. Cat	Skin colour length of hair	Tabby Short hairs	Black or blue Long hairs
2. Dog	Skin colour Tail	Grey Stumpy	Black normal tail
3. Cattle	Colour of face Horn	White Polled or Hornless	Horned
4. Horse	Skin colour Movement	Black trotting	Red Pacing
5. Sheep	Hair or wool or fleece	White	Black
6. Swine or Pig	Skin colour Hoof	Black Uncleft	Red Normal
7. Salamander	Body colour	Dark	Light
8. Drosophila	Wings Eye colour Body colour	Flat and yellow Red Grey	Curled and white White Black
9. Land snail	Shape of shell	Unbanded shell	Banded shell

(c) Dominant and recessive characters in man:

(i).Normal characters

	Body part	Character	Dominant	Recessive
1	Hair	Form Colour	Curly Dark	Straight Light
2	Skin	Colour Pigment	Dark Normal	Light None
3	Eyes	Colour	Brown	Blue

(ii) Abnormal characters:

	Body Part	Dominant	Recessive
1	Hair	Absent	Present
2	Skin epidermis	Thickened	Normal
3	Fingers	Short webbed extra digits	normal normal normal
4	Ear	Normal hearing	Deaf mutism
5	Ear lobe	Free ear lobe	Attached ear lobe
6	Eyes	Opaque lens Glaucoma	normal normal
7	Teeth	Absent	Present
8	Tongue	Ability to role	Inability to role
9	Taste of phenyl thiocarbamide (PTC)	Taste bitter to PTC	Tasteless to PTC

iii) Sex-linked characters:

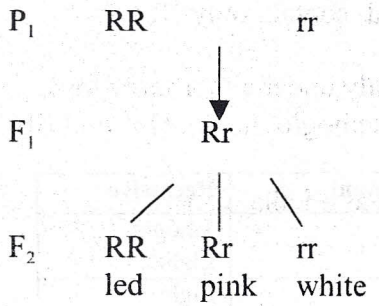
Character	Dominant	Recessive
1. Colour vision	Normal	Colour blind
2. Blood clotting	Normal	Hemophilia

4.1.7.1 Variations in Phenomenon of Dominance:

Mendel reported full dominance and recessiveness for all seven pairs of characters. In some cases the phenotypes of heterozygotes are found to be different from either of the homozygotes.

4.7.1.2 Incomplete Dominance:

When a red flowered pea plant (RR) is crossed with white flowered pea plant (rr), then F_1 hybrids are pink flowered. It shows that red colour could not completely dominate the gene for white colour.



In the same pattern, four-o'clock plant, *Mirabilis jalapa*, when a pure homozygous plant with red petals (C_1C_1) is crossed with homozygous white petals (C_2C_2), F_1 are in pink petals (C_1C_2).

4.7.1.3 Codominance:

When both alleles of a gene in a heterozygote lack the dominant and recessive relationship i.e., each allele is capable of some degree of phenotypic expression, then codominance phenomenon exists.

Eg: i) Coat colour of shorthorn breed of cattle.

When a cattle of red coat (C^RC^R is crossed with cattle of white coat (C^WC^W) F_1 is found to possess roan coat (C^RC^W). But no hair has intermediate colour of red and white.

P_1 C^WC^W x C^RC^R

↓

F1 C^WC^R x C^WC^R

↓

F2 C^WC^W C^WC^R C^WC^R C^RC^R

White Roan Roan Red

ii) M.N. Blood groups: In humans, 3 types of genotypes are present L^ML^M, L^NL^N and L^ML^N.

iii) Inheritance pattern of Sickle-cell anaemia shows incomplete dominance (at cellular or cell shape level) and codominance (at molecular i.e., hemoglobin level). The gene pair concerned, Hb^A (for hemoglobin A) and Hb^S (for hemoglobin S) affects the oxygen transport molecule hemoglobin – the major constituent of red blood cells (erythrocytes). The three genotypes have different phenotypes, as follows:

Hb^AHb^A : Normal, Red blood cells, contain only Hb^A

Hb^SHb^S : Fatal anaemia, red blood cells are sickle shaped, contain only Hb^S

Hb^AHb^S : No anaemia. Red blood cells sickle shaped, only under abnormally low oxygen concentrations; contain both types of hemoglobin i.e., Hb^A and Hb^S.

Thus, Hb^S Hb^S only in homozygous condition acts as a lethal gene.

Hb^A Hb^A x Hb^S Hb^S

↓

Hb^A Hb^S x Hb^A Hb^S

↓

Hb^A Hb^A ← Hb^A Hb^S → ~~Hb^A Hb^S~~ → Hb^S Hb^S

Disease free Normal — Carriers Normal — Sickle shaped erythrocytes, dies

4.1.8 Variations in phenomenon of Dominance

(Law of purity of Gamets)

The hybrids or heterozygotes of F_1 generation have two contrasting characters of dominant and recessive nature. These alleles though remain together for long time, but do not contaminate or mix each other and separate or segregate at the time of gametogenesis, so that each gamete receives only one allele of a character either dominant or recessive.

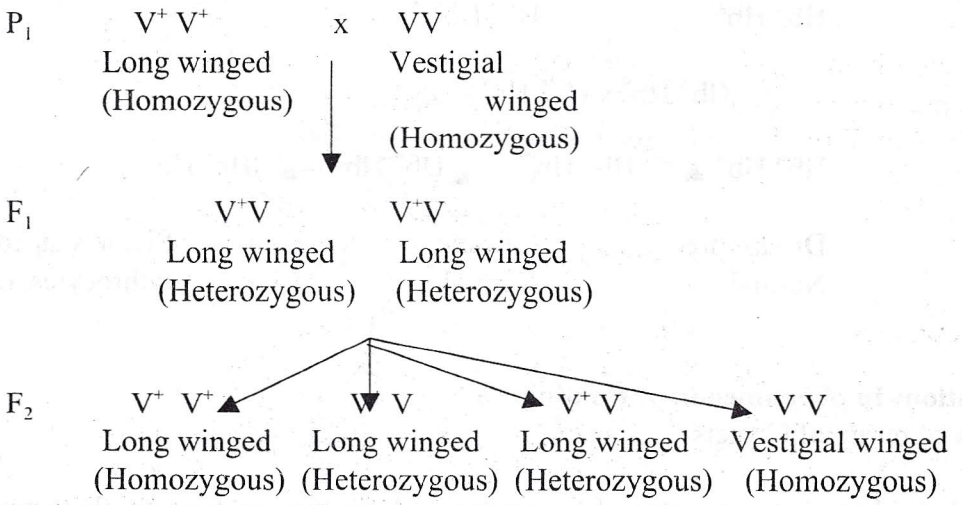
Mechanism of Segregation:

Mendel crossed a homozygous red flowered pea plant with a homozygous white flowered pea plant. The F_1 heterozygotes were pink or purple flowered, thus incomplete dominance prevails over red or white colour, when F_1 were self-fertilized, the progeny were in 3:1 ratio of red and white colours. The reappearance of white colour in F_2 generation indicates the process of segregation. When we represent RR for homozygous red flowered pea plant and rr for whiteness, which produces R and r alleles in their gametes. The gametes unite to form Rr, and due to phenomenon of incomplete dominance, allele R partially expresses over recessive. Both the allele R and r remain together for long time, but they do not effect each other. Neither they mix nor they contaminate each other. At the time of gametogenesis, two types of gametes are produced by F_1 hybrids in equal numbers. Where half the gametes carry allele R and other the allele r. During the process of fertilization, three possible combinations namely RR, Rr and rr are produced in F_2 generation. Thus, in F_2, 75% individuals have coloured flowers and 25% white flowers. the appearance of white colour in F_2 generation indicates that in the hybrid the allele (r) for white colour remains along with allele (R) for red colour but does not mix with it or contaminated by it and it separates or segregates during gametogenesis.

Examples of Law of Segregation:

1. Cross between long-winged and vestigial winged Drosophila:

When a homozygous long-winged (wild type), Drosophila is crossed with a homozygous vestigial winged (mutant) Drosophila, then F_1 were found to be long winged. When F_1 are self-crossed, long-winged and vestigial winged in 3:1 ratio in F_2 generation.



The gametes of both parents V^+V^+ and VV are V^+ and V which produce V^+V as a hybrid in F_1 generation. At the time of gametogenesis, V^+ and V are separated along with chromosomes to form two types of gametes, where half gametes have V^+ and others have V. Three possible combinations

namely V^+V^+, V^+V, VV in 1:2:1 ratio in F_2 generation were observed. Thus, the dominant and recessive alleles remain together for long time without contaminating or mixing with one another and segregate during gametogenesis.

2. Albinism in humans:

A and a represent dominant allele and recessive allele, where three genotypes and two phenotypes are possible.

AA – Homozygous dominant - Normal
Aa – Heterozygous - Normal
aa - Homozygous recessive - Albino

The Albino phenotype person has recessive aa genes, where tyrosinase enzyme for melanocyte synthesis is not present.

4.1.9 Principle of Independent assortment

Dihybrid cross:

Mendel crossed two varieties of pea plants which were different in two pairs of contrasting characters, such type of crosses yielded dihybrids and at a time two pairs of contrasting characters had been considered.

Crossing Technique:

When a homozygous pea plant having yellow round seeds is crossed with homozygous pea plant having green wrinkled seeds. Then F_1 were yellow round in the ratio of 9:3:3:1, where Yellow Round-9, Yellow Wrinkled-3, Green Round-3, Green wrinkled-1.

P_1 YYRR (Yellow Round) yyrr (Green wrinkled)

P_1 Gametes YR yr

F_1 YyRr
Yellow Round
Heterozygotes

F_1 gametes	YR	Yr	yR	yr
YR	YYRR Yellow Round	YYRr Yellow Round	YyRR Yellow Round	YyRr Yellow Round
Yr	YYRr Yellow Round	Yyrr Yellow wrinkled	YyRr Yellow Round	Yyrr Yellow wrinkled
yR	YyRR Yellow Round	YyRR Yellow Round	yyRR green Round	yyRr green Round
yr	YyRr Yellow Round	Yyrr Yellow wrinkled	yyRr green Round	yyrr green wrinkled

Beside getting the ratio of 3:1 of monohybrid crosses, Mendel got 9:3:3:1 ratio. This was explained as – "when the parents differ from each other in two or more pairs of contrasting characters or factors then the inheritance of one pair of factors is independent to that of the other pair of factors".

Mechanism of Independent assortment:

When we assume that YY and RR for yellow colour and roundness of the seed, which is crossed with another plant homozygous with green yy and wrinkled rr seeds, then the P_1 gametes are YR and yr, which combine in a hybrid to form Yy Rr, which is heterozygous yellow round. When F_1 were self-fertilized, then the F_1 gametes which are involved are. YR, Yr, yR, yr. Thus, four types of alleles are assorted independently to produce four types of gametes. These four types of gametes of F_1 unite at Roundom and produce sixteen types of individuals in F_2 generation.

Number of individuals	**Genotype class**	**Phenotype class**
1	YYRR	Homozygous yellow Round Heterozygous yellow Round Yellow Round = 9
2	YYRr, YYRr	
2	YyRR, YyrR	
4	YyRr, YyRr, YyRr, YyRr	
1	Yyrr	Homozogous yellow wrinkled Heterozygous yellow wrinkled Yellow wrinkled=3
2	Yyrr, Yyrr	
1	yyRR	Homozygous Green Round Heterozygous Green Round Green Round = 3
2	yyRr, yyrR	
1	yyrr	Homozygous Green wrinkled Green wrinkled = 1
16		9 : 3 : 3 : 1

Dihybrid cross in Drosophila:

When a homozygous long-winged and black-bodied Drosophila was crossed with vestigial-winged, grey bodied Drosophila, then F_1 progeny were heterozygous long-winged grey bodied. When F_1 were self-crossed, then F_2 progeny involves -

Long winged grey bodied – 9
Long-winged black bodied - 3
Vestigial winged grey bodied – 3
Vestigial-winged black bodied - 1
The 16 individuals were in the ratio of 9:3:3:1.

4.1.10 Back cross and Test cross

When F_1 individuals are crossed with one of the two parents from which they were derived, then such a cross is called back cross. In such back crosses, when F_1 is back crossed with phenotypically dominant parent, then no recessive progeny are obtained. On the other hand, when it is crossed with recessive parent, both phenotypes appear in the progeny. While both these crosses are back crosses, only the cross with recessive parent is known as Test cross.

It is called test cross because, it is used to test whether an individual is homozygous (pure) or heterozygous (hybrid). Monohybrid test cross yields 1:1 and Dihybrid test cross, 1:1:1:1.

Examples:
Monohybrid Back cross and Test cross:

In a cross between homozygous tall (DD) and homozygous dwarf (dd) pea plants, F_1 is heterozygous Dd, dominant. When F_1 hybrid (Dd) is crossed with DD then homozygous and heterozygous tall progeny is obtained.

P1 Homozygous Tall (DD) x Homozygous Dwarf (dd)

↓

F1 Dd
Heterozygous Tall

Back cross F_1 Tall (Dd) x P_1 Tall (DD)

↓

Progeny ½ DD Homozygous ½ Dd Heterozygous

Test cross is done by crossing F_1 hybrid (Dd) with recessive parent (dd). Then progeny were in 1:1 ratio of tall and dwarf.

Test cross	F_1 Tall Dd	x	P_1 Dwarf dd
Progeny	½ Dd Heterozygous Tall		½ dd Homozygous dwarf
	1	:	1

Dihybrid Test cross:

The test cross of a heterozygous yellow round seeded pea plant with a double recessive parent, yields the test cross genotypic and phenotypic ratio of 1:1:1:1.

Parents	F_1 Heterozygous Yellow round YyRr	F_1 Homozygous x green wrinkled yyrr
Gametes	YR, Yr, yR, yr	yr
Progeny	¼ YyRr : ¼ Yyrr : ¼ yyRr : ¼ yyrr	
	1 : 1 : 1 : 1	

4.1.11 Summary

Gregor Johann Mendel (1822-1884) is called father of Genetics. He did his experiments on garden pea. Before Mendel, J. Kolreuter conducted his experiments in tobacco. Different parameters like variation, reproduction, controlled mating, short life cycle, large number of offsprings were considered by Mendel. Seven pairs of contrasting characters chosen by Mendel include – seed shape, cotyledon colour, coat colour, pod shape, pod colour, position of pod, plant height. The population obtained as a result of crossing plants showing contrasting characters is called F_1 generation. The progeny of F_1 plants were obtained by self-fertilization and it forms F_2 generation. Similarly, F_3, F_4 etc., generations can also be obtained.

In the phenomenon of Dominance, when pure (homozygous) organisms for contrasting characters of a pair are crossed, only one character of the pair appears in the first filial generation. A number of examples have been quoted for dominance in plants and in animals. Variations in phenomenon of dominance includes incomplete dominance and codominance.

In the principle of segregation, alleles of the dominant and recessive nature remain together but do not contaminate or mix each other and separate or segregate at the time of gametogenesis. Examples include a cross between long winged and vestigial winged Drosophila and albinism in humans.

In the principle of Independent assortment, which can be explained using Dihybrid cross, when a cross between 2 parents which differ in two or more pairs of contrasting characters, then the inheritance of one pair of characters is independent to that of other pair of characters. This can be best explained by crossing homozygous yellow round pea plant with homozygous green wrinkled plant which gave F_1 (YyRr), when they are inbred, F_2 progeny were in the ratio of 9:3:3:1, for yellow-round, yellow wrinkled, green round and green wrinkled respectively.

A back cross involves crossing F_1 hybrid with one of their parent. A Test cross is a backcross involving cross between F_1 hybrid and recessive parent.

4.1.12 Terminology

Pisum sativum
Hybridization experiments
Tall
Dwarf
Variation
Reproduction
Controlled mating
Offsprings
Self-fertilization
Dominance
Segregation
Independent assortment
Incomplete dominance
Codominance
Albinism
Back cross
Test cross

4.1.13 Self-Assessment Questions

1. Why did Mendel choose pea plant as his experimental material?
2. Define an explain Mendel's law of segregation.
3. A black mouse mates with a brown mouse, and all the offspring are black. Why areno brown offspring produced?
4. Illustrate phenomenon of dominance in plants.
5. What is meant by Dihybrid cross. Illustrate in garden pea.
6. Define law of Independence assortment, how is it deduced from Dihybrid cross.

4.1.14 Reference Books

1. **Genetics** – P.K. Gupta
2. **Genetics** – Strickberger
3. **Genetics** – Gardener (Indian edition)
4. **Genetics** – Simmons, Gardener and Jenkins
5. **Cell Biology, Genetics, Molecular Biology, Evolution and Ecology** – P.S. Verma and V.K. Agarwal.

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UNIT - IV

4.2. GENE INTERACTIONS

4.2.1. Introduction

Genes are not necessarily separate elements. Each gene is responsible for the expression of a single character. There are some genes which will interact with each other and produce a phenotype, which is the result of that gene interaction. So, this is a deviation from Mendel's Laws of inheritance.

Metabolism is the sum of all the physical and chemical processes by which energy is made available for the use of the organism. These biochemical reactions occur as step-wise conversions of one substance into another, each step being mediated by a specific enzyme. All the steps that transform a precursor substance to its end products constitute a biosynthetic pathway.

Several genes are required to specify the enzymes involved in each step in these pathways. Gene interaction occurs whenever two or more genes specify the enzymes that catalyze the steps in a common pathway.

$$P \text{ (Precursor) } P \xrightarrow{g^+_1} A \xrightarrow{g^+_2} B \xrightarrow{g^+_3} C \text{ (end product) (g=gene)}$$

Here each metabolite (A, B, C) is produced by the catalytic action of different enzymes, which are specified by different wild type genes (g_1 , g_2 , g_3). If the substance 'C' is essential for the production of a normal phenotype, then a mutant phenotype would result from a genotype that is homozygous recessive at any of the three loci. The recessive mutant alleles are g_1 , g_2 and g_3 which will produce defective genes.

If g_3 contains a mutation, the conversion of B to C does not occur and the substance B tends to accumulate in excessive quantity. If g_2 contains a mutation, the substance B will not be produced and substance 'A' will accumulate. Thus the gene mutations are said to produce " metabolic blocks ". An organism with a mutation in only g_2 could produce a normal phenotype, if it is were given either substance B or C, but an organism with a mutation in g_3 has a specific requirement for "C". Thus the gene g_3 becomes dependent upon gene g_2 for its expression as a normal phenotype. If the genotype is homozygous for the recessive g_2 allele, then the pathway ends with the substance 'A'. Thus the genotype g_2g_2 can hide or mask the phenotypic expression of the alleles at the g_3 locus. So, g_2 is epistatic to g_3, that means it masks any expression of alleles at the g_3 locus. The gene or locus that masked the action of a gene at another locus is called epistatic and the gene or locus that was suppressed is called hypostatic.

The Classical phenotypic ratio of 9:3:3:1 observed in the progeny of dihybrid parents becomes modified by epistasis into ratios that are various combinations of the 9 : 3 : 3 : 1 groupings.

4.2.2. Objectives

- To explain the concept of gene interaction.
- To explain the different types of gene interactions in detail.
- The explain the concept of abbreviated genotypic ratio.
- To explain the concept of Lethality.
- To explain modifier genes, pleiotropic genes.
- To give the concept of penetrance and expressivity.

4.2.3. Definition

According to Mendel's Laws of Inheritance a single character is controlled by one gene only. But there are some characters which are controlled by more than one gene. In such a situation two or more than two genes may interact to give rise to a particular phenotype.

Eg:- If gene 'A' is responsible for phenotype 'A' and gene 'B' is responsible for phenotype 'B', when both 'A' and 'B' are present together will give rise to a new phenotype 'C'. However, these genes still obey the principle of segregation and independent assortment. If A and B are the two genes which are

normally dominant over their respective recessive alleles 'a' and 'b' then the interaction will depend upon,

1. the presence of both dominant alleles 'A' and 'B'
2. absence of 'A'
3. absence of 'B'
4. absence of both 'A' and 'B'

——Dominance involves intra-allelic gene suppression i.e., the masking effect of one allele upon the expression of another allele at the same locus.

——Epistasis involves inter-allelic gene suppression or the masking effect of one gene locus upon the expression of another gene locus.

——Now the term epistasis has come to be synonymous with almost any type of gene interaction that involves the masking of one of the gene effects.

4.2.4. Abbreviated Genotypic Ratio

The abbreviated genotypic ratio is very much useful in predicting the phenotypic ratio for a particular kind of gene interaction. We can give an abbreviated genotypic ratio that is expected in F_2 as follows:

9AB : 3Ab : 3aB : 1ab

——Here AB means, the individuals will have both A and B either in homozygous or in heterozygous condition. It includes 4 genotypes.

1AABB : 2AaBB : 2AABb : 4AaBb

——Ab means absence of 'B' but presence of 'A' either in homozygous or heterozygous condition. It includes 2 genotypes.

1AAbb : 2Aabb

——aB includes two genotypes

1aaBB : 2aaBb

——If the abbreviated genotype is understood and practiced, no checker boards or forked line methods will be needed for working out phenotypic ratios.

4.2.5. Epistatic Gene Interactions

When epistasisis is operative between two gene loci, the no. of phenotypes appearing in the off-spring from dihybrid parents will be less than four. There are six types of epistatic ratios commonly recognized, three of which have 3 phenotypes and the other three have only two phenotypes.

4.2.5.1. Dominant Epistasis

When the dominant allele at one locus for example, the allele A, produces a certain phenotype, regardless of the allelic condition of the other locus, then the 'A' locus is said to be epistaticto the 'B' locus. Further more, since the dominant allele 'A' is able to express itself in the presence of either B or b this is a case of dominant epistasis. Only when the genotype of the individual is homozygous recessive at the epistatic locus (aa) can the alleles of hypostatic locus B or b expressed.

Thus the genotypes A_B_ and A- bb produce the samephenotype, where as aaB- and aabb produce two additional phenotypes. Here thedominant A (white) masks the effect of yellow or green. So, the classical Mendelian ratio 9 : 3 : 3 : 1 becomes modified into 12 : 3 : 1.

4.2.5.1 Dominant Epistasis (12:3:1)

A A B B (white) X a a b b (green) ——— P Generation

(white) A a B b ——— F1 Generation
(Selfing)

——— F2 generation

♀ / ♂

	AB	Ab	aB	ab
AB	AABB White	AABb White	AaBB White	AaBb White
Ab	AABb White	AAbb White	AaBb White	Aabb White
aB	AaBB White	AaBb White	aaBB Yellow	aaBb Yellow
	AaBb White	Aabb White	aaBb Yellow	aabb Green

Eg :- Fruit colour in summer squash
Interaction : Dominant - A (White) masks the effect of Yellow - B or Green

4.2.5.2. Recessive Epistasis

If the recessive gene at 'A' locus eg., (aa) suppresses the expression of alleles at 'B'locus, the 'A' locus is said to exhibit recessive epistasis over the 'B' locus.

——only if the dominant allele is present at the 'A' locus, can the alleles of the hypostatic 'B' locus be expressed.

——The genotypes A-B- and A-bb produce two additional phenotypes. Here the classical 9 : 3 : 3 : 1 ratio becomes a 9 : 3 : 4 ratio.

——In mice, the wild body colour is known as agouti, characterized by banding of individual hairs. The agouti colour is controlled by a gene 'A', which is hypostatic to the recessive allele 'C'.

——The dominant allele 'C' in the absence of 'A' gives coloured mice. In the presence of dominant allele 'C', 'A' gives rise to Agouti.

2. Recessive Epistasis (9:3:4)

(Coloured) CC a a X c c A A (Amino) —— P Generation

↓

C c A a —————— F1 Generation
(Agouti)
Selfing

↓

—————— F2 generation

♀ ♂

	CA	Ca	cA	ca
CA	CCAA Agouti	CCAa Agouti	CcAA Agouti	CcAa Agouti
ca	CCAa Agouti	CCaa Black	CcAa Agouti	Ccaa Black
cA	CcAA Agouti	CcAa Agouti	ccAA Albino	ccAa Albino
Ca	CcAa Agouti	Ccaa Black	ccAa Albino	ccaa Albino

Eg :- Skin colour in mice
Interaction : Agouti colour is controlled by gene 'A' **which is hypostatic to the** recessive allele 'c'→ **ccAA (albino).**

The dominant allele 'C' in the absence 'A' gives coloured mice.
In the presence of A, C gives Agouti.

4.2.5.3. Duplicate gene interaction between two dominant genes to give new phenotype:

Here the dominant condition at either locus (but not both) produces the same phenotype and the interaction between two dominant genes give new phenotypes.

Eg:- Fruit shape in summer squash

Here the classical ratio becomes modified to 9 : 6 : 1.

3. Duplicate gene interaction between the two dominant genes to give new phenotype: 9:6:1.

A A B B (Disc) X a a b b (Long) ——— P Generation

A a B b (Selfing) ——— F1 Generation

F2 - Generation

♀ ♂

	AB	Ab	aB	ab
AB	AABB Disc	AABb Disc	AaBB Disc	AaBb Disc
Ab	AABb Disc	AAbb Sphere	AaBb Disc	Aabb Sphere
aB	AaBB Disc	AaBb Disc	aaBB Sphere	aaBb Sphere
ab	AaBb Disc	Aabb Sphere	aaBb Sphere	aabb long

Eg :- Fruit Shape in Summer squash
Interaction : Both the dominant alleles A&B together give disc shape.
Sphere shape is dominant over longshape.

4.2.5.4. Duplicate Dominant Gene Interaction:

Here the dominant alleles of both the loci will produce the same phenotype without cumulative effect. Here the classical ratio gets modified into a 15 : 1 ratio.

Eg., Fruit shape in Capsella.

——Here if A and B are the two genes controlling the shape of the fruit, top shaped capsules will be obtained on the plants with the genotype aabb.

——So, it is obvious that top shaped capsules result from double recessive genotypes (aabb).

——Plants with triangular capsules can beof AABB, AAbb, aaBB and other genotypes with heterozygosity.

——It shows that a single dominant gene is enough to give rise to triangular shape and also the presence of both the dominant genes have the same effect.

4. Duplicate Dominant Gene Interaction (15:1)

A A B B (Triangular) X a a b b (Topshaped) ——— P Generation

A a B b (Triangular) Selfing ——— F1 Generation

——— F2 - Generation

♀ ♂

F2

	AB	Ab	aB	ab
AB	AABB Triangular	AABb Triangular	AaBB Triangular	AaBb Triangular
Ab	AABb Triangular	AAbb Triangular	AaBb Triangular	Aabb Triangular
aB	AaBB Triangular	AaBb Triangular	aaBB Triangular	aaBb Triangular
ab	AaBb Triangular	Aabb Triangular	aaBb Triangular	aabb Top shaped

Eg : Fruit shape in Shepherd's purse (capsella).

Interaction : Presence of both the dominant genes have the same effect. A single dominant gene is enough to give rise to triangular shape.

4.2.5.5. Duplicate Recessive Gene Interaction

Here identical phenotypes are produced by both homozygous recessive genotypes. Here the genotypes aaB-, A-bb produce one phenotype and both the dominant alleles, when present together, complement each other and produces adifferent phenotype.

Eg., Flower colour in Sweet Peas.

——It is obvious from the above example that both the dominant alleles 'C' and 'P' are necessary for the production of pigment in the flowers.

——Here each of the two parents lacks one of the two dominant alleles and therefore both will bear white flowers only.

——The two dominants are brought together i.e complementing each other in the F1-generation and therefore coloured flowers are produced.

5. Duplicate Recessive Genes (9:7) (Complementary Genes)

While X White —— P Generation
CCpp ccPP

Purple C c P p —————— F1 Generation
(selfing)

—————— F2 - Generation
F2

♀ ♂

	CP	Cp	cP	cp
CP	CCPP purple	CCPp purple	CcPP purple	CcPp purple
Cp	CCPp purple	CCpp White	CcPp purple	Ccpp white
cP	CcPp purple	CcPp purple	ccPP white	ccPp white
cp	CcPp purple	Ccpp white	ccPp white	ccpp white

Eg:- Flower colour in sweet peas (possum sativum)

Interaction : Both the dominant alleles e & P are necessary for the production of pigment.

In the parents both are lacking one of the two dominant alleles. They are showing white fls – Identical phenotypes are produced by both the homozygous recessive genotypes.

4.2.5.6. Dominant and Recessive Gene Interaction

Here only two F2 phenotypes will result when a dominant genotype at one locus (A-) and recessive genotype at the other produce the same phenotypic effect. Thus A-B-, A-bb and aabb produce one phenotype and aaB- produces another in 13 : 3 ratio.

Eg., Feather colour in Fowls

'I' - Colour inhibitor (epistatic on 'C')

'C'- Colour dominant to white, so colour will be expressed.

——Here dominant colour inhibitor 'I' prevents colour when colour gene is present.

——Colour gene when in homozygous recessive condition prevents colour expression though the dominant colour inhibitor gene is absent.

6. Dominant and Recessive Gene Interaction (13:3)

While IICC X iicc (White) —— P Generation
(Leghorn) (Wyandotte)

↓

(White) IiCc ———————— F1 Generation
(selfing)

↓

———————— F2 - Generation

♀ ♂

	IC	Ic	iC	ic
IC	IiCC White	IICc White	IiCC White	IiCc White
Ic	IICc white	IIcc white	IiCc white	Iicc white
iC	IiCc White	IiCc White	iiCC Coloured	iiCc Coloured
ic	IiCc white	Iicc white	iiCc coloured	iicc white

Here 2 phenotypes will result when a dominant phenotype at one lows and the recessive phenotype at the other lows produce the same phenotypic effect.

I → colour inhibitor (epistatic on C).
E → colour dominant to white, so, colour will be expressed.

Eg:- Feather colour in Fowls.

Interaction : Dominant colour inhibitor I, prevents colour when colour gene is present. Prevents colour expression even in the absence of dominant inhibitor gene.

4.2.6. Other types

Modifiers: Some genes which modify the effect of other genes without any characteristic form are called modifiers. These modifiers normally change the phenotypic effect of other genes in quantitative manner. Many genes responsible for dilution of body colour belong to this category. There are modifiers which show minor effected on quantitative characters like yield or height etc. There are also modifiers, which will not allow mutant allele of another gene to expressfully or partially. These modifiers are called suppressors. This results in the expression of wild type Modifiers may also influence the degree of dominance expressed by another gene.

Eg:- A moth, Abraxas grosssulariata it couldbe possible to establish lines with same gene, tutea, dominantin one case and recessive in the other.

There are some other genes, which in addition to their main effect, may also act as modifiers for another entirely different gene. Such genes having more than one effect are called pleiotropic genes.

Lethality

There are certain genes which control certain phenotypic traits, and at the same time also influences the viability of individuals.

Example: Drosophila with white eyes and vestigial wings have lower viability than wild type flies.

There are some other genes which have no effect on the phenotype of their carrier individuals but influences their viability. Such genes which reduce the viability of an individual or the gene which cause the death of the individual carrying them are called "Lethal genes" and the phenomenon of their action is called Lethality.

Example: 1. In Drosophila genes like curly wings (Cy), plum eyes (pm) and stubble bristles (sb) influences the viability of the flies when present in homozygous condition.

2. In mice, an incompletely dominant allele 'Y' for yellow coat has been found lethal in homozygous condition.
3. In man homozygous recessive genesfor Amaurotic idiocy are found to be lethal genes.

Penetrance

The ability of a given gene or gene combination to be expressed phenotypically to any degree is called Penetrance. There are two types of penetrance.

1. Complete Penetrance: The dominant genes and the recessive genes in homozygous condition and many completely dominant genes even in heterozygous condition give their complete phenotypic expression.

   Example:1. In Pea the alleles (RR) for red flowers and the alleles (rr) for white flowers have complete penetrance in homozygous conditions.

2. In Drosophila the recessive alleles for vestigial wings in homozygous conditions have complete penetrance.

3. In guinea pigs the dominant allele 'B' for black coat hascomplete penetrance both in homozygous and heterozygous conditions.

2.Incomplete Penetrance: Some genes in homozygous as well as in heterozygous conditions fail to express their phenotype completely. Such genes are said to have incomplete penetrance.

Examples: 1. polydactyly in man.

2. Genes controlling the development of diabetes mellitus.

The environmental factors and genetical background have some definite effect on the degree of penetrance of a gene.

Expressivity

A trait though penetrant, may be quite variable in its phenotypic expressions. The degree of effect produced by a penetrant genotype is called Expressivity.

Example: In man the polydactylous condition may be penetrant in the left hand and not in the right hand or it may be penetrant in the feet and not in hands.

——The expressivity of a given gene is often influenced by the environmental conditions.

Example: The expressivity of completely penetrant gene for vestigial wings in Drosophila is influenced by the temperature at which the fly develops, and the effect will be most obvious at low temperature.

Pleiotropism

Each gene has its relation with a single phenotypic trait. But there are some genes which often influences more than one phenotypic trait. This phenomenon of multiple phenotypic expressions of a single

gene is called Pleiotropism.

Examples:

1. In Drosophila the recessive gene for vestigial wings cause vestigial wings in homozygous condition. The same gene will affect the other traits like, the tiny wing like balancer behind the wings, structure of reproductive organs, certain bristles, egg production and reduction of life time etc.

2. In human beings, the gene for phenylketonuria disease has pleiotropic effect and produces various abnormal phenotypic traits, like short stature, mental retardation, widely spaced incisors, pigmented patches on skin, excessive sweating and non-pigmented hairs and eyes, etc.

4.2.7. Summary

* Mendel's experiments suggested that each character is controlled by a single gene.

* However, a gene does not carry a character, but controls a developmental process. It is due to its influence on the development the gene brings about the desired effect on phenotype.

* Genes may sometimes interfere with the development and may cause the death of the individual. Such genes are called Lethal genes.

* Genes are not necessarily separate elements producing individually distinct effects. In some cases they may interact and produce a phenotype which is the result of the interaction of these genes.

* Epistasis involves the masking effect of one gene locus upon the expression of another gene locus.

* Now the term epistasis has come to be synonymous with almost any type of gene interaction that involves the masking of one of the gene effects. When epistasis is operative between two gene loci, the no. of phenotypes that are observed in the off-spring from dihybrid parents will be less than four.

* There are six types of epistatic ratios, three of them have three phenotypes that are observed in the off-springs of F2 and the other three have only two phenotypes.

* In case of dominant epistasis the dominant allele (A) of one gene (epistatic) masks the effect of both the alleles of the hypostatic gene i.e., (B or b). Here the Mendelian ratio 9 : 3 : 3 : 1 becomes modified to 12 : 3 : 1.

* In the case of recessive epistasis the recessive alleles of 'A' locus (aa) suppresses the expression of alleles at 'B' locus (B,b). Here the classical ratio 9 :3 : 3 : 1 gets modified to 9 : 3 : 4.

* In the case of the duplicate gene interaction between two dominant genes, the dominant condition at either locus (but not both) produces the same phenotype and the dominant condition at

both the loci results in the interaction between two dominant genes which gives new phenotype. Here the classical ratio 9 : 3 : 3 : 1 becomes modified to 9 : 6 : 1.

* In the case of duplicate dominant genes, the dominant alleles of both the loci will produce the same phenotype without cumulative effect. Here the classical ratio 9 : 3 : 3 : 1 gets modified to 15 : 1.

* In the case of duplicate recessive genes, identical phenotypes are produced by both the homozygous recessive genotypes. Here each of the two parents lacks one of the two dominant alleles. So, both are bearing white flowers. In the F_1-generation the two dominant genes are brought together. So, they are bearing coloured flowers. Here the classical ratio becomes modified to 9 : 7.

* In the case of dominant and recessive gene interaction, a dominant genotype at one locus and a recessive genotype at the other locus produce the same phenotypic effect. Here the classical ratio gets modified to 13 : 3.

* Thus the interactions between two or more genes is resulting in the expressionof phenotypes which are deviating from the classical Mendelian ratio 9 : 3 : 3 : 1.

4.2.8. Terminology

Lethal genes

Epistasis

Modifiers

Pleiotropic genes

Expressivity

Agouti

Albino

4.2.9. Self Assessment Test

1. Discuss the modifications in dihybrid ratios (9 : 3 : 3 : 1) due to different kinds of interactions of genes? Can you explain these modifications on the basis of Mendel's Laws of Inheritance?

2. Write short notes on the following:

   (i) Complementary genes

   (ii) Epistasis and hypostasis.

   (iii) Duplicate genes.

   (iv) Abbreviated genotypic ratio.

3. Write short notes on the following:

(i) Lethal genes

(ii) Meiotic drive

(iii) Segregation distortion

(iv) Modifiers

(v) Suppressors

(vi) Penetrance and expressivity.

4. What is gene interaction? Discuss different types of gene interactions?

4.2.10. Additional readings

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UNIT-IV

LESSON-4.3

MULTIPLE ALLELES

4.3.0 INTRODUCTION

Phenotypic trait of an individual depends on a single pair of genes, which occupies a specific position called the gene locus individually on a homologous chromosome. Each gene had two alternative forms, also called allelomorphs, one being dominant and the other recessive. One being wild form and the other being mutant. This mutant has developed from wild form of allele due to mutation and there is equal chance for the mutant allele to mutate once again to give rise to another mutant form. Therefore, a gene can have more than two allelomorphs. These several states or variants of one gene are called multiple alleles. Although only two actual alleles of a gene can exist in a diploid cell, total number of possible different allelic forms that might exist in a population of individuals is often quite large. This is called multiple allelism and the set of alleles itself is called a multiple allelic series.

Several alleles which exhibit and express themselves within the same phenotypic range are called isoalleles. If the phenotype is wild, these are normal or wild isoalleles and if it is a mutant, then those are called mutant isoalleles.

4.3.1 OBJECTIVES

- To study the alternative states or variants of one gene.
- To study various examples of multiple alleles.
- To study multiple alleles responsible for various coat colours in rodents.
- To study multiple alleles in A, B, O blood groups, MN series and Rh blood groups.
- To study the cause of erythroblastosis fetalis.
- To study different multiple alleles involved in the eye color of drosophila.
- To study self sterility in Nicotiana (tobacco).
- To study different multiple alleles contributing colours to corn seed and plant.

4.3.2 CHARACTERS OF MULTIPLE ALLELES

The most important and distinguishing features of multiple alleles are –

a) multiple alleles of a series always occupy the same locus in the chromosome.
b) because, all the alleles of multiple series occupy same locus in chromosome, no crossing-over occurs within the alleles.
c) multiple alleles always influence the same character.
d) the wild type allele is nearly always dominant, while the other mutant alleles in the series may show dominance or there may be an intermediate phenotypic effect.
e) when any two of the mutant multiple alleles are crossed, the phenotype is mutant type and not the wild type.

4.3.3 EXAMPLES OF MULTIPLE ALLELES

A capital letter is commonly used to designate the allele which is dominant to all other alleles in the series. The corresponding lower case letter designates the allele which is recessive to all others in the series. Other alleles which are intermediate in their degree of dominance between these two extremes, are usually assigned the lower case letter with some suitable superscript. A superscript plus (+) sign after the letter for the gene is generally used to represent wild-type alleles.

