APPOLO STUDY CENTRE CHENNAI

APPOLO STUDY CENTRE

Scorates Weekly test 4

Cell Biology		
6th term -2	Unit - 5	The Cell
7th term -2	Unit - 4	Cell Biology
10th book	Unit - 16	Plant And Animal Hormones
11th botany	Unit - 6	The Unit of Life
	Unit - 7	Cell Cycle
	Unit - 8	Biomolecules
GENETICS		
10th book	Unit – 18	Heredity
	Unit – 19	Origin & Evolution of Life
	Unit – 20	Breeding & Biotechnology
12th zoology	Unit- 4	Principles of Inheritance and Variation
	Unit - 5	Molecular Genetics
	Unit - 6	Evolution
	Unit - 10	Application of Bio Technology
12th botany	Unit- 2	Classical Genetics
	Unit- 3	Chromosomal Basis of Inheritance
	Unit- 4	Principles and processes of Biotechnology
	Unit- 5	Plant Tissue Culture
Human Disease		
6th term 1	Unit- 6	Health & Hygiene
7th Term 1	Unit- 6	Health & Hygiene
8th book	Unit- 6	Microorganisms
9th book	Unit - 21	Nutrition & Health
10th book	Unit - 21	Health & Disease
11th zoology vol -1	Unit - 5	Digestopm And Absorption
12th zoology vol -1	Unit - 7	Human Health & Disease
	Unit - 8	Immunology
	Unit - 9	Microbes in Human Welfare

6th Term (II)
Unit 5. The Cell

Introduction

- Observe the two pictures given above. Do you observe any similarity between them?
- Close your eyes and imagine a brick wall. What is the basic building block of the wall? A single brick, of course.
- Like a brick wall, your body is composed of basic building blocks, and are named as "Cells".
- The cell is the basic structural and functional unit of every living organism.
- The cell is self-sufficient to carry out all the fundamental and essential functions of an organism.

The Cell

- All living things are made of one or more cells. There are variety of cell types however, they all have some common characteristic features.

Discovery of the cell

- The Englishman Robert hooke was a scientist, mathematician, and inventor. He improved microscope which was usedin those days, and built a compound microscope. He placed water-lens beside the microscope to focus the light from an oil-lamp on specimens to illuminate them brightly. So that he able to see the minute parts of the objects clearly.
- One day Hooke made thin sections of the cork and observed them through his microscope. He observed many small identical chambers which were hexagonal in shape. He was surprised.
- After that he saw many objects like Butterfly's wings, Bee's compound eyes etc.,
- Based on this observations Hooke published a book named Micrographia in the year 1665, where he first used the term Cell . He describe the structure of tissue using the term cell.
- In Latin the word 'cellua' means a small chamber.
- The branch of science that deals with the study of cells is called 'Cell Biology'.

The Structural Organization Of The Cell

A typical cell consists of three major parts:

1. An outer cell membrane.
2. A liquid cytoplasm.
3. A nucleus.

- Analogous to the body's internal organ, like eyes, heart, lungs organelles are specialized structures and perform valuable functions necessary for normal cellular operation. Many of miniscule but distinct structures called Organelles lie within the cell.

Size of the cell

- The size of cells may vary from a micrometer (a million of a metre) to a few centimeters. Most cells are microscopic and cannot be seen with the nacked eye. They can be observed only through the Microscope.

- Smallest size of the cell is present in Bacteria. The size of the bacterial cell ranges from 0.01 micrometer to 0.5 micro meter.

- On the other hand the largest cell is the egg of an ostrich with 170 millimeter width. We can see this with the nacked eye. In Human body the nerve cells are believed to be the longest cells.

Cell size has no relation to the size of an organism. It is not necessary that the cells of, say an elephant be much larger than those of a mouse.

Shapes

- Cells are of different shapes. For example some shapes are given in the below pictures.

Number

- The number of cells present in different organisms may vary. Organisms may be either unicellular (single cell) or multicellular. Organisms such as Bacteria, Amoeba, Chlamydomonas, and Yeast are unicellular. On the other hand, organisms such as Spirogyra, Mango, and Human beings are multicellular. (i.e) made up of a few hundreds to million cells.