4.3.3.1 Coat color in rabbits

In rabbits, four kinds of skin colour are known and so, they are classified accordingly as coloured (agouti or wild type), chinchilla, himalayan and albino. In coloured rabbit, the genes for full color represented as 'c' or c^+. In chinchilla, the gene responsible for silvery-grey coat is represented as c^{ch}. The himalayan type coat is white except for black extremities like nose, ears, feet, tail and is represented as c^h and the albino coat totally lacks coat pigmentation on the eyes remain pink. These rabbits are represented by 'c'.

Crosses of homozygous coloured (c^+c^+) and albino (cc) produce a uniform coloured F_1 generation. Interbreeding the F_1 generation yielded F_2 ratio of 3 coloured : 1 albino.

P_1 : Coloured (Agouti) c^+c^+ X Albino cc

F_1 : Agouti c^+c

F_2 : 1 c^+c^+ : 2 c^+c : 1 cc

3 Agouti 1 Albino

Two-third's of F_2 agouti are found to be heterozygous by test crosses. Thus, it is a case of monohybrid inheritance, with agouti completely dominant to albino.

Crosses between chinchilla and agouti produced all agouti individuals in F_1 by a 3 agouti : 1 chinchialla in F_2 generation.

P_1 : Agouti c^+c^+ X Chinchilla $c^{ch}c^{ch}$

F_1 : Agouti c^+c^{ch}

F_2 : 1 c^+c^+ : 2 c^+c^{ch} : 1 c^{ch} c^{ch}

3 Agouti 1 chinchilla

Complete dominance of agouti individuals also occurs on himalayan individuals.

P_1 : Agouti c^+c^+ X Himalayan c^hc^h

F_1 : Agouti c^+c^h

F_2 : 1 c^+c^+ : 2 c^+c^h ; 1 c^hc^h

3 Agouti 1 Himalayan

c^{ch} allele for chinchilla, though is recessive to c^+ alleles, it is incompletely dominant over himalayan (c^h) and albino (c) alleles.

P_1 : Chinchilla $c^{ch}c^{ch}$ X Himalayan c^hc^h Chinchilla $c^{ch}c^{ch}$ X Albino cc

F_1 : $c^{ch}c^h$ Light grey $c^{ch}c$ Light grey

F_2 : 1 $c^{ch}c^{ch}$: 2 $c^{ch}c^h$: 1 c^hc^h 1 $c^{ch}c^{ch}$: 2 $c^{ch}c$: 1 cc

1 Chinchilla : 2 light grey : 1 himalayas Chin hilla : 2 light grey : 1 albino

In the same way, c^h allele for himalayas is recessive to c^+ and c^{ch}, but dominates over albino.

P_1 : Himalayan c^hc^h X Albino cc

F_1 : Himalayan c^hc

F_2 : 1 c^hc^h : 2 c^hc : 1 cc

3 Himalayas : 1 Albino

These results tells us that c^+, c^{ch}, c^h, c are allelic to each other and the alleles of this multiple allelic series have the dominance hierarchy as $c^+ > c^{ch} > c^h > c$.

4.3.3.2 A, B, AB and O Blood groups in humans

Basing on the presence or absence of certain antigens, Landsteiner established ABO blood groups. Along with these A and B antigens, there are also some antibodies in the serum of the blood. Antibodies in a particular individual will be found only for those antigens which are absent in blood of that individual. So, the antibodies in blood group A will be able to agglutinize red blood corpuscles of blood group B and the antibodies in blood group B will be able to agglutinize red blood corpuscles of blood group A. AB blood group will not agglutinize any other group, since no antibodies are present. In the same way, O blood group will be able to agglutinize all other three groups except its own.

Blood groups (Phenotype)	Antigen in red blood cells	Antibodies in plasma	Can give blood to groups	Can secure blood from groups	Genotype
O	None	Anti-A Anti-B	O, A, B, AB	O	ii
A	A	Anti-B	A, AB	O, A	I^AI^A or I^Ai
B	B	Anti-A	B, AB	O, B	I^BI^B or I^Bi
AB	A & B	None	AB	O, A ,B, AB	I^AI^B

Table: Human blood groups

The genetics of these blood groups indicates that three alleles are present (i) I^o or i or + (ii) I^A or A and (iii) I^B or B. I^A and I^B are mutant alleles and are dominant over the wild allele I^o or i or +.

4.3.3.3. MN series of blood groups in humans

Landsteiner and Levine first discovered three general types of blood groups called M, N and MN respectively. The M-type produced antibodies, specific for M which could not agglutinate N, while the N red blood cells caused the production of antibodies specific for N. Both types of antibodies however could agglutinate the MN red blood cells.

By analyzing the relationship that these blood groups appear in the people, we can understand that genes responsible for M and N appeared to be alleles to each other, and were named as L^M and L^N respectively. $L^M L^M$ individuals had the M phenotype and produced only L^M gametes, $L^N L^N$ individuals had the N phenotype and produced both L^M and L^N gametes. As there are three different MN phenotypes, there are six different possible matings.

Parents	Offspring (ratios)		
	M	MN	N
$L^M L^M \times L^M L^M$	all	--	--
$L^M L^M \times L^M L^N$	1	1	--
$L^M L^M \times L^N L^N$	--	all	--
$L^M L^N \times L^M L^N$	1	2	1
$L^M L^N \times L^N L^N$	--	1	1
$L^N L^N \times L^N L^N$	--	--	all

"Rhesus" alleles in humans

Some individuals have on the surface of erythrocytes contain one more type of antigen called Rh factor besides A and B antigens. It was named after the Macaca rhesus monkey in which Rh factor was first discovered by Landsteiner and Wiener. It was found that humans contain eight different types of Rh antigens. The individuals possessing the Rh antigen are called Rh-positive (Rh^+) and those lacking it are Rh-negative (Rh^-). Both these types of persons are normal and none has natural anti-Rh antibodies in their blood plasma.

But in blood transfusion, we can see that Rh-negative person can develop antibodies on receiving Rh antigens of Rh-positive blood. This process can be safe when the recipient encounters with Rh-positive blood for the first time. But if already exposed, the recipient anti-Rh antibodies will agglutinate the donor's RBC. The high concentration of anti-Rh causes severe agglutination of RBC which sometimes proves fatal.

4.3.3.4.1 Erythroblastosis fetalis

This disorder results due to the incompatibility of Rh-positive and Rh-negative bloods which may lead to death of the child before or as soon as after birth. If a Rh-negative women marries a Rh-positive man and in such cases, all children will be Rh-positive if the father is homozygous and half

of the children will be Rh positive if he is heterozygous. If Rh-negative mother carries a Rh-positive foetus in the first case of pregnancy, even when some of the foetal RBC carrying Rh antigens may pass into her own blood stream and cause the production of anti-Rh antibodies, the concentration of anti-Rh antibodies is gradually built up in mother and she becomes sensitized only at or just before birth of her first Rh-positive child. But in a second pregnancy involving a Rh-positive child, these anti-Rh antibodies may return to the foetus through the placenta and destroy the Rh antigen carring RBC of foetus. Then the child may suffer with hemolytic anaemia, accompanied by jaundice as the liver capillaries become clogged with RBC's and bile is being absorbed by the blood. This collective symptoms lead to death of foetus before or after birth, is called erythroblastosis fetalis.

R and r was postulated for Rh^+ and Rh^- respectively. The Rh^+ blood type is found to be composed of several antigens such as C, c, D, d, E and e all of which indicate the possibility of multiple allelism of gene R. According to Wiener, gene R contains eight alleles – r, Ro, R', R", R_1, R_2, Rx or Rz, Ry. But according to fisher, cde, cDe, Cde, cdE, CDe, CDE, CDE, CdE alleles.

4.3.3.5 Eye colour in Drosophila

Normal eye in drosophila is red, which is determined by a X-linked wild type gene. White colour eye was one of the first mutants in these fruit flies (drosophila). Red eye and white eye showed simple dominant recessive relationship. Subsequently, different shades between the red and white colour eye were recovered. Different alleles were – red or wild type (w^+), coral (w^{co}), blood (w^{bl}), eosin (w^e), Cherry (w^{ch}), appricot (w^a), honey (w^h), buff (w^{bf}), tinged (w^t), Pearl (w^p), Ivory (w^i) and White (w). On the basis of F_2 ratios, wild being dominant to all others and white is recessive to all and the dominance hierarchy is – $w^+ > w^{co} > w^{bl} > w^{ch} > w^a > w^{bf} > w^t > w^t > w^i > w$.

w^e

When any two recessive alleles were brought together, intermediate types called compound is obtained.

4.3.3.6 Self sterility alleles

Self sterility means, pollen from a plant will not be able to bring about fertilization in the ovules of the same plant. A series of multiple alleles for self sterility in tobacco was designated as S_1, S_2, S_3 . . . . to Sn. The presence of self sterility provides the cross pollination in many plants. A tobacco plant have any two of these alleles, since they are located opposite to each other in a pair of chromosomes. Fertilization could be accomplished only by a pollen grain with one of the alleles not present in the mother tissue. Thus, the pollen of S_1 S_2 cannot fertilize the stigma of another S_1 S_2. But a cross between S_1S_2 (female) and S_2S_3 (male) yields offspring of classes S_1S_3 and S_2S_3 only. In a cross of S_1S_2 with S_3S_4, the progeny were of four types S_1S_3, S_1S_4, S_2S_3 and S_2S_4.

In *Oenothera lamarckiana*, 37 different self-sterility alleles have been found. In red clover, more than 200 alleles for self-sterility were found.

4.3.3.7 Colour loci in corn

Several series of multiple alleles are known in corn.

a) C locus on chromosome 9

The C locus on the chromosome 9 controls colour and has atleast three alleles C^I (an inhibitor of colour), c^i (responsible for full colour) and c (expressing colourless phenotype).

b) R locus on chromosome 10

The R locus on chromosome 10 controls anthocyanin colour of seed and the plant. Four alleles R^r, R^g, r^r, r^g were described previously but later, due to their two independent functions, they were now designated as SP, sp, sP, sp respectively.

R^r = SP = coloured seed and cloured (red) plant
R^g = Sp = coloured seed and green plant
r^r = sP = colourless seed and coloured (red) plant
r^g = sp = colourless seed and green plant

4.3.4 SUMMARY

The mendelian concept that genes exist in not more than two allelic states had to be modified when genes with three, four or more alleles were discovered. A number of illustrations are provided to prove the concept of multiple alleles. The multiple alleles occupy the same locus in the chromosome and no crossing over occurs between them. All these alleles influence same character. Wild being dominant to most others, four different coat colours were seen in rabbits due to multiple allelism, having the dominance hierarchy as agouti > chinchilla > himalayan > albino.

The ABO blood groups were mainly based on the presence or absence of antigens on the surface of red blood cells. A blood group contain A antigen on its surface and have Anti-B antibodies in the serum. B-blood group contain B antigen on its surface and have anti-B antibodies. AB blood group contain A & B antigens and do not contain any antibodies. But O blood group do not contain any antigens but have anti-A and anti-B antibodies. Three alleles I^o, I^A and I^B are present for ABO blood groups. I^A and I^B are mutant alleles, and are dominant. In the MN series of blood groups L^M and L^N are two alleles which can give rise to six different possible matings. The surface of red blood cell also contain Rh-factor in some individuals which are designated as Rh-positive and the others as Rh-negative. The incompatibility of these two blood groups lead to death of child in the name of erythroblastosis fetalis. Multiple allelism of gene R of Rh-positive individuals have been observed.

Different shades between red and white colour eye of drosophila were observed due to the presence of multiple alleles. The dominance hierarchy for colour of eye include – $w^+ > w^{co} > w^{bl} > w^e > w^{ch} > w^a > w^{bf} > w^t > w^p > w^i > w$.

The presence of self-sterility led to cross pollination. A series of multiple alleles for self sterility in tobacco has been designated as S_1, S_2, S_3 to Sn.

The colour of seed and plant in corn was governed by multiple alleles on 'C' locus on chromosome 9 and R locus on chromosome 10.

4.3.5 TERMINOLOGY

Multiple alleles
Isoalleles
allelomorphs
multiple allelism
Agouti
chinchilla
himalayan
albino
ABO blood groups
MN series
Rh factor
erythroblastosis fetalis
Dominance hierarchy
Self sterility

4.3.6 SELF-ASSESSMENT QUESTIONS

1. What are multiple alleles? Give a brief account of multiple allelism?
2. Is it possible to cross two agouti rabbits and produce both chinchilla and himalayan progeny?
3. Discuss the control and inheritance of skin colour in rodents. Give phenotypic ratios expected in the following crosses in rabbits.

   (i) C/c^a x C/c^h (ii) c^{ch}/c^a x c^{ch}/c^a (iii) C/c^h x c^hc^h (iv) c^{ch}/c^L x C^h/c^a

4. Write short notes on:
   (a) Multiple alleles
   (b) Antigen and antibody
   (c) Universal donor & Universal recipient
   (d) ABO blood groups
   (e) MN blood groups
   (f) Rh blood groups

5. What do you understand by self-incompatibility? Explain this phenomenon by giving a suitable example.
6. Given a series of self-incompatibility alleles S_1, S_2, S_3, S_4. What genotypic ratios would be expected in embryos and in endosperms of seeds from the following crosses?

	Seed parent	Pollen parent
a	$S_1 S_4$	$S_3 S_4$
b	$S_1 S_2$	$S_1 S_2$
c	$S_1 S_3$	$S_2 S_4$
d	$S_2 S_3$	$S_3 S_4$

4.3.7 REFERENCE BOOKS

1. *Genetics* - Strickberger
2. *Genetics* - P.K. Gupta
3. *Genetics* - A.V.S.S. Samba Murthy
4. *Cell biology, Genetics, Molecular biology, Evolution and Ecology* - P.S. Verma and V.Agarwal.
5. *Principles of Genetics* - D. Peter Snustard, Michael J. Simmons and John B. Jenkins.

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UNIT IV

4.4 SEX DETERMINATION

4.4.1. Introduction

Sex is occurring universally in all organisms both in plants and animals. Before 1900, many biologists puzzled, regarding the problem what determines whether an off-spring will be a male or female. After 1900, Genetics was established with the discovery of Mendelism. Later it was observed that in some insects the males had an odd number of chromosomes and the females had an even number.

The males had unequal partners---------- XY
The females had similar chromosomes----- XX

Males will give rise to two kinds of spermatozoa X and Y. Females will give rise to only one kind of eggs X. So, the male is said to be heterogametic.

The sex of the individual is determined at the time of fertilization. The union of an egg with X-carrying spermatozoa gives a zygote with two X-chromosomes, which develops

into a female. The spermatozoa containing Y-chromosome after fertilizing with the egg, give rise to a zygote which will develop into a male individual.

The phenomenon of sex determination is worked out by Wilson, Sutton, Stevens, Montgomery and others. There are several variations in the determination of sex among animals.

Eg :

(i) In birds, some reptiles, amphibians, butterflies, moths and certain insects, the females are XY and the males are XX.

(ii) There is another type i.e., XO in females and XX in males.

(iii) In Drosophila and many insects, man and in mammals, males have XY and the females have XX chromosomes. This is the predominant system of sex determination.

Function of X and Y - Chromosomes

Morgan's investigations of sex linked inheritance in Drosophila revealed that many sex linked characters were carried in the X-chromosome and Y-chromosome carried no genes. In 1916, Bridges discovered certain males of Drosophila lacking Y chromosome (XO). Such males are sterile but normal in appearance and behaviour. Females carry extra Y chromosome (XXY) but are normal and fertile. This situation proves that the females have two X-chromosomes and the males have one X-chromosome for the determination of sex.

Based on embryological studies, there are three possibilities of sex determination.

1. Progamic: Sex is determined before fertilization.
2. Syngamic: Sex is determined at the time of fertilization.
3. Epigamic: Sex is determined after the zygote has been formed.

Syngamic sex determination has been popularly considered by modern workers.

4.4.2. Objectives

- To study the different types of sex-determining mechanisms.
- To study to basic principles involved in each type of sex determining mechanism.
- To study the sex determining mechanisms operating in man and Drosophila.
- To study the sex-determining mechanisms operating in plants.

4.4.3. Sex determining Mechanisms

Modern Geneticists have reported many different mechanisms of sex determination in living organisms. Important and common mechanisms of sex determination are as follows:

A. Genetically controlled sex determining mechanisms.
B. Environmentally controlled sex determining mechanism.
C. Metabolically controlled sex determining mechanism.
D. Nutritional theory of sex determination.
E. Hormonally controlled sex determining mechanisms.

A. Genetically controlled sex determining mechanisms

Most of the mechanisms of the determination of sex are under genetic control. They are classified as follows:

1. Chromosomal theory of sex determination.
2. Genic Balance theory.
3. Male haploidy or haplodiploidy mechanism.
4. Single gene effects.

4.4.3.A1: Chromosome theory of sex determination: According to this theory, male and female individuals would differ in their chromosome constitution. In dioecious organisms, two types of chromosomes were recognized viz., autosomes and sex chromosomes. In a diploid individual there are (2n-2) autosomes and two sex chromosomes. In females the two sex chromosomes are similar i.e., homomorphic (XX) and in males the two sex chromosomes are heteromorphic (XY). In birds usually female is designated as ZW and is heterogametic and male is designated as ZZ and is homogametic. To distinguish the heterogametic female in birds (ZW) from the heterogametic sex (XY) in man and Drosophila, different symbols were used.

The role of sex-chromosomes in the determination of sex became evident for the first time due to the discovery of sex linked genes, whose inheritance did not follow the expected segregation patterns in both sexes, instead it followed a criss-cross type of inheritance. The experiments of C. B. Bridges on non-disjunction of X-chromosomes proved that a diploid set of auto somes with two X-chromosomes will always give rise to a female individual irrespective of whether the two X-chromosomes came from same parent or from different parents. This example suggested that in Drosophila, Y-chromosome does not carry any sex determining factor, but it is essential for the fertility of male sex. Presence of one or two X-chromosome is more important than the presence or absence of Y-chromosome.

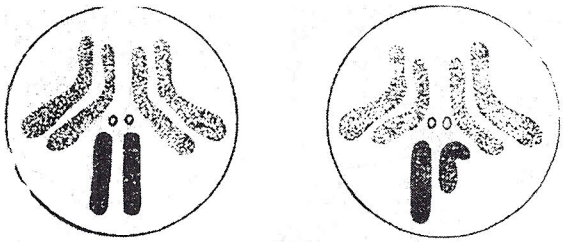


Fig. 1 Chromosomal set of male and female Drosophila. Autosomes are shown dotted while sex chromosomes completely blck. X chromosomes are solid rod shaped while Y is curved, small and inverted comma-like. The two small spheres in centre indicate diploid nature of organisms.

In dioecious diploid organisms, 2 systems of sex chromosomal determination of sex have been identified

a. Heterogametic Males
b. Heterogametic Females.

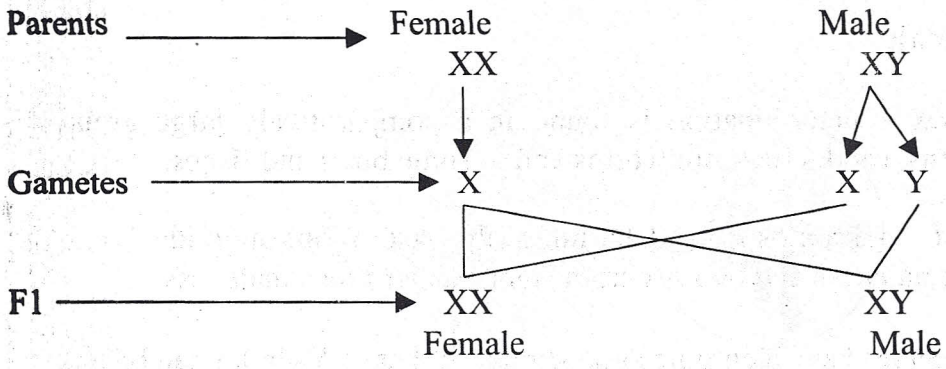
a. Heterogametic Males: In this type of sex chromosomal determination, of sex, the female sex has two X-chromosomes and the male sex has only one X-chromosome. In human beings and most of the mammals the males show 2 different sex chromosomes, viz., the X and the Y-chromosomes. So, during gametogenesis it produces two types of gametes and is said to be heterogametic sex. The female sex produces similar type of gametes and it is called homogametic sex. There are two types of heterogametic males.

1. XX – XY type

2. XX – XO type.

1. XX – XY type of Sex determination

In man, other mammals, Drosophila, and in certain angiospermic plants like Melandrium, the female possesses two homomorphic X-chromosomes in their body cells called homogametic and produces one kind of eggs, each with one X chromosome. The males of these organisms possesses one X-chromosome and one Y-chromosome. The males are having two heteromorphic sex chromosomes and they produce two kinds of sperms, half with X-chromosomes and half with Y-chromosome. The sex of the embryo depends on the kind of sperm which fertilizes the egg. An egg fertilized by a X-bearing sperm produces a female and the fertilized by Y- bearing sperm produces a male.



2. XX – XO type of sex determination

In some insects, especially those of the order Hemiptera and Orthoptera, males are also heterogametic, but they produce either X-bearing sperm or gametes without a sex chromosome.

In the males of these species, the X-chromosome has no homologous pairing partner because, there is no Y-chromosome. Thus, males exhibit an odd number in their chromosome complement.

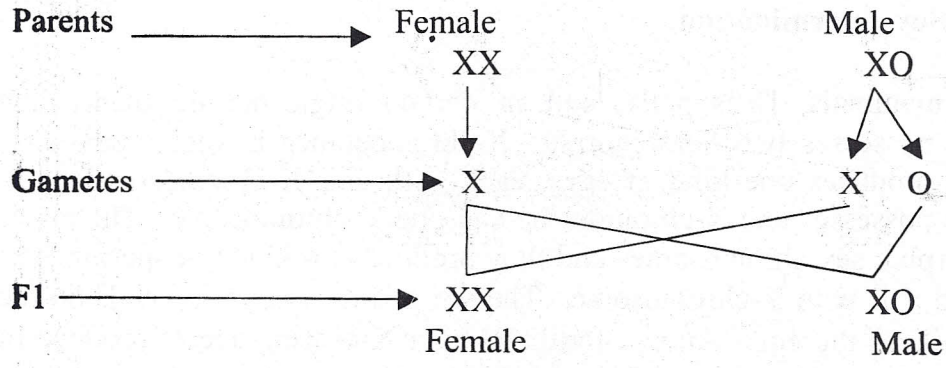
The no. of X-chromosomes determines the sex of an individual.

Example :- One X --- Male
Two X --- Female

If a single X-chromosome of the male is always included in one of the two types of gametes formed, then a 1: 1 sex ratio is predicted in the progeny.

This mode of sex determination is commonly referred to as XO method. Here 'O' symbolizes the lack of a chromosome analogous to the Y of XY – system.

Example : XO method :



b. Heterogametic Females

This method of sex – determination is found in a comparatively large group of insects like butterflies, moths, caddis flies, silkworms and in some birds and fishes.

---- Here also the no. of X-chromosomes determines the sex of an individual one X chromosome determines femaleness and two X chromosomes determines maleness.

---- The females of some species have a chromosome similar to that of Y- in human beings.

---- In these cases, the chromosomes are some times labeled Z and W instead of X and Y respectively.

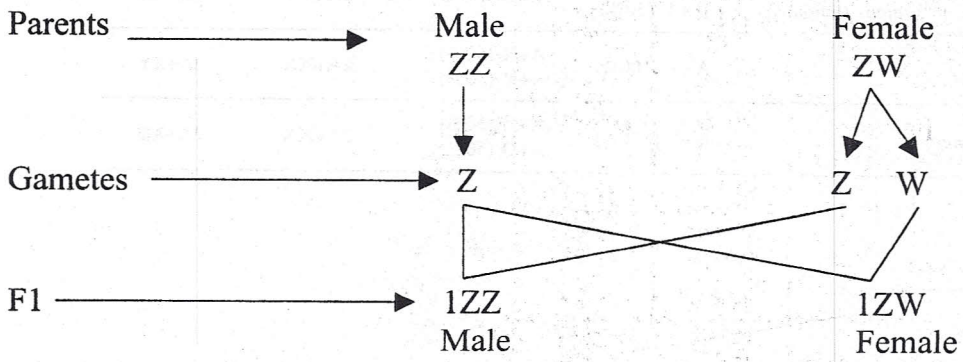
Generally the female exhibits the heterogametic sex (ZW) and the male exhibits homogametic sex.

---- In heterogametic females also 2 types sex determination systems are present.

1. ZW – method of sex determination
2. ZO – method of sex determination.

1. ZW – method of sex determination

Here two different sex chromosomes are present viz. Z and W i.e., heteromorphic. The presence of W chromosome determines femaleness. The female is said to be heterogametic and the male is said to be homogametic.
Example:



2. ZO method of sex determination

The females of some species have no homologue to the single sex chromosome as in the case of XO mechanism. To point out this difference the symbols ZZ and ZO may be used to designate males and females respectively.

In the females of these species, the Z-chromosome has no homologous pairing partner because, there is no W- chromosome present. Thus the females exhibit an odd number in their chromosome complement. The number of Z-chromosomes determines the maleness and femaleness. If the single Z-chromosome of the male is included in one of the two types of gametes formed, then a 1 : 1 sex ratio is predicted in the progeny.

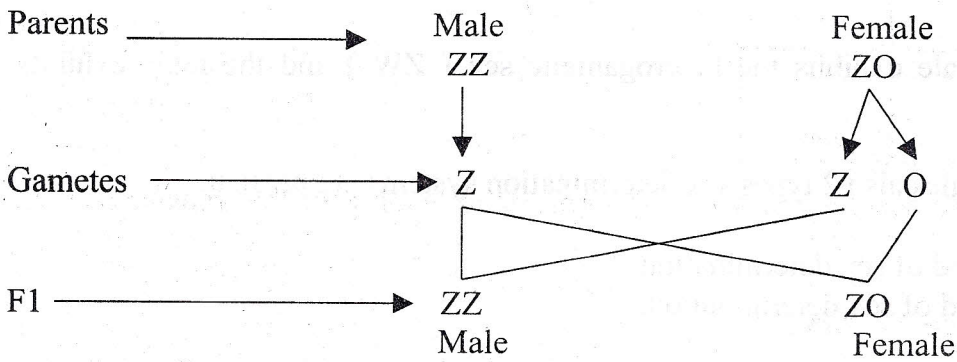


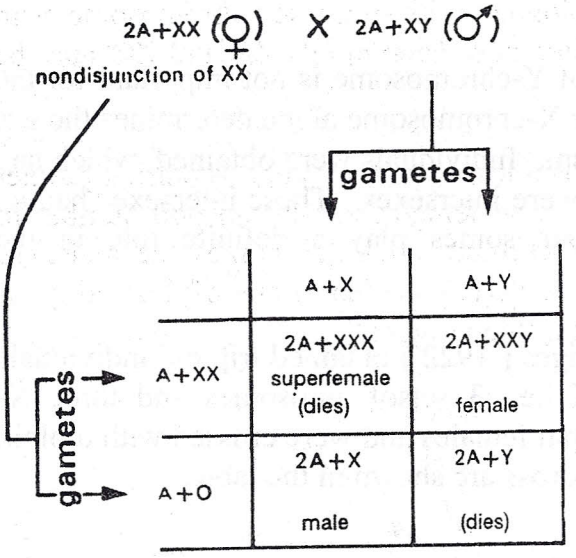
Table **Different systems based on chromosome mechanism of sex determination in animals (A = one set of autosomes).**

	System	Gametes		Zygotes	
		Sperms	Eggs	Males	females
Male heterogametic	XO ♂ (protenor type), e.g. *Protenor*	A+X (50%) A+O (50%)	A+X (100%)	2A+XO	2A+XX
	XY ♂ (Lygaeus type), e.g., *Drosophila, Homo sapiens* (humans)	A+X (50%) A+Y (50%)	A+X (100%)	2A+XY	2A+XX
Female heterogametic	XY ♀, e.g. birds	A+X (100%)	A+X (50%) A+Y (50%)	2A+XX	2A+XY
	XO ♀, e.g. *Fumea*	A+X (100%)	A+X (50%) A+O (50%)	2A+XX	2A+XO

BRIDGE"S Experiment

The experiment of Bridges on non-disjunction of X-chromosomes demonstrated that a diploid set of autosomes with two X-chromosomes will always give rise to a female individual irrespective of whether the two X-chromosomes came from the same parent or from different parents. This example suggested that in Drosophila, Y-chromosome does not

carry any sex determining factor. The presence of one or two X-chromosomes is more important than the presence or absence of Y-chromosome.



Non-disjunction of X chromosomes in a female Drosophila leading to the transfer of both X's to the daughter.

4.4.3.A.2. Genic Balance Theory

This theory is proposed by C. B. Bridges in 1921. According to this theory there seems to exist a delicate balance of masculine and feminine tendencies in the hereditary compliment of an individual and the mechanisms like XY, serve to trip the balance in one direction or another.

Sex determination in Drosophila

In Drosophila, the presence of Y chromosome has been found to be essential for the fertility of male sex but, it has no role in the determination of sex. In this fly the sex is determined polygenecally. The sex determining genes were distributed in such a way that the net effect results in the autosomes determining maleness and the X-chromosomes determining femaleness.

The sex of an individual depends upon the ratio of X-chromosomes to autosomes. If each haploid set of autosomes carries factors with a male-determining value equal to one (1), then each X-chromosome carries factors with a female determining value of one and half (1 ½).

If A represents a haploid set of autosomes in a normal male (AAXY), the male and female determinants are in the ratio of 2 : 11/2. So, the genic balance is in favour of maleness. A normal female (AAXX) has a male and female ratio of 2 : 3. So, the genic balance is in the favour of femaleness.

The experiment of Bridges demonstrated that Y-chromosome is not important for the determination of sex and it did not indicate whether X-chromosome alone determines the sex or the autosomes also play any role in the mechanism. Individuals were obtained, which had two X-chromosomes as in the normal female, but were intersexes. These intersexes had an extra set of autosomes (A) indicating that autosomes play a definite role in the determination of sex.

Suddenly while working on Drosophila, Bridges (1922) obtained triploid individuals containing 3 sets of chromosomes i.e., 3A : 3X i.e., 3 setsof autosomes and three X-chromosomes. These triploid individuals were normal females and were crossed with diploid males (2A + XY). The results obtained from such cross are shown in the table.

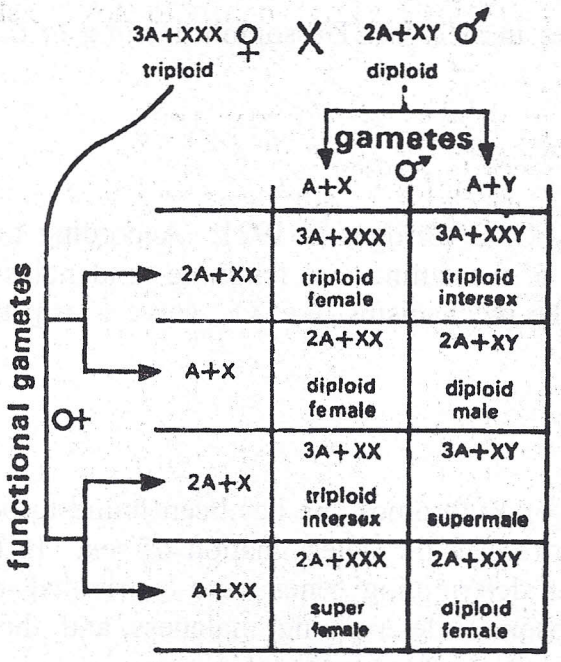


Fig. 17.3. Results obtained from a cross of a triploid (3A + XXX) ♀ fly, and a diploid ♂ (2A + XY) fly in *Drosophila*.

From the table it is obvious that from such a cross, normal diploid males, triploid females, intersexes, supermales, and super females were obtained. The chromosomal constitution of these phenotypically different sexes had an important role in the determination of sex.

Triploid intersexes in Drosophila and Genic Balance Theory

The presence of Triploid intersexes in the experiment is a proof that autosomes also carry factors for sex-determination. These intersexes are sterile individuals, which are intermediate between male and female. The cause of these results, was interpreted by Bridges in the form of Genic Balance theory of sex-determination. According to this theory, the ratio between the number of X-chromosomes and the number of complete sets of autosomes will determine the sex.

----The X-chromosome is believed to carry female tendency genes, while autosomes carry male tendency genes. Both these sets of genes start functioning and there has to be a balance between them for an individual to become male or female. In one complete set of chromosomes (A + X), female tendency genes are more. Here the ratio betweern X and A is 1.0, it will be a female individual.

The different ratios between the no. of X-chromosomes and the no. of sets of autosomes i.e., X/A ratios and their corresponding phenotypes for sex are shown in Table. 2.

Table **Different doses of X-chromosomes and autosome sets and their effect on sex determination in *Drosophila*.**

Ploidy level	Number of X chromosomes	Sets of autosomes (A)	X/A ratio	Sex
Diploid	3	2	1.50	Superfemale
Triploid	4	3	1.33	
Haploid	1	1		Female
Diploid	2	2	1.00	
Triploid	3	3		
Tetraploid	4	4		
Triploid	2	3	0.67	Intersex
Tetraploid	3	4	0.75	
Diploid	1	2	0.50	Male
Tetraploid	2	4	0.50	
Triploid	1	3	0.33	Supermale

When X/A ratio is 1.0, the individual will be female, when it is 0.5, the individual is male. When this balance is disturbed the individual deviates from normal male or normal female. For example, if the ratio (X/A) falls between 1.0 and 0.5, it would be inter sex, when it is below 0.5 it would be a super male and when it is above 1.0 the individual would be a super female

Table **A summary chart showing different ratios of X-chromosomes and autosome sets (X/A ratio) controlling sex in *Drosophila*.**

X/A ratio	Sex
> 1.0	Superfemale
1.0	Female
1.0–0.5	Intersex
0.5	Male
< 0.5	Supermale

X/A ratio and gynandronorphs in Drosophila

In Drosophila, occasionally flies are obtained which have female characters in one part of the body and male characters in the remaining parts. Such individuals are known as gynandromorphs. They are formed due to the loss of an X-chromosome in a particular cell during development. If this event happens during first mitotic division of the zygote, then one of the two cells of two celled proembryo will have 2A + XX with X/A = 1.0 and the other cell will have 2A + X with X/A = 0.5. The fly derived from such a situation will have

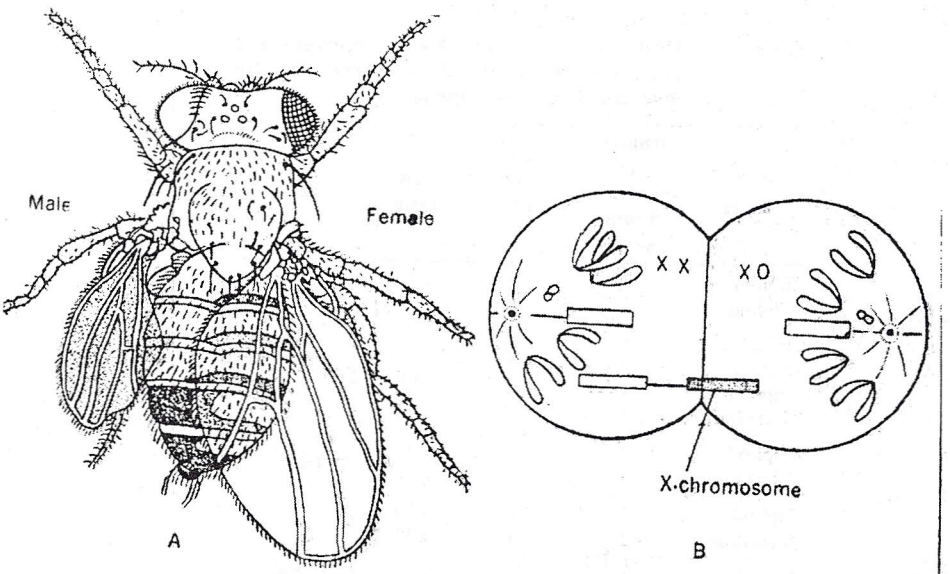


Fig. 13.6 : $A = a$ gynandromorph of *Drosophila* showing left half male and right half female parts and B = the loss of X chromosome during mitosis resulting from $2A + XX$ into $2A + XX$ and $2A + X$ (daughter cells). It explains one of the possible causes of gynandromorphism. The X-chromosome labelled below and dotted will be lost during cell division.

half of its body as female and the other half as male. The cytological examination of gynandromorphs suggested that Y-chromosome does not play any role in the determination of sex in Drosophila.

X/A ratio in Coenorhabditis elegans

Coenorhabditis elegans is a nematode. In this two types of individuals are found.

1. XX – self fertilizing hermaphrodites
2. XO – males

The hermaphrodites are believed to be female in its somatic tissue and mixed (male and female) in its germline. When the X/A ratio was observed in this nematode, a pattern similar to Drosophila with minor variations was observed.

In this nematode an X/A ratio less than 0.67 leads to male sex and a ratio greater 0.75 leads to a hermaphrodite. In case of Drosophila this ratio is 1.0 for female and 0.5 for male. A comparison of these ratios in the nematode and fruitfly suggests that a threshold is in operation in each case.

Sex determination in Man

In man like Drosophila XX – XY type of sex-determining mechanism operates but, here the Y-chromosome contains potent male determining genes which can overcome the feminizing action of the rest of the genotype. The evidence that Y chromosome is a determiner of fertility and sex of male individual comes from Barr body. This is reported by Barr and Bertram in 1949. This is a chromatin body, which is called sex chromatin or Barr body. This is observed in most of the body cells of man and other mammals. Human females have the Barr body in the nuclei of their body cells in higher proportion than males. So, they are called sex chromatin positive. The human males are called sex chromatin negative. In 1959 Ohno, Kaplan and Kinoshita demonstrated the relation between sex chromatin and sex chromosomes. It is demonstrated that the sex chromatin is derived from only one of the two X-chromosomes. The other X-chromosome behaves like an autosome and it is not heteropycnotic at interphase. The number of corpuscles of sex-chromatin at interphase is equal to nX – 1. It means that there is one Barr body less than the no. of X-chromosomes.

Role of X and Y chromosomes in determination of sex in man

Barr body seems to have some relation with female sex. The sex chromosomal aberrations have provided strong evidences that Y chromosome in man have some role in the determination and fertility of male sex.

For example, the individuals having at least one Y chromosome are males at least as to the external genitalia, though they may be sterile. The persons with one or more X-chromosomes are phenotypically female as long as no Y chromosome is present, though

sometimes infertile. Therefore, it is concluded that in man genes for maleness reside in Y chromosome and those for femaleness on the X-chromosomes. The autosomes have no sex determining role in man unlike Drosophila and the Y chromosome determines maleness even a single one outweighing the no. of X-chromosomes. The X-chromosome determine the female sex only in the absence of Y-chromosome.

4.4.3.A.3. Male haploidy or haplodiploidy Mechanism

Male haploidy or haplodiploidy or arrhenotokous parthenogenesis is particularly common in the insect order Hymenoptera, which includes ants, bees, wasps and saw-flies etc. In these insects fertilized eggs develop into diploid females and unfertilized ones into haploid males. So arrhenotoky is a both form of reproduction and a means of sex determination. Meiosis is normal in females but in males crossing over and reduction in chromosome number fail to occur during spermatogenesis due to their haploidy.

Thus, arrhenotokous parthenogenesis determines the sex in hymenopterons. Here the sex chromosomes have no identity like the Drosophila. It has been found that the quantity and quality of food available to the diploid larva determines whether that female will become a sterile worker or a fertile queen. Thus, here the environment determines the sterility or fertility but does not alter the genetically determined sex. The sex ratio of the off spring is under the control of the queen. Most of the eggs laid in the hive will be fertilized and developed into worker females. Those eggs which the queen chooses not to fertilize will develop into fertile haploid males. Queen bees usually mate only once during their life time.

4.4.3.A.4. Single Gene Control of Sex

In our previous discussion, sex was determined by individual sex chromosomes (X or Y) or by a complete set of autosomes. In such cases, the whole chromosome having large no. of genes, controlled the sex. There are some cases where individual single genes were found to be responsible for the determination or expression of sex.

1. **Sex determination in Asparagus**

Asparagus is a dioecious form. Rarely the female flowers bear rudimentary anthers and the male flowers bear rudimentary pistils. Rare male flowers having poorly developed pistils may set seeds. In one such case, when seeds obtained from a rare male flower, were raised into plants, male and female plants were found to be present in 3 : 1 ratio. When male plants raised thus were used to pollinate female flowers on female plants, only 2/3 of them showed segregation indicating that sex is controlled by a single gene. In this case, maleness should be dominant over femaleness and male plants should ordinarily be

Tassel Seed (ts) and Silkless (sk) genes in Maize:

Maize is monoecious with male inflorescence (tassel) and female inflorescence (silk) located on the same plant. A gene tassel seed (ts) is known which will convert the tasse in to seed bearing inflorescence. Another gene silkless is responsible for the absence of silks. Therefore, a plant sk/sk will be a male plant and a plant ts/ts will be a female plant. By using these genes it has been possible to convert maize from a monoecious to dioecious form, where plants will bear only male or only female inflorescence. For example if we use females with genotype tsts sksk (seeds on tassels, silkless) and male with genotype Tsts sksk (Normal tassel and silkless) on crossing, they will give males having the same genotypes as parents in 1 : 1 ratio. This is the stable dioecious situation.

Transformer gene (tra) in Drosophila:

In Drosophila, a transformer gene (tra) has been identified, when it is present in homozygous condition (tra/tra) converts a female into a sterile male, but does not act upon normal male individual.

Sex-determination in Neurospora:

Neurospora has two sexes exactly equivalent and designated as A and a. Mating occurs only between opposite sexes. The mating type A or a is determined by a pair of autosomal alleles and follows simple Mendelian inheritance.

4.4.3.B. Environmentally controlled sex determining mechanism

In some organisms, the environment determines the sex of the individual. This is reported in the variety of animal species which belong to different groups such as Rotifera, Annelida, Arthropoda, Echiuroidea, Mollusca etc. The environemental control of sex can be studied in the following examples.

Sex in Bonellia:

An example of environmental determination of sexual phenotype is afforded by Bonellia viridis. It is a marine echiuroid worm studied by F-Baltzer (1935). The adult female is about an inch long and has fairly complex anatomical organization. Male is of the size of large protozoan and has rudimentary organs. The males live as parasites in the uterus of the females. All larvae of Bonellia are genetically and cytologically similar.

If a particular larva settles on the proboscis of an adult female, it becomes a male individual. On the other hand, if a larva develops in isolation i.e., in water it develops into a

female. And also if an incompletely developed male is detached from the proboscis of female, it becomes an intersex. Thus it is evident that proboscis of adult female secretes some hormone like substance which suppresses femaleness and initiates maleness in the larvae which are attached with it.

Sex in Crepidula:

In 1943, Coe demonstrated in the molluscan genus crepidula that its every zygote contains all the genes which are necessary for the development of male and female reproductive systems. But, in this species it has been found that young crepidula remains functional male and produce only sperms but no ova.

As it matures the male phase is changed by transitional phase to a functional female phase. The female phase continues for the remaining life of the animal. So, it is suggested that the environment somehow regulates the activity of certain genes to produce only male phenotype during young stages, and inhibits them during adult stages and simultaneously initiating other genes to produce female phenotype.

4.4.3.C. Metabolically controlled sex determining mechanism:

Some suckers have seen the possibility of involvement of the role of metabolism in the determination of sex. Crew suggested that sex is a physiological equitable division between anabolic and catabolic individuals A. F. Shull and D. D. Whitney have shown that by increasing metabolic rate in rotifers the occurrence of male individuals increases than females.

Riddle found that metabolism had some definite role in the determination of sex in Pigeons and Doves, because, increased rate of metabolism developed the male potency, while decreased rate of metabolism caused femaleness.

4.4.3.D. Nutritional Theory of Sex-determination:

This is proposed by Sharp in 1934. It states that the sex determination depends upon the constitution of protoplasm concerned as well as upon the condition in which this protoplasm is compelled to react such as external and internal changes in habitat, nutrition, influence of hormones, parasitism, disease, etc.

4.4.3.E. Hormonally controlled sex determination:

In many cases it has been observed that sexual differentiation is controlled by hormones. The classical examples for hormonal control of sex determination are,

1. Sex reversal in hen,
2. Free martins.

1. Sex reversal in hen (Crew's hen):

Another case of sex determination which is controlled by hormones is that of Crew' s hen. It is an example of sex reversal and is named after the discoverer, Crew in 1923. He observed that a female fowl, which earlier laid off-spring changed over to a fully fertile male. It happened due to the damaged ovary in the female which secretes a male suppressing hormone under normal conditions. When the ovary of the hen is damaged or removed, the steroid cells of adrenal become active and provoke the development of rudimentary gonad into testes. Thus in the absence of the ovary testes could develop.

2. The Free martin:

In cattle, when twins are produced, one of them being male and the other female, the female is a sterile inter sex called a freemartin and the male is normal. These are known as freemartins after the name of the discoverer and are produced only when there is a vascular connection between the two embryos. The freemartin has external female genitalia but the internal sex organs are more or less like that of a male. The male twin is usually normal.

In 1917, F. R. Lillie suggested that the formation of freemartin was due to the fusion of foetal membranes of the twin calves, when they were in the uterus of mother. The fusion of foetal membranes permitted the blood of each twin to circulate in the blood vessel of the other. The male hormones produced by the male twin are presumed to suppress the differentiation of the female internal sex organs of the co-twin.

4.4.4. Sexdetermination in Higher Plants:

1. Melandrium: It is a dioecious plant belonging to the family Caryophyllaceae. In this plant the sex is determined by a pair of XY chromosomes. It contains 11 pairs of autosomes and XY chromosomes. Morphologically X and Y chromosomes are quite different. The pistillate plants are XX and the staminate plants are XY. Here X-chromosome is smaller than Y-chromosome. In 1949,Warmke noticed that when polyploidy is induced in this plant, Y-chromosome is most important in determining sex and the autosomes have no role in this aspect.

The detailed study of X and Y chromosomes of Melandrium have been made. Y-chromosome is longer than X-chromosome. It has been possible to divide these into 5 different segments.

1. Segment I is suppressing femaleness (FS).
2. Segment II promotes maleness i.e., initiates anther development (MP).
3. Segment III controls fertility or late stage of another development (MF).
4. Segment IV is common in both X and Y chromosomes and helps in pairing and regular disjunction of X and Y chromosomes during meiosis.
5. Segment V is the differential region of the X-chromosome, which should promote femaleness in the absence of female suppressing segment I and Y-chromosome.

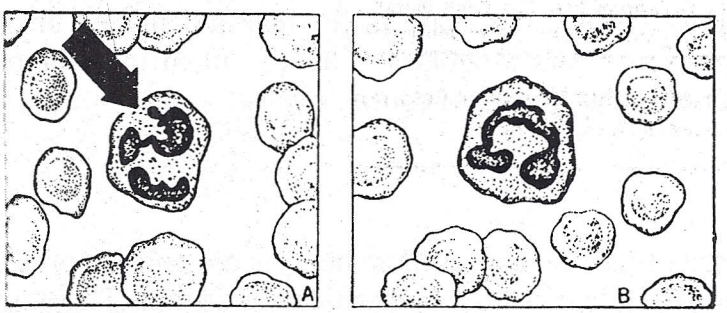


Fig. 13.4: Diagram showing the presence of drum stick in the white blood cell of normal human female (A) (indicated by thick arrow) and white blood cell of normal human male (B).

In case of Melandrium, diploids, triploids and tetraploids having different doses of X and Y chromosomes were studied with respect to their sex. The results are summarized in the tables 3 and 4.

Table 13. II Showing balance of *X* and *Y* chromosomes in *Melandrium*

No. of autosomes	*XY complement*	*Sex of plant*
2*A*	*XX*	Female plant : Normal pistillate and no anthers
2*A*	*XY*	Male plant : Normal staminate plant,
2*A*	*XXY*	Male plant : Occasional hermaphrodite blossom
3*A*	*XXY*	Male plant : ,, ,, ,,
4*A*	*XXY*	Male plant : ,, ,, ,,
4*A*	*XY*	Male plant : Staminate
4*A*	*XXXY*	Male plant : Occasional hermaphrodite, blossom
4*A*	*XXXXY*	Hermaphrodite plant : Occasional staminate blo

Table 13.III. Showing lack of relationship between autosome & *X* chromosomes in *Melandrium*.

Autosomes	*X-chromosome constitution*	*X/A ratio*	*Sex*
4*A*	*XX*	0·5	Pistillate
3*A*	*XX*	0·67	,,
4*A*	*XXX*	0·75	,,
2*A*	*XX*	1·0	,,
3*A*	*XXX*	1·0	,,
4*A*	*XXXX*	1·0	,,
4*A*	*XXXXX*	1·25	,,
2*A*	*XXX*	1·5	,,

Sex determination in Coccinia:

The mechanism of sex determination in *Coccinia indica*, a member of family Cucurbitaceae was studied by Prof. R. P. Roy and his Co-workers at Patna University.

They studied the sex in diploid, triploid, and tetraploid plants with and without Y-chromosome and observed that irrespective of the no. of X-chromosomes and/or autosomes, presence of a single Y-chromosome gave a male individual.

Thus in Coccinia, a plant is male when one or more Y-chromosomes are present and is female when Y-chromosome is absent. Female potential in the presence of Y-chromosome showed expression only when the ratio of Y : X reached 1 : 4. The number of autosomes did not visibly affect the sex expression. Thus the male determining genes are present on Y-chromosome in this plant, like that of Melandrium.

Bryonia and Rumex:

Correns was the first person who demonstrated the genetical differences in the genus Bryonia by managing reciprocal crosses. Bryonia dioica is dioecious and Bryonia alba is monoecious. The following crosses were made.

B.dioica ( ) * B. alba ( ) = off springs all female plants
B. alba ( ) * B. dioica ( ) = off springs 50% male and 50% female.

The results indicate that male B. dioica is heterozygous for sex.

In case of Rumex acetosa and Rumex acetocella, male has 15 chromosomes while the female has 14. During meiosis 6 bivalents and one trivalent are formed. During anaphase X-chromosome disjoins from Y-chromosomes. As a result two types of gametes are formed, one with 6 + X and the other with 6 + Y1 Y2. Thus plant would be 12 + XX and the male 12 + Y1 Y2.

The sex chromosomal mechanism of sex determination has also been observed in certain monoploid bryophytes. Example: Spaerocarpas. In 1919, Allen found that the sporophyte of spaerocarpos contains 2 sex chromosomes (XY) and it produces two kinds of meiospores- some with X and other with Y-chromosomes. The meiospores with X-chromosome germinate into sexually reproducing female gametophyte and the meiospores with Y chromosome germinate into sexually reproducing male gametophyte.

4.4.5. Summary:

Sex determination in plants and animals deals with the study off actors which are responsible for making an individual male, female or a hermaphrodite.

Modern Geneticists have reported many different mechanisms of determination of sex in living organisms.

Some important and common mechanisms of sex determination are,

1. Genetically controlled sex determining mechanisms.
2. Metabolically controlled sex determining mechanism.
3. Hormonally controlled sex determining mechanism
4. environmentally controlled sex determining mechanism.

Genetically controlled sex determining mechanism includes different types viz.,

a. Chromosomal theory of sex determination or Heterogamosis.
b. Genic balance theory.
c. Single gene effects.
d. Male haploidy or haplodiploidy.

----Chromosomal theory of sex determination states that in sexually dimorphic dioecious organisms besides morphological and behavioural differences between both sexes, the sexual diversity also occurs at the level of chromosomes.

----In dioecious organisms two types of chromosomes were recognized, viz., autosomes and sex chromosomes. Autosomes have no relation with sex and contain the genes which determine the somatic characters of the organisms. Allosomes or Sex chromosomes are responsible for the determination of sex and they are known as Sex-chromosomes. Eg., X and Y chromosomes.

----There are two types of sex chromosomal mechanism of sex determination viz,
Heterogametic Males.
Heterogametic Females.
----In case of heterogametic males, the female has two X-chromosomes and the male has only one X-chromosome. Here the male produces two different types of gametes in terms of sex-chromosomes. So, it is called heterogametic sex. The female produces similar type of gametes, so it is called homogametic sex.

----The heterogametic males may be of two types viz.,
XX - XO type and
XX - XY type.

----In case of XX –XY type males are having two heteromorphic sex chromosomes and produces 2 kinds of sperms, half with X-chromosome and half with Y-chromosome. The sex of the embryo depends on the kind of sperm which fertilizes the egg. An egg fertilized by X-bearing sperm produces a female and the egg fertilized by a Y-bearing sperm produces a male.

----In case of XX - XO type, the male is having only one X-chromosome which is unpaired and hence it is referred to as XO. It produces 2 types of sperms; half with X- chromosome and half without X-chromosome. The sex of the offspring depends upon the sperm that fertilizes the egg.

----In case of heterogametic females, the male sex contains two X-chromosomes and is homogametic. The female sex contains one X and one Y chromosome and it is heterogametic. To avoid confusion with that of XX – XY and XX – XO type of sex

determination mechanism, here instead of X and Y alphabets we are using Z and W alphabets respectively. So heterogametic females are of the following two types viz., ZW – ZZ type and ZO – ZZ type.

----Genic balance mechanism of sex determination was studied in Drosophila by C. B. Bridges in 1921. According to this theory, there seems to exist a delicate balance of masculine and feminine tendencies in the hereditary compliment of an individual and like the XY, ordinarily serve to trip the balance in one direction or another.

----So, according to this theory each haploid set of autosomes carries male determining factors whose value is equal to one (1), and each X-chromosome carries a female determining value of one and half (11/2).

----In a normalmale, the male and female determinants are in the ratio of 2 : 11/2, so the genic balance is in the favour of maleness.

----In a normal female the male and female determinants are in the ratio of 2 : 3 and the balance is in favour of femaleness.

----In the insect order Hymenoptera, the male individuals arise parthenogeneticlly and they will have a haploid chromosome number and the females will show diploid chromosome number.

Example: Honey Bee Males (n) = 16
Females (2n) = 32.

----In some cases single genes were found to be responsible for the determination of sex.
Example: Asparagus, Maize, Drosophila.

----Metabolically controlled sex determining mechanism is reported in Pigeons and Doves by Riddle.

Hormonally controlled sex determination is also reported in some cases.

Example: 1. Sex reversal in hen reported by Crew in 1923.
2. Freemartins in cattle reported by F. R. Lillie (1917).

----In some organisms,the environment determines the sexual phenotype of the individuals. This is reported in many animal species belonging to Rotifera, Annelida, Arthropoda, Echiuroidea, Mollusca, etc.

----Most of the flowering plants are monoecious and they have no sex chromosomes. But in case of dioecious plants usually the sex is under the control of a single gene locus. However, chromosomal sexuality has been reported in some plant species viz., Melandrium and Spaerocarpus.

----The Melandrium has XX – XY chromosomal mechanism of sex determination like man. Here the pistillate plants are XX and the staminate plants are XY.

4.4.6 Terminology:

Autosomes
Gynandromorphs
Genic balance theory
Criss – Cross inheritance
Hermaphrodites
Dioecious
Monoecious
Heterogametic
Sex reversal
Free-martin
Barr body
Male haploidy
Arrhenotokous parthenogenesis
Staminate
Pistillate

4.4.7. Self- Assessment Test:

1. Compare and contrast the chromosome theory and the genic balance theory of sex determination. Describe experiments which indicated a balance between sex chromosomes and autosomes in Drosophila.

2. Write concise account on sex determination

3. Write short notes on

   i. Gynandromorphs
   ii. Y-chromosome in Drosophila
   iii. Sex determination in human beings.
   iv. Role of Drosophila Triploids in Sex determination.

4. What is dosage compensation? How this is achieved? Discuss the difference in the mechanisms involved in humans, Drosophila and birds.

5. Discuss the phenomenon of sex reversal in mammals. How did the availability of XX-males and XY-females help in understanding and locating the genes responsible for sex determination.

6. Discuss the sex determining mechanism in Coccinia and Melandrium and compare it with that in human beings.

4.4.8 Additional readings:

1. Principles of Genetics -----8th edition by Gardner/ Simmons/ Snustad
John Wiley and Sons (ASIA) Pte. Ltd.

2. Genetics by Susan Elrad and William Stanfield -----4th edition
Tata Mc Graw-Hill Edition (Schaum's outlines)

3. Genetics by P. K. Gupta ----- 3rd edition.
Rastogi Publications.

4. Genetics by A.V.S. S. Samba Murthy
Narosa Publishing House.

5. Genetics by Monroe W. Strickberger------ 3rd edition
Prentice Hall of India (Private Limited).

UNIT-IV

LESSON-4.5

SEX-LINKED INHERITANCE

CONTENTS

4.5.0 INTRODUCTION

Sex makes no difference in Mendel's crosses, as the progeny of a cross between two individuals of pure lines is the same regardless of which individual is female or which individual is male. But Mendel's laws are not applicable on those genes which are exclusively located either in X or Y chromosome. In dioecious individuals, there can be two kinds of characters, namely (i) characters which do not show a difference in reciprocal crosses. (ii) Characters which show a difference in reciprocal crosses. The former type of characters are located on autosomes and the latter are located on specialized chromosomes known as sex chromosomes. Traits, which are carried on sex chromosomes are known as sex-linked traits. Characters whose expression in a particular genotype depends on whether the individual is male or female are known as sex influenced traits and the trait which can be expressed in one sex only and not in the other are called sex limited traits.

The genes which occur exclusively on x-chromosome (eg. mammals, Drosophila, Melandrium etc.) or on the analogous Z chromosome (eg: birds etc.) are called X- or Z-linked genes. The genes which exclusively occur in Y chromosome are called holandric genes. The inheritance of X- or Z- and holandric genes is called sex-linked inheritance.

4.5.1 OBJECTIVES

- To study different characteristics of sex-linked inheritance.
- To study sex-linked inheritance in humans, drosophila, fowls and moths.
- To study X-lined, Y-linked and XY-linked types of inheritance of genes.
- To study inheritance of hemophilia, colour blindness in humans
- To study inheritance of x-linked recessive gene.
- To study inheritance of x-linked dominant gene.
- To study inheritance of x-linked gene in drosophila.

In the widespread type of sex determination, female has 2X chromosomes and in male one X and one Y chromosomes. If we take human as example, there are 23 pairs of chromosomes of which in male there are XY and in female XX pair of sex chromosomes are present. So, the male is heterozygous or heterogametic sex because in sperms there are two types and the female is homogametic because, all eggs are sexually alike.

In the sex-linked inheritance, X-linked, Y-linked and XY-linked inheritance has been observed.

X-linked inheritance

It is performed by those genes which are localized in the non-homologous sections of X-chromosome, and that have no corresponding allele on Y-chromosome. The X-linked genes are commonly known as sex-linked genes.

Y-linked inheritance

It is performed by those genes which are localized in the non-homologous sections of Y-chromosome and that have no alleles on X-chromosome. These genes which are Y-linked are commonly known as holandric genes.

XY-linked inheritance

This type of sex-linked inheritance is performed by those genes which are localized in homologous sections of X and Y chromosomes.

4.5.2 INHERITANCE OF X-LINKED GENES

Experimental evidence linking the inheritance of genes to chromosomes was given by chromosome theory of heredity given by Morgan. According to this theory, all genes were located

on chromosomes and the Mendel's principles could be explained by the transmissional properties of chromosomes during reproduction. This theory paved the way for all studies of inheritance.

4.5.2.1 Characteristics of sex-linked inheritance

1. The differential region of each chromosome contain genes that have no counterparts on the other kind of sex chromosome.

2. X-linked recessive genes show criss-cross pattern of inheritance i.e., a X-linked recessive gene is transmitted from P_1 male parent (father) to F_2 male progeny (grandsons) through its F_1 heterozygous females (daughters) which are carriers (Fig. 1).

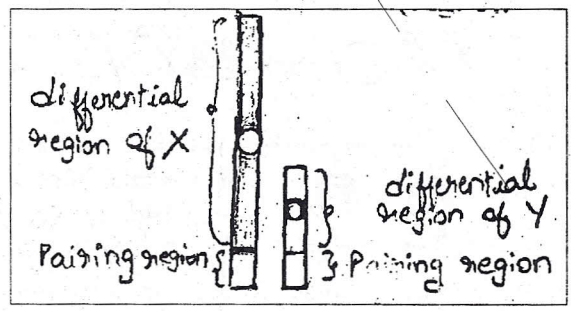


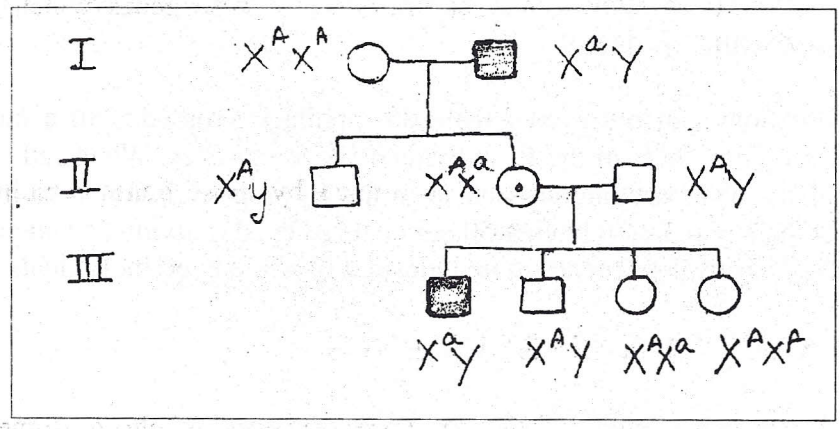
Fig.1. Homo sapiens

3. Reciprocal crosses of X-linked recessive genes can give different F_1 and F_2 ratios.

4. The X-linked recessive phenotype is usually found more frequently in the male than in the female, because an affected female can result only when both mother and father bear the X-linked recessive allele, whereas an affected male can result when only the mother carries the gene.

5. Offspring of affected male will not be affected, but all his daughters will carry gene in masked heterozygous condition, so one half of their daughter's sons (grandsons) will be affected **(Fig. 2).**

Fig.2. Pedigree showing expression of X-linked recessive genes in **males**



6. Affected male will not inherit the X-linked recessive gene to any one of his sons and even will not pass the gene along to their offspring.

7. Dominant X-linked genes are more frequently found in female than in male.

8. The affected males pass the condition on to all of their daughters but to none of their sons (Fig. 3).

9. Female usually pass the condition on to one-half of their sons and daughters (Fig. 4).

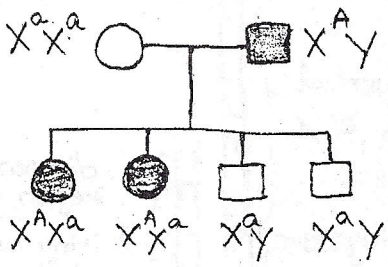


Fig. 3. Pedigree chart showing X-linked dominants condition

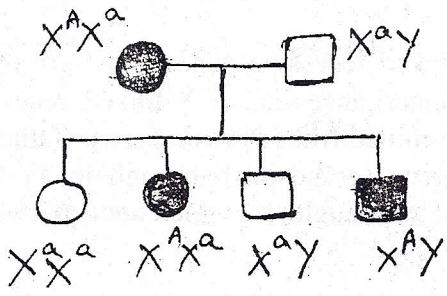


Fig. 4. Pedigree chart showing females affected by X-linked dominant condition

10. X-linked dominant gene fails to be transmitted to any son from a mother which did not exhibit the trait itself.

4.5.2.2 Examples of Inheritance of X-linked recessive gene

4.5.2.2.1 Inheritance of X-lined gene for eye colour in Drosophila

In Drosophila, the gene for white eye colour is X-linked and recessive to X-linked dominant gene for red-eye colour. The characteristic feature of sex linked inheritance, criss-cross inheritance of gene is observed.

a) Red eyed female X white eyed male

When wild or dominant red eyed female drosophila is crossed with a mutant or recessive white eyed male drosophila, then all the F_1 individuals have red eyes. When red eyed male and red eyed female individuals of F_1 are intercrossed, then the F_2 progeny is found to include all females having red eyes and male population having 50 per cent red eyed individuals and another 50 per cent white eyed individuals. Thus, F_2 generation includes red to white eyed individuals in the ratio of 3:1 (Fig. 5).

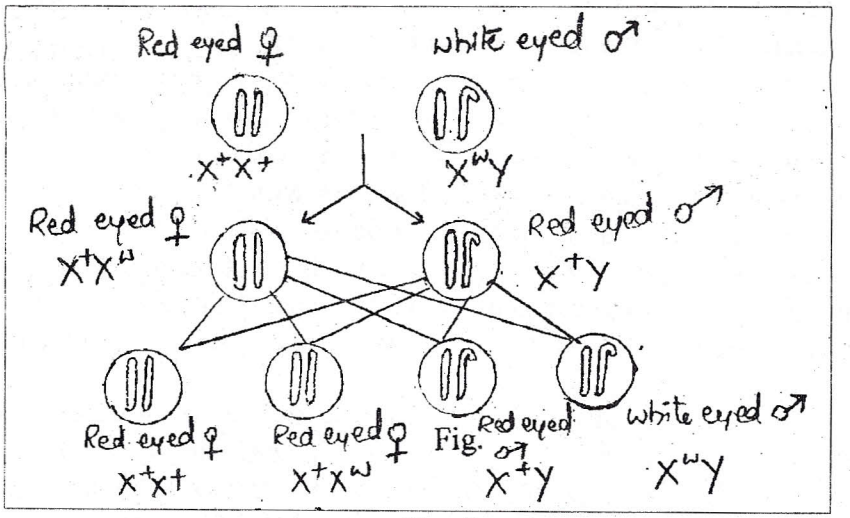


Fig. 5.

b) White eyed female X red eyed male

When a white eyed female drosophila is crossed with a red eyed male drosophila, all the female individuals in the F_1 generation are red eyed. When these red eyed female individuals and white eyed male individuals are intercrossed, female population of F_2 generation is found to include 50 per cent red eyed and 50 per cent white eyed flies (Fig. 6).

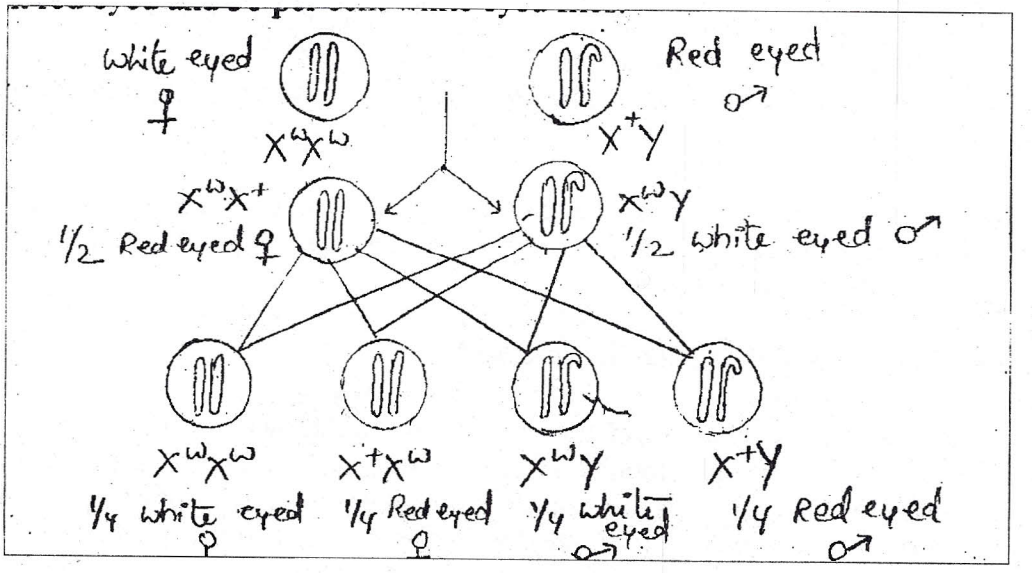


Fig. 6

In the cross between red eyed female and white eyed male drosophila, red-eyed female contains the gene + + for red colour of eye. The white eyed male contains a single gene w for white colour of eye which remains located in X chromosome. This one allelic condition of male is termed hemizygous condition in contrast to the homozygous or heterozygous possibilities in the female. The female being homogametic produces only one type of gametes or eggs with the gene + for red coloured eyes. The male being heterogametic produces two types of gametes, 50 per cent sperms with X-chromosome containing w gene, 50 per cent sperms with 'Y' chromosome without w or + gene. The gametes of both parents unite in fertilization to produce F_1 progeny. The F_1 hybrids which receive X-chromosome with + gene from the female and a X-chromosome with w gene from the male becomes red-eyed female because gene '+' is dominant over gene 'w'. The hybrids which receive a gene '+' from mother and Y chromosome from father produce red eyed males.

The F_1 red eyed female with the gene '+w' when crossed with F_1 red eyed having the gene '+', the female hybrids produce two types of eggs, 50 per cent eggs carry gene '+' and the remaining 50 per cent carry gene 'w'. The males produce two types of sperms, half carry '+' and half carry no such gene on Y chromosomes. The union of sperms and ova of F_1 offsprings may produce four possible types of F_2 individuals – X^+X^+ (red eyed females), X^+Y (red-eyed males), X^WX^+ (red eyed females), X^WY (White eyed males).

In the same way, when white eyed female is crossed with red-eyed male, similar X-linked inheritance of recessive gene for white eye colour is revealed. The white eyed female contains the gene 'ww' located on both X chromosomes. The red eyed male contains the gene '+' located on single x-chromosome. The female being homogametic, produces single type of eggs with single 'w' gene for whiteness, while the male being heterogametic produces two types of sperms, 50 per cent sperms carry the gene '+' and remaining 50 per cent sperms carry no such genes on the Y chromosomes. Two kinds of F_1 hybrids are produced $X^W X^+$ (red eyed female) and X^WY (white eyed male) and a cross between the two F_1 individuals breeded four types of individuals in F_2 generation, such as X^WX^W (white eyed female), X^+X^W (red-eyed female), X^WY (white eyed male) and X^+Y (red eyed male).

As we can see in the above reciprocal crosses, the trait located on a sex chromosome would alternate sex from one generation to next generation. In other words, character is transferred from mother to son and never from father to son.

4.5.2.2.2 Inheritance of X-linked genes in humans

In humans, more than 150 confirmed X-linked traits are known, most of them are recessives. Some examples include red-green colour blindness, haemophilia, Duchenne's muscular dystrophy, deficiency of glucose-6-phosphate dehydrogenase during allergic reactions for sulphonamides or for *Vicia faba*, two forms of diabetes insipidus, one form of anhidrotic ectodermal dysplasia (absence of sweat glands and teeth), absence of central incisors, certain forms of deafness, spastic parapelagia (partial paralysis of lower extremities with increased irritability and spasmodic contraction of muscles), uncontrollable rolling of eye balls (nystagmus), a form of cataract, night blindness, optic

atrophy, juvenile glaucoma (hardening of eye ball), juvenile muscular dystrophy and white frontal patch of hair.

4.5.2.2.2.1 Colour blindness

In human beings, colour perception is mediated by light absorbing proteins in the specialized cone cells of the retina in the eye. Three such proteins have been identified, one to absorb blue light, one to absorb green light and one to absorb red light. Dominant X-linked gene is necessary for formation of colour sensitive cells and any abnormality in any of these receptor proteins and are unable to distinguish between green and red colours lead to colour blindness, which is most frequently seen in males than in females. Lack of chloroble pigment in the retinal cones results in an inability to discriminate green colour and the defect is known as deuteranopia or deutan colour blindness. When the lack of erythrolable pigment in the cones, lead to indiscrimination in red end of spectrum which leads to protanopia or protan colour blindness. The two genes encoding for green and red colour perception evolved from an ancient colour receptor gene. Third gene for colour perception, the one encoding the receptor for blue light is located on an autosome.

The inheritance of colour blindness can be studied in the following types of marriages.

i) Marriage between colour blindness man and normal visioned woman

When colour blindman marries a normal visioned woman, they will produce normal visioned male and female individuals in F_1 generation. In the F_2 generation, as a result of marriage between F_1 normal visioned woman and normal visioned male, two normal visioned female, one normal visioned male and one colour blind male are observed.

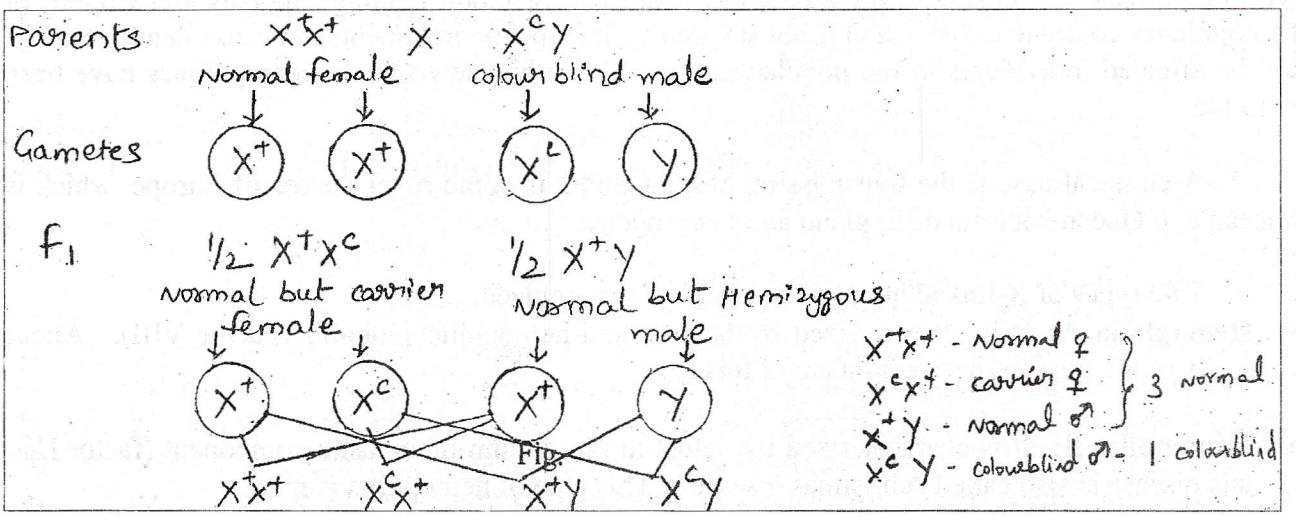


Fig. 7.

ii) Marriage between normal visioned male and colour blind female

When a colour blind woman marries a normal visioned male, all F_1 sons will be colour blind and daughters will be normal visioned, because male receives one X-linked recessive gene for colour blindness from colour blind mother. The daughter receives one X-linked dominant gene from father by one X-linked recessive gene for colour blindness from mother. In F_2 generation, a colour blind homozygous daughter, a normal visioned heterozygous daughter, a normal visioned hemizygous son and a hemizygous colour blind son are observed.

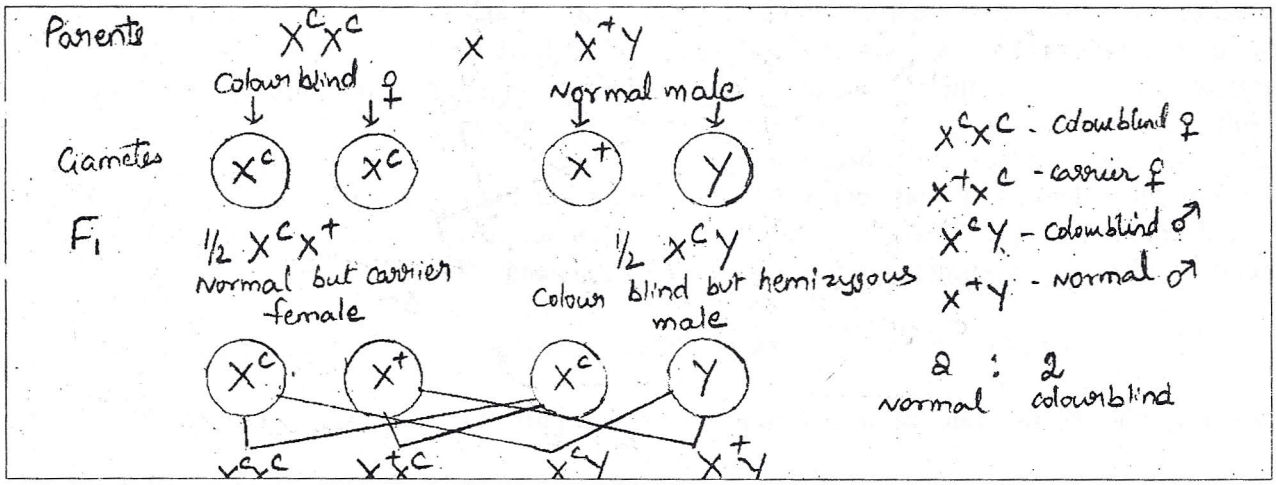


Fig. 8

4.5.2.2.2.2 Hemophilia

In human beings, hemophilia is one of the best known examples of an X-linked trait. People with this disease are unable to produce a factor needed for blood clotting; the cuts and wounds of hemophiliacs continue to bleed and if not stopped by therapeutic treatment, can cause death. Nearly, all the affected individuals in the population are male, only a few female hemophiliacs have been reported.

A classical case is the transmission of hemophilia in some royal houses of Europe, which is traceable to Queen Victoria of England and her progeny.

Two types of X-linked hemophilia have been recognized.

- **Hemophilia A:** It is characterized by lack of anti-hemophilic globulin (Factor VIII). About $^4/_5{}^{th}$s of the cases of hemophilia are of this type.

- **Hemophilia B:** It is characaterized by defect in plasma thromboplastic component (factor IX), this disease is also called "christmas disease". This type of hemophilia is mild.

Hemophilia is caused by recessive X-linked gene and so, a lady may carry the disease. When a carrier woman marries a normal individual, 50 per cent of her sons were affected, even if father is normal.