Approximate number of cells in the human body is 3.7 X1013 or 37,000,000,000,000

TYPES OF CELL

- Generally cells are classified into two types. First one is Prokaryotic cell .It has No true nucleus consisting of no nuclear membrane. Another one is Eukaryotic cell. It has True nucleus consisting of nuclear membrane.

Prokaryotic cell

- The unicelluar organisms like Bacteria has Prokaryotic cells. It has No true nucleus. This type of nucleus is called as nucleiod. No nuclear membrane is around this nucleiod. These cells were the first form of life on earth. It is ranging from 0.003 to 2.0 micro meter in diameter.

Eukaryotic cell

- Cells which has true nucleus is called as eukaryotic cell. It is bigger than prokaryotic cells. It's organelles bounded by membrane.

- Ex. Plants,animals, most of the fungi and algae.

Differences between Prokaryotic cell Eukaryotic cell

Prokaryotic cell	Eukaryotic cell
It's diameter ranges from 1 to 2 micron	It's diameter ranges from 10 t0 100 micron
Absence of membrane bound organelles	Presence of membrane bound organelles
Nucleus consisting of no nuclear membrane	True nucleus consisting of nuclear membrane
Absence of nucleoli	Presence of nucleoli

Plant cell and Animal cell

- Both plant and animals are made up of cells. Both cells are eukaryotic in nature, having a well defined membrane – bound nucleus.

Plant cell

- ❖ It is usually larger in size. It is hard in nature.
- ❖ Plant cell have a cell wall in addition to their cell membrane.
- ❖ Plant cell have chloroplast which contain chlorophyll
- ❖ Plant cells have large vacuoles. Centrioles are absent.

Animal cell

- ❖ Animal cells are generally smaller than plant cells. It is not so hard as plant cell.
- ❖ A cell wall is absent.

- ❖ Chloroplast is usually absent.
- ❖ An animal cell may have many small vacuoles.
- ❖ Centrioles are found in animal cells

Dimension - cell structure

1. How does a cell look like?

2. What is its shape and size?

- The above cell has a three dimensional view. We can see the three sides of the cell structure. You can also view the size, shape and location on the organelles of the cell also.

- 3-D view is appealing because it is more like reality

- In 3-D, We can see the entire view of the cell. It exposes the accurate size and shape and shows the correct location of the cell organelles.

Cell components and their functions

S. No	Cell Components	Main Functions	Special Name
1.	Cell wall	• Surrounds and protects the cell • Make the cell stiff and strong	Supporter and protector
2.	Cellmembrane	• Holds and protects the cell • Controls the movement of materials in and out of the cell	Gate of the cell
3.	Cytoplasm	• A watery, gel-like material in • which cellparts move	Area of movement
4.	Mitochondria	• Produce and supply most of Theenergyfor the cell	Power house of the cell

5.	Chloroplasts	• Contain green pigment chlorophyll • Capture the energy of sunlight and use it to produce food for the cell by photosynthesis.	Food producers for the cell (Plant cell)
6.	Vacuoles	• Store food, water, and chemicals	Storage tanks
7.	Nucleus	• Acts as 'brain' of the cell • Regulates and controls all the cell activities	Control centre
8.	Nucleusmembrane	• Surrounds and protects the nucleus control the movement materials in and out of the nucleus	Gate of the nucleus

7th Std term(II)
Unit – 4 Cell Biology

Introduction

- Sona had a dinner, some hour later; sheexperienced a stomach pain and wentto aclinic. After examination, the Doctor told Sonathat she had eaten foodcontaminated with atype of bacteria which might have caused foodpoisoning.Bacteria are micro-organisms thatcan be seen only under microscope and notseenthrough nacked eyes. Salmonella species isa bacterium that can cause food-borneinfection.

- Our earth is a beautiful place where indifferent types of organisms happilycoexist.From minute mosses to huge conifers, invisiblebacteria to huge blue whale,all have a basic unitcalled Cell. Let us study about the cell.

Cell as a fundamental unit of life:

- The building wall is made up of numerousbricks. In the similar manner, a beehive iscomposed of numerous hexagonal units. Someof the organisms arerepresented by a single cell.Therefore, they show a simple organization. Thebasicfunctional unit of an organism is called, a cell.Structures of a cell represent thearrangementof parts or organells in a cell. Function is theactivity of each part ororganell in a cell. Cellsare the basic building blocks of an organism.You learnt thatatoms are the basic buildingblocks of matter in chapter three. Likewise,humanbody is made up of animal cell and plantis made up of plant cell.