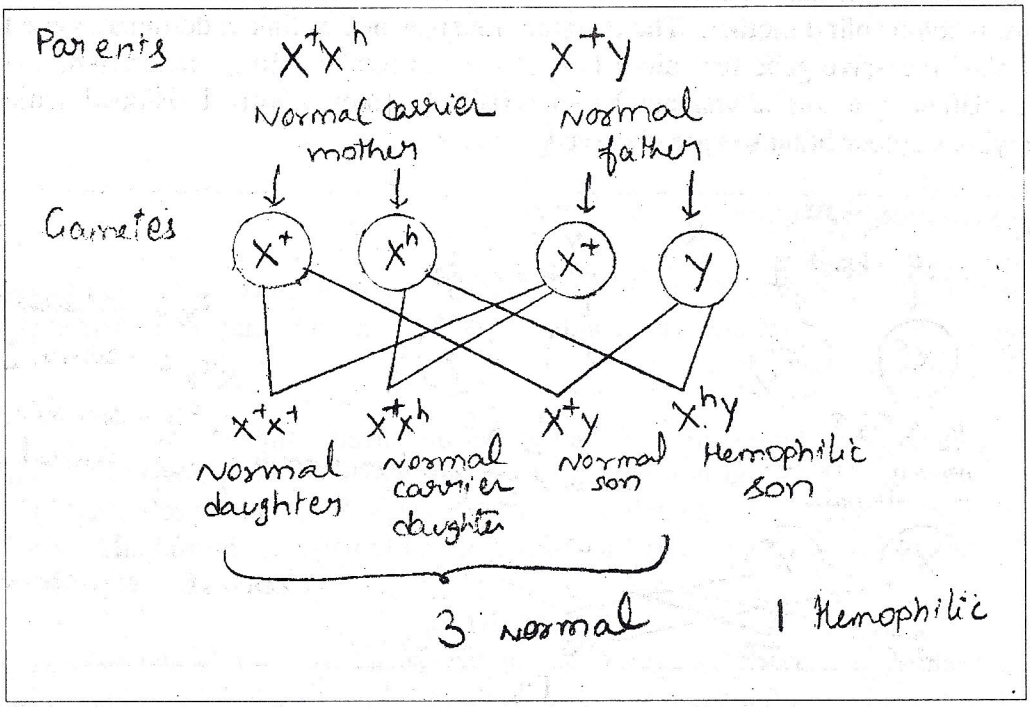


Fig. 9

4.5.2.2.3 Inheritance of Z-linked genes in moths

In birds, moths, butterflies etc., females are heterogametic and males are homogametic. Criss-cross pattern of inheritance occurs from mother through heterozygous F_1 sons to grand daughters of F_2. Best example is ZW-ZZ sex linkage is plymouth rock chicken. When the light coloured winged magpie moth (Abraxas) is crossed with dark winged males, all the progeny of F_1 generation are dark irrespective of sex. This shows that light colour is recessive trait to dark colour which is a dominant trait. But when we do reciprocal cross, i.e., now with dark winged female with light winged male in the F_1 generation, female progeny with light wings and male progeny with dark wings were observed.

As these two pairs of reciprocal crosses does not give similar results, and by observing the inheritance pattern in the second cross, we can understand that the wing phenotypes are associated with the sex of the moths.

First Cross:

Parents Z^LZ^L X $Z'w$

Dark male Light female

gametes Z^L Z^L Z' W

F_1 ½ Z^LZ' ½ Z^LW

Dark male Dark female

Second Cross:

Parents Z′Z′ X Z^LW

Light male Dark female

↓ ↓ ↓ ↓

gametes [Z′] [Z′] [Z^L] [W]

F_1 ½ Z′ Z^L ½ Z′W

Dark female Light male

4.5.2.2.4 Sex linkage in Poultry

In poultry, female individual is heterogametic having only one X-chromosome (XO-condition) and male is in homogametic condition (XX). The inheritance of barred plumage is a classical example for this category.

Barred plumage is dominant over black or red unbarred plumage. The gene for the barred plumage is represented by B and non-barred by b. The cross between non-barred hen (♀) and a bared cock (♂) produces only barred offspring of both sexes. When they are inbred, they produced only barred males in F_2. Approximately half of the F_2 (♀) females are barred and other half is non-barred.

Fig. 10

In the reciprocal cross of barred hen (♀) and non-barred cock (♂), the F_1 progeny contains barred males and non-barred females. In F_2, equal numbers of barred and non-barred in both sexes are seen. So, barring in birds follows criss-cross inheritance, with father transmitting the gene to both his sons and daughters. The gene thus follows the X-chromosome.

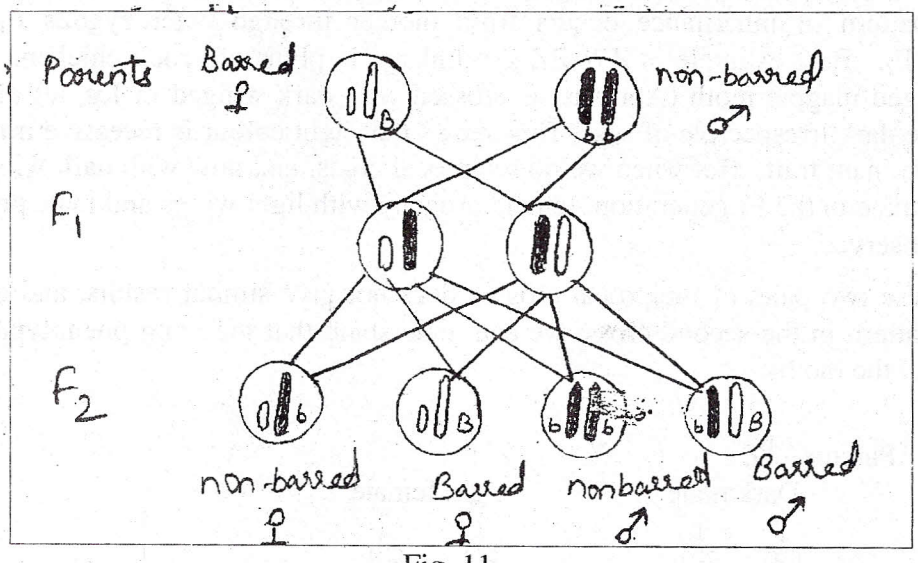


Fig. 11

4.5.2.3 Examples of Inheritance of X-linked dominant gene

4.5.2.3.1 Fragile X Syndrome and Mental retardation

In human beings, many cases of mental retardation appear to follow an X-linked pattern of inheritance. Most of these are associated with a cytological anomaly (e.g. absence of certain nucleotides etc.). This anomaly, i.e., a constriction near the tip of long arm of X-chromosome gives the impression that the tip is ready to detach from the rest of the chromosome, hence the name fragile X chromosome. The clinical manifestations of this syndrome vary considerably, making diagnosis difficult. Most patients show significant mental impairment and some show facial and behavioural abnormalities, where both sexes can be affected.

The fragile X syndrome has been described as an X-linked dominant disorder with incomplete penetrance. Affected females are heterozygous for fragile X chromosome and affected males are hemizygous for this chromosome.

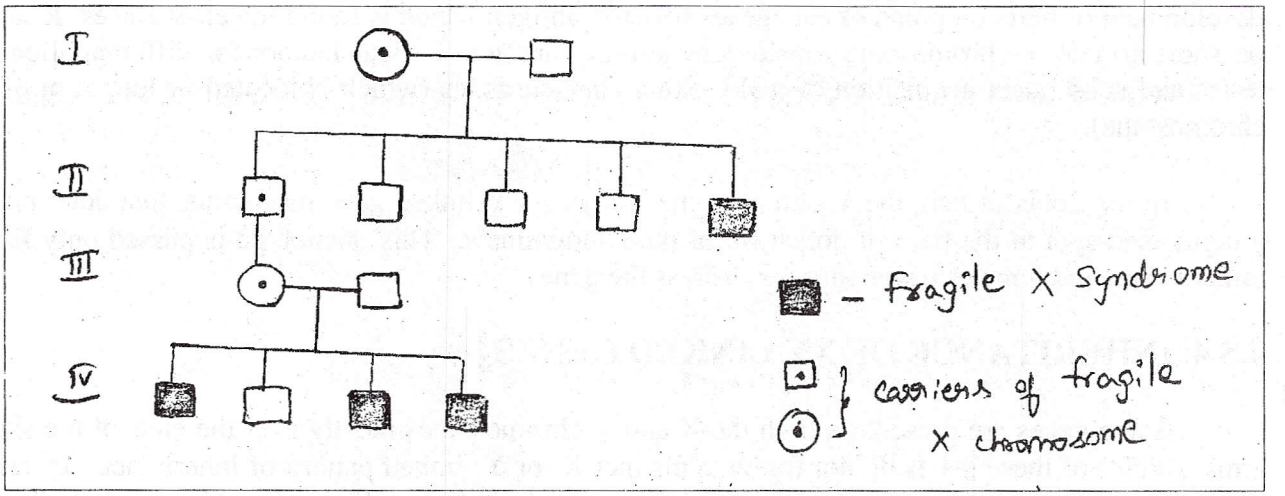


Fig. 12

4.5.3 Inheritance of Y-linked genes

Genes in the non-homologous region of the Y-chromosome pass directly from male to male (son).

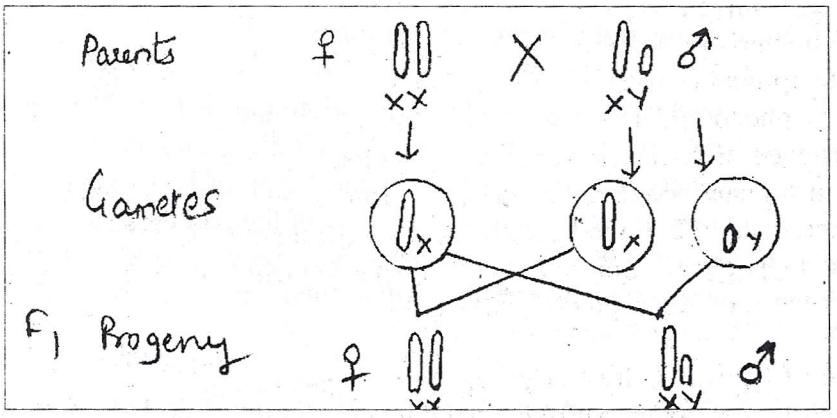


Fig. 13

In man Y-linked genes include ichthyosis hystrix gravis hypertrichosis (excessive development of hairs on pinna of ear) genes for H-Y antigen which is found in cell-surfaces (located on short arm of Y-chromosome), histo-compatibility antigen, critical factors for differentiation of testes and subsequent acquisition of male sexual characteristics (which is located or long arm of Y chromosome).

In the Lebistes fish, the Y-chromosome contains a Y-linked gene maculatus, that determines a pigmented spot at the base of dorsal fin of male individuals. This phenotype is passed only from father to son and females never carry or express the gene.

4.5.4 INHERITANCE OF XY LINKED GENES

Some genes are present on both the X and Y chromosomes mostly near the ends of the short arms. Alleles of these genes do not follow a distinct X- or Y- linked pattern of inheritance. Instead, they are transmitted from mothers and fathers to sons and daughters alike, mimicking the inheritance of an autosomal gene, called pseudo autosomal genes.

In humans several diseases are XY-linked. Examples include – total colour blindness, *Retinitis pigmentosa*, skin diseases like, *Xeroderma pigmentosum* and *Epipermolysis bullosa*.

4.6 SUMMARY

The genes which occur exclusively on X-chromosome or on the analogous Z-chromosome are called X- or Z-linked genes. The genes which exclusively occur in Y-chromosome are called

holandric genes. The inheritance of X or Z-linked holandric genes is called sex-linked inheritance. Female has 2X chromosomes and male has one X and one Y chromosomes. Out of 23 pairs of chromosomes, 22 are autosomes and one pair either XX in female, XY in male are sex chromosomes. Sex linked inheritance are of 3 types – X-linked, Y-linked and XY-linked inheritance. Characteristic features of sex linked inheritance include, criss-cross pattern of inheritance; and reciprocal crosses of X-linked recessive genes give different F_1 and F_2 ratios. The X-linked recessive phenotype is usually found more frequently in males than in females. In Drosophila, inheritance of X-linked gene for eye colour is an example for inheritance of X-linked recessive gene. In humans, colour blindness and Hemophilia, the examples for inheritance of X-linked genes. Inheritance of Z-linked genes was demonstrated in moths. Sex linked inheritance was also demonstrated in poultry. Inheritance of fragile X syndrome and mental retardation are the examples for inheritance of X-linked dominant gene.

Inheritance of ichthyosis hystrix gravis hypertrichosis, criticals factors for spermatogenesis, genes for H-Y antigen, histocompatibility antigen are the examples for inheritance of Y-linked genes.

Total colour blindness, *Retinitis pigmentosa* and skin diseases like xeroderma pigmentosum and epipermolysis bullosa are the examples for inheritance of XY linked genes.

4.7 TERMINOLOGY

Reciprocal crosses
Sex-linked traits
Sex-influenced traits
X-linked genes
Z-linked genes
Holandric genes
Heterogametic
Homogametic
Alleles
Recessive
Dominant
Hemizygous condition
Hemophilia
Colour blindness

4.8 SELF-ASSESSMENT QUESTIONS

1. What are the characteristic features of sex-linked inheritance.
2. Describe the inheritance of X-linked recessive gene by giving some examples in humans.
3. What is Hemophilia? How is it transmitted?
4. Describe in detail the inheritance of X-linked genes in Drosophila.
5. What are Y-linked and XY-linked inheritance of gene? Give some illustrations.

6. A male Drosophila with reduced eyes was crossed to a female with normal eyes. The F_1 consisted of a total of 67 males, all with normal eyes, and 65 females, all with reduced eyes. How would you explain the inheritance of this character?

7. In the domestic fowl (*Gallus domesticus*), the gene for plumage colour is sex-linked. The dominant allele and determines gold-coloured plumage, and its recessive allele of determines silver coloured plumage. A cross is made between a homozygous gold coloured male and a silver coloured female. The F_1 males are mated with F_1 females. Give the genotypes and phenotypes for each sex in the F_1 and F_2.

8. Red-green colour blindness in humans is recessive and sex-linked. If a woman heterozygous for colour blindness marries a colour blindman, what is the probability that their first child will be a colour blind daughter?

9. 'Bent', a dominant sex-linked allele B, in the mouse, results in a short, crooked tail, its recessive allele, b produces normal tail. If a normal-tailed female is mated to a bent-tailed male, what phenotypic ratio should occur in the F_1?

4.9 REFERENCE BOOKS

1. *Principles of Genetics* – D.Peter Snustad, M.J. Simmons, John B. Jenkins.
2. *Genetics* – Strickberger.
3. *Genetics* – P.K. Gupta
4. *Genetics* – A.V.S.S. Sambamurthy
5. *Cell Biology, Genetics, Molecular Biology, Evolution and Ecology* – P.S. Verma and V.K. Agarwal.

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Lesson 5.1

THEORIES OF EVOLUTION
THEORY OF SPECIAL CREATION
LAMARCKISM

Contents

5.1.1 INTRODUCTION

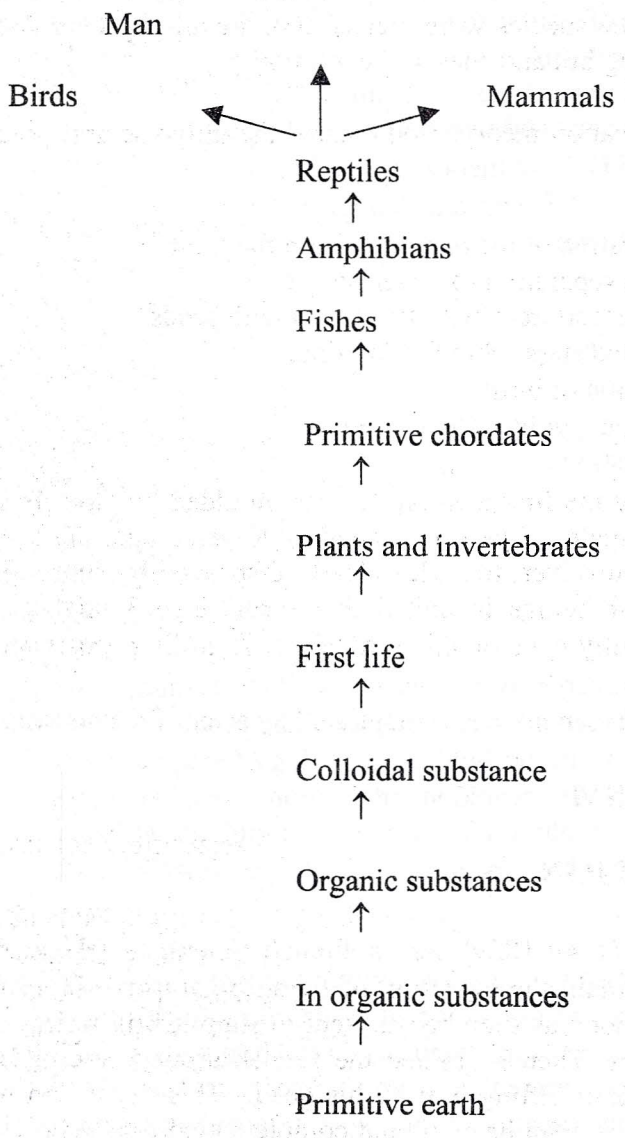
In millions of diverse living species we find around us in modern world descended from a common ancestor that lived in the remote past. The processes that have brought this diversity are collectively called evolution. In the field by of biology the term evolution was first proposed by Herbert Spencer and it refers to the development of more complex forms of life from simpler and earlier forms. Thus the organic evolution is the branch of biology which is concerned with the origin and differentiation of organism which exists at present and which existed in the past from the pre-existing organisms.

The theory of organic evolution convincingly explains how the wide variety of plants and animals came in to existence in to this world. According to this theory, the world has not been crected but evolved. Life originated spontaneously in the remote past from the non-living in organic substances. These substances reacted to form organic compounds. The organic compounds developed in to colloidal system capable of step wise improvement in order to give rise to simple

life. The simple life formed in the remote past subsequently gave rise to more complex living beings. The present day plants and animals are the modified descendents of the first formed life. The theory of organic evolution says that higher animals came from protozoans; Amphibians came from fishes; reptiles descended from amphibians; Birds and mammals came from reptiles. Man descended from ape like ancestors.

Origin of life and organic evolution are separately dealt with. The origin of life deals with the formation of simple life in the remote past from chemical substances. On the other hand, organic evolution deals with the formation of new species of plants and animals from the first formed. The origin of life and organic evolution may be illustrated as shown in the table below.

Table; Showing the origin of life and organic evolution



Theories of organic evolution explained by

1. Theory of special creation
2. Lamarckism
3. Darwinism
4. Hugo Devries mutation theory
5. Modern synthetic theory

5.1.2 Special Creation Theory

This theory was proposed by Father Sudrez. (1548-1617) a Spanish monk. He opined that the world was created by super natural power of God. It is based on the Biblical book of Genesis. Accordingly, the sun, earth plant, animals and man were created by God. i.e., Man as Man, Horse as horse and Insects as Insects as soon. This theory postulates the whole world originated in six days. Thus this theory says that all the species were created individually by God and they have not under gone any change. This is nothing but anti-thesis of evolution.

According to special creation theory, God created the universe and organisms in 6 days. The events are as follows.

I. Creation of light. Separation of the dankness from the light
II. Formation of sky by the separation of water bodies
III. Creation of earth, oceans and trees with fruits also with seeds
IV. Creation of sun, moon and stars. Sun for day time
V. Oceanic creatures, creation of birds
VI. Creation of cattles, insects, wild animals & man

According to this theory the first man Adam was moulded by God from in animate material and given the soul and later female – Eye was created 6000 years ago. This is as per the Christian the theologians calculations. However, this idea clearly disproved by scientific facts. Because ne fossil record shows that human-beings inhabited this earth since 3 million years. The gigantic animals like dinosaurs successfully lived on this earth about 75 million years ago.

So this theory of special creation can not be substantiated by scientific evidences and facts.

5.1.3 LAMARCKISM

5.1.3.1 INTRODUCTION

Jean Bapiste Lamarck (1744-1829) was a French Scientist. He was a first biologist to propose a definite theory to explain the evolution of living organisms. He was born in 1744. His father wished him to be priest hood as such he was sent to study Christianity. As soon as his father died he discontinued his course. Then he joined the French army. One of his colleagues caused injury to the glands of his neck by lifting h n by his head. This made him unfit for military life. Then he studied medicine in Paris. But he could not complete medical career. However at the age of

50 Lamarck became a professor of invertebrate Zoology. He became blind for 10 years in his last days. But he died in a miserable conditions in 1829. Lamarck published many books. Out of these, Philosophic Zoologique published in 1809 provides the evolutionary ideas of Lamarck. His evolutionary theory is popularly called Lamarckism or inheritance of acquired characters. Osborn stated that Lamarck was the most popular figure between Aristotle and Darwin.

5.1.3.2 Prominent Features of Lamarckism

I. According to Lamarck, the environment does not remain constant. It changes. The change in the environment in turn provides new needs for the organism. In response to the new needs, organisms develop new structures. The new structures developed by the organisms in response to the environment are called acquired characters. These acquired characters transmitted to off spring generation after generation and by this way a new species in produced. Thus Lamarck strongly believed that changes in the environment bring about the development of new characters.

II. Variations in organism arise through the effects of use and disuse. Thus the constant use makes the structure greatly developed and disuse makes the structure atrophied or disappeared. Or degenerated.

5.1.3.3 Principles of Lamarckism

Lamarckism consists of 4 principles. They are as follows:

1. Internal urge of the Organism
2. Environment and new ideas
3. Use and Dis-use theory
4. In heritance of acquired characters

5.1.3.3.1 Internal urge of the organism

In nature each organism has the ability to grow and to increase in size to attain the maximum-size. For this not only the body but also each and every part of the organism increases in volume. According to Lamarck this increase in size is mainly due to an internal urge and inherent ability of the animal it self. Here it is to be said that some sort of an internal urge of animal involves to increase in size.

5.1.3.3.2 Environment and new needs

According to Lamarck, environment plays an important role in evolution. When ever there is a change in environment the animals respond to the changes. The change in the environment brings about the new needs for the animals. The new needs lead to the development of new structures. Thus Lamarck strongly believed that changes in the environment bring about the new needs for the animals. The new needs lead to the development of new structures.

In this regard Lamarck quoted many examples of the influence of environment on animals. He observed difference in the same species of plants grown in different environmental conditions. The plants are grown on fertile soils they are healthy and luxuriant. If the same plants grown on unfertile soils are weak and thin. Here the nature of the soil and other environmental factors bring about changes in the plants.

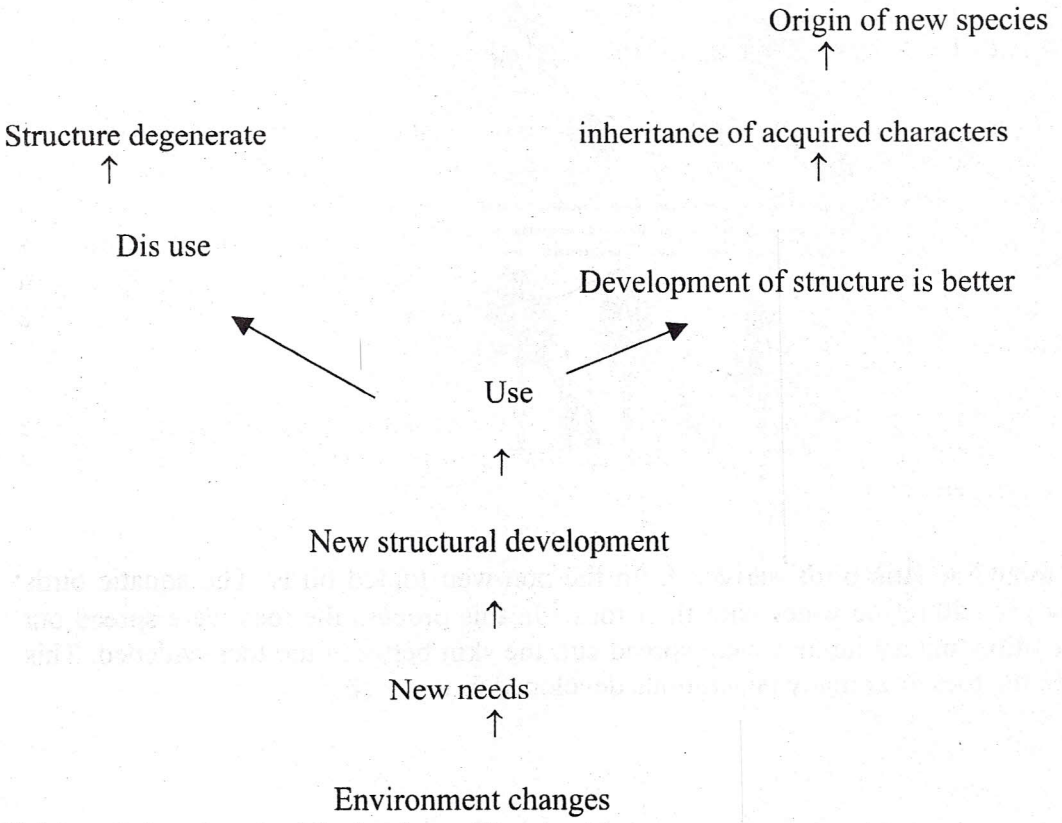


Table – 1 showing the Mechanism of Lamarckism

5.1.3.3.3 Use and Dis-use Theory

This theory says that when an organ is put to constant use then it develops very well. If an organ is not used for along time it gets reduced and in due course it degenerates and disappears completely from the organism. Lamarck quoted many examples in support of his use and dis-use theory they are as follows.

Examples for use theory

1. **Giraffe**

Lamarck believed that the long neck and long forelimbs of giraffes are the results of continuous use he assumed that the ancestral giraffes were provided with short neck and the short limbs and hind limbs with uniform length. They are grazing animals which feed on grass. On those days due to scarcity of grass the ancestral giraffes were forced to feed on the foliage of trees. So

they tried to strengthen their neck and tore limbs to reach the foliage of trees. This results in a slight increase in the length of the neck. The process of stretching the neck, continued generation after generation to get the foliage of still taller trees and this resulted in longer neck leading to the origin of modern giraffe.

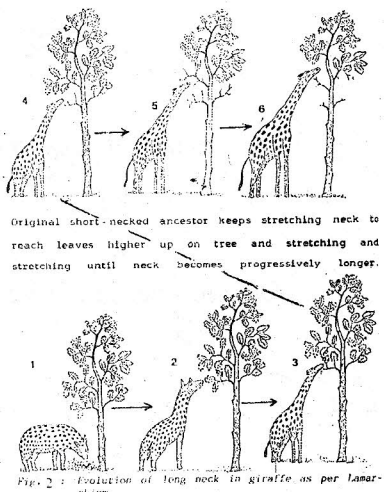


Fig. 2 : Evolution of long neck in giraffe as per Lamarckism.

2. **Web Foot**

Web footed aquatic birds derived from the non-web footed birds. The aquatic birds float on the water by propelling the water with their toes. In this process the toes were spread out generation after generation and as the toes were spread cut, the skin between the toes widened. This widened skin between the toes after many generations developed in to a web.

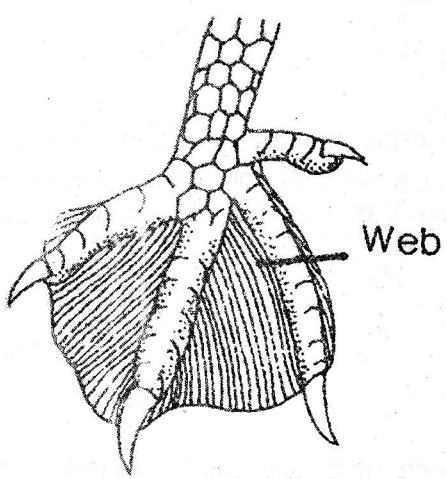


Fig. 3 : Web-foot of an aquatic bird.

3. **Biceps muscles**

In blacksmiths the biceps muscles (the muscles in the upper arm) are very well developed. This is because the black smith has to put more effort to his hands than to any other parts of the body.

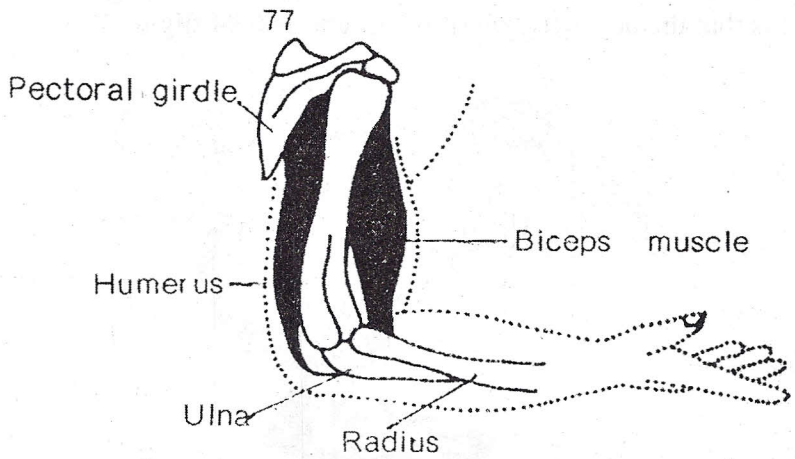


Fig.4 Hand showing biceps muscles.

4. **Water birds**

Wading birds (water birds) always try to keep their body above the water level. The continuous stretching of the leg in order to keep their body above water results long legs.

Examples for disuse theory

1. Eyes of cave animals

Eg; Proteus arguineus (Amphibian); this animal lives in caves. The cave is identified by complete darkness. In the absence of light, the cave animals cannot use their eyes. Since the eyes are not used for many years, the eyes became degenerated and in extreme cases eyes disappears completely.

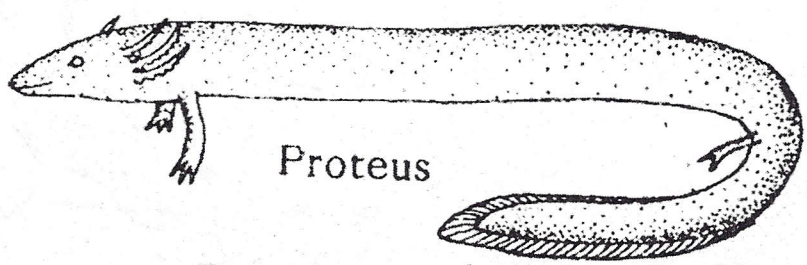


Fig. 5 : Proteus. The eyes become rudimentary becaus of dis-use.

2. **Flight less ness**

Eg:- Kiwi, a flightless bird of New Zealand and other such of flight less birds are the good examples. Evidence informs that kiwi has descended from normal flying birds. When they first came to New Zealand they were good fliers during that time these were no enemies on the land in New Zealand. Hence they lead a peaceful life and had no chance to use their wings. This happened for many generations. Result is that the degeneration of wings and loss of flight.

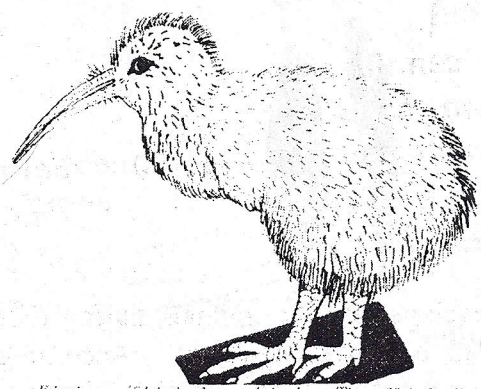


Fig. 6 : Kiwi, a flightless bird. The flightless condition is the result of dis-use of wings.

3. **Vestigial organs**

The vestigial organs of man and other animals are the result of continuous dis-use eg:- Coccyx, ear muscles, limbs in snakes, Plica semilunaris in man.

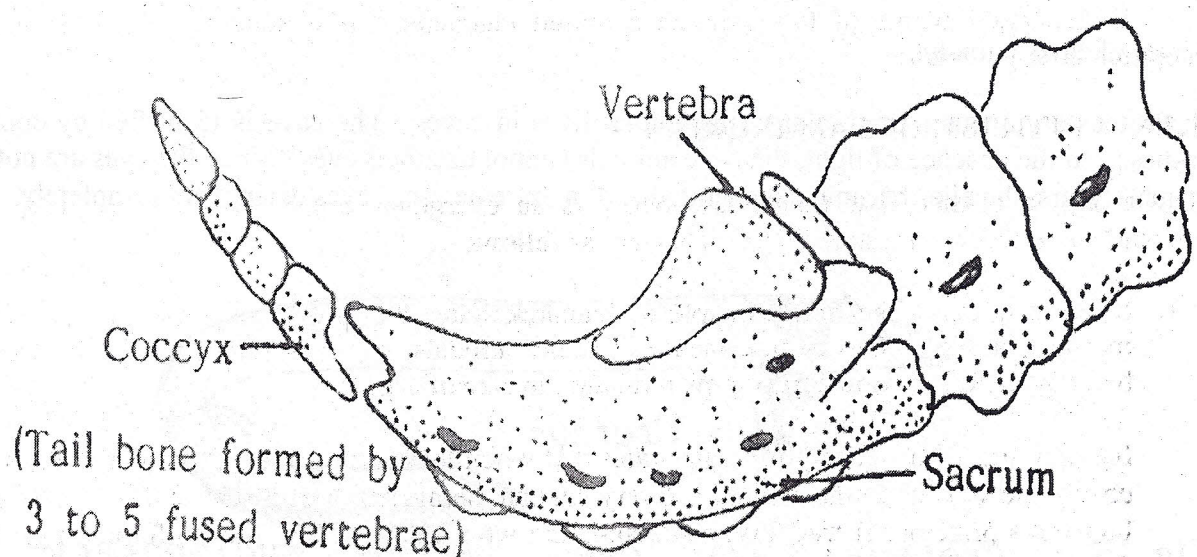


Fig. 7 : Coccyx in man.

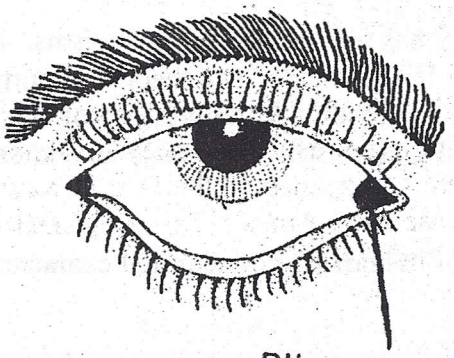


Fig. 8 Eye of man to show rudimentary nictitating membrane.

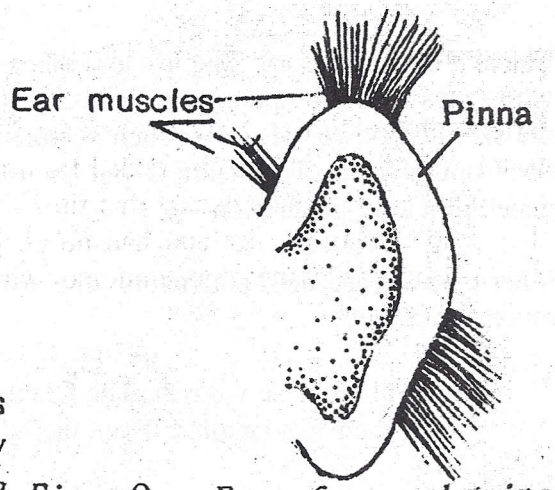


Fig. 9 Ear of man showing vestigial muscles.

4. **Inheritance of acquired characters**

The characters developed by the animals during their life time in response to the environmental changes are called acquired characters. According to Lamarck, these acquired characters are transmitted to the off spring this process is known as inheritance of acquired characters.

Eg; Giraffe

Long neck of giraffe is an acquired character it is transmitted to the off springs generation after generations.

Eg; Kiwi

Degenerated wings of kiwi are an acquired character. It is inherited by their progeny generation after generation

5.1.3.4 CRITICISM OF LAMARCKISM.

C.D Darlington opened that Lamarck's theory is an evergreen superstition. There were many criticisms for and against Lamarckism. They are as follows.

1. Increase in size: The first principle of Lamarck states that organisms have the tendency to increase in size. This is true incase of many animals, but it is not universally applicable because there are instances to show reduction in size of organs.

2. Internal urge: The second principle states that when there are new needs new structures form up on the desire of the animal. Every human being has the desire to fly in the air. If Lamarcks principle is true, every human-being who desires to fly, should form wings; but it does not occur.

3. Use and dis-use theory: The use and dis-use theory has met with strong criticisms. A person who reads books frequently, uses his eyes more. If Lamarck's "use theory" is correct, then the size and power of eye in such persons should increase with the increase of age; but this does not happen. The heart is put to use continuously; still it remains in the same size generation after generation.

4. Inheritance of acquired characters: The principle of in heritance of acquired characters is also much contradicted.

    a. Feet of Chinese woman: The Chinese girls are allowed to wear iron shoes; it order to keep their feet smaller. Even then the young ones have their feet in normal size.

    b. Circumcision: Circumcision (cutting off the prepuce of penis) is practiced by Jews and muslims even from the very ancient times. Still muslims are born with normal prepuce and everybody has to repeat circumcision.
    c. Bored ears: The ancient Indian women bored their ears to wear ornaments. If Lamarck's principle is correct then almost all the Indian girls of the present day should have inherited bored ears from their parents.

    d. Scars: The scars, acquired by the soldiers in the battle field are not inherited by their sons.

    e. Tail of mice: August Weismann cut the tail of mice continuously for about twenty generations. If Lamarcks principle of inheritance of acquired characters was correct then in the 21st generation all the mice should have been tail less. But the mice were born with tails with normal length.

Germplasm theory: The germplasm theory of August weismann gives another big blow to Lamarckism. The germplasm theory states that each organism is formed of 2 types of cells, viz, Somatic cells; germ cells, the somatic cells constitute all the body cells except the germ cells of gonads. They perish with the death of the animal. Any character, acquired by somatic cells cannot be transmitted to the off spring that is why the scar of a soldier is not inherited by his son. The germ cells (gametes of parents) alone are transmitted from parents to off springs, According to weismann any change that occurs in the germ cells alone is transmitted to the off spring. But Lamarck could not differentiate between the germplasm and the somatoplasm. He said that all acquired characters are heritable. But, according to germplasm theory, the characters or changes that occur in the germ cells alone are inherited.

5.1.4 Neo – Lamarckism

5.1.4.1 Introduction

Lamarck was the first biologist to postulate a theory on evolution. Despite strong objections to the

who contributed their own experiments modified the original theory of lamarck in the light of recent developments in biology rectifying almost all the defects. This modified theory of lamarckism is called Neo-lamarckism. The biologist who contributed their ideals for Neo lamarckism are called Neolamarckians. A few of them are spencer, cope, Giard Naegali, MC Dougall Osborn, Cannon, Tysenko, Summer etc.

5.1.4.2 Experimental evidences

Lamarckism is supported by a number of experiments conducted by biologists. A few are given below:

1. Inheritance of Bony plates in stickle back; the best example that illustrates the inheritance of acquired characters is provided by the fish stickle back, Gasterosteus. This fish inhabits fresh water, seawater and brackish water. The marine stickle back has 20 to 30 long plates on the mid dorsal line of the trunk; these bony plates seem to project the fish from tides and waves and salt. The fish form tides and waves and salt. The brackish water fish has 3 to 15 bony plates; the fresh water fish has no bony plates. When a fresh water stickle back is brought in to the fresh water, it loses all the bony plates. This plate less condition is also inherited by the off spring.

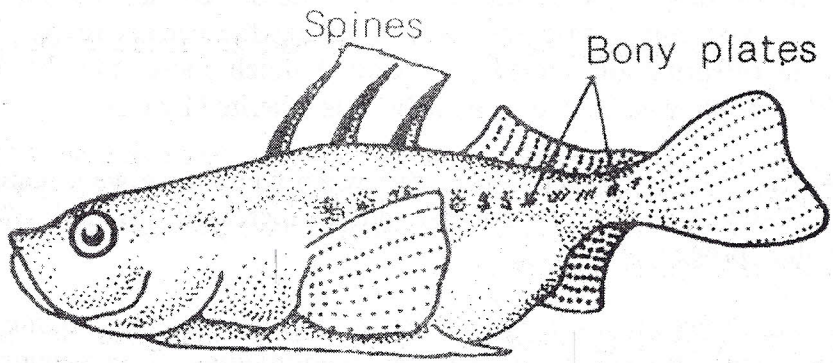


Fig. 10: A stickle back showing bony plates.

2. **Effect of Antiserum**: Guyer and Smith injected the lens extract of rabbit in to a fowl. This antiserum was injected into pregnant rabbits. Some off springs of these treated rabbits exhibited defects of eyes and these defects were inherited for many generations.

3. **Rats on Rotating Cages**: Griffith and Detleofsun conducted a fascinating experiment in support of inheritance of acquired characters. They reared rats on a rotating table for several months. In the beginning the rats found it difficult to live on the rotating table; but in course of time, they adjusted to live in that condition. After several months, when the rotation was stopped the rats exhibited dizziness, and they appeared to be mentally confused. These rats

were allowed to reproduce. The off spring also exhibited dizziness and irregularity in the gait. This is an acquired character inherited by the off spring.

4. **Inheritance of Pigments and Eye;** Kammerer conducted experiment in the urodele amphibian Proteus anguineus. It inhabits caves where there was no light. Hence proteus is colorless and the eyes are rudimentary and non-functional Kammerer brought the proteus to day-light. The skin gradually became black by the development of black pigments and eyes became prominent and functional. These characters were inherited by the off spring. (Fig – 5-2; p.g-81; publications).

5. **Training Rats**: MC Dougall in 1938 performed an experiment using rats to prove the inheritance of acquired characters. He allowed a few rats in to a trough containing water. The trough had 2 routes for the rats to escape; one route was lighted and the other remained dark. The lighted route was provided with on electric shock and the dark route was free from shock. The rats tried to come out through the lighted route. But they could not succeed because of electric shock. After repeated trials the rats learned to come out through. The dark route. Then the off spring of these trained rats were given the same type of training. The experiment was repeated for 45 generations. MC Dougall found that there was a gradual decrease in the number of trails to find out the correct route generation after generation. In the first generation the rats received about 35 shocks before coming out and in the 45the generation they received only 3 to 5 shocks in selecting the correct route. MC. Dougall concluded that the offspring of trained rats learned much more quickly than those of untrained rats. Thus he showed how learning habit was inherited by rats.

6. **Effect of temperature;** F.13 summer showed that when white mice were reared at a higher temperature (20 -30^{0}C) their body, hind limbs and tail increased in length. This character was inherited by their progeny.

7. Potato Beetle; Tower showed that when potato beetles were exposed to extreme conditions of moisture and temperature, the off spring exhibited abnormalities. These abnormalities were inherited generation after generations.

8. Germ Plasm Theory: The Neo-Lamarckians considered the germ plasm theory of August Weismann to be in their favour. They argued that hormones secreted by the gonads (Germ cells) bring about changes in the somatoplasm similarly the somatic cells are influenced by the environment, and in response, somatic cells secrete some secretions; the secretions are taken to the germ plasm of the germ cells where they bring about changes in the germ cells and these are inherited. Thus Neo-Lamarckism argues that profound changes, brought by environment, in course of time, get imprinted in the germplasm, and thus become heritable changes, which, in turn, be passed on to off spring of succeeding generations.

9. Jennings made many experiments up on paramecium. He noted that of the environment was changed the characters had modified and if again the original environment was provided, the modified disappeared.

10. Kammeres also studied the effect of colored surroundings on the coverer of slain of spotted salamander he placed some salamander in black surroundings while few others in yellow surroundings. The surroundings developed their respects colors in salamander and the same character was then inherited to their off springs. William Bateson questioned the validity of the kammeres work. Kingsley noble reported the fraud done by kammeres by colouring them with Indian ink that is why kammerer committed suicide. The above facts indicate that there is definitely some truth in Lamarckian theory. The modern view is that only those characters are illusted which affect the germplasm while others affecting somatoplasm are not inherited to their progeny

The appeal of Lamarckism has persisted because it seems that many of the more remarkable adaptations could have arisen only in direct response to the environment or to the needs of the organism. These are phenomena in evolution which some believe to suggest that what ever may be said against it, there is some truth in Lamarckism of inheritance and it is possible in future, truth may be formed in it and the Neo-Lamarckians may be justified.

5.1.5 Summary of the Lesson

Jean Baptiste Lamarck (1744-1829) was a French evolutionist. He had proposed an evolutionary theory is popularly known as Lamarckism or inheritance of acquired characters. According to Lamarck the environment plays an important role in the evolution of living organisms. Environments provide a new need to the organisms. In response to new needs, the organism develops a new structure. The developments of new structures during its life time in response to the environment are called acquired characters. These acquired characters are transmitted to off spring. Another aspect to be dealth with by Lamarck, was that use and disuse theory. According to this theory if an organism uses the organ, it develops very well. If the organisms does not use or disuse the organ it disappears or degenerates from the organism it self.

Another principle of Lamarck is the "Internal urge" of the organism. He says each organism has the desire to develop new structures in response to the environment. This is happened due to the internal desire of the organism.

The first principle of Lamarck says that each organism has the ability to increase in size and in volume. It could be applied to many of the animals and plants except a few.

5.1.6 KEY TERMINOLOGY

1. Acquired Characters: The characters developed by animals during their life time in response to environmental changes.

2. Evolution: A culminate change in the character tics of populations or organisms occurring in the course of successive generations related by decent.

3. Lamarckism: An evolutionary theory proposed by Lamarck which states that acquired characters during the life time of animals are inherited by their descendant.

4. Internal urge: A strong desire esp. one which is difficult or impossible to control.

5.1.7 SELF ASSESSMENT QUESTIONS

1. Why do creation hypothesis not form research tools that lead to new understandings?
2. Lamarckism is purely based on the assumptions that the environments are the determining factor in evolution of living organism.
3. Lamarckian theory of inheritance of acquired characters has many contradictions.

5.1.8 Reference books

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UNIT-V

LESSON-5.2

DARWINISM

CONTENTS

5.2.1 INTRODUCTION

Charles Darwin (1809-1882) was an English Naturalist. He was the first person profounded the theory of Natural selection or Darwinism. He was born in Shrewsberry, on February 12, 1809 in England. The career of Charles Darwin began with his voyage on HMS Beagle (December 27, 1831 to October 2, 1836) across the globe. He visited Cape Verde and other Atlantic islands, New

1831 to October 2, 1836) across the globe. He visited Cape Verde and other Atlantic islands, New Zealand, Australia, Tasmania, Mauritius and Brazil. He spent about 5 weeks in the Galapagos Archipelago. In 1858, Charles Darwin and Alfred Wallace presented a paper to the Linnaean society of London in which they coined the term evolution to describe the progressive changes in successive generation of living organisms. Charles Darwin published more than 15 journals on books and various branches, like Geology, Botany and Zoology. Of these, the most important one to Darwin is the origin of species in 1859. The evolutionary idea determined by Charles Darwin is called 'Darwinism' or 'Natural Selection Theory'. This theory forms the mechanism of evolution. Thus, the theory of evolution helps to explain two things:

(a) Similarities between related organisms.

(b) The differences between the organism due to the result of variation inherited from one generation to the other.

Fig. 5.2.1 Charles Darwin in 1840. By this time he had worked out the main points of the paradigm of evolution. He is 31

Darwinism consists of five principles. They are as follows:

1. Prodigality of production
2. Struggle for existence
3. Variations
4. Survival of the fittest, and
5. Origin of species

5.2.2 DARWINISM OR NATURAL SELECTION THEORY

5.2.2.1 Prodigality of production

Every organism has potentiality to increase its number in geometrical ratio. This is called prodigality of production. This leads to over production. The examples are as follows:

1) **House fly**: A pair of common house-flies breeds in April and if all eggs hatched and all the resultant individuals alive in turn by reproduction gives 191, 01 x 10^{16} descendants by August.

2) **Drosophila**: Each female lays 200 eggs and the fly completes the life cycle in 10-14 days. Therefore, if the production goes as such, in 40 days there will be about 200,000,000 flies.

3) **Paramecium**: Paramecium under goes binary fission every 16 hours. If all the daughter paramoecia of a single paramecium survive and reproduce at the rate of 3000 generation in five years, if all the descendant existed, their protoplasm would approximately equal to 10 times, the volume of the earth.

4) One mosquito fly reproduces 200 billion descendants in one summer.

5) **Oyster:** An oyster lays about 114,000,000 eggs in her life span. If all these eggs hatch out, and if all the offsprings reproduce for 5 generations, they will form a volume about eight times, the size of the earth.

6) **Ascaris**: A single Ascaris lays about 27 million of eggs in her life span. Among the lower vertebrates where no parental care is given to the young potential productivity is necessarily enormous.

7) **In** four herrings, number of eggs varied from 20,000-47,000 but in a **Cod**, there may be as many as 6 crore eggs. In **Turbot**, 9 crores and in Ling 28 crores eggs in a season.

8) **Salmon:** A Salmon (fish) produces 28,000,000 eggs in a season.

9) **Female** toad usually lays as many as 12,000 eggs.

5.2.2.2 Struggle for existence

Struggle for existence is most important check to keep the numbers constant. Due to excessive rate of production of organisms, there is an everlasting competition or struggle between the various individuals for food, space and other requirements. This competition is called struggle for existence. There are 3 types of struggles:

a. Intraspecific struggle
b. Interspecific struggle
c. Environmental struggle

a. Intraspecific struggle

This is the struggle among the individuals of same species. When the members of the same species are living in the same environment, their needs and requirements are also same. Hence the competition is keen.

Eg. When there is a piece of flesh, dogs fight among themselves to get it. Similarly the seedlings growing under a tree compete among themselves for food, light and other requirements.

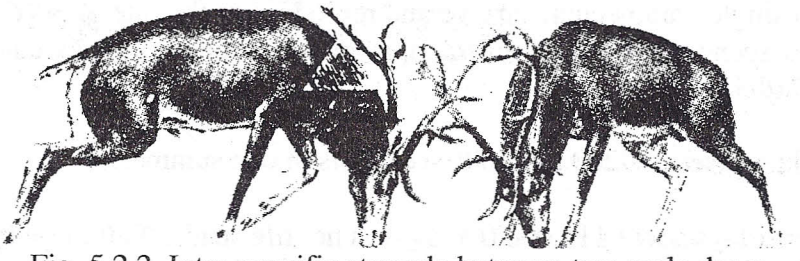

Fig. 5.2.2 Intra-specific struggle between two male deers

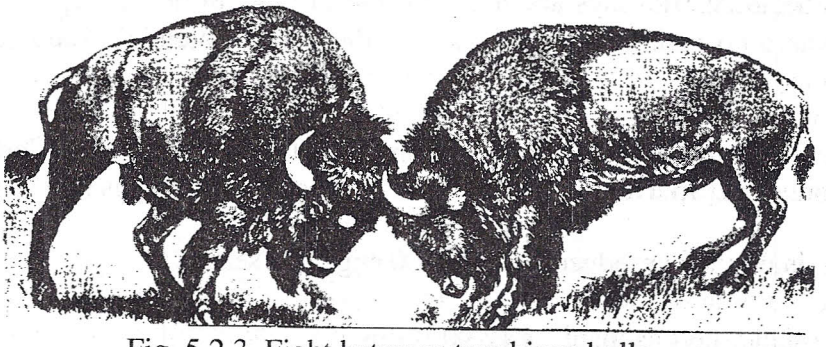

Fig. 5.2.3 Fight between two bison bulls

b. Interspecific struggle

This is the struggle among the individuals of different species. For **eg.** Rabbit is preyed upon by a Fox, beiging eaten away by Tiger. In this way, a struggle continues between aggressor and a victim. Another example for interspecific struggle is between an Ostrich and a Lion.

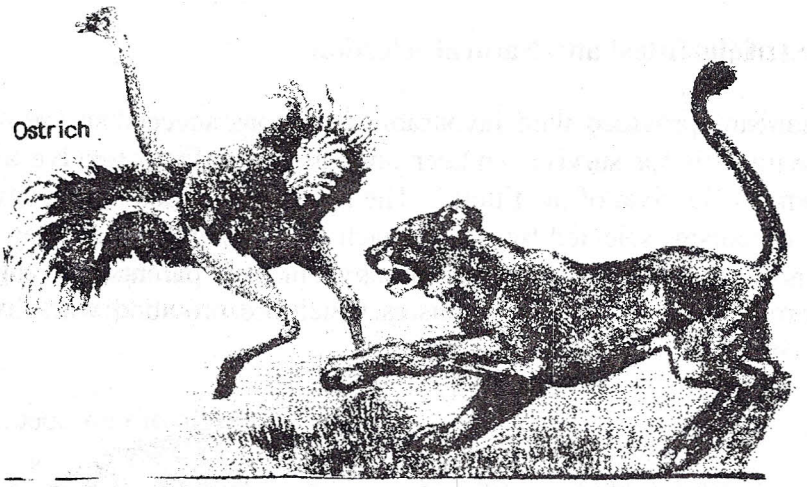

Fig. 5.2.4 Inter-specific struggle between an Ostrich and a lion

c. Environmental struggles or Extra Specific

The competition between animals and environmental factors is called environmental struggle. The environmental competition is caused by the following factors.

1) Shortage of food, water and oxygen, 2) Lack of light, 3) Predators, 4) Lack of shelter, 5) Disease, 6) National calamities.

All these factors constitute environmental resistance. It checks the reproductive potential of the population and keeps the population from growing indefinitely.

5.2.2.3 Variation and Heredity Transmission

Variation is defined as the difference in character between individuals. No two individuals are exactly alike. Variation occur among the offsprings of the same parent. Even in the same organism variation can be observed. **For eg**. in plants, one leaf is different from the other in size, shape, venation etc. The length of the same fingers of the two hands may differ in some persons. Darwin considered these variations are transmitted to the next generation while unfavourable variations are eliminated. Darwin described two types of variations. They are favourable and unfavourable variations.

a. Favourable variations: These are the characters which are useful to the possessor in the struggle for existence.

eg:- The strength and aggressiveness of dogs, the high speed of a deer.

b. Unfavourable variations: These are the characters which are useless or harmful to the possessor in the struggle for existence.

5.2.2.4 Survival of the fittest and Natural selection

The organisms provided with favourable variations succeed in the struggle for existence. Those organisms are fit for survival. Other animals are unfit to survive and they perish. This process is known as "Survival of the fittest". The fitness is decided by the environment and also by the nature. The organisms selected by nature reach sexual maturity and they reproduce offsprings. These offsprings receive advantageous variations from their parents. So the environment acts as the selecting force. It selects those organisms which are provided with favourable variations is called "Natural Selection".

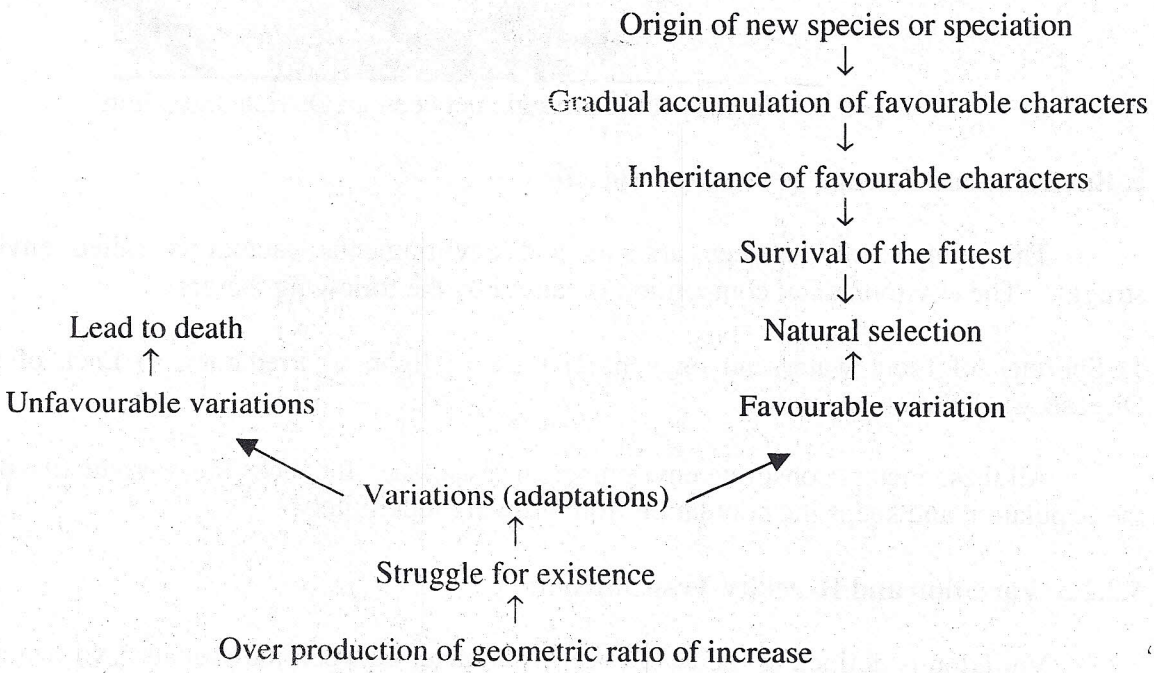

Fig. 5.2.5 Central theme of Darwinism

5.2.2.5 Speciation or Origin of the new species

According to Darwin, adaptation of survivors to new environment may lead to the formation of new structure and modes of behaviours. Thus, the organisms generation after generation, will show new forms and thus latest forms will be regarded (0-6) as new species. As such Adaptations with respect to the environment variation will lead to the origin of new species. As such, Darwin thought that possible new species might have arisen from the old ones with the difference of lines of descent, which produced varieties, incipient species and then species themselves.

5.2.3 DARWIN'S EXAMPLE OF NATURAL SELECTION

Darwin quoted **Giraffe** as an example. In ancient times, the Giraffes were grazing animals, eating grass. When there was scarcity of grass, they had to depend on the leaves of trees. Darwin believed that there were short necked and long-necked Giraffes in ancient time. In other words, there were variations in the length of the neck of giraffes. The Giraffe provided with a long neck and long fore limbs were able to reach the leaves of trees and were getting food, the giraffes which were provided with a short neck and short fore limbs could not reach the leaves of trees and hence starved and died.

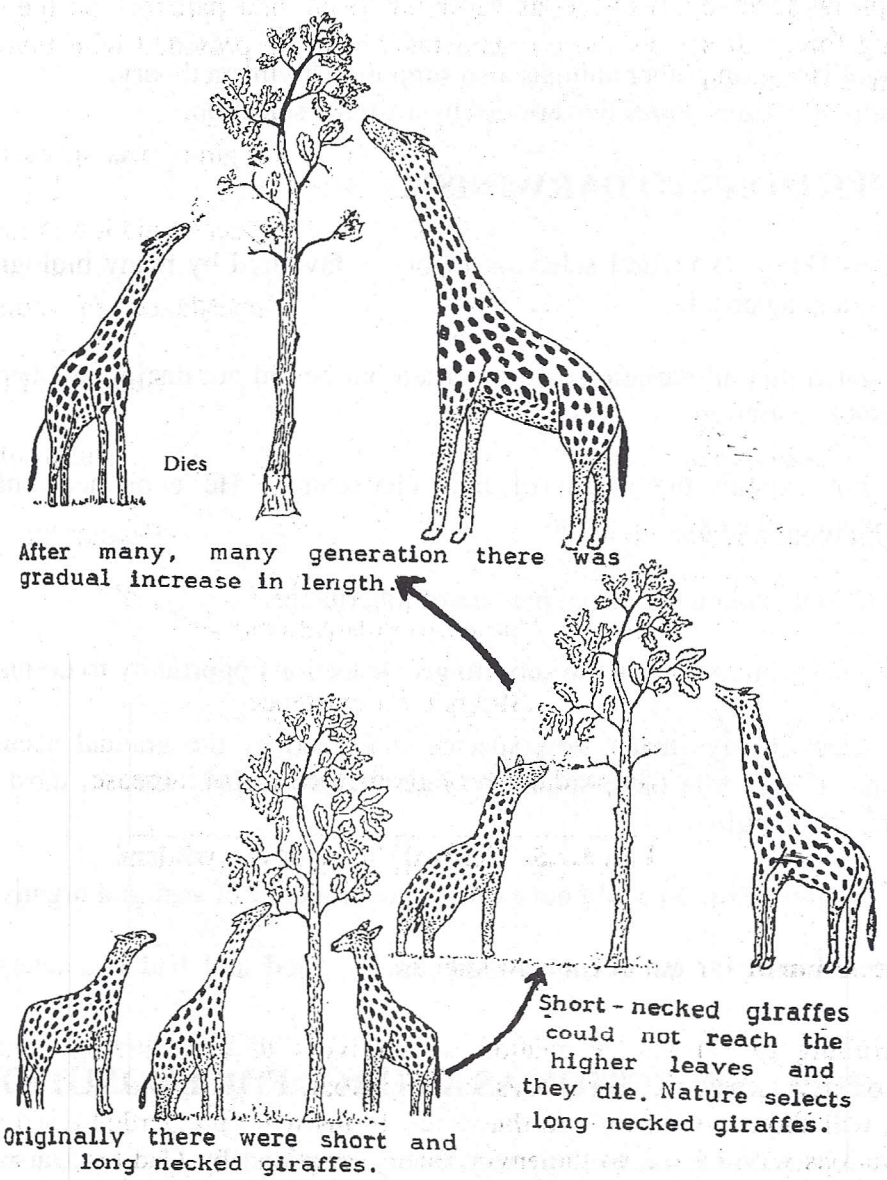

Fig. 5.2.6 Evolution of long neck in giraffe as per Darwinism

Thus in the struggle for existence the Giraffe which were provided with favourable variations long neck and long forelimbs were selected by nature and were allowed to survive and others were eliminated from the earth. This happened generation after generation and that led to the development of long neck and long forelimbs of the present day Giraffes.

5.2.4 EVIDENCES IN FAVOUR OF DARWINISM

There are a number of evidences which favour of Darwinism. Some of them are given below:

1. Pedigree of Horse and other animals also support Darwinism theory.
2. Production of various domestic varieties by artificial selection.

5.2.5 OBJECTIONS TO DARWINISM

Charles Darwin's natural selection theory is favoured by many biologists. Still there are objections for and against it.

1. Darwin stated that all variations are inherited but he did not distinguish between heritable and non-heritable variations.

2. He did not explain the origin of new characters. He explained only the progressive improvement of the existing organs.

3. Darwin did not explain the actual process of inheritance.

4. The geological time has been too short to give selection opportunity to do his work.

5. Natural selection says that a new species is formed by the gradual accumulation of useful variations. If it is true there should be intermediate forms. But, in most cases intermediate forms are not identified.

6. Darwins natural selection could not explain the frequence of vestigial organs in some animals.

7. Selection depends on the organisms of sum of good and bad characters and not a single character.

5.2.6 NATURAL SELECTION AS A FORCE FOR EVOLUTION

Natural selection is an evolutionary theory proposed by Charles Darwin. It explains the mechanism of evolution and the origin of species. Natural selection refers to the process of selection of better adapted organisms by the environment. The better adapted organisms are provided with favourable variations. The nature of environment allows them to reproduce. Hence

more and more adapted organisms are produced. The synthetic effect of this process leads to the formation of a new structure that in turn leads to the origin of species.

Types of natural selection

There are three basic types of natural selections. They are:

1. Stabilizing selection (or) normalizing selection
2. Directional selection (or) Progressive selection
3. Disruptive selection (or) diversifying selection

5.2.6.1 Stabilizing selection

It is one of the natural selections, which plays a role in the unchanging environment. It operates in the population when the environment remains constant or normal. Mutations are of no use to a species living in that environment. This maintains a genetically constant population. Stabilizing selection favours the normal individuals, but it eliminates the individuals with specialised characters. Stabilizing selection arrests evolutionary change. As this selection is maintaining a constancy in the gene pool of the population, it is called stabilizing selection.

Salient features:

The stabilizing selection has the following salient features:

1. Stabilizing selection operates when the environment remains constant.
2. It promotes a genetically constant population.

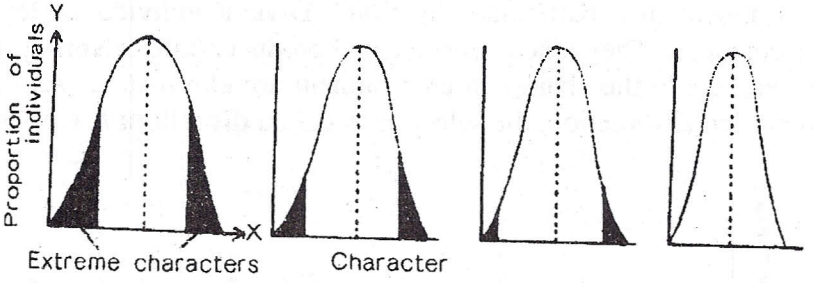

Fig. 5.2.7 Stabilizing selection. It eliminates extremes

1. Differential mortality in sparrows:

In the winter of 1898, the english sparrows were subjected to severe snow, rain and acute storm. Bumpux brought 136 affected English sparrows into his laboratory at Brown University. Out of these birds, 64 birds died and the other 72 birds revived. He measured the weight and length of the body, wings, head and beak of all the dead and living birds. He calculated the mean value of

weight and length submitting for the dead and living birds. He found that the measurements of surviving birds were closer to the mean. He concluded that the death of the sparrows was due to their weight and length which extremely deviated from the mean.

2. Differential mortality in Babies

Karn and Penrose weighed a large sample of human babies. He calculated the mean value of the weight of babies. The mean values was 8 pounds. They found that the high percentage of mortality was among those whose weight was much greater than 8 pounds or lesser than 8 pounds.

3. Experiment on Red checker moth

In red checker moth *Panaxia dominula.* "Pale wing" is an abnormal character. F.B. Ford raised a popualtion of this moth with pale wings by artificial selection. These moths were released in an area where none of this species existed. After five years, the descendents produced a population having normal wings. The genes for pale wings were reduced in frequency and finally eliminated by stabilizing selection.

3. Swiss starlings

The ornithologist David Lack observed that the optimum and model number of eggs in the nest of Swiss starlings is five. He observed higher mortality in the nests. Where there were more than the normal number of 5 fledgelings. This mortality was due to poor nutrition. To operate stabilizing selection, the optimum number of nestlings was always maintained even though the reproductive ability was greater.

5.2.6.2 Directional selection (Progressive selection)

Directional selection produces a shift of the population in one direction. This is due to a change in the environment in a particular direction. Diviant individuals develop adaptation in response to the environment. They tend to survive and produce more offspring; However, the other individuals do not respond to the change in environment are eliminated. As this selection brings about changes in a particular direction, the selection is called **directional selection.**

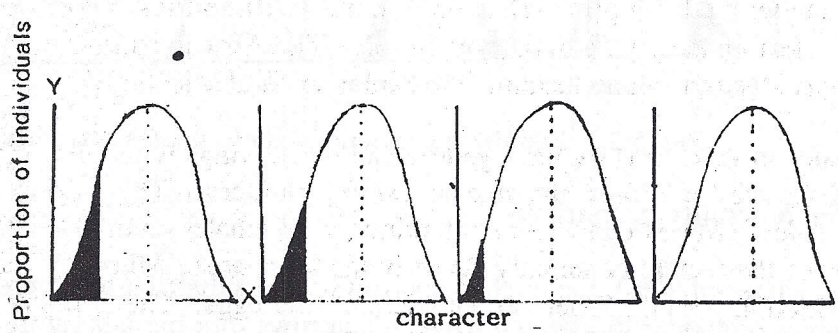

Fig. 5.2.8 Directional selection. It produces adaptive change in a particular direction by the gradual elimination of one phenotype in favour of other.

To summarise, the directional selection produces a genetically changed population favours the accumulation of mutations that would increase the fitness of species in the changing environment, and also favours the specialised individuals, but eliminates the normal individuals. It brings about progressive evolutionary changes.

1. Industrial melanism:

The best example to illustrate directional selection is industrial melanism. It is exemplified by the peppered moth *Biston betularia* of Great Britain. About 145 years ago, the surroundings were light coloured. This light colour is inhabited by white coloured moths. As the industries were developing, soot deposited on the surroundings. This makes the environment from light to dark colour. In modern days that area is pre-occupied by a population of black moths replacing the original white moths.

2. Resistance of Bacteria to Drugs

Directional selection was illustrated by the experiments of L.L. Cavalli and G.A. maccacro on the colon bacteria *E. coli.* Bacteria which do not show resistance to chloramphemical died when exposed to the drug while the resistant varieties of the bacterium continued to live even at 250 times the concentration. This is an example of directional selection according to natural selection.

3. Resistance of Insects to DDT

The development of resistance to DDT by house flies stands as another example of directional natural selection.

5.2.6.3 Disruptive selection

When natural selection favours two (or) more phenotype modes, it is called **disruptive selection**. It occurs when the environment is heterogeneous consisting of different microhabitats. Disruptive selection increases genetic divergence.

For example: the Disruptive selection in mimetic Butterflies. In many natural populations, the disruptive selection is playing an important role. The African species of swallow tail butterfly, *Papilio dardanux* stands as a good examble to explain natural selection.

The males of this butterfly have yellow and black wings with tails. Tails are absent in the females. The female butterflies are able to exhibit mimicry. This is one of the characteristic features of females. Males cannot exhibit mimicry. Females mimic in different ways several different forms of the female commonly occur in the same area. Mimicry in females is associated with the reproduction period. When the female is laying eggs. The mimicry in females offers an adoptive values of protection during egg laying. The absence of mimicry in males makes them sexually acceptable to the females.

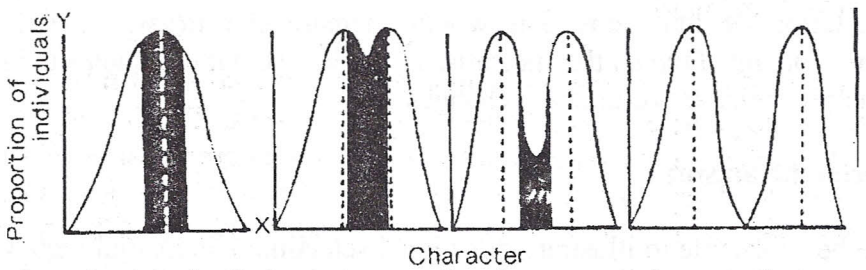

Fig. 5.2.9 Disruptive selection. It eliminates intermediate forms producing two divergent populations

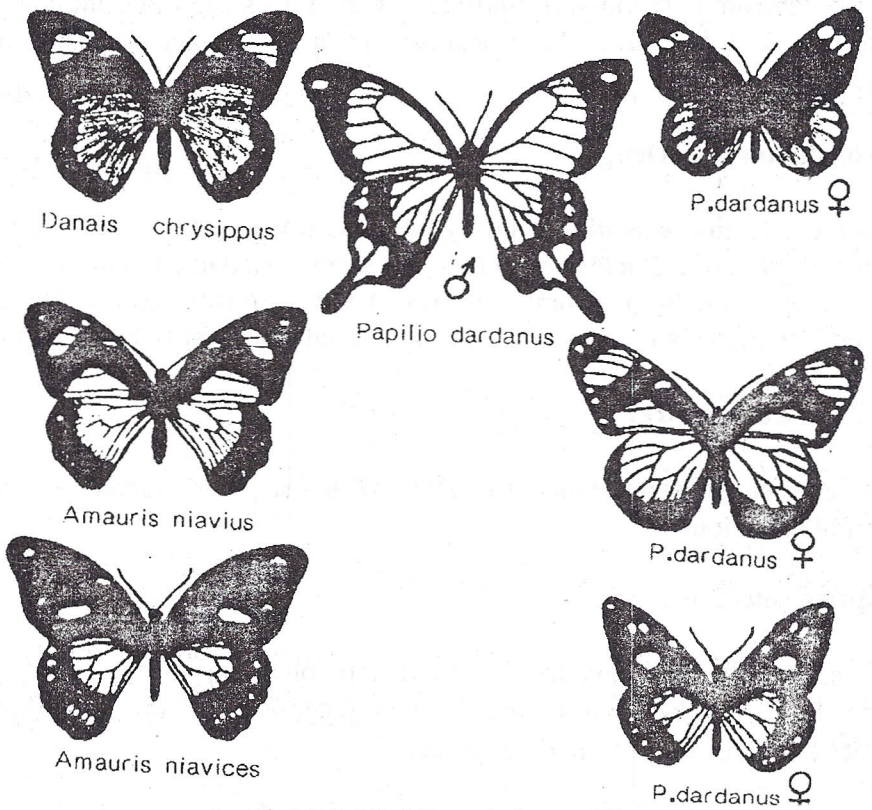

Fig. 5.2.10 Mimetic butterflies

5.2.7 SUPPLEMENTARY THEORIES OF CHARLES DARWIN

Darwin proposed three additional theories to explain certain facts which cannot be explained by the natural selection theory. They are:

1. Theory of Pangenesis
2. Sexual selection theory, and
3. Artificial selection theory

5.2.7.1 Theory of Pangenesis

This theory explain the process of Inheritance. According to this theory, each cell of the body produces minute particles called **Pangenes** or **gemmules**. The Gemmules are produced by all the cells through out the life of an individuals. The gemmules are transported and deposited into the germ cells by the blood stream. Thus the germ cell contains a sum total of gemmules. During development each gemmule controls the development of the cell. This theory is not at all accepted now-a-days.

5.2.7.2 Sexual selection theory

In some species of animals, males differ from the females of the same species in their morphology. This is due to the presence of some specialized characters in males alone. These specialized characters or organs assist in reproduction. Hence, they are called secondary sexual characters. Some of the secondary sexual characters available in animals are as follows:

a. The brilliantly coloured features of pea-cock.
b. The vocal sacs
c. The nuptial pads of frogs
d. The mane of lions
e. The moustache and beard of man
f. The scent glands of mammals
g. The brood pouch of Hippocampus.
h. Musical sounds of birds, insects etc.
i. The antlers of male deer
j. The beautiful bands of colour on the body of tiger

The above characters cannot be explained on the basis of natural selection theory. If natural selection operates, here only males will be allowed to survive and all the females will be doomed to death. Hence, Darwin proposed the sexual selection theory to explain the existence of all these characters.

It states that females select males for mating. The selection is based on the presence of desirable qualities or attractive characters. Females give chance to mate. There is also competition among males to get the females. This competition leads to fights among the males in some species like **sea lions, Walruses** etc. In the fight, the males with unfavourable variations are driven away or killed.

(ii) Salient features of sexual selection theory

1. Males attract females by the development of secondary sexual characters.

2. The males provided with brilliant colours, conspicuous ornamentation, pleasing behaviour, mating dances, melodious songs and sounds are ore attracted by the females.

3. According to this theory, sexual selection is operated by only one sex i.e. the male. Hence, the males alone develop more desirable characters. But natural selection operates on both the sexes equally.

4. Sexual selection is concerned with reproduction while natural selection is concerned with the survival of the individual.