Unicellular organisms

- Some simple organisms are made upof only one cell. They are calledunicellularorganisms, which can be seen with the help ofa microscope. There aremany single - celledmicroscopic organisms.

- Have a look at the image. Chlamydomonasand an Amoeba, a single cellorganism which carryout entire functions the bodyof all organisms are made upof tiny buildingblocks called, cells. Bacteria are also one celledunicellularorganisms.

Multicellular Organism

- The cells are organized into tissues, organsand organ systems in amulticellular organism.Macroscopic organisms are visible and consist of manyells. The body of macroscopicorganisms involves various functions. Youcan see cells of onion and human through amicroscope. Onion and man are examples formulticellular organism.

Cell to organism

- Many cells function together to formtissues, different tissues combinedtogether toform an organ and different organs to form anorgan system, whichleads to form an organism.

Organisms

- Many types of organ systems functiontogether in a body, e.g. respiratorysystem,digestive system, excretory system circulatorysystem etc.

Organ System

- Many organs together form an organsystem, which is concerned with aspecificfunction. For example, Respiratory system,which has organs like nostrils,nasal chamber,wind pipe and lungs that helps in the process ofrespiration. In aplant, the root system consistsof primary root, secondary root and tertiaryroot,which does the function of conduction ofwater, mineral and also fixation.

Organ

- A collection of different tissues workedtogether to perform a specific functionorfunctions is called an organ. Human body hasdifferent organs like stomach, eye,heart, lungsetc., are made up of different type of tissues.Plants have organs such asleaves, stems, androots.

Tissue

- Tissue is a group of cells, organized for aspecific function. Tissues havefollowing featureslike same shaped cells or different shapedcells to perform acommon function. Humanand other animals are made up of nervous,epithelial,connective and muscle tissues. Plantshave transport, protective and ground tissues.

Cell

- The cell is a basic structural and functionalunit of life. Cell is the building unitof livingorganisms. You can see in a hand, how manytypes of cells are there towork together toperform its functions. So, cell is known as thebasic unit of life.

Plant and Animal cell comparison

- Why do plant cellsdiffer from animal cells?They differ from eachother becausethey have toperform different functions.Now you know that thereare many main similarities between plant andanimal cells. Let us see how they differfrom oneanother as given in the picture.

Human cells related to functions Different types of cells

- Our body is made up of many differentkinds of cells. Each type of cell isspecialized toperform a specific function. Depending on thefunction, cell hasspecific shape, size and mayhave some components which other type ofcells do nothave. Have a look at the differencesbetween nerve cells and red blood cells in theimages. Even though there are many differenttypes of cells, there are somecomponentscommon to all type of cells. Let us take a look atthis in the next section.

What's inside a cell?

- Inside a cell, there are many tiny structurescalled cell organelles. Theseorganelles areresponsible for providing needs of the cell.They work to bring infood supplies, get ridof waste, protection and repair of the cell, andhelp it to growand reproduce. Each one has aspecific function to do for the cell. And, if anyoneorganelle stops its function, then the cell isprogrammed to die.

Cell Structure

- As we have mentionedbefore, all cells have somecommon structure.

These are

1. Cell membrane
2. Cytoplasm, and
3. Nucleus (In most eukaryotic cells).

- The structure of a typical plant and animal cellshows following peculiarities:

Cell membrane

- The boundary of an animal cell is theplasma membrane, which is also calledas cellmembrane

Cell wall - "Supporter and Protector"

- All animal and plant cells are enclosed orsurrounded by a cell membrane asyou learnedbefore. However, as you might have noticedpreviously that, animalcells often have anirregular shape, whereas plant cells have a muchmore regular and rigid shape.

- Plant cells have an additional layer on theouter side of the cell membrane.This is calledas the cell wall that provides a frame work forsupport and stability.The cell wall is formed from variouscompounds, the main one being cellulose.Cellulose helps to maintain the shape of theplant cell. This allows the plant toremain rigidand upright even if it grows to great heights.Each cell is interconnectedwith its neighbouringcells through openings called Plasmodesmata.