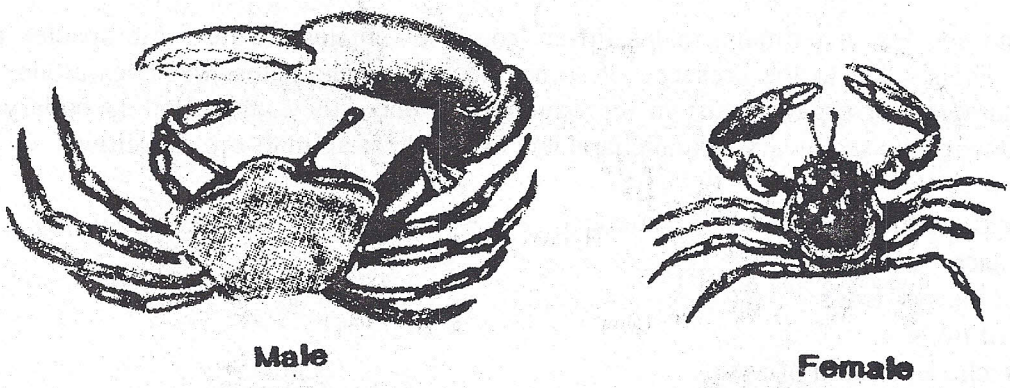

Fig. 5.2.10 Secondary sexual characters in fiddler crabs

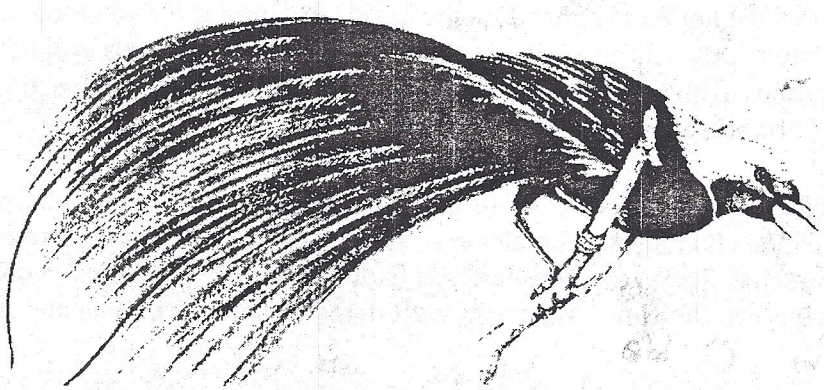

Fig. 5.2.11 Male paradise bird showing secondary sexual characters

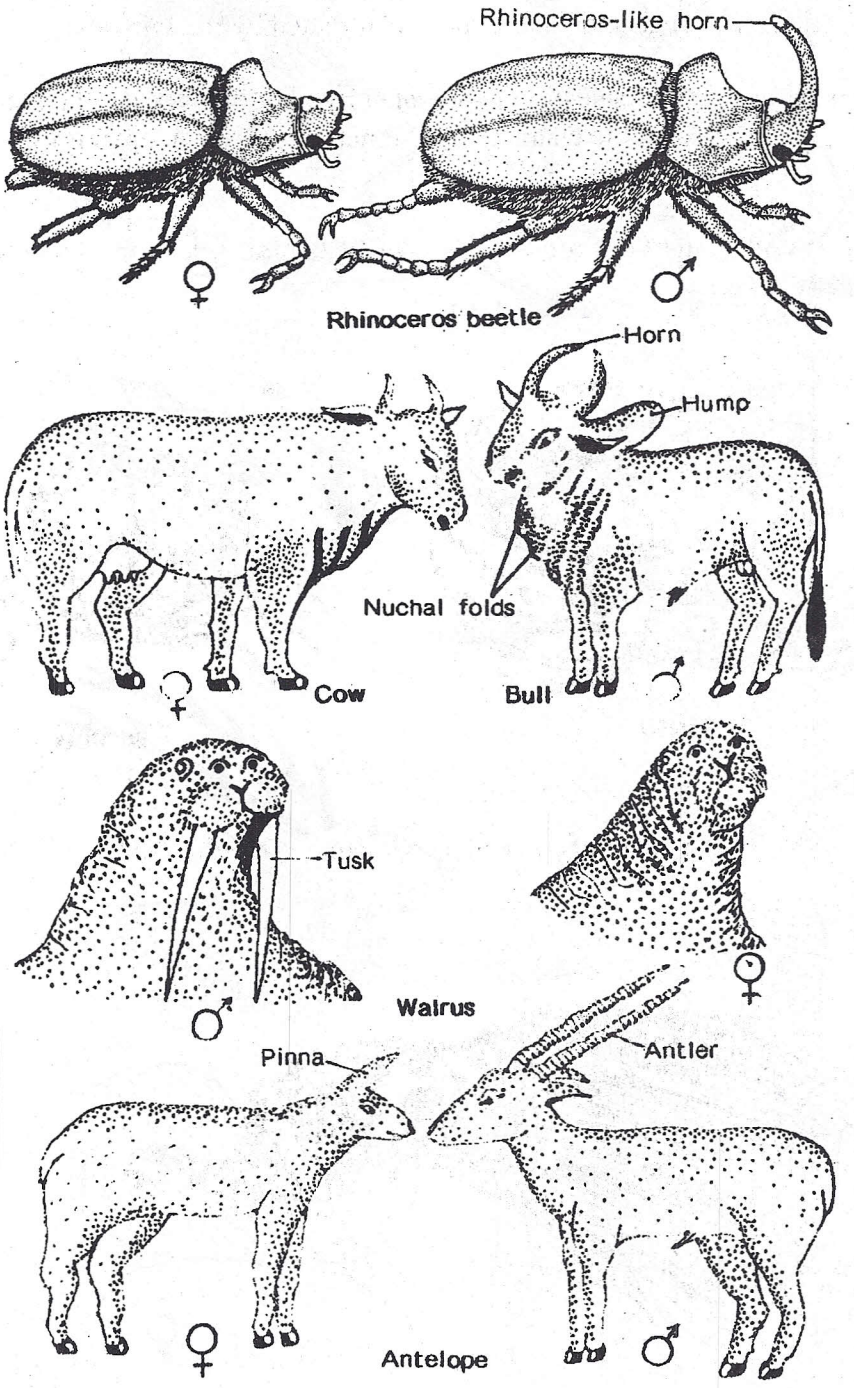

Fig. 5.2.12 Secondary sexual characters

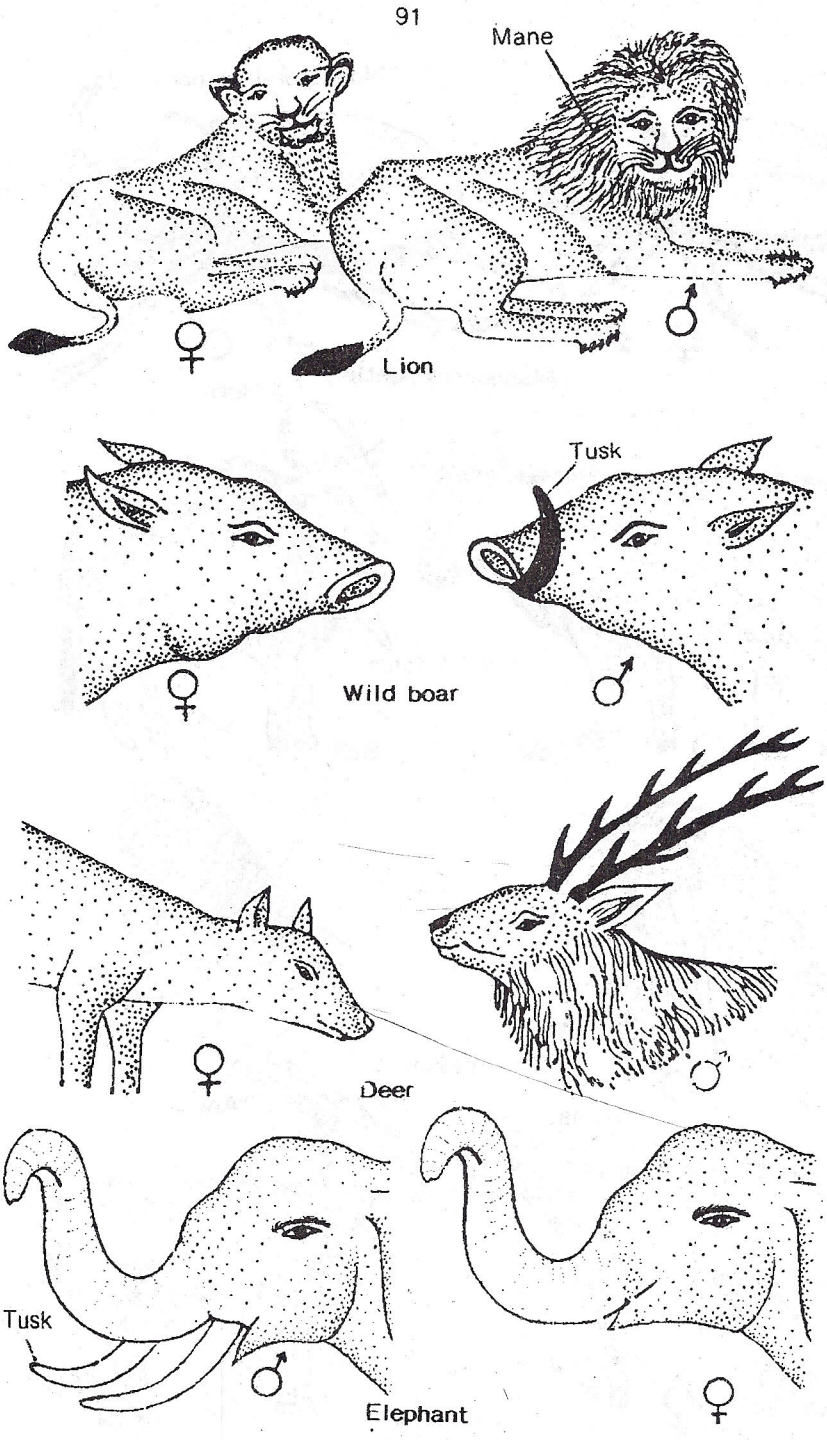

Fig. 5.2.13 Secondary sexual characters

5.2.7.3 Artificial Selection Theory

It is another supplementary theory proposed by Darwin. It resembles natural selection that the environment is the operative force and selects the animals with favourable and desirable characters. In artificial selection man selects the animals and plants with desirable characters. The desirable characters means the characters which are useful for the welfare of human beings.

For example, the production of a large number of eggs by hen, more quantity of milk by cow, good yields in crop etc. Desirable characters obtained by crossing different varieties.

Eg: Cows, poultry, poultry birds, dogs, crops and vegetables.

Darwin stated that if man can produce new varieties by crossing different varieties, then there is a possibility for the formation of new species in nature.

5.2.8 ALFRED RUSSEL WALLACE, THE FORGOTTEN EVOLUTIONIST

Wallace was a coworker of Darwin. He was a co-discoverer of natural selection theory. He was poor. He left school at 14. He was a school master. He contributed a paper titled *on the tendency of varieties to depart indefinitely from the original type.* Wallace went on an expedition of nature. He spent 8 years in the islands of Malayan Archipelago. He got the friendship of Darwin. They jointly published two research papers in 1859. The whole credit went to Darwin. Hence, he did not get honour and he became a forgotten evolutionist.

5.2.9 H.M.S. BEAGLE

HMS Beagle was a British ship. It was set for a voyage. The voyage was for 5 years (december 27th, 1831 to October 2, 1836). The ship went around the world and visited Cape Verde, galapagos islands, New Zealand, Australia, S. America, Tasmania, Mauritius, Brazil etc.

74 people travelled and Charles Darwin was one among them.

Darwin was the naturalist in the group. He was recommended and deputed by Dr. Henslow.

Darwin observed widely, carefully and took elaborate notes on a fauna, flora and the geological conditions of the various places. He spent 5 weeks in Galapagos islands. He was astonished by the giant land tortoises, finches (birds) and plant of sunflower family. He conceived his idea on natural selection only in Galapagos islands. In 1936, he returned to England. He published his observations in the form of scientific papers and books. The most important one is the origin of species by means of natural selection. Natural selection theory was born only because Darwin had the chance to travel in the ship H.M.S. Beagle.

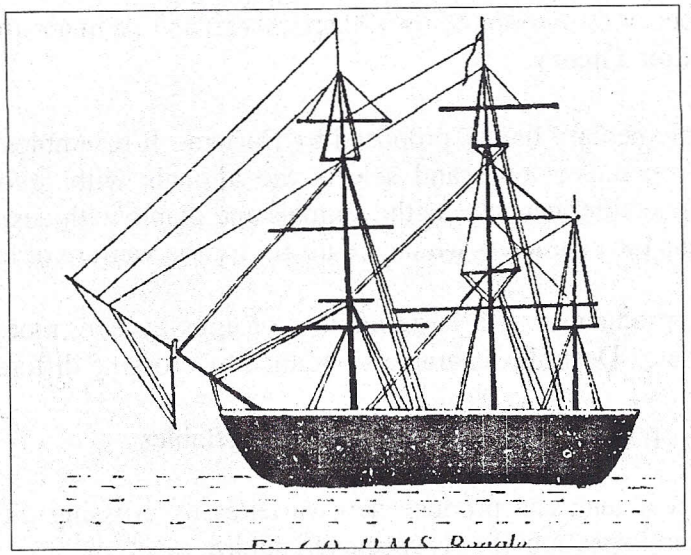

Fig. 5.2.14 H.M.S. Beagle

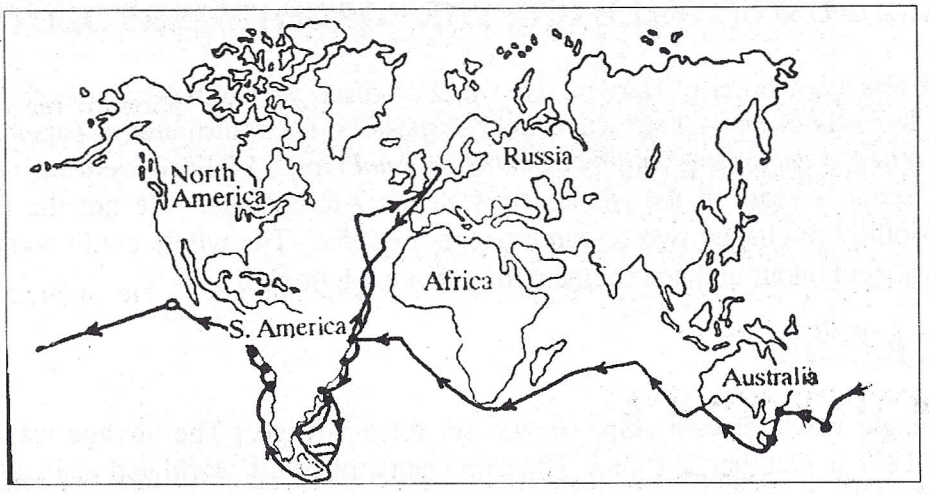

Fig. 5.2.15 The route sailed by H.M.S. Beagle

5.2.10 GALAPAGOS ARCHIPELAGO

Galapagos islands located in the Pacific Ocean in the west of South America. It is situated on the equator. The island gets its name from its giant land tortoises. It consists of 19 islands.

It is a group of oceanic islands. The islands have no land connections with the neighbouring continents. They originated by volcanic erruptions.

This archipelago became internationally famous after Charles Darwin visited in 1835. Amphibians are completely absent in these islands. Only one marine lizard, the marine iguana and it is living here only. The birds are represented only by 80 species. The bulk of land birds are

Darwin's finches. Insects are represented by 700 species. The mammals are represented by only 7 species.

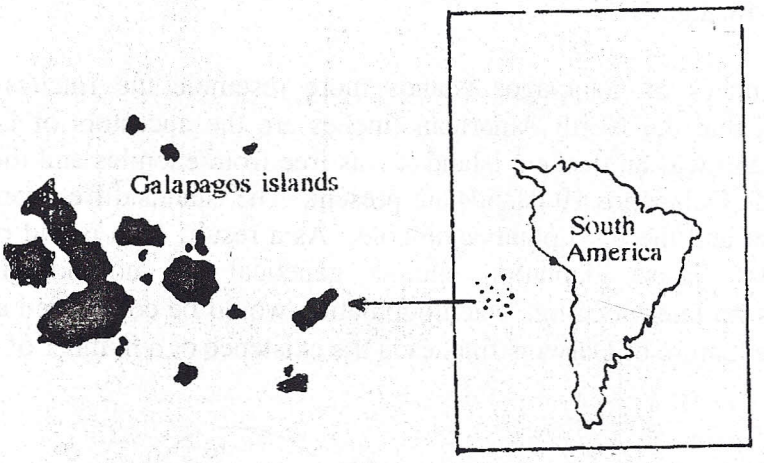

Fig. 5.2.16 Map showing the Galapagos islands

Galapagos animal life shown is of keen interest because of the following reasons:

1. Species have developed subspecies.
2. It contains high percentage of endemic forms.
3. The Darwin finches have developed adaptive radiation.
4. Though the islands are situated on the equator, species of antarctic origin, such as penguins, and fur seals live on these islands.

5.2.11 DARWIN'S FINCHES

Finches forms a group of small birds like sparrow included in the family *Fringillidae*.

The finches living in Galapagos islands are called *Galapagos finches*. Charles Darwin visited Galapagos islands in 1835 during his voyage on H.M.S. Begle. He spent there approximately 5 weeks. Charles got the inspiration for this views on the natural selection from the observation of these finches. The Galapagos finches are also called as "*Darwin's finches*". The Darwin's finches are known for their varying feeding habits. According to their feeding habits the beaks are variously modified.

Lack stated that "Darwins finches are dull in look. Only the variety of their beaks, and number of their species excite attention". According to their beak modification, the Darwin's finches are grouped into the following types:

a. Ground finches
b. Cactus ground finches

c. Vegetarian tree finches
d. Insectivorous tree finches
e. Woodpecker finches
f. Warbler finches

Darwin's finches of Galapagos islands more resemble the finches of North America. Hence, he believed that the North American finches are the ancestors of Darwins finches. As Galapagos archipelago was an oceanic island it was free from enemies and there were a variety of ecological niches. In Galapagos 19 islands are present. The islands differ from each other in many features of the event and the food plants available. As a result, each island population developed its own adaptations. These adaptations include genetical and morphological and behavioural changes. There was no interbreeding. Each population would be considered as a separate species. Another remarkable feature of Darwins finches is the existence of a number of sub-species.

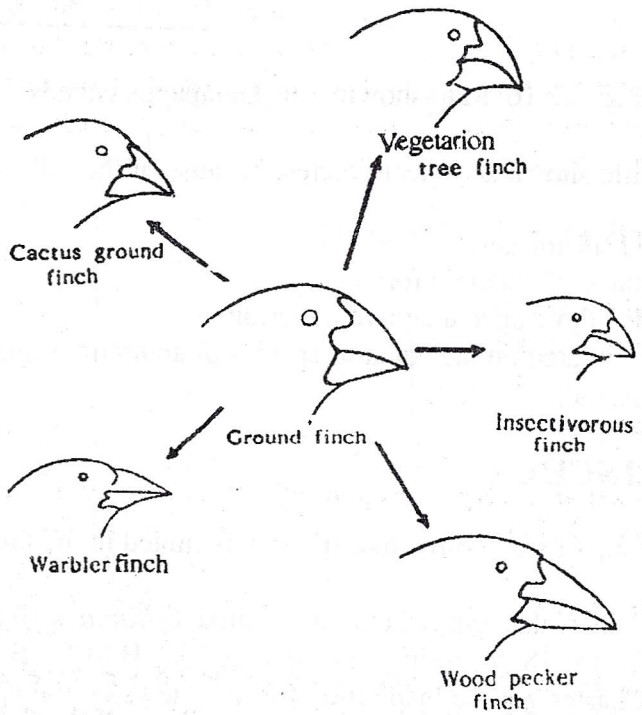

Fig. 5.2.17 Adaptive radiation in Darwin's finches

Significance of Darwin's finches

1. Darwin's finches laid the impression in the minds of Darwin for the germination of world famous natural selection theory on evolution.
2. Darwin's finches form clear evidences for evolution. By observing Galapagos finches Darwin stated that species are not immutable.
3. Darwin's finches clearly show that isolation brings about evolution and the origin of new species.

4. Darwin's finches show adoptive radiation. All the species and sub-species of Darwin's finches originated from 9 single ancestral stock.

5.2.12 NEO-DARWINISM

Though the Darwins natural selection theory is generally accepted it has some limitations. The recent supporters of Darwin modified, the theory according to the latest developments in biology. This modified theory is called Neo-Darwinism or Modern theory of Natural selection. But around 1910, some biologists raised the objections about the Darwinism due to the fact that Darwinism became purely speculative involving selection to explain anything and everything without having any proof and without providing any explanations.

Darwin's natural selection theory had a number of defects which are unable to explain the entire process of evolution. For example, it failed to differentiate acquired characters and inheritable variations. Secondly natural selection is a limiting but not an initiating force. In this light of modern developments, Huxley, Haldane, Goldschmidt, Dobzhansky, Fisher Ernst Haeckel, Mendel, Herbert Spencer Romances, Wallace and others put forth the theory which supported Darwinism and it is named as Neo-Darwinism.

The Neo-Darwinism has the following ideas:

(a) Experimental evidences
(b) Answers to the objections

(a) Experimental support

The natural selection theory is supported by a number of experiments conducted by biologists. A few are given below:

1. Industrial melanism

The peppered moths stands as a good example for industrial melanism. Industrial melanism is a phenomenon where the moths living in the industrial areas, develop black colour to match body to suit the covered background, on the bark of trees. The industrial melanism is observed and worked out by a number of evolutionists like Fisher, Ford and Kettle Well.

The peppered moths exist in two forms, namely melanic forms and non-melanic forms. The melanic forms are black in colour because they contain melanin pigments. The non-melonic forms are light coloured due to the absence of **melanin pigments**. Genetical studies showed that melanism is produced by a single dominant gene. Cross breeding experiments indicate that the melanic genes follow the typical mendelian inheritance.

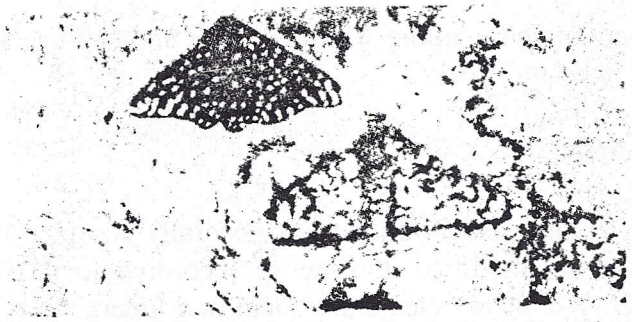

Fig.5.2.17 Melanic and non-melanic moths in an polluted environement

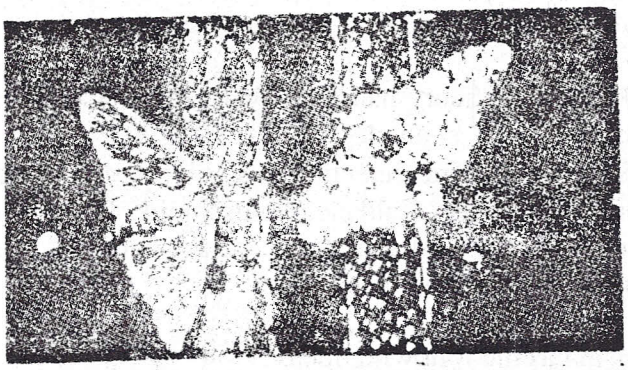

Fig.5.2.18. Melanic and non-melanic moths in an polluted environement

2. Resistance to DDT

According to Darwin evolutionary change in animals bring small variations and suitable variations under the force of Natural selection. For example, if an area of mosquitoes is sprayed with D.D.T. to kill them, mutant forms have been found to have evolved which showed great tolerance to D.D.T. Thus, Neo-Darwinism has a genetic basis and it lays special emphasis on the occurrence of mutations. This experiment shows that Neo-Darwinism involves the germinal mutations.

3. Weldon's experiment on shore crab

Weldon conducted his experiment on shore crab *Carcinus meanus*. The large break water was laid in the mouth of the river plymouths sound. The break slowed down the rate of water flow. This resulted in the deposition of silt. The deposition caused the death of numerous crabs living in that area. He analysed both dead crabs and the living ones and found that the living crabs were provided with narrow carapace and dead ones with broad carapace. He assumed that, due to the accumulation of silt in the gills, crabs were died. This experiment shows that the natural selection operates in the changed environment and it selects the narrow carapaced crabs.

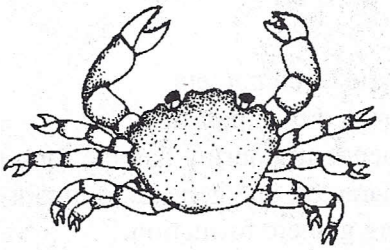

Fig. 5.2.19 A shore crab

4) Devenporte's experiment on chicks

Devenporte painted chicks with different colours of white, grey, black etc. He painted some of chicks with bars. In the fields, they were allowed to wander freely. He observed that most of the chicks except the barred chicks, were captured and killed by enemies like hawks, kites, etc. This is because the colour of barred chicks merges with the surroundings. This shows that the nature selects the animals with favourable characters.

5) Resistance of Bacterium to drugs

L.L. Cavalli and G.A. Maccacro (1952) practically demonstrated that the colon bacteria *Escherichia coli* develop resistance to the antibiotic chloramphenicol - 250 times as great as that tolerated by normal bacteria by exposing the bacteria to increase concentration of the drug. When the resistant strains are cultured in chloramphenicol free medium, they grow much more slowly than the susceptible ones. This experiment proves that population's will be made to respond adaptively to control the changes in the environment. These adaptive changes are due to mutations.

B. Explanations against the objections

Neo-Darwinians tried to give convincing evidences and explanations against the objections and criticisms raised against Darwinism. These are as follows:

1) **Germplasm theory:** The important objection levelled against Darwinism without Darwin did not distinguish between heritable and non-heritable variations. This objection is answered by germplasm theory. According to the germplasm theory proposed by August Weismann (1904), the characters appearing in the Somatoplasm disappears with the death of the possessor; so they are not inherited. The characters appearing in the germplasm alone are inherited generation after generation.

2) **Mendel's experimnets:** Another objection raised against darwinism is that it does not explain the process of inheritance. Mendel explained the process of inheritance through experiments. He said that characters determined by factors. The factors are transported through the gamets to the offspring. He made a number of experiments on pea plant. The results of his experiment

are formulated in the form of laws, collectively called as Mendel's laws. Mendel's concept has the following principles:

(i) Each animal has a bundle of characters
(ii) Each character is controlled by a pair of genes.
(iii) Genes assort out independently during gamete formation.
(iv) Of the two genes of character, one gene is dominant and the other is recessive.
(v) Alleles segregate during gamete formation.

(3) Variation: A third objection against Darwinism is that it does not explain the origin of variation. Variation is produced by the following factors. After the discovery of Mendel's laws the origin of variation in clearly understood.

(1) Mutation, (2) Recombination, (3) Hybridization, (4) Isolation, (5) genetic drift, (6) Founder's effect, (7) Migration and gene flow.

(1) Mutation: Mutation is a spontaneous change in a gene or chromosome. Dobzhansky stated that mutation is a mistake or misprint in cell division. The change that occurs in a gene are called gene mutation (or) point mutation; the change that occurs in a chromosome are called chromosomal mutations or chromosomal aberration. Thus mutation produce variations.

(2) Recombination: The formation of gene combinations not present in the parental type is called Recombination. It is a process of mixing up of available genes. Moody (1970) states that recombination involves the re-assorting and re-combining of genes already present. Recombinations are produced by the following process:

(a) Chromosomal aberrations
(b) Interbreeding
(c) Random union of gamets at the time of fertilization

It does not produce new genes in recombination, the genes are re-arranged and hence they are brought in a close association with new genes. This causes position effect and *Epistatis*. Recombination is a primary source of variation. It assists the spreading of mutant genes in the population.

3. Hybridization

It refers to the crossing of two species resulting in the formation of hybrids. The hybrids share the genetic materials from two different species. Hence, they develop new characters.

Eg: 1) Mole is a hybrid between horse and donkey.
2) *Raphano brassia* is a hybrid between Raphanus (radish) and Brassica (cabbage).
3) Pomato is a hybrid between potato and tomato.

4. Genetic drift

The random changes in gene frequency by chance in a small population is called genetic drift. This view was proposed by sewall wright. Hence, it is also called sewall wright effect. This results, a new mutation arising in a small population is either fixed or lost.

5. Isolation

Isolation is the separation of individuals of a species by some barrier, which prevents interspecific interbreeding. Geographically separated populations are called allopatric populations. Mayr (1963) states that geographic isolation is usually a necessary first step in the development of diversity between populations and the subsequent origin of species. Elementary forces of revolution, namely mutations, recombinations, natural selection and genetic drift occur independently and differentlyin each population. This leads to phosnessive genetic divergence.

6. Founder's Principle

This principle was proposed by Mayr (1963) and Sheppard (1960). It states that when a new population is in isolation its gene pool is not identifical with that of parent population because it sampling error. This difference is further, improved by different types of evolutionary. Founders principle leads to the following evolutionary changest.

(1) Small isolated populations have come to possess unusual characteristics as compared to the characteristics shown by large populations of their relatives.

(2) Large populations descended from a few immigrants may differ from the population from which the immigrants came.

For example, a large population has in its gene pool equal numbers of genes M and m.

Such population is expected to consists of 25% MM individuals 50% mm individuals and 25% mm individuals (1:2:1 ratio). Assume that 10 members of this individuals migrate to an island. These 10 members are called the founders because they are going to give rise to a new population. The founders are drawn by chance that is by migration and hence they may not show the 1:2:1 ratio of the parent population. However, they may be 7 mm, 2 mm and 1 mm individuals in any other combination. In extreme cases, the 10 individuals may be MM and mm. So it is an error of sampling when all the 10 individuals are MM, the island population is send to be descended from the 10 individuals which lack completely them gene. So, in this manner, the gene pool of the island popultion might be very different from the gene pool of the population from which the founder cause.

7. Migration and Gene flow

When the isolation disappears in empiele when the animals exhibit fast migration. Gene pool of a population is changed. When the animals belonging to different population or species brought together followed by interbreed, results gene flow town the population to another. This brings about variation.

Gene flow is a source of variation. Mutation and recombination produce genetic variations within a population, whereas gene flow introduces genetic variation from one population into another population. Gene flow keeps adjacent populations similar to each other by the mixing of genes.

5.2.13 SUMMARY

Charles Darwin (1809-1882) was born in England in 1809. He was a English naturalise. He was the first who founded the theory of organic evolution. The evolutionary idea determined by Charles Darwin is called Darwinism of Natural selection theory. He began his career with his voyage on HMS Beagle for five years across the globe. He published so many books. Out of these, the origin of species by Natural selection in 1859 is the most important one.

The evolutionary theory stated by Charles Darwin known as Darwinism. It consists of five principles. They are as: 1. Prodigality of production, 2. struggle for existences, 3. Variation, 4. Survival of the fittest, 5. Origin of species.

The above principles reveals that each organism has the ability to reproduce and toe increase its number in geometric ratio. Out of these, some organisms able to survive and the others died off due to the unfavourable environment. The organism which able to survive and to withstand the adverse conditions will remain others perish. According to Darwin, the organisms are able to develop new structures or variations to sustain within the environment. This organism in due course of time generation after generations develop new structures favoured by environment ultimately developed into a new species.

Though there are some objections to the Darwinism's theory, it is more or less universally accepted by the biologists to understand the mechanism of evolution and the origin of species. No doubt, Darwin's theory is a speculative without any experimental proof in the 18^{th} century, but it forms a basis to understand the origin of new species. Natural selection as a force for evolution put forwarded by Charles Darwin explains the speciation supplementary theories profounded by Charles Darwin though not acceptable but the theory of pangenesis forms the clue how the pangenas of gemmules are more or less equivalent to the today's experimental work of Alleles or genes. Sexual selection theory explains how the sexual selection is concerned with reproduction. It is also explained how the artificial selection leads to the formation of new species. In Neo-Darwinism, the major defects of Darwinism are rectified. Darwin's supporters gave their experimental evidence of Natural selection.

5.2.14 KEY TERMINOLOGY

Neo Darwinism : Name sometimes given to modern evolution theory which combines the theory of natural selection with the discoveries of modern genetics.

Genetic Drift : Random genetic changes in small population by chance.

Gene flow : The movement of genes as a result of mating and gene exchange within or between population.

Founder principle : It states that when a new population is established by isolation, its gene pool is not identified with that of the parent population because of sampling error. These differences are enhanced because of different evolutionary pressures in the different areas. This leads to increased genetic divergence.

Isolation mechanism : It is a barrier which prevents interbreeding of animals.

Artificial selection : Selection by man of new genotypes of better variety for the welfare of him.

Directional selection : A type of natural selection which produces a shift of the population in one direction owing to change in the environment.

Disruptive selection : It is a diversifying selection which favours two or more phenotypic modes in the heterogenous environment.

Mimicry : Protective similarity in appearance of one species of animal to another.

Natural selection : The principal mechanism of evolutionary change, originally suggested by Darwin. It states that environment selects the animals provided with favourable, characteristics and hence they have more opportunities to breed and produce more offspring. It leads to non-random or differential

5.2.15 SELF ASSESSMENT QUESTIONS

1. Give an account of theory of Darwinism or Natural selection theory.
2. Explain the Natural selection as a force for Evolution.
3. Explain briefly the supplementary theories of Charles Darwin.
4. Write short notes on:

a) Neo Darwinism
b) Industrial melanism
c) HMS Beagle
d) Describe Neo-Darwinism principles about evolution
e) Darwin's finches

5.2.16 REFERENCE BOOKS

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Lesson 5.3
HARDY-WEINBERG LAW

5.3.1 INTRODUCTION

The hardy-Weinberg law is the fundamental law of population genetics and provides the basis for studying the mendelian population. This law was independently developed by the British mathematician G.H. Hardy (1908) and the German physician W.Weinberg (1909).

5.3.2 OBJECTIVE OF THE LESSION

Hardy-Weinberg law is useful for predicting genotype frequencies from allele frequencies.

5.3.3.1 **Knowledge of Gene pool and Gene frequency**

5.3.3.2 Hardy Weinberg law

5.3.3.3 Explanation with Example

5.3.3.4 Significance of Hardy Weinberg law

5.3.3.5 Salient feature of Hardy Weinberg law

5.3.3.6 Applications of Hardy-Weinberg law.

5.3.3.7 Factors upsetting Hardy-Weinberg equilibrium

5.3.3.1 Knowledge of Gene pool and gene frequency

For understanding Hardy-Weinberg law, knowledge of gene pool and gene frequency is essential.

(a) **Gene pool**: Gene pool is defined as "The sum-total of genes present is a mendelian population". It includes all the genes of all the individuals of population. A study of the gene pool of a population gives the number of genes, the variety of genes and the type of genes present in a population. Gene pool helps to understand the ratio between the different types of genes. The gene pool of each population maintains its integrity as long as there is no interbreeding between populations.

(b)

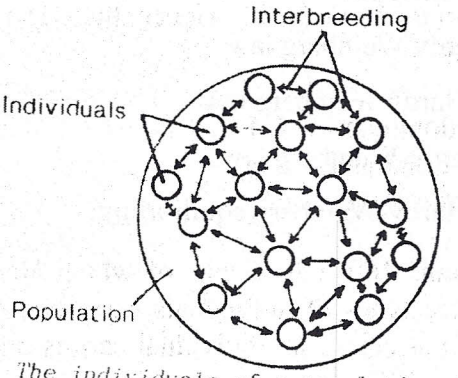

Fig.5.3.1 The individuals of a population inter-breed.

When there is interbreeding between populations the genes of one gene pool enter another and vice versa. The transfer of genes from one gene pool to another is called gene flow. Gene flow leads to the mixing and shuffling of gene pools.

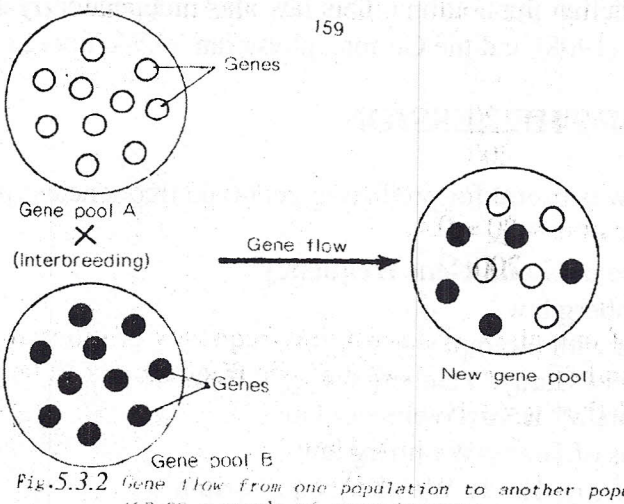

Fig.5.3.2 Gene flow from one population to another population as a result of inter-breeding.

The gene pool is not static. It maintains a dynamic equilibrium between the inflow and outflow of genes. Gene pool may become larger or smaller depending upon the various external and internal factors.

The gene pool becomes larger by the addition of genes to the gene pool. This is brought about by immigration and mutation. The gene pool becomes smaller by the removal of genes from the gene pool. This is brought about by emigration, natural selection and genetic drift.

(b) Gene frequency: The ratio of a gene in a gene pool or a population is called gene frequency. In other words, gene frequency is the proportion of one allele in the gene pool to the other alleles of the same locus.

In a hypothetical hamster population, black and grey colour of hair is controlled by two alleles "M" and "m" located on the same locus. Hence, there is a possibility for the existence in a population.

(i) Homozygous dominant – MM
(ii) Heterozygous dominant – Mm
(iii) Homozygous recessive – mm

Assume that the population contains 100 individuals, of which 40 are MM, 40 are Mm and the remaining 20 are mm. These three types of individuals carry two types of genes. As such MM individuals carry two M genes and as each Mm individual carries one M gene, the total number of M genes in population is 2x40+40 = 120.

As each mm individual carries two "m" genes and as each Mm individual carries one "m" genes in a population is 2x20+40=80. Thus a population of 100 individuals contains 100x2=200M and m alleles in the gene pool.

Now the frequency or proportion of any one allele in the population is calculated by dividing the number of the given allele by the total number of alleles located on the same locus in the population, thus,

The gene frequency of allele M is = $\frac{120}{200}$ = 0.6

The gene frequency of allele m is = $\frac{80}{200}$ = 0.4

When the gene frequency of one allele is known, the frequency of other allele in the population is calculated by using the formula P + Q = 1. Let the gene frequency of M be 'p' and that m be 'q' in the above example if p = 0.6 then q = 1-p

= 1-0.6
= 0.4

5.3.3.2 Hardy –Weinberg law

Hardy –Weinberg law states that the gene and genotype frequencies in a mendelian population remain constant, generation after generation, if there is no selection, mutation, migration or random genetic drift,

5.3.3.3 Explanation with example

Hardy –Weinberg law can be explained by taking PTC inheritance as example in human population. The individuals which carry mendelian gene can taste weak solution of phenyl thiocarbamide (PTC) and reported it to be bitter are called as tasters. Whereas the individuals which cannot taste PTC are called non-tasters. Let us assume that a population contains equal numbers of tasters (TT) and non-tasters (tt). They are as follows:

(a) TT x TT
(b) tt x tt
(c) TT x tt

As the tasters and non-tasters are in equal numbers, among the tasters there will be 50% (1/2) males and the other 50% will be females. Similarly among the non-tasters 50% (1/2) will be males and the other half will be females. The frequencies of these matings and the resultant offspring (F_1 generation) are represented in the checker board. Thus in the F_1 generation, three types of individuals are produced; they are TT, Tt and tt. These individuals are produced in the ratio 1: 2: 1

Males/Females	0.5 TT	0.5 tt
0.5TT	0.25 TT	0.25 Tt
0.5tt	0.25 Tt	0.25 tt

Fig: Marriages between males and females.

Frequency of individuals in F_1 generation

TT = ¼ = 0.25
Tt = 2/4 = 0.5
Tt = ¼ = 0.25

The same result is obtained more simply considering the union of gametes. The population produces gametes with T and t genes in equal numbers. As the TT and tt individuals are present in equal numbers, 50% gametes will be carrying T genes and the remaining 50% gametes will be carrying t genes.

Frequency of genes in F_1 generation

T = 50%
t = 50%

Sperms/ Eggs	0.5T	0.5t
0.5T	0.25 TT	0.25 Tt
0.5t	0.25 Tt	0.25 tt

Fig: combinations of sperms and eggs.

When the F_1 individuals are male, as there are three types of individuals, 9 types of matings are possible. The matings, their frequencies and the frequencies of off springs in the F_2 generation are given in the table.

Types of matings With frequency	Frequency of matings	Types of off springs frequency
¼ TT x ¼ TT	¼ x ¼ = 1/16	1/16 TT
¼ TT x ½ Tt	¼ x ½ = 1/8	1/16 TT + 1/16 Tt
¼ TT x ¼ tt	¼ x ¼ = 1/16	1/16 Tt
¼ TT x1/2 Tt	¼ x ½ = 1/8	1/16 TT + 1/16 Tt
½ Tt x ½ Tt	½ x ½ = ¼	1/16 TT+ 1/8 Tt + 1/16 tt
½ Tt x ¼ tt	½ x ¼ = 1/8	1/16 Tt + 1/16 tt
¼ TT x ¼ tt	¼ x ¼ = 1/16	1/16 Tt
½ Tt x ¼ tt	½ x ¼ = 1/8	1/16Tt+ 1/16 tt
¼ tt x ¼ tt	¼ x ¼ = 1/16	1/16 tt
Total	1	¼ TT ½ Tt ¼ tt

Fig: Showing the frequency of marriages and off spring.

This table clearly shows that the frequency of off spring in the F_2 generation is also ¼ TT : ½ Tt: ¼ tt, ie 1:2: 1. Thus the frequency remains constant. The same constancy is expected in the succeeding generations provided the population is large, mating is at random and it is free from mutations and selection.

The above example can be more simplified by substituting the letter p for T gene and q for t. The homozygous dominant TT is represented by pp or more simply by p^2. Similarly homozygous recessive tt is represented by qq or more simply by q^2. This is simplified as $(p+q)^2$ The off springs of this mating can be obtained by expanding $(p+q)^2$ ie

$$(p+q)^2 = p^2 + 2pq + q^2.$$

This formula is referred to as Hardy –Weinberg formula. In this formula p represents gene T, q represents t gene, p^2 represents T^2 i.e., TT individual, q^2 i.e., tt individual and 2 pq represents 2Tt individuals.