Stem Cells
Stem cells are quite amazingas they can divide andmultiply while at the sametime with their ability to develop into anyother type of cell. Embryonic stem cellsarevery special as they can become absolutelyany type of cell in the body, forexample,blood cell, nerve cell, muscle cell or glandcell. So they are utilized by theScientistand Medicos, to cure and prevent somediseases like Spinal cord injury.

Cytoplasm - I am the "Area of Movement".

- When you look at the temporary mountsof an onion peel, you can see a largeregion ofeach cell an enclosed by the cell membrane. Thisregion takes up very littlestain. It is called thecytoplasm.

- The cytoplasm includes all living parts ofthe cell with in the cell membrane,excluding thenucleus. The cytoplasm is made up of the cytosoland cell organelles.The cytosol is a watery, jellylikemedium made up of 70% - 90% water andusuallycolourless.

- Cell organelles and structures presentin a cell are endoplasmic reticulum,vacuole,ribosome, Golgi body, lysosome, mitochondria,centriole, chloroplast,surrounded by plasmamembrane and cell wall.

Protoplasm vs. Cytoplasm

- In particular, the material inside and outsidethe nuclear membrane is knownas ProtoplasmThe fluid inside the nucleus is known as thenuclear fluid or nucleoplasm and outside thenucleus is called as cytoplasm.

Inside the cytoplasm Mitochondria - "Power house of the Cell"

- Do you remember learning about the foodas the energy source for the body?Just as woodis burnt to release the stored potential energyto make a fire to heatsome water The food thatyou ate to be broken down in order to releasethe energywhich can be used by your body tofunction. Mitochondria are responsible to dothisfunction.

- Very active cells have more mitochondriathan cells that are less active. Whichtype of cell,do you think, will have more mitochondria, amuscle cells or a bonecell?

- Mitochondrian is an oval or rod shapeddouble membrane bounded organelle.Aerobicrespiratory reactions take place within themitochondrion to release energy.So it is knownas "the Power House" of the cell. The energyproduced within themitochondrion is used forall the metabolic activities of the cell

Chloroplast- "Food Producers"

- Do you notice the green organellespresent in plant cells and absent in animalcells.Chloroplasts are the only cell organelles thatcan produce food from thesunenergy. Only plants withchloroplast are able to dophotosynthesis because theycontain the very importantgreen pigment, chlorophyll.Chlorophyll can absorbradiant energy fromthe Sun and convert it to the chemical energywhich can beused by the plants and animals.Animal cells lack chloroplasts and are unable todophotosynthesis.

Golgi complex- I need a break

- Membrane bounded sacs are stacked on topof the other with associatedsecretory vesicles arecollectively known as Golgi complex. Functionsof Golgicomplex are the production of secretorysubstances, packaging and secretion. This is thesecret behind the change in the colour and tasteof fruits

Lysosome- "Suicidal Bag"
Everything I touch, I destroy

- You will find organelles called aslysosomes, which are very small to viewusinga light microscope. They are the main digestivecompartments of the cell. Theylyse a cell, hencethey are called "suicidal bag".

Centrioles

- They are generally found close to thenucleus and are made up of tube-likestructures.Centrioles or centrosomes are present only inanimal cells and absent inplant cells. It helpsin the separation of chromosomes during celldivision.

Endoplasmic reticulum - You guys, be quiet, I have so much work to do

- It is an inter membranous network madeup of flat or tubular sacs within thecytoplasm.Endoplasmic reticulum is of two types. Theyare rough endoplasmicreticulum and smoothendoplasmic reticulum.

- **Rough endoplasmic reticulum**: are rough dueto the ribosomes attached to themembrane.which helps in the synthesis of protein.

- **Smooth endoplasmic reticulum**. It is a networkof tubular sacs withoutribosomes on themembrane. They play a role in the synthesis oflipids, steroids andalso transport them withinthe cell.

Nucleus - Everyone do what I say. Acting like the "Brain" of the cell

- Plant and animal cells have a nucleus insidethe cytoplasm. It is surrounded by anuclearenvelope. One or two nucleolus and thechromatin body are present insidethenucleus. During cell division, the chromatinbody is organised into achromosome.Storage of genetic material and transfersheredity characters fromgeneration togeneration are the functions of chromosome.