5.3.3.4 Significance of Hardy – Weinberg law.

(a) This law states that the gene frequencies in a large population remain constant generation after generation when mating is at random and when there is no selection and mutation.
(b) In small populations this equilibrium can not be maintained.
(c) When the population is in equilibrium there is no possibility for evolutionary change and hence the rate of evolution is zero.
(d) Evolution occurs only when this equilibrium is upset or altered.
(e) The equilibrium is detrimental and it prevents the evolutionary progress.
(f) The equilibrium tends to conserve gains (acquired) that have been made in the past and to prevent too rapid changes.
(g) The equilibrium keeps a store of recessive gene continually in existence in the population.
(h) The equilibrium maintains heterozygotes in the population.

5.3.3.5 Salient features of Hardy –Weinberg law.

In the absence of all evolutionary forces

(a) The gene and genotype frequency of each allele in a large population remain in equilibrium generation after generation.
(b) In a population, the mating is complementary random phenomenon.
(c) All the genotypes (individuals) in a population reproduce equally and successfully.
(d) Particular allele will be neither differentially added to nor differentially subtracted from a population.

5.3.3.6 Applications of Hardy –Weinberg law

(a) Hardy – Weinberg law is useful to predict the genotype frequencies from allelic frequency in a population.

Let us apply Hardy-Weinberg formula to a human population containing equal number of T genes (Taster) and equal number of t genes (Non-tasters).

The frequency of T gene in the population = 50% = ½

So p = ½ = 0.5

The frequency of t gene in the population = 50% = ½ so q = ½ = 0.5

$$(p+q)^2 = p^2 + 2pq + q^2$$
$$= (0.5)^2 + 2x0.5\ x0.5 + (0.5)^2$$
$$= 0.25 + 0.5 + 0.25$$
$$= 25\%\ TT : 50\%\ Tt : 25\%\ tt$$

The natural populations will not contain the genes in equal numbers always. Most of the populations have different proportions of genes. The above formula can be applied to any population containing any proportion of genes. When the proportion of genes in a population is known, the proportions of the off springs and genotypes can be easily calculated by applying Hardy – Weinberg formula. Let us take human population containing 90% T genes and 10% t genes. Here let us assume mating is random and the population is free from selection and mutation.

The frequency of T gene is p = 90% = 0.9

The frequency of t gene q is = 10% = 0.1

$(p+q)^2 = p^2 + 2pq \_q^2$

$= (0.9)^2 + 2x0.9x0.1 + (0.1)^2$
$= 0.81 + 0.18 + 0.01$
$= 81\%\ TT : 18\%\ Tt : 1\%\ tt.$

(b) Hardy – Weinberg method is useful to predi . the frequency of individuals homozygous for a deleterious recessive allele. The frequency of phenyl ketonuria (P .U) is estimated in a population

(c) Cases of multiple alleles can also be handled by the Hardy – Weinberg method.

5.3.3.7 Factors upsetting Hardy –Weinberg equilibrium

As long as population maintains Hardy-Weinberg equilibrium, the evolutionary processes cannot occur; naturally, the rate of evolution is zero. Evolution progresses only when the population deviates from or upsets the equilibrium. This deviation of the equilibrium is brought about by the following evolutionary forces.

(a) Mutation
(b) Natural selection.
(c) Non-random mating.
(d) Genetic drift
(e) Migration and gene flow.

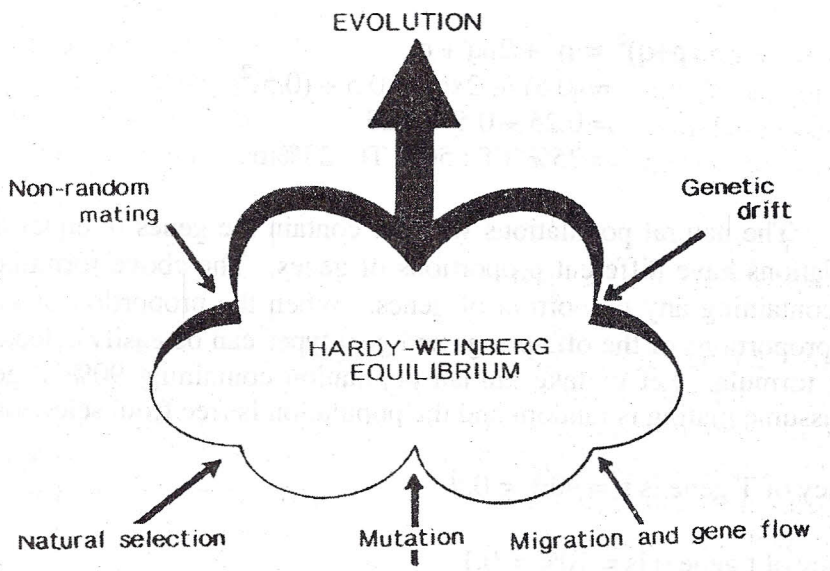

Fig.5.3.3: Factors upsetting Hardy-Weinberg equilibrium.

(a) **Mutation**: Mutation is a biochemical change which alters the genetic material. The mutation occurring in a gene is called gene mutation (or) point mutation. Mutation occurs continually in a population. When there is mutation in a population, it tends to alter the gene frequencies in the gene pool and hence the equilibrium is upset. This paves way for evolutionary change.

(b) **Natural selection**: Environment favors the animals having favorable characters. These animals have ample opportunities for mating and they produces more off spring. Hence the genes controlling the favorable characters spread more rapidly than other genes. This leads to the differential reproduction of genes by which some genes are favored over others. This differential reproduction of genes upsets Hardy – Weinberg equilibrium and helps evolution to progress.

(c) **Non-random mating**: Hardy – Weinberg law is maintained only when the mating in a population is at random. But in most of the natural populations mating does not occur at random, mating is a selective process. As a result of this selective, non-random mating the frequency of heterozygotes in the population will decrease generation after generation. Hence, non-random mating results in an abundance of certain genotypes at the expense of other genotypes. Hence, when mating is on a non – random basis, Hardy-Weinberg equilibrium is upset.

(d) **Genetic drift**: Genetic drift is an evolutionary force operating in small populations. In small populations gene frequency is found to fluctuate purely by chance. This change in gene frequencies of genes purely by chance is called genetic drift. In small populations the effect of genetic drift is significant. As a result of this in small populations some genes may be reduced in frequency or even lost by chance and others may be increased in frequency by chance. Thus the gene pool of population is changed leading to evolution.

(e) **Migration and Gene flow**: Animals are not static. When they migrate, they interbreed with individuals of other population. Thus, the genes of one population are transferred into another population. This is called gene flow. Gene flow brings about an addition of or loss of genes in the gene pool. Thus the gene frequency is altered and Hardy – Weinberg law is upset.

5.3.4 Summary of the Lesson

In a randomly mating large population the frequency of genotypes and genes remain cons. generation after generation when the forces of evolution are not operating. When Hardy – Weinberg equilibrium exists in a population the chance of evolution is zero and hence the forces of evolution tries to change the equilibrium. When the population is in equilibrium the deleterious recessive alleles maintain their frequency constant for several generations, which is the def characteristic with the Hardy – Weinberg method frequency individuals carrying defectiv es can be predicted.

5.3.5 KEY TERMINOLOGY

1. Alleles: one of a pair of a gene that occur at a given locus on the chromosome.
2. Locus: A fixed position on a chromosome that is occupied by a particular gene or one of its allele.
3. Random mating population: The population in which individuals have equal opportunity for mating with any other individual of that population. Such a population is known as mendelian population or panmictic population.
4. Gene pool : The sum total of genes presents in a mendelian population.
5. Gene flow: The transfer of genes from on gene pool to another.
6. Gene frequency: The ratio of a gene in a gene pool or a population is called gene frequency.

5.3.6 SELF ASSESMENT QUESTION

1. Explain Hardy – Weinberg law of equilibrium with suitable example.
2. Explain Hardy – Weinberg law with phenyl thiocarbamide (PTC) inheritance.
3. How Hardy – Weinberg law of equilibrium is disturbed?
4. Describe applications of Hardy – Weinberg's law along with its significance.

5.3.7 Reference books

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UNIT-V

LESSON - 5.4 A

THEORIES OF ORGANIC EVOLUTION
MUTATION THEORY OF HUGO DE VRIES
MODERN SYNTHETIC THEORY

CONTENTS

5.4.1.1 INTRODUCTION

The mutation theory was formulated in 1902 by Hugo De Vries. This theory is somewhat recent and convincing upto some expectation. De Vries was one of the three re-discoverers of Mendelism. He was a Dutch botanist. He was the director of the Botanical gardens at Amsterdam. His conclusions were based upon careful observations on evening primrose - *Oenothera lamarckiana*.

5.4.1.2 OBJECTIVE

Mutation theory explains how new species are formed? According to this theory, new species are formed from pre-existing ones in a single step suddenly without any intermediate stage. This marked differences in organisms is brought about by sudden changes in the genetic material. The sudden change in the genetic material is called mutation and the organisms which are formed as a result of mutations are called mutants. The main theme of this theory is that "evolution is a discontinuous and jerky process, rather than a continuous and gradual one as held by Lamarck and Darwin. In other words, there is jump from one species to another."

5.4.1.3 De Vries's Experiment

Hugo De Vries postulated the mutation theory based on his observations on an ornamental plant called evening primrose, *Oenothera lamarckiana*. This is wild plant native of America. It is a biennial plant of about 5-6 feet height. It bears bright yellow flowers at the tips of the branches. The

flowers blossom in the evening, hence named "evening primrose". During his work in the gardens, de vries observed not only the original *Oenothera lamarckiana* but also three other varieties which he named as "*Oenothera brivistylis, Oenothera leavifolia* and *Oenothera nannelia*". Out of these, *O. brevistylis* produces short-styled flowers. *O. leavifolia* produces smooth leaves. *O. nannelia* is very short with broader and stouter leaves. Out of curiosity, he cultivated three different plants in his garden for a period of 8 years and collected 54,343 plants, out of which 837 were different from the original wild parental variety. This markedly different forms were found to breed true. They gave rise to a few different plants in each generation. From this, de vries held that the new types were appearing in evening primrose and that he was actually seeing evolution going on. He called the marked differences as "mutations" and the plants bearing them as "mutants". He found that the mutations appeared suddenly and were inherited by the offspring.

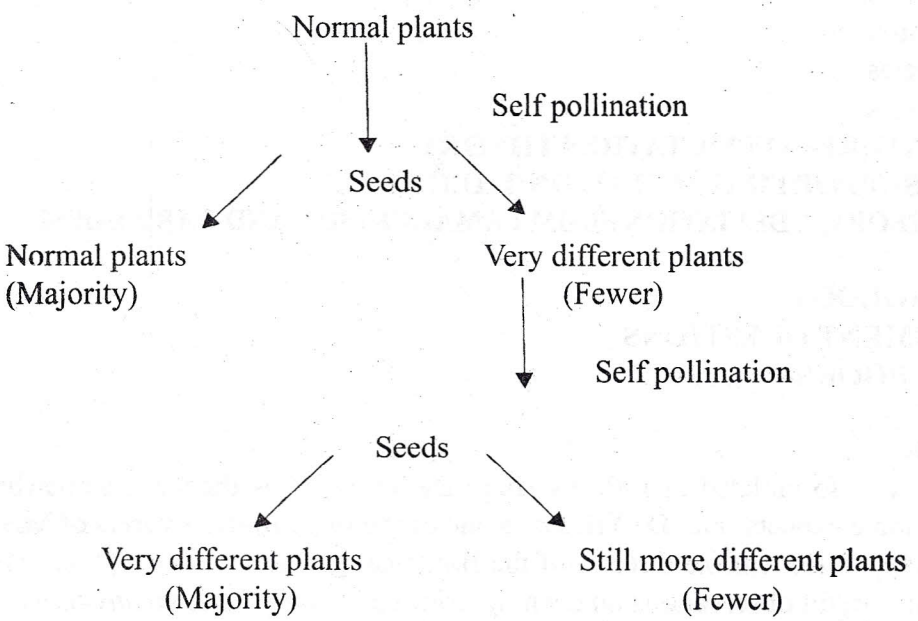

Fig. 5.4.1 De Vries Experiment (Arora, p.No. 69).

5.4.1.4 Classification of mutant species

Hugo De Vries classifies the mutant species into four types viz.,

1) Progressive species
2) Retrogressive species
3) Degressive species
4) Inconstant species

5.4.1.4.1 Progressive species

The species having one (or) more new characters which are quite different from the original plants.

Eg: *O. gigas* and *O. rubinervis*.

O. gigas is a giant variety. It has a stout stem having twice the thickness of lamarckiana. Its leaves are more in number and broader. Its flower buds are stouter and its seeds are large in size.

O. rubinervis is a red-veined slender variety. The fruits contain red veins and red streaks. The leaves are thin and more brittle.

5.4.1.4.2 Retrogressive species

When in mutant there is loss of one (or) more characters of the parental nature, the variety is called as retrogressive species. **Eg:** *O. laevifolia, O. brevistylis* and *O. nannellia.*

5.4.1.4.3 Degressive species

In this mutant species one (or) more vital characters are lost and due to this, their survival is difficult. **Eg:** *O. albida, O. oblonga.*

In *O. albida*, the chlorophyll becomes defective hence could not survive. *O. oblonga* is a weak variety where the leaves are oblong and needle-like.

5.4.1.4.4 Inconstant species

These are the mutants which behave just like the parents and occasionally give rise to mutants. These species do not breed true. **Eg:** *O. lata* and *O. scintillians. O. lata* produce only pistillate flowers. Pollen grains are absent; so cross pollination is the rule. *O. scintillans* produce flowers of its own variety as well as of original variety.

5.4.1.4.5 Salient features of mutation theory:

(i) De Vries believes that new species originate as a result of large discontinuous variations which appear suddenly and form new species at once.
(ii) New species are formed by sudden changes at a single stroke. The animals exhibiting mutations are called mutants.
(iii) There are no intermediate stages because new species are formed in a single step.
(iv) Mutation can take place in any direction.
(v) Mutations are recurring in nature.
(vi) A large number of the same type of mutations appear at the same time.
(vii) Mutations are subjected to natural selection.

5.4.1.4.6 Evidences supporting mutation theory

Besides objections, there are certain sure points which go in favour of mutation theory. There are definite examples of mutations giving specific characteristics. Some of them are:

(a) Ancon sheep: In 1891, in the flock of Seth Wright, a farmer in England, appeared a male lamb with short, bowed legs. Wright reared this lamb and bred from it, the Ancon breed of sheep. It

was so short that it could not jump over an ordinary stane fence. This breed became extinct about 80 years ago.

Later, another short-legged lamb appeared in the flock of a Norwesian farmer representing probably a new occurrence of the same situation. From this, a new strain of short-legged sheep has been bred.

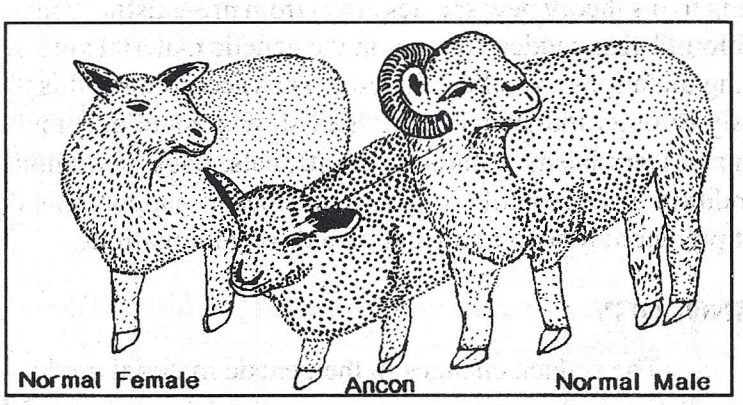

Fig. 5.4.2 Normal and mutant sheep

(b) Hornless cattle was born for a normal cattle in 1889.
(c) Albinism in rats, cats, dogs, rabbits, guinea pigs, man etc., is another mutation.
(d) Multinippled condition in sheep is due to mutation.
(e) Hare-lip in man is an instance of mutation.
(f) White-eyed condition in Drosophila is an example of mutation.

5.4.1.7 Mutation theory, a deviation from Lamarckism and Darwinism

According to Lamarckism, organisms develop variations because of their internal urge in the changing environment. These variations are inherited from one generation to another generation and consequently these variations accumulate in organisms leading to the evolution of new species. Thus evolution is a slow process.

According to Darwinism, organisms develop new structures to overcome the struggle for existence and if these structures are useful for organisms to survive in the nature then the nature selects such organisms. These structures are inherited from one generation to another and those variations responsible for these structures accumulate and lead to the evolution of new species. As a result, there are intermediate stages. Darwin also made mention of the large variations in his book "origin of species". He called them sports (or) saltations. But he considered mutations as rare and as having no evolutionary significance.

According to mutation theory of Hugo De Vries, new species were developed from wild ones suddenly. These sudden changes are due to changes in the genetic material of the wild variety. As per recent knowledge, mutations contribute less to the evolution of new species. Most of the mutations are harmful leading to the extinction of species, only few mutations are beneficial which contribute to

the adaptability of newly evolved species in the changing environment. Thus evolution of new species occurs as a result of large, conspicuous, discontinuous mutations.

5.4.1.8 SUMMARY

Hugo De Vries proposed mutation theory by observing the evening primrose, *Oenothera lamarckiana*. According to his theory new species arose from pre-existing species by sudden changes in the genetic composition. These sudden changes in the genetic material are known as mutations and the organisms containing mutations are called mutants. Thus according to this theory, new species are evolved discontinuously through mutations rather than slow gradual accumulation of variations as stated by Lamarckism and Darwinism. Albinism in rats, cats, dogs etc., multi nippled condition in sheep, white-eyed condition in Drosophila are the evidences to support this theory. As per recent knowledge, we cannot predict new species are evolved through mutations.

5.4.1.9 KEY TERMINOLOGY

(1) Mutations : The sudden changes in the genetic material are known as mutations.

(2) Mutants : The organisms which evolved through mutations are called mutants.

(3) Progressive species: The newly evolved species by the addition of one (or) more characters through mutations.

(4) Retrogressive species: The newly formed species by the lost of one (or) more characters through mutations.

5.4.1.10 SELF ASSESSMENT QUESTIONS

(1) Describe Mutation theory.
(2) Write a short on classification of mutant species.
(3) Write a short note on salient features of mutation theory.

5.4.1.11 REFERENCE BOOKS

Strickberger, M.W.Z. 1994. **Evolution**. CBS Publishers and Distributors, ISBN Shahdra, Delhi (India).

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Lesson 5.4. B

THEORIES OF ORGANIC EVOLUTION
MUTATION THEORY OF HUGO DEVRIES
&
MODERN SYNTHETIC THEORY OF EVOLUTION

Contents

5.4.2.1 INTRODUCTION

The term evolution was first used by English Philosophers, Herbert Spencer. Charles Darwin was the father of evolution. He defined evolution as "descent with modification". In other words, evolution is the process by which stated populations diverge from one another, giving rise to new species. Nobody has seen how new species are evolved within a population. But some famous biologists by observing the nature closely proposed some famous theories viz.

1. Darwin's theory of natural section.
2. Lamarck's theory.
3. Mutation theory-Hugo de Vries.
4. Mendel's law of heredity.

Among the above theories Darwin's theory of natural selection was predominant and most widely accepted up to 19^{th} century. After the discovery of Mendel's laws of heredity and mutation theory, natural selection of Darwin was exploited to some defects. Hence some followers of Darwin, who are called as Neo-Darwinists proposed modern synthetic theory of evolution. The synthetic theory revolves around Darwin's natural selection, theory. Mays states that the modern synthetic theory of evolution amplified Darwin's theory of natural selection in light of Mendelism, mutation theory, population genetics, biological concept of species. The Neo-Darwinists who proposed modern-synthetic theory of evolution around 1930 were S.Wright, H.J. Muller, Th. Dobzhansky, R.B. Goldschmidt, J.S. Huxley, R.A Fisher, J.B.S Haldane, Ernst Mays and GL Stebbins.

5.4.2.2 OBJECTIVE OF THE LESSON:

Modern synthetic theory is the combination of all the theories of evolution. Thus the effect and integration of various forces responsible for evolution is clearly understood from modern synthetic theory of evolution.

5.4.2.3 FUNDAMENTAL FORCES OF EVOLUTION

The modern synthetic theory involves five fundamental processes of evolution. Their impact on a population leads to the evolution of new species.

1. Mutation.
2. Variation (Recombination)
3. Heredity.
4. Natural selection.
5. Isolation.

5.4.2.3.1 Mutation:

Mutation theory was proposed by Hugo deVries mutation theory changes the Darwinian theory. Mutations are the raw materials for organic evolution. In nature mutations occur at slow rats. In nature physical factors are responsible for mutation. Mutations are of two types.

(a) Gene nutation (or) point mutations.

(b) Chromosomal mutations.

Sudden change in the chemistry of gene is called gene mutation (or) point mutations. The effect of point mutation is more significant than any other effect. Change in the chemistry of chromosomes is called chromosomal mutations. Mostly mutations are lethal but occasionally some nutations are beneficial. Forward mutation change the dominant able to recessive allele. The recessive allele express Phenotypically in homozygous condition. Backward mutation change recessive allele to dominant allele. Thus mutations change the genotype of individuals in a population and consequently individuals having new phenotype are produced. In this way mutations lead to evolution of new species.

5.4.2.3.2 Variation (Recombination):

Reshuffling of already existing genetic material in an individual is called re-combinations. Re-combinations are possible only in sexually reproducing organisms. Diploid organisms which produces gametes by meiosis exhibit re-combinations at the stage of Pachytene of meiosis – I. This type of recombination in which exchange of chromosome arms during meiosis – I is called crossing over. This recombination leads to phenotypic variations in organisms. Chromosomal mutations such as a polyploidy, deletion, duplication, inversion and translocation also result in variation. These variations are accumulated in organisms generation after generation leading to the evolution of new species.

5.4.2.3.3 Heredity:

The transmission of characteristics (or) variations from parent to off spring is an important mechanism of evolution. This process of transmission is called inheritance. According Lamarck's theory of inheritance of acquired characters, the characters acquired by an organism in its life time are transferred to its progeny. But this theory was disproved by August Weismann's germplasm theory. He proposed this theory by conducting decasualization experiment on mice. According to this theory, the changes which occur in sonatoplasm are not inherited, but the changes which occur in germplasm are inherited to next generation loading to the evolution of new species which is complitely different from its ancestors.

5.4.2.3.4 NATURAL SELECTION

Theory of natural selection was proposed by Charles Darwin. According to Darwin. Nature (environment in which organism living) is the predominant evolutionary force which determine the direction of evolution. The changes which occurred in organisms are subjected to selection by the nature. Thus the nature selects changes in organisms which are more suitable to the existing environmental condition. The organisms having characters selected by nature survive and other organisms perish. In this way natural selection dilate the course of evolution of species with its

specific selectivity for the characters in organism. This specific selectivity by the environment is inherited to future offsprings through differential reproduction to produces adaptable new species.

5.4.2.3.5 Isolation:

Separation of organisms from one another is called isolation. Isolation of organisms of a species into several populations (or) groups under physiological (or) geographical barriers is supposed to be one of the most significant factors responsible for evolution. Reproductive isolation is the most effective isolation because these isolations do not allow the interbreeding (gene flow) between separated organisms for maintaining their individuality. In the course of time isolated organisms may emergy out as new species differing from each other.

5.4.2.4 ACCESSORY FORCES OF EVOLUTION :

The accessory forces responsible for evolution to modern synthetic theory are,

(a) Migration

(b) Hybridization.

(c) Genetic drift.

5.4.2.4.1 **Migration:**

Movement of organisms from one habitat to another is called migration. There are two types of migrations.

(i) **Immigration**: Movement of new organisms into the population is called migration. These new organisms bring new genes into the population, changing the gene pool of that population. This changed gene pool triggers the evolution of new species within the population by various processes such as selection etc.

(ii) **Emigration**: Outward movement of organisms from the population is called emigration. These organisms carry some genes from the population, decreasing the gene pool of that population. These emigrated organisms settles at another place and thus remain as small population. These

small populations in the course of time adapt to changing environment. In this way new species (subspecies) are evolved from the existing species through emigration.

5.4.2.4.2 Hybridization:

Interbreeding between two genetically distinct individuals of a species for the production of individuals having two genetic traits is called hybridization. Such hybridization may bring many new alleles into a population and may initiate a new trend in the evolution of receiving population.

5.4.2.4.3 Genetic drift:

Genetic drift is a random change in the gene frequency of small populations. This change occurs by chance even under constant environmental conditions. Genetic drift leads to the fixation (or) the loss of certain genes regardless of their adaptive value. Genetic Drift directs the evolution in a population in an unpredictable manner. Genetic drift increases the homozygosity in small populations. In course of time these homozygous small population merges out to form new population.

5.4.2.5 INTEGRATION AMONG THE FORCES OF EVOLUTION.

The three processes mutation, genetic recombination and natural selection are equally indispensable for evolutionary change to take place. Speculation as to which of the three is the most important is completely pointless. An evolutionary line of organisms which is changing through million of years can be linked to an automobile being driven along the high way. Mutations then correspond to the fuel in the tank. These mutations are raw materials for the evolutionary vehicle. As without fuel the vehicle does not move, without mutations evolution does not occur. Since mutation is the only possible source of new genetic variation, it is essential for continued progress, but is not the immediate source of motive power. This source (genetic variation) undergoes genetic recombination, through the shuffling of genes and chromosomes and goes on during sexual cycle. Since this process provides the immediate source of variability upon which selection exerts its primary action, it can be compared to the engine of the automobile. Natural selection, which directs the genetic variability

toward adaptation to Zygotic mortality isolation: When there is inter specific interbreeding the resulting hybrids do not survive. It is exemplified as Warwick and Berry showed that the cross between goat and sheep produces normal embryos, but they die much before death.

The environment, can be compared to the drives of vehicle. Various lines of evidence indicate the structural changes in the chromosomes, which change the order of arrangement of the genes along them, and have profound effects upon the inter-relationship between genetic recombination and natural selection, and can be compared to the transmission and accelerator of the automobile. Finally reproductive. isolation which includes all the barriers to the gene exchange between populations, has a canalizing effect similar to that which the highway, with its limits and directive signs, exerts upon the driver of the automobile, thus permitting several vehicles to drive in the same direction at the same time.

In this way various forces of evolution are integrated for the evolution of new species.

5.4.2.6 OBJECTIONS TO THE MODERN SYNTHETIC THEORY:

The synthetic theory of evolution is on original construction, supported by several facts. May of its aspects are valuable and positive, and it opens all new approaches to the problem. Mutations, the genetics of populations and selection are indisputably elements of importance in evolution. But it has some following objections:

I. The genetics of populations, analyzing genetic structure statistically, is a science which grants wide scope to mathematical speculation. From one generation to the next, the forces capable for modifying structure are variable and they can be represented by mathematical formula.

II. While admitting that the evolutionary value of nutations is great, we have no unimpeachable proof that the variations of fossil forms well identical to those variations of present day forms which we can produce experimentally.

III. Within populations which exist today as new species, do induced, arise; but no profound change modifying basic structure has been observed.

IV. Again, is mutation the only manner in which variations today occur? The study of directed nutations has scarcely begun. Nucleic acids give same expression when transferred from one cell to another. Then the influence of somatic cells on germ cells would no longer sum impossible.

V. The order in which mutations appear is purely random. The paths which evolution follows and the apparent sense of direction, is difficult to explain by the simple interplay of random nutations and selection.

VI. The phenomenon of convergence leading to similar organs in animals which are unrelated can not be explained.

VII. The synthetic theory of evolution, like classic Darwinism, is based on the principle of utility. Now, all that exists in living creatures is not necessarily useful; and that which is useful to the individual may not be of value to the species. Sexual selection favors the most attractive and vigorous males; but are these always the most fertile? The principle of utility implies a judgement of value, which is incompatible with the purely mechanistic interpretation of evolution which the Neo-Darwinists defend.

VIII. P.P Grasse remarked that the synthetic theory of evolution helps too much on one string to give a general explanation of evolution. The mechanisms of evolution are many and their causes are no less numerous.

5.4.2.7 SUMMARY OF THE LESSON

The modern synthetic theory of evolution revolves around the Darwin's natural selection. It explains all the forces of evolution. This theory explains how integration is brought between various evolutionary forces. At present this is widely accepted theory.

5.4.2.8 KEY TERMINOLOGY;

1. **Genetic drift**: Random genetic changes in small populations by chance.
2. **Hybridization**: It is the crossing of individuals of different species; the resulting offspring is called a hybrid. This leads to gene flow between populations resulting in genetic divergence.

5.4.2.9 SELF ASSESMENT QUESTIONS.

1. Describe modern synthetic theory of evolution?
2. How integration is brought about by various forces of evolution?
3. What are the defects of modern synthetic theory?

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LESSON-5.5

SPECIES CONCEPT

CONTENTS

5.5.1 INTRODUCTION

Species is the basic unit of classification. Species is a latin word. The meaning of the term species is kind (or) appearance. John Ray coined the term species. Species is defined as the smallest unit of classification having the organisms which show similarities in body construction, physiological aspects, genetical aspects and also successfully interbreed among themselves.

- Du Rietz called the species "a syngameon separate from all others by sexual isolating mechanism".

- Mayr defined species as "groups of actually (or) potentially interbreeding natural population which are reproductively isolated from other such groups.

- Simpson gave the definition "a genetic species is a group of organisms so constituted and so situated in nature that a hereditary character of any one of these organisms may be transmitted to a descendent of any other".

- Dobzhansky defined the species as "the largest and most inclusive reproductive community of sexual and cross fertilizing individuals which share in a common gene pool.".

- Tate Regan defined the species as "a community (or) a number of related communities having distinctive morphological characters.
- Lotsy defined species as "a group of genetically identical individuals".
- Goldschmidt stated that groups which can be successfully crossed should be treated as a single species.
- Eimer defined species as "groups of individuals which are so modified that successful interbreeding is no longer possible".
- Von Baer defined the species as "the sum of the individuals that are united by common descent".
- Illinger defined the species as "a community of individuals which produce fertile offsprings".
- Brauer told natural tie of blood relationship through which the individuals of a species are held together.

5.5.2 OBJECTIVES

Species concept provides fundamental information for the classification of organisms. This concept explains how the new species are evolved from the pre-existing species.

5.5.3 STRUCTURE

5.5.3.1 Definitions of different species
5.5.3.2 Salient features of species
5.5.3.3 Sub-species
5.5.3.4 Differences between species and sub-species
5.5.3.5 Significance of sub-species in speciation
5.5.3.6 Sibling species
5.5.3.7 Speciation
5.5.3.8 Different concepts of species

5.5.3.1 Definitions of different species

Allopatric species : The species inhabiting different geographical areas.

Sympatric species : The species normally occupying the same geographical areas.

Morpho species : These are ones established by the morphological similarity regardless of the other considerations.

Biospecies and genetic species : A group of interbreeding population which are reproductively isolated from other such groups.

Sibling species : It is a term applied to pairs (or) groups of very similar and closely related species. When applied to closely related species (in phylogenetic sense) this expression refers to hypothetical species, these can not be dealt within taxonomy but can be useful in speculations on evolution.

Taxonomic species : A species which has been provided a specific name under the International Rules of Nomenclature.

Evolutionary species : These are lineages (ancestral descendent sequences of populations) evolving separately from each other and with their own unitary evolutionary roles and tendencies.

Polytypic species : Polytypic species are those which consists of two (or) more sub-species.

Monotypic species : Monotypic species consist of a single sub-species.

5.5.3.2 Salient features of species

(1) The members of a species possess distinctive morphological characters.

(2) A species is a Mendelian population.

(3) All the individuals of a species contain more or less similar number of chromosomes.

(4) They share a common gene pool.

(5) Members of a species interbreed freely and produce fertile progeny.

(6) They are reproductively isolated from other species. The different species of a genus also can interbreed, but usually their progeny are sterile.

(7) Each species fills an ecological niche.

(8) The individuals of a species resemble each other in the structure of their organs, their chemical composition, genetic make-up and physiological aspects.

5.5.3.3 Sub-species

Each species is formed of two or more geographically isolated populations. These populations are called sub-species or geographical races. A sub-species is defined as geographically isolated populations of a species, which are capable of interbreeding and producing fertile hybirds.

A sub-species has the following salient features.

(1) A species is formed of many populations. Each population is called a sub-species.

(2) Each sub-species has a geographical area and is geographically isolated from other sub-species.

(3) The similarity between sub-species of a species is greater when compared to related species.

(4) The morphological difference between sub-species is so slight that only expert can recognize the different species.

(5) The sub-species differ from each other in the frequencies with which certain genes occur rather than in the possession of certain genes by all members of one sub-species and the absence of those genes from all the members of a second sub-species.

(6) The different sub-species in a species are not reproductively isolated, they interbreed whenever they came into contact.

(7) In areas between the ranges of neighbouring sub-species intermediate forms are found. These are formed by hybridization between the neighbouring sub-species.

Examples of sub-species:

(1) The bird Golden Whistler pachycephala pectoralis inhabiting solomon islands is a good example of sub-species. The different sub-species develop different patterns of plumage.

(2) Another example is deer mouse.

(3) The third example is provided by zebra.

5.5.3.4 Differences between species and sub-species:

(1) The members of different species do not interbreed when they come into contact; but members of different sub-species of a species interbreed.

(2) Different species occupy separate territories, but the territories may overlap; different sub-species may occupy separate territories, they do not overlap.

(3) Intermediate forms do not arise between species, but intermediate forms arise between sub-species.

(4) Structural differences between species are usually greater; but smaller between sub-species.

5.5.3.5 Significance of sub-species in speciation

It seems that sub-species is a small step in the origin of the species. The different populations of a species are separated by distance or by geographical barriers. These separate groups are forced to experience different environmental conditions. So each group develops differences gradually but independently. In course of time, the different populations begin to differ from the original stock. Now each group attains the rank of a sub-species. By further development of differences and reproductive isolation, the sub-species is transformed into a new species. Thus it is assumed that a sub-species is a step in the development of a species.

5.5.3.6 Sibling species

Sometimes two or more closely related species live in the same geographical area. Morphologically these species are more or less similar, but reproductively they are isolated; they can be identified by their breeding time, breeding habit and behaviour. Mayr named these species as sibling species. The sibling species are defined as "sympatric species that are morphologically similar but reproductively isolated."

Examples of Sibling species

(1) A good example of sibling species is the malaria-carrying mosquito of Europe, Anopheles maculipennis and its relatives. *A. atroparvus, A. ianbranchial, A. sacharovi* and *A. subalpinus* are all morphologically similar, but they are reproductively isolated and they cannot produce viable hybrids.

(2) Another example of sibling species is given by the genus Drosophila. *D. psudoabscura* and *D. persimilis* are identical in their morphology. The salivary gland and the gene arrangement in these two species are different. They cannot produce hybrids owing to the difference in the Y-chromosome.

Significance of Sibling species: The sibling species have some importance in biology.

(i) They help in the understanding of the process of speciation. The geographically isolated population accumulates the newly formed changes in the gene pool. Owing to genetic drift and natural selection, the populations are reproductively isolated.

(ii) They provide an opportunity for testing the validity of biological species concept with reference to morphological species concept.

5.5.3.7 Speciation

The formation of new species is called speciation. New species are formed in a variety of ways. There are four main patterns of speciation. They are as follows:

(a) Allopatric speciation
(b) Sympatric speciation
(c) Quantum speciation
(d) Parapatric speciation

(1) Allopatric speciation:- When two related populations occupy geographically or spatially separated areas, they are called allopatric populations. The evolution of allopatric populations into separate species is called allopatric speciation. It is a speciation in different places.

A species is formed of many interbreeding populations or denes. They share a common gene pool. When there appears a geographic barrier, the species is split or fragmented into two or more groups. These groups separated by geographic barriers are called allopatric populations.

In sexually reproducing animals geographic isolation is the first step in speciation. This brings about separation of populations. The degree of separation needed is still not clear; it differs from organism to organism.

Tack explained with a suitable example the importance of geographical isolation for speciation. He observed the finch inhabiting cocos island. This finch was so unlike those of Darwin's finches that it was placed in a separate genus suggesting that it had been on the island for a long time. Yet this genus contained but one species, which was not even divided into subspecies. Tack wrote "cocos resembles the Galapagos in providing varied habitats and in having a great paucity of other land birds; but it differs in one essential respect. It is a single island, not an archipelago. Hence, there has been no opportunity for the geographic isolation of populations and hence no evolution of new sub-species or an adaptive radiation. This shows that some effective means of spatial isolation is essential as an initial step for speciation.

In the beginning, the gene pool of each allopatric population is similar. Since there is geographical barrier, there is no possibility for the mixing up of genes between populations. Similar populations are thus forced to inhabit different environmental conditions. The environmental factors influence the different populations differently. Hence each population

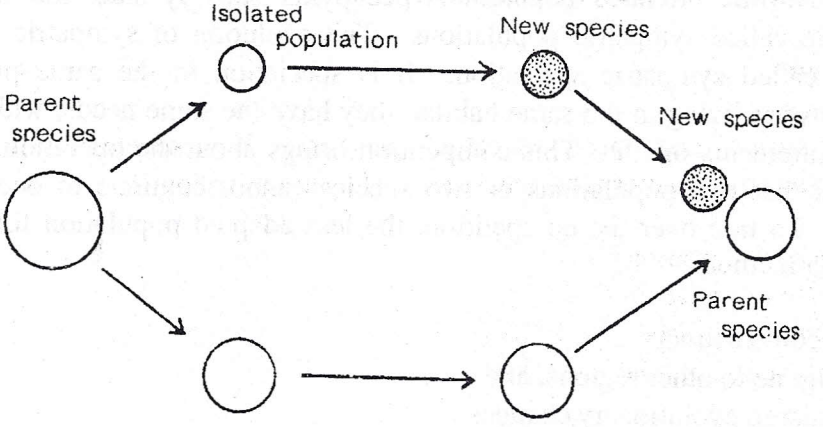

Fig. 5.5.1 Allopatric speciation

develops mutations and recombinations. The different populations as under the operation of natural selection and genetic drift. This leads to genetic divergence. The nature of mutation, of recombination, of natural selection and of genetic drift differs from one population to another. Hence different populations develop different types of genetic divergence. When genetic divergence is developed, the populations which once made effective interbreeding fail to interbreed when they are brought together by migration or by the disappearance of barriers. Thus reproductive isolation comes into existence. When the populations first come into contact, the reproductive isolations may be only partial. So a few hybrids are formed. These hybrids may be sterile or they may not be well adapted to the existing conditions. Such hybrids constitute biological wastage. Natural selection will tend to eliminate the wastage by improving and intensifying the means of reproductive isolation until the populations no longer interbreed at all. When this point is reached, the populations may live in the same area and still retain the integrity of their respective gene pools. They are now designated as separate species.