Functions of Nucleus

- In controls all the processes and chemicalreactions that take place inside the cell
- Inheritance of character from one generation to another

Red blood cells

Red bloodcells do notcontain anucleus. Without a nucleus,these cells diequickly; about two millionred blood cells die every second! Luckily,the bodyproduces new red blood cellsevery day.

10thstd
Unit 16 - PLANT AND ANIMAL HORMONES

Introduction

- The word hormone is derived from the Greek word "hormon" meaning " to excite". The function of control and coordination in plants is performed by chemical substances produced by the plants called plant hormones. In plants several cells are capable of producing hormones. These phytohormones are transported to different parts of the plants to perform various physiological functions.

- Endocrine glands in vertebrate animals possess a diversified communication system to co-ordinate physiological and metabolicfunctions by chemical integration. The endocrine system acts through chemical messengers known as hormones which are produced by specialized glands. Physiological processes such as digestion, metabolism, growth, development and reproduction are controlled by hormones.

Plant Hormones

- Plant hormones are organic molecules that are produced at extremely low concentration in plants. These molecules control morphological, physiological and biochemical responses.

Types of Plant Hormones

- There are five major classes of plant hormones. They are:

1. Auxins
2. Cytokinins
3. Gibberellins
4. Abscisic Acid (ABA)
5. Ethylene

- Among all these plant hormones auxins, cytokinins and gibberellins promote plant growth while abscisic acid and ethylene inhibit plant growth.

Auxins

- Auxins (Gk. auxein = to grow) were the first plant hormones discovered. The term auxin was introduced by Kogl and Haagen- Smith (1931). Auxins are produced at the tip of stems and roots from where they migrate to the zone of elongation. Charles Darwin (1880), observed unilateral growth and curvature of canary grass (Phalariscanariensis) coleoptiles. He came to the conclusion that some 'influence' was transmitted from the tip of the coleoptile to the basal region. This 'influence' was later identified as Auxin by Went.

Went's Experiment

- Frits Warmolt Went (1903– 1990), a Dutch biologist demonstrated the existenceand effect of auxin in plants. He did a series of experiments in Avena coleoptiles.

- In his first experiment he removed the tips of Avena coleoptiles. The cut tips did not grow indicating that the tips produced something essential for growth.In his second experiment he placed the agar blocks on the decapitated coleoptile tips. The coleoptile tips did not show any response. In his next experiment he placed the detached coleoptile tips on agar blocks. After an hour, he discarded the tips and placed this agar block on the decapitated coleoptile. It grew straight up indicating that some chemical had diffused from the cut coleoptile tips into the agar block which stimulated the growth.

- From his experiments Went concluded that a chemical diffusing from the tip of coleoptiles was responsible for growth, and he named it as "Auxin" meaning 'to grow".

- Types of Auxins: Auxins are classified into two types, namely natural auxins and synthetic auxins.

- **Natural Auxins:** Auxins produced by the plants are called natural auxins. Example: IAA (Indole – 3 - Acetic Acid)

- **Synthetic Auxins:** Artificially synthesized auxins that have properties like auxins are called as synthetic auxins. Example: 2, 4 D (2,4Dichlorophenoxy Acetic Acid).

- Physiological effects of auxins: Auxins bring about a variety of physiological effects in different parts of the plant body.

1. Auxins promote the elongation of stems and coleoptiles which makes them to grow.

2. Auxins**induce root formation** at low concentration and inhibit it at higher concentration.

3. The auxins produced by the apical buds suppress growth of lateral buds. This is called **apical dominance**.

4. Seedless fruits without fertilization are induced by the external application of auxins. (**Parthenocarpy**). Examples: Watermelon, Grapes, Lime etc.

5. Auxins**prevent** the formation of **abscission layer**.

Phenyl Acetic Acid (PAA) and Indole 3 Acetonitrile (IAN) are natural auxins. Indole 3

Butyric Acid (IBA), Indole-3- Propionic Acid, α-Naphthalene Acetic Acid (NAA), 2, 4, 5-T (2,4,5Trichlorophenoxy Acetic Acid) are some of the synthetic auxins.

Cytokinins

- Cytokinins (Cytos - cell; kinesis - division) are the plant hormones that promote cell division or cytokinesis in plant cells. It was first isolated from Herring fish sperm. Zeatin was the cytokinin isolated from Zea mays. Cytokinin is found abundantly in liquid endosperm of coconut.