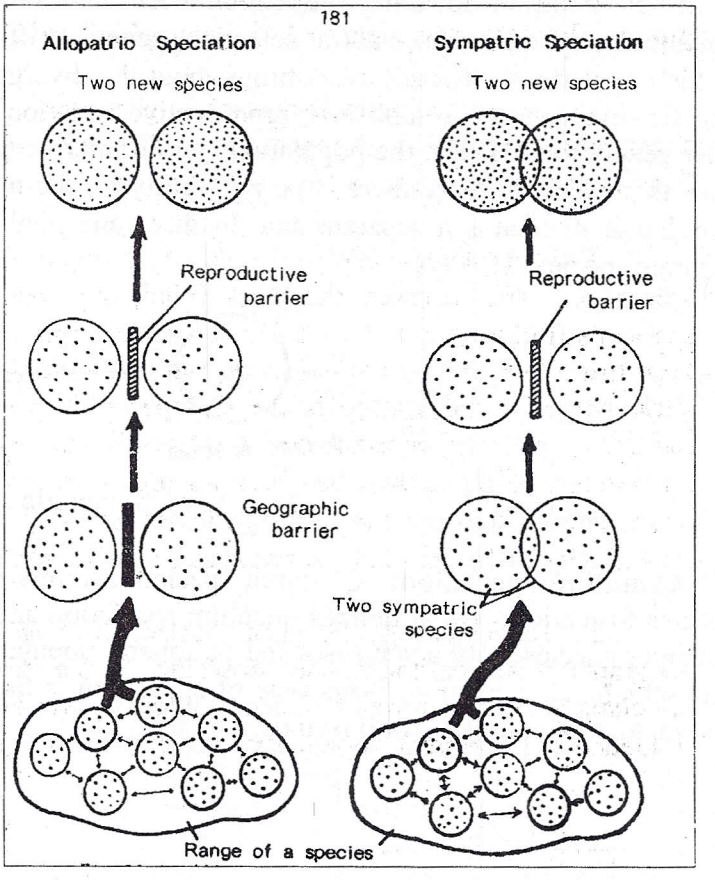

Fig. 5.5.2 Allopatric and sympatric speciation

(b) Sympatric speciation: Related populations occupying side by side, the same territory or geographical area are called sympatric populations. The evolution of sympatric populations into separate species is called sympatric speciation. It is speciation in the same place. Since the sympatric populations are living in the same habitat, they have the same needs, with regard to food, space and other requirements of life. This competition brings about the operation of Gause's law. This principle states that two populations or two species cannot continue to occupy. The same habitat indefinitely. To tide over the competition, the less adapted population follows one or the other of the following methods:

(1) It may become extinct.
(2) It may migrate to other regions, and
(3) It may undergo evolutionary changes

For example, if it is a population of seed eating birds, the birds will develop a stronger and heavier beak so that they can cope with larger seeds that can be utilized by their competitor or they may change their food entirely. Thus populations develop variations.

In addition to this competition there are other factors like mutation, recombination, polyploidy, hybridization, natural selection, genetic drift, isolation, founder principle etc., operating in the populations. These factors bring about the divergence in the once similar gene pools. When genetic divergence is established, reproductive isolation is established step by step and generation after generation. Hence, the populations fail to interbreed. When there is no interbreeding, the gene flow is prevented; hence there is no possibility for the mixing up of the genes. Consequently, each population maintains a separate and distinct gene pool. Now the populations are ranked as two separate species.

Fig. 5.5.3 Sympatric speciation

(C) Quantum speciation: Quantum speciation refers to a more rapid and more abrupt mode of species formation. Grant defines quantum speciation and "the budding off a new and very different daughter species from a semi-isolated peripheral population of the ancestral species". Ayala called this saltational speciation. This type of speciation is based on the observations of H.L. Carson on Drosophila inhabiting Hawaii islands.

These islands are of geologically recent origin. They are not older than 7 million years. Nevertheless, they contain about 700 species of Drosophila. Still there are no sub-species in any species.

It is said that Hawaii islands are characterized by volcanic eruptions. These volcanoes kill the vegetation and pre-existing animals. Occasionally gravid female flies are carried to this area affected by volcanoes. In such habitats, the fly population increases rapidly. Since this area is free from enemies, natural selection is temporarily relaxed; this leads to the development of new genetic variations. As the environment becomes saturated this expansion may be followed by a population crash which will eliminate almost all the individuals. A few flies recover from the crash. They are acted upon by natural selection and genetic drift. This leads to the formation of genetic divergence and the failure of interbreeding with the original population by the development of reproductive isolations.

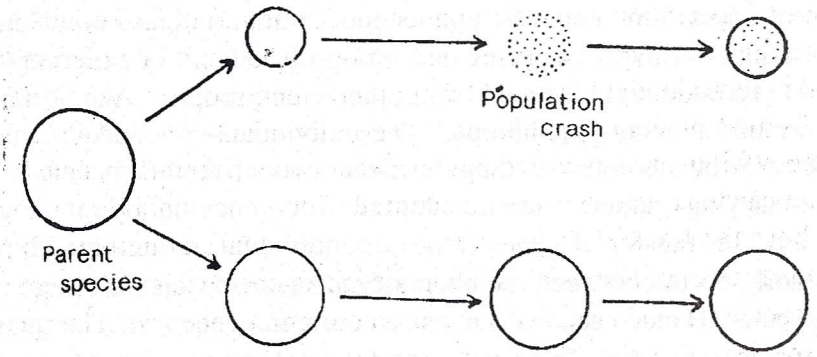

Fig. 5.5.4 Quantum speciation

The quantum speciation can be summarised as follows:

(i) It is a sudden and rapid speciation.
(ii) The ancestors of the new species do not include a large proportion of the populations and may consist of only one or a few individuals.
(iii) It does not produce sub-species or intermediate stages.
(iv) Genetic drift or chance plays a major role.
(v) The quantum speciation resembles geographic speciation in that spatial isolation is necessary for speciation.

(d) Parapatric speciation: This type of speciation is intermediate between allopatric and sympatric speciations. In this form, the divergence takes place between continuous, rather than separated or overlapping populations.

According to Bush, "Parapatric speciation may occur whenever species evolve as continuous populations in a continuous cline". It is a rapid process. It involves only a relatively few individuals of the parent population. No geographical isolation is involved here. Reproductive isolation is the operative force. Reproductive isolations are produced by natural selection.

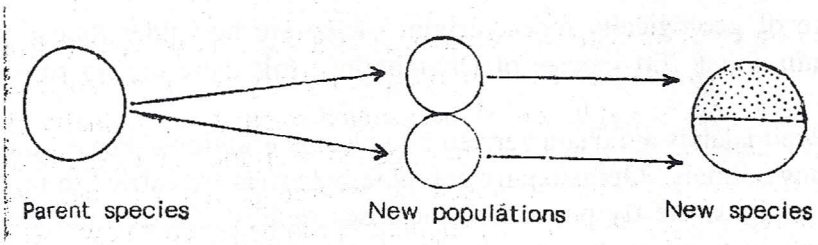

Fig. 5.5.5 Parapatric speciation

Mechanism of speciation:

The origin of new species requires a sequence of events over a very long span of time. It takes place only when both ecological conditions and the genetical make up of a population are favourable.

The mechanism of speciation cannot be understood without a clear knowledge of biological species concept. According to Mayr, "a species is a group of actually or potentially interbreeding natural population that is reproductively isolated from other such groups". According to this species concept, a species is formed of many populations. The individuals of a species interbreed among themselves. But there will be no interbreeding between two different species. When there is interbreeding, the gene-carrying gametes are transmitted from one individual to another. Hence interbreeding brings about the transfer of genes from one individual to another. This is called gene flow. The gene flow readily occurs between members of the same species; but there is no gene flow between two different species. Hence each species has a common gene pool. The quality of the gene pool differs from one species to another. Thus each species maintains the integrity as long as the gene pool does not mix with other species. The mixing of gene pools is not possible without interbreeding. Interbreeding is not possible when there are genetic differences between the two groups. Thus species are evolved owing to the development of genetic divergences and isolating mechanisms.

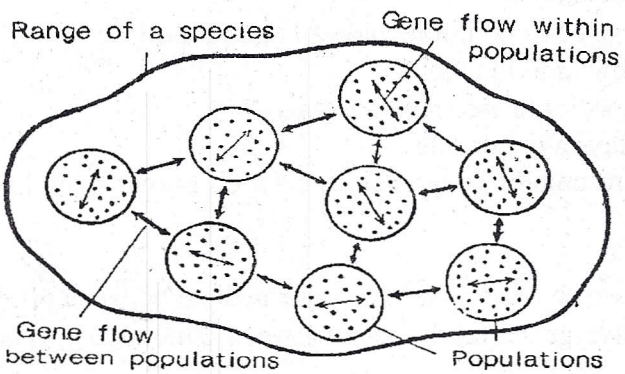

Fig.5.5.6 Inter-breeding and gene flow in a species

When a population is isolated from the parental species, it is prevented from interbreeding with its parental species. This leads to the failure of gene flow between the isolated population and the parental species. The genetic constitution of the isolated population gradually changes from the parental population. This leads to the development of genetic variation and genetic divergence in the isolated population. After the development of genetic divergence, the isolated population fails to mate with the parental species. Now the isolated population can be called a new species.

The following sequence in speciation is now universally accepted for sexually reproducing animal species:

The following sequence in speciation is now universally accepted for sexually reproducing animal species:

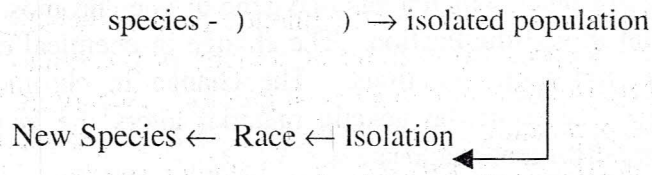

Fig. 5.5.7 Stages in speciation

Factors influencing speciation:

Speciation is the production of new gene complexes capable of ecological shifts. New gene complexes are produced by changes in the genetic materials. These changes are nothing but variations or genetic differences or genetic divergences. These are produced by a large number of factors. They are as follows:

(1) Mutation

(2) Recombination

(3) Natural selection

(4) Genetic drift

(5) Hybridization

(6) Polyploidy

(7) Founders principle

(8) Isolation

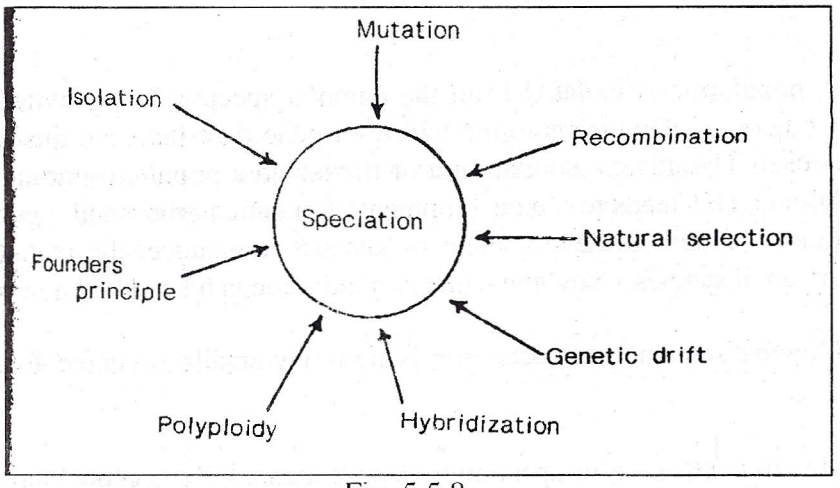

Fig. 5.5.8

(1) Mutation: Mutation is a sudden change which alters one gene or one chromosome into another form. It is a nitrate or misprint in cell duplication. The change in chemical composition of a gene is called gene mutations or point mutations. The change in chromosome is called chromosomal aberration. Any change in the genetic material alters the gene pool and the characters of the animals. Hence divergences appear.

Mutations are important sources of variations. Variations are the raw materials for evolution. Mutations supply variations continuously. Variations produced may be useful or useless or harmful or neutral to the population. The population will benefit by the production of useful mutations. Mutational changes occur regardless whether they are useful or not. But it should be noted that mutations which are disadvantageous in a given environment may be valuable and useful in a changed environment. The injection of variation by mutation into the population is only the first step in the process of evolution. the second step is the influence of natural selection.

(2) Recombinations: The formation of new gene combinations not present in the parental type is called recombination. It is a process of the mixing of the available genes. Recombinations are produced by crossing over at the time of meiosis, free assortment of genes at the time of gametogenesis, random union of gametes at the time of fertilization, chromosomal aberrations and interbreeding.

Recombination does not produce new genes; but it does produce new gene combinations or genotypes. Recombinations assists the spreading of mutant genes in the population. It is a primary source of variations. It is more potent than mutation in producing variations. In recombination genes are rearranged and hence they are brought in close association with new

genes. This causes position effect and epistasis. Hence the original character of the animal is changed and new characters appear by recombination.

Recombination of genes brings about recombination of characters. These characters are processed by natural selection. Natural selection favours the animals possessing characters that are beneficial.

(3) **Natural selection:** Mutations and recombinations bring about variations which may be advantageous or disadvantageous. Animals which are provided with favourable variations have more survival value. Therefore such animals are selected by nature and are allowed to mate. Since these animals have more opportunities for breeding, they produce more young ones. But animals with disadvantageous characters have less chances of reproducing. So they yield lesser number of offspring. this results in rapid spreading of genes producing advantageous characters; the disadvantageous gene combinations and mutations are gradually eliminated from the population. Thus the unfavourable genes are prevented from spreading in the population. This leads to differential reproduction of genes. Thus the gene frequency is altered from one generation to the succeeding one.

Natural selection does not produce genetic change, but it favours the spreading of one type of gene more rapidly than the other. Again natural selection encourages the genes that produce the highest adaptation in the environment. When a population contains two or more gene combinations, selection favours the gene combination which is more efficient in the environmental condition. Natural selection operates over millions of years to facilitate the development of new adaptive characters. Thus the interaction of mutation, recombination and natural selection results in new adaptive characters.

(4) **Genetic drift:** Genetic drift is defined as a random genetic change in small populations taking place purely by chance. It is also called sewall wright effect. It operates in small populations; in small populations the genetic changes occur purely by chance. It acts on characters irrespective of their adaptive nature. It is an operative force responsible for fixing non-adaptive or neutral characters in populations. Genetic drift leads to homozygosity in a few generations. The homozygous individuals may be fixed in the population or lost from the population. Since most of the natural populations are smaller, genetic drift has a considerable role in micro-evolution.

(5) **Hybridization:** Interbreeding between the members of different species is called hybridization. the resulting offspring are called hybrids; the hybrids share the genetic materials from two different species. Hence they develop new characters and are reproductively isolated from the parental populations and they constitute new species. Hybridization leads to sympatric speciation.

(6) **Polyploidy:** The increase in the number of chromosome sets is called Euploidy. The increase in number of chromosome sets more than two is called polyploidy. If the increase is due to the union of gametes belonging to same species, is called autopolploidy. If the increase in number of sets due to the union of gametes belonging to different species is called allopolyploidy.

Polyploidy is a kind of chromosomal aberration. Polyploidy brings about instantaneous speciation. Thus reproductive isolation appears at one stroke without any geographic isolation. Hence polyploidy produces sympatric speciation.

(7) **Founder's Principle:** This principle was proposed by Mayr and Sheppard. It states that when a new population is established in isolation, its gene pool is not identical with that of the parent population because of sampling error; this difference is further improved by the different types of evolutionary pressures independently in the two populations; this leads to genetic divergence. This principle is similar to genetic drift. It brings about genetic divergence in small populations. It may help to explain how small isolated populations have come to possess unusual characteristics they sometimes exhibit. It explains how a large population descended from a few immigratns may differ from a population from which the immigrants came.

(8) **Isolation:** Isolation is the seggregation or separatiòn of populations by some barriers which prevent interbreeding. When interbreeding is prevented, gene flow between populations is also prevented. In other words, isolation prevents the exchange or mixing of genes between populations. Each population is isolation develops genetic divergence independently leading to the formation of new species. Geographic isolation brings about allopatric speciation. Reproductive isolation bring about sympatric speciation.

5.5.3.8 Different concepts of species

Depending upon the choice of criteria there are different types of species concepts. Mayr listed out five types of species concepts. They are:

(a) Practical species concept
(b) Morphological species concept
(c) Genetic species concept
(d) Sterility species concept
(e) Biological species concept

All the ideas of various biologists regarding species is grouped into three concepts. They are:

(a) Typological species concept (or) essentialist species concept
(b) Nominilistic species concept
(c) Biological species concept

(a) **Typological species concept:** Typological species concept mainly reflects the philosophies of Plato, Aristotle, Linneus and their followers. According to this concept each species has an essential and an accidental properties. Essential properties are the defining characteristics of a

given species. Accidental properties are characteristics that vary from one individual to another. According to this concept, species can be recognised by their essential characters and these are expressed in their morphology. In its practical application, typological species concept is usually called the morphological species concept.

(b) Nominilistic species concept: According to Nominilistic species concept only individuals exist, while species are man-made constructs. Bessey expressed this view point particularly well, "nature produces individuals and nothing more, species have no actual existence in nature. They are mental concepts and nothing more. Species have been invested in order that we may refer to great numbers of individuals collectively". This concept says, species are nothing but a product of human imagination.

(c) Biological species concept: The major concept which currently in use is biological species concept. This is based on reproductive isolation between groups of organisms. Reproductive isolation is a key to the process of speciation and therefore the biological species concept reflects the actual organisation of organisms in nature.

5.5.4 SUMMARY

Species is a group of actually or potentially interbreeding natural population which is reproductively isolated from other such groups. Species is a mendelian population having equal number of chromosome and also have common physiological and morphological aspects. Polytypic species have more than one sub-species. A sub-species is defined as geographically isolated populations of a species, which are capable of interbreeding and producing fertile hybrids. The formation of new species is called speciation. New species are formed in four patterns.

(a) Allopatric speciation
(b) Sympatric speciation
(c) Quantum speciation
(d) Parapatric speciation

The factors causing speciation are mutation, recombination, natural selection, genetic drift, hybridization, polyploidy, founders principle and isolation. Geographical isolation leads to allopatric speciation and reproductive isolation leads to sympatric speciation. The major species concepts are:

(a) Typological species concept
(b) Nominilistic species concept
(c) Biological species concept

5.5.5 KEY TERMINOLOGY

1) **Genetic** drift: It is defined as a random genetic change in small populations taking place purely by chance.

2) **Isolation:** Isolation is the segregation or separation of populations by some barriers which prevent interbreeding.

3) **Mutation:** Mutation is a sudden change which alters one gene or one chromosome into another form.

4) **Recombination:** The formation of new gene combinations not present in the parental type is called recombination.

5) **Sibling species:** Sympatric species that are morphologically similar but reproductively isolated.

6) **Speciation:** The formation of new species is called speciation.

7) **Species:** A group of actually or potentially interbreeding natural population which is reproductively isolated from other such groups.

8) **Sub-species:** A sub-species is defined as geographically isolated populations of a species, which are capable of interbreeding and producing fertile hybrids.

5.5.6 SELF ASSESSMENT QUESTIONS

1. Describe species concept.
2. Write an essay on speciation.
3. What are the factors leading to speciation?
4. Write a short notes on mechanism of speciation.

5.5.7 REFERENCE BOOKS

1. Dobzhansky, 1964. **Genetics and the Origin of Species**. Oxford Book and Stationery Company, Calcutta.
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Lesson 5.6

ISOLATION

CONTENTS

5.6.1 INTRODUCTION

Separation of organisms from one another is called isolation. Isolating mechanism is a barrier which prevents interbreeding between populations. The occurrence of new mutations, natural selection, re-combination and genetic drift in one population has no effect on other populations. Dobzhansky first coined the term "isolating mechanism". It plays a major role in the evolution of species. Romans stated that without isolation or the prevention of interbreeding, organic evolution in no case is possible.

5.6.2 OBJECTIVE OF THE LESSION

When interbreeding is prevented by means of isolation, the gene flow between populations is also prevented. In other words, isolation prevents the exchange (or) mixing of genes between populations. In the due course of time, isolated organisms by the effect of various forces of evolution develop into new species (speciation). In this way isolation plays an important role in speciation.

5.6.3 Types of isolating mechanisms

Modern evolutionists such as Mayr, Mecham, Muller, Patterson, Huxley, Stebbins and others have classified and studied the isolating mechanisms. Isolating mechanisms are broadly classified into two main groups. They are : I Geographic isolation II Reproductive isolation

I Geographic isolation:

The separation in space of two populations of a species by geographic barriers such as landmass, water, mountains, deserts etc is called geographical isolation. In this, geographic factors prevents interbreeding between populations. The importance of this isolation in speciation was first stressed by Wagner. Geographic isolation plays a significant role in allopatric speciation. Allopatric species are the related groups occupying different geographical areas. They have no chance to meet. It is assumed that a geographic barrier such as ocean (or) mountain comes to stand between two populations of the same species. This means that a single common gene pool is split into two gene pools by the barriers, thus there is no possibility for interbreeding. Now each population is affected by separate type of environmental factors. This leads to the development of genetic divergences. The main factors causing divergences are evolutionary forces. After the development of genetic divergences, the two populations' will not interbreed even after the original barrier disappears. Hence, the two populations can be designated as two separate species. Geo-graphical isolation can be explained by following examples.

a. The classic example of continuing evolution by geographical isolation is that described by Darwin for finches. Darwin found that there were 26 groups of finches among the Galapagos Islands. Only five of these groups were the same as the finches found on the mainland. The other 21 types were peculiar to the groups of islands. Each of the groups became isolated by migration. Each group evolved separately from the continental (or) mainland forms as well as from other isolated groups on the islands, forming a series of species and subspecies.

b. The southern elephant seal, *Miroungo leonina* occurs in the cold waters of the southern coasts of South America, South Africa, Australia and New Zealand. A close relative of this is northern elephant seal, *Miroungo angustirostris*. It is found in cold waters along the coasts of western North America. The two forms are very similar to one another. However the two forms are separated by about 3000 miles of warm tropical seas and hence are incapable of genetic exchange. The two kinds of elephant seals are allopatric species genetically isolated by an Eco-geographical barrier.

c. The common wood pigeon, *Carpophaga novaezealandise* of New Zealand resembles *Carpophaga chathamensis* of Chatham Islands. Similarly the New Zealand lizard *Lygosoma moco* resembles the Chatham Island lizard L*lygosoma dendyl*. In these animals inter breeding is prevented by the geographical barriers (sea).

d. The Indian giant squirrel, *Ratufa indica* lives in forest. It has different colors in different forests. Here forest act as a barrier and this leads to the formation of sub-species with different colors within a species. For example the giant squirrel is yellowish colour in the deciduous forests of Gujarat, light brown in the deciduous forests of Maharashtra and deep brown in the ever green forests of Mysore.

II Reproductive isolation:

Mayr coined the term "reproductive isolation". Reproductive isolating mechanism is defined as "any genetically determined agency that prevents the interbreeding of mendelian populations". In reproductive isolation, the gene exchange between populations is prevented because of genotypically conditioned differences between the populations. The reproductive isolations are responsible for the formation of sympatric species. The reproductive isolation is broadly classified into two types,

1. Premating (or) prezygotic isolation.
2. Postmating (or) post zygotic isolation.

1. **Primating (or) Pre-zygotic isolation**: Premating (or) prezygotic isolation are those that prevent contact between the species when they are reproductively active (or) which prevent (or) restrict the union of gametes after mating or cross pollination has occurred. Premating (or) prezygotic isolating mechanisms prevent wastage of gametes (germ cells) and so are highly susceptible to improvement by natural selection. This is of following types.

I. Ecological isolation
II. Seasonal isolation
III. Ethological isolation
IV. Mechanical isolation
V. Physiological isolation.
VI. Gametic mortality isolation.

I. Ecological isolation:

The difference between habitat function as a barrier, which prevents the interbreeding of populations. This is called ecological (or) habitat isolation. The following are examples for ecological isolation.

a. The pig frog, *Rana grylio* is extremely aquatic and it lives in deep waters while the gopher frog, *Rana areolata* lives inside burrows dug by mammals and tortoise. They never interbreed in the natural condition because they live in two different habitats.

b. The toads' *Bufo fowleri* and *Bufo woodhousii* are fresh water in habitat. But interbreeding between them is prevented because of the difference in habitats. *B. fowleri* breeds mainly in the stream and *Bufo woodhousii* in the ponds.

c. The water snake, *Natrix sipedon* found in Florida has two races. One race lives in fresh water and another in salt water. They do not mate with each other because of the differences in habitats. Habitat isolation is not a very effective isolating mechanism in mobile animals.

II Seasonal isolation:

Follows Two groups may exist in exactly the same ecological area but don't interbreed because they become sexually mature at different times of the year or under different conditions. Seasonal barriers are particularly frequent among aquatic animals. It is exemplified as.

a. In the northeastern united states, three species of frog, *Rana pipiens*, *R. sylvatica* and *R. clamitans* all mate in the same pond at different times. The different temperatures of pond water stimulate the breeding in these frogs. *R. sylvatica* breeds when the temperature of water is about 44^0F, *R. pipens* breeds when the temperature of water is about 55^0F and *R. clamitans* breeds when the temperature of water is about 60^0F.

b. The salamanders *Amblystoma tigrinum* completes breeding before the end of March but *A. maculatum* does not breed before march.

III ETHOLOGICAL ISOLATION (OR) BEHAVIOURAL ISOLATION:

Ethological isolating mechanisms are barriers to mating due to incompatibilities in behaviour. It is based on the production and reception of stimuli by the sex partners. The males of every species have specific courtship behavior and females of the same species are receptive. Courtship involves an exchange of stimuli between male and female continuing until both have reached a state of physiological readiness in which successful copulation can occur. These specific stimuli are responsible for ethological isolation. It is exemplified as follows.

a. The grey tree frog *Hyla versicolor* and the pine wood tree frog *Hyla femoralis* breed in the same pond but they do not interbreed because of behavioral differences. The mating calls produced by these males are recognized only by their corresponding females.

b. In the case of *Drosophila*, males of related species of *Drosophila melanogaster* and *Drosophila simulans* have specific patterns of courtship movements. The females identify their partners by these specific characters during mating.

IV Mechanical isolation or Morphological isolation

Morphological difference in the external genital organs prevents interbreeding. This is called mechanical isolation (or) morphological isolation. The differences in genital structures of different species of insects was first recognized by Dufour and stated that these genital armatures act like lock and key preventing hybridization between individuals. It is exemplified as follows.

a. In Drosophila interspecific mating causes injury to the genital organs and in severe cases death occurs.

b. Federley has stated that when the male moth's *Chaerocampa elpenor* copulate with the females of *Metopsilus porecellus*, they are sometimes unable to withdraw the penis, making egg deposition impossible.

V PHYSIOLOGICAL ISOLATION:

Sometimes two different species living in the same area are getting chance to mate. In such cases fertilization is prevented by physiological differences between the species. This condition most frequently seen in plants which are showing both self and cross pollination mechanisms. In animals this condition is rarely found it is exemplified as follows.

a. When the spermatozoa are introduced into the reproductive organs of foreign species they find this new environment unsuitable for them. Patterson observed the cross insemination between the related species *Drosophila virlis*, *Drosophila americana*, *D. montana* and *D. lacicola*. The mobility of the sperm in the sperm receptacles of the females of foreign species is lost rather rapidly, while in the same species the mobility is retained for a long time.

b. The sea urchin *strongylocentrotus purpuratus* and *S. franciscanus* inhabit the same area but interbreeding between them is prevented because of physiological differences.

VI GAMETIC MORTALITY ISOLATION:

When there is interspecific mating, the gametes are killed in some species because of an antigenic reaction in the genital tracts. It is exemplified by Warwick and Berry and they showed that the cross between goat and sheep produces normal embryos, but they die before birth.

In another example Volpe shows that when mating occurs *between Bufo fowleri* and *Bufo valliceps* the gametes will die.

2. POST MATING (OR) POST ZYGOTIC ISOLATION:

Post mating (or) post zygotic isolating mechanisms are those which prevent the growth of hybrid individuals after fertilization has occurred (or) which reduce the fertility of the F_1 hybrids (or) the viability of their descendants. Postmating (or) post-zygotic isolating mechanisms do not prevent wastage of gametes and their improvement by natural selection is indirect. This is of following type.

I. Cytological isolation
II. Zygotic mortality isolation
III. Hybrid inviability isolation.
IV. Hybrid sterility isolation.
V. Hybrid break down isolation.

I. **Cytological isolation**: When there is interspecific mating, the isolating mechanism operates at the level of fertilization. Fertilization can not occur successfully between the gametes because of differences in the chromosome number. It is exemplified as,

The bronze frog. *Rana clamitans* and its close relative, the bullfrog *Rana catesbiana* do not produce hybrids because of cytological differences in the gametes. The chromosomes of these two species are incompatible and hence fertilization control occurs.

II. **Zygotic mortality isolation**: The development of the fertilized hybrid egg is often irregular and development may cease at any stage between fertilization and adulthood, zygotic mortality can be caused due to several cytological, genetical, embryological, biochemical and physiological reasons in most animals. It is exemplified as

a. Hybrid zygotes of *Ambystoma mexicanum* lack nucleolus and in the absence of nucleolus, such zygotes die without undergoing cleavage.

b. When the sea urchin *Paracentrotus lividus* and *Psammechinus microtubercutalus* are crossed, most of the embryos die before the gastrula stage is reached.

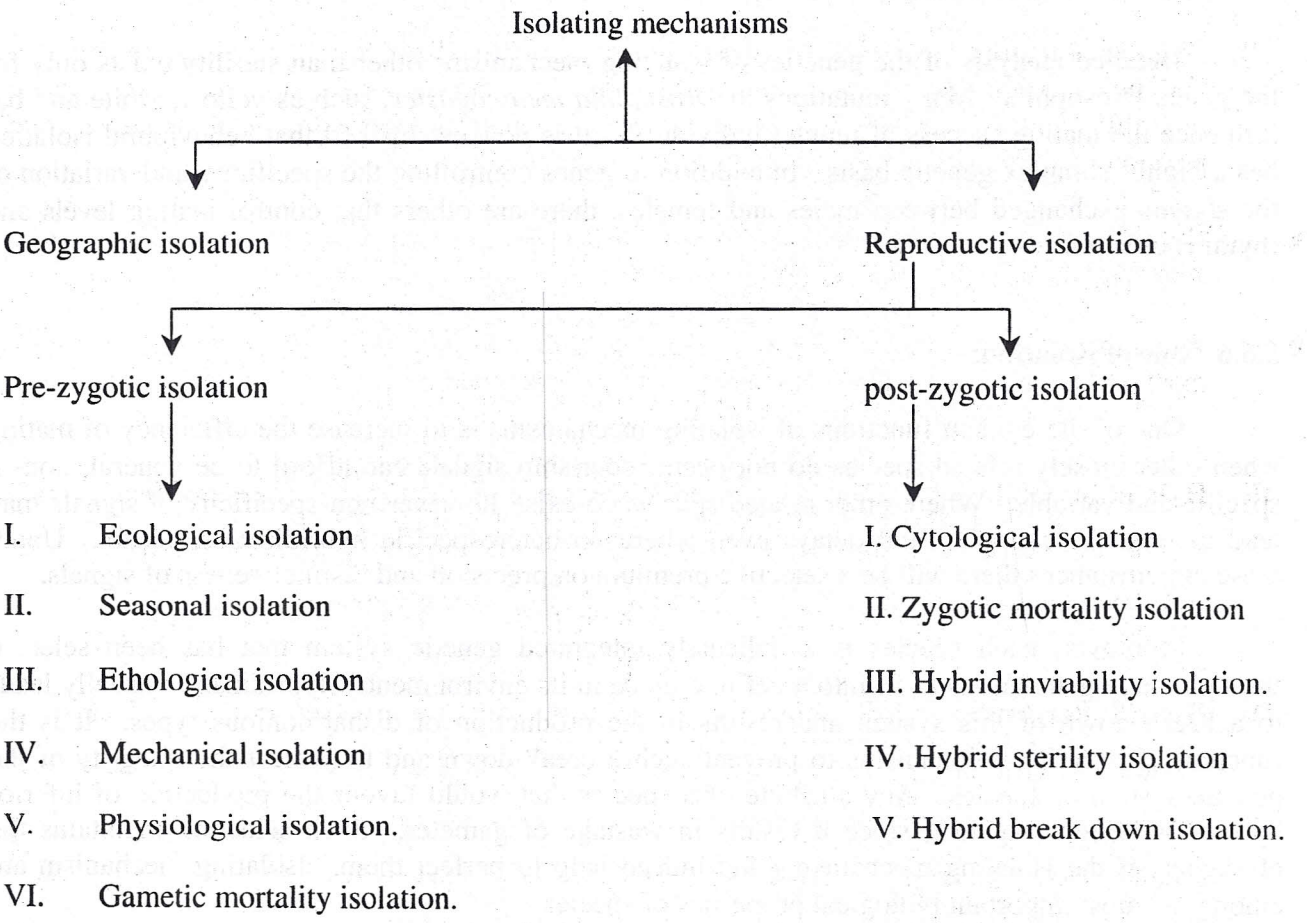
III. **Hybrid inviability isolation**: When there is interspecific interbreeding the resulting hybrids do not survive. It is exemplified as Warwick and Berry showed that the cross between goat and sheep produces normal embryos, but most of them die before death.

IV. **Hybrid sterility isolation**: Sometimes postzygotic isolation involves in the production of vigorous but sterile species hybrids. This type of hybrid sterility involves the abnormal development of gonads (or) abnormal meiosis (or) abnormality in gamete formation. It is usually most common in animals and plants. It is exemplified as, the mule formed as a hybrid for a female horse and a male ass.

V. **Hybrid break down isolation**: In both animals and plants, there are examples of hybrids which are highly (or) atleast partly fertile, but which give rise to weak, abnormal (or) sterile progeny in the second (F_2) generation. This phenomenon is called hybrid break down. It is exemplified as follows.

a. Harland, Hutchinson and Stephens found that species of *Gossypium hirsutum*, *G. barbadense* and *G. tomentosum* intercross freely to give fertile and vigorous F_1 hybrids. But the F_2

hybrids appear to be as vigorous as the parental species, although the hybrid males are sterile, F_1 hybrid female produce numerous eggs and are fertile when back crossed to either parental species. The general viability of this back cross hybrids is low in comparison with parental species is and F_1 hybrids.



5.6.4 The Co-action of isolating mechanisms:

The interbreeding of closely related sympatric species of animals is prevented by a whole series of ecological, behavioral and cytogenetical factors, usually several for each pair of species. One factor (isolation) is often dominant, such as acoustic stimuli in certain frogs, grass hoppers, cicadas and mosquitoes; chemical contact stimuli in some insects, sex stuffs in Lepidoptera and certain marine organisms (Bonellia etc) and visual stimuli in certain birds, fishes and insects. The sterility barrier may be strong (or) weak but is rarely tested except when the other isolation mechanisms break down. Thus, the isolating mechanisms are arranged like a series of hurdles,. If one breaks down, another must be overcome. For example, if the habitat barrier is broken, individuals of two species may still be separated from each other by behaviour patterns. If these also fail, the mates may be unable to produce viable hybrids (or) if hybrids are produced they may be sterile.

5.6.5 The genetics of isolating mechanisms:

From the complexity of isolating mechanisms that protect every species, one can conclude that a considerable number of genes is involved. Almost any gene that changes the adaptation of a population may have an incidental effect on the interaction between male and female. Conversely, a mating advantage of a male will become established in a population provided it does not seriously lower the fitness of off-spring.

Detailed analysis of the genetics of isolating mechanisms other than sterility exists only for the genus Drosophila. Many mutations in *Drosophila melanogaster*, such as yellow, white and bar influence the mating success of mutant individuals. It is now established that behavioural isolation has a highly complex genetic basis. In addition to genes controlling the specificity and variation of the signals exchanged between males and females, there are others that control mating levels and rhythms of activity.

5.6.6 Role of isolation:

One of the evident functions of isolating mechanisms is to increase the efficiency of mating when other closely related species do not occur, courtship signals can afford to be general, non- – specific and variable. Where other related species co-exist, however non-specificity of signals may lead to wasteful courtship and delays even where no heterospecific hybridization occurs. Under these circumstances there will be a selective premium on precision and distinctiveness of signals.

Moreover, each species is a delicately integrated genetic system that has been selected through many generations to fit into a definite niche in its environment. Hybridization usually leads to a break down of this system and results in the production of disharmonious types. It is the function of isolating mechanisms to prevent such a break down and to protect the integrity of the genetic system of species. Any attribute of a species that would favour the production of inferior hybrids is selected against, since it results in wastage of gametes. Such selection maintains the efficiency of the isolating mechanisms and indeed help to perfect them. Isolating mechanism are among the most important biological properties of species.

5.6.7 Origin of isolation:

Following two theories have been proposed about the origin of isolation.

a. Wallace has proposed the first theory. According to him, isolation mechanisms are selected by natural selection to root out lowly fitted hybrids between species and allow only those genotypes that have developed better isolation mechanism. There are instances where hybrids survive better under vigorous environmental stresses than the pure races. The serious objection to this theory principally comes from Darwin. The backcrossing and introgression by a certain hybrid population would lead to the steady state deterioration of the pre-existing mechanism. Thus, Wallace's argument of the improvement of isolation is failed.

b. Poulton and Muller put forwarded the second idea regarding origin of isolating mechanisms. According to their idea, isolation mechanism originates as a bye product of genetic divergence of isolated populations. Genetical studies reveals that hybrids between two species must be effectively sterile (or) inviable. Natural selection then picks out such instances, and improves accessory isolating mechanisms, such as failure of sterile species survival and such a selection ultimately renders hybrid production lesser and lesser in nature.

5.6.8 Summary of the lesson:

Separation of closely related populations from interbreeding by means of different barriers is known, as isolation .The isolation is biologically important phenomenon for speciation. Isolation of population is mainly brought by geographic and reproductive barriers. Geographical isolation is perfect in most of the cases but it fails for organisms which share overlapping habitats and also have strong locomotory capacity. Geographical isolation leads to allopatric speciation. Reproductive isolation is of two types (I) Premating (or) prezygotic isolation (ii) post mating (or) post zygotic isolation. Reproductive isolation leads to symapatric speciation. The isolating mechanisms act in a co-operative fashion. If one mechanism fails then the other mechanism plays its established role. The theory put forward by. Poulton and Muller is the most convincing one to explain origin of isolation.

5.6.9 KEY TERMINOLOGY

1. **Isolation** : Separation of closely related populations from interbreeding by means of different barriers is known as isolation.

2. **Allopatric species**: Closely related populations, which are separated by geographical barriers.

3. **Symmetric species**: Closely related populations, which are separated by reproductive barriers.

5.6.10 SELF-ASSESSMENT QUESTIONS.

1. Write an essay on isolation.
2. Write a note on Premating or pre zygotic isolation mechanisms.
3. Write a note on post mating or post zygotic isolation mechanisms.
4. Describe reproductive isolation mechanisms.
5. Write a short notes on origin of isolation.

5.6.11 Reference

1. Stebbins, G.L. 1970. Processes of Organic Evolution, Prentice Hall.

2. Strickberger, MW. Z 1994. Evolution. CBS Publishers and distributors. ISBN Shahdra, Delhi (India)