Physiological effects of cytokinins

1. Cytokinin induces cell division (cytokinesis) in the presence of auxins.
2. Cytokinin also causes cell enlargement.
3. Both auxins and cytokinins are essential for the formation of new organs from the callus in tissue culture (Morphogenesis).
4. Cytokinins promote the growth of lateral buds even in the presence of apical bud.
5. Application of cytokinin delays the process of ageing in plants. This is called Richmond Lang effect.

Gibberellins

- Gibberellins are the most abundantly found plant hormones. Kurosawa (1926) observed Bakanae disease or foolish seedling disease in rice crops. This internodal elongation in rice was caused by fungus Gibberellafujikuroi. The active substance was identified as Gibberellic acid.

Physiological effects of gibberellins

1. Application of gibberellins on plants stimulate extraordinary elongation of internode. e.g. Corn and Pea.

2. Treatment of rosette plants with gibberellin induces sudden shoot elongation followed by flowering. This is called bolting

3. Gibberellins promote the production of male flowers in monoecious plants (Cucurbits).

4. Gibberellins break dormancy of potato tubers.

5. Gibberellins are efficient than auxins in inducing the formation of seedless fruit - Parthenocarpic fruits (Development of fruits without fertilization) e.g. Tomato.

Abscisic Acid

- Abscisic acid (ABA) is a growth inhibitor which regulates abscission and dormancy. It increases tolerance of plants to various kinds of stress. So, it is also called as stress hormone. It is found in the chloroplast of plants.

Physiological effects of abscisic acid

1. ABA promotes the process of abscission (separation of leaves, flowers and fruits from the branch).
2. During water stress and drought conditions ABA causes stomatal closure.
3. ABA **promotes senescence** in leaves by causing loss of chlorophyll.
4. ABA **induces bud dormancy** towards the approach of winter in trees like birch.
5. ABA is a powerful **inhibitor of lateral bud growth** in tomato.

Ethylene

- Ethylene is a gaseous plant hormone. It is a growth inhibitor. It is mainly concerned with maturation and ripening of fruits. Maximum synthesis of ethylene occurs during ripening of fruits like apples, bananas and melons

Physiological effects of ethylene

- Ethylene promotes the ripening of fruits. e.g. Tomato, Apple, Mango, Banana, etc
- Ethylene inhibits the elongation of stem and root in dicots.
- Ethylene hastens the senescence of leaves and flowers.
- Ethylene stimulates formation of abscission zone in leaves, flowers and fruits. This leads to premature shedding.
- thylene breaks the dormancy of buds, seeds and storage organs

Human Endocrine Glands

- Endocrine glands in animals possess a versatile communication system to coordinate biological functions. Exocrine glands and endocrine glands are two kinds of glands found in animals. Endocrine glands are found in different regions of the body of animals as well as human beings. These glands are called ductless

- glands. Their secretions are called hormones which are produced in minute quantities. Th e secretions diff use into the blood stream and are carried to the distant parts of the body. They act on specifi c organs which are referred as target organs.

The branch of biology which deals with the study of the endocrine glands and its physiology is known as 'Endocrinology'. Thomas Addison is known as Father of Endocrinology. English physiologists W. M. Bayliss and E. H. Starling introduced the term hormone in 1909. They fi rst discovered the hormone secretin.

- Exocrine glands have specifi c ducts to carry their secretions e.g. salivary glands, mammary glands, sweat glands.

- Endocrine glands present in human and other vertebrates are

❖ Pituitary gland
❖ Thyroid gland
❖ Parathyroid gland
❖ Pancreas (Islets of Langerhans)
❖ Adrenal gland (Adrenal cortex andAdrenal medulla)
❖ Gonads(Testes and Ovary)
❖ Thymus gland

- Pituitary Gland The pituitary gland or hypophysis is a pea shaped compact mass of cells located at the base of the midbrain attached to the hypothalamus by a pituitary stalk. The pituitarygland is anatomically composed of two lobes and perform different functions. They are the anterior lobe (adenohypophysis) and the posterior lobe (neurohypophysis). The intermediate lobe is non-existent in humans.

- The pituitary gland forms the major endocrine gland in most vertebrates. It regulates and controls other endocrine glands and so is called as the "Master gland".
- Hormones secreted by the anterior lobe (Adenohypophysis) of pituitary

- The anterior pituitary is composed of different types of cells and secrete hormones which stimulates the production of hormones by other endocrine glands. The hormones secreted by anterior pituitary are

❖ Growth Hormone
❖ Thyroid stimulating Hormone
❖ Adrenocorticotropic Hormone
❖ Gonadotropic Hormone which comprises the Follicle Stimulating Hormone and Luteinizing Hormone
❖ Prolactin

- Growth hormone (GH) GH promotes the development and enlargement of all tissues of the body. It stimulates the growth of muscles, cartilage and long bones. It controls the cell metabolism. Th e improper secretion of this hormone leads to the following conditions.

Dwarfism:

- It is caused by decreased secretion of growth hormone in children. The characteristic features are stunted growth, delayed skeletal formation and mental disability.

Gigantism:

- Over secretion of growth hormone leads to gigantism in children. It is characterised by overgrowth of all body tissues and organs. Individuals attain abnormal increase in height.

Acromegaly:

- Excess secretion of growth hormone in adults may lead to abnormal enlargement of head, face, hands and feet.

Thyroid stimulating hormone (TSH)

- TSH controls the growth of thyroid gland, coordinates its activities and hormone secretion.

Adrenocorticotropic hormone (ACTH)

- ACTH stimulates adrenal cortex of the adrenal gland for the production of its hormones. It also influences protein synthesis in the adrenal cortex.

Gonadotropic hormones (GTH)

- The gonadotropic hormones are follicle stimulating hormone and luteinizing hormone which are essential for the normal development of gonads.

Follicle stimulating hormone (FSH)

- In male, it stimulates the germinal epithelium of testes for formation of sperms. In female it initiates the growth of ovarian follicles and its development in ovary.

Luteinizing hormone (LH)

- In male, it promotes the Leydig cells of the testes to secrete male sex hormone testosterone. In female, it causes ovulation (rupture of mature graafian follicle), responsible for the development of corpus luteum and production of female sex hormones estrogen and progesterone.

Prolactin (PRL)

- PRL is also called **lactogenic hormone**. This hormone initiates development of mammary glands during pregnancy and stimulates the production of milk after child birth.

Hormones secreted by the posterior lobe (Neurohypophysis) of pituitary

- The hormones secreted by the posterior pituitary are

1. Vasopressin or Antidiuretic hormone
2. Oxytocin

Vasopressin or Antidiuretic hormone (ADH)

- In kidney tubules it increases reabsorption of water. It reduces loss of water through urine and hence the name antidiuretic hormone.

- Deficiency of ADH reduces reabsorption of water and causes an increase in urine output (polyuria). This deficiency disorder is called Diabetes insipidus.

Oxytocin

- It helps in the contraction of the smooth muscles of uterus at the time of child birth and milk ejection from the mammary gland after child birth.

Thyroid Gland

- The thyroid gland is composed of two distinct lobes lying one on either side of the trachea. The two lobes are connected by means of a narrow band of tissue known as the isthmus. This gland is composed of glandular follicles andlined by cuboidal epithelium.The follicles are filled with colloid material called thyroglobulin.

- An amino acid tyrosine and iodine are involved in the formation of thyroid hormone. The hormones secreted by the thyroid gland are

1. Triiodothyronine (T_3)
2. Tetraiodothyronine or Thyroxine (T_4)

Functions of thyroid hormones

- The functions of thyroid hormones are

❖ Production of energy by maintaining the Basal Metabolic Rate (BMR) of the body.
❖ Helps to maintain normal body temperature.
❖ Influences the activity of central nervous system.
❖ Controls growth of the body and bone formation.
❖ Essential for normal physical, mental and personality development .
❖ It is also known as personality hormone.
❖ Regulates cell metabolism.

Thyroid Dysfunction

- When the thyroid gland fails to secrete the normal level of hormones, the condition is called thyroid dysfunction. It leads to the following conditions

Hypothyroidism

- It is caused due to the decreased secretion of the thyroid hormones. The abnormal conditions are simple goitre, cretinism and myxoedema.

Goitre

- It is caused due to the inadequate supply of iodine in our diet. This is commonly prevalent in Himalayan regions due to low level of iodine content in the soil. It leads to the enlargement of thyroid gland which protrudes as a marked swelling in the neck and is called as goitre.

Cretinism

- It is caused due to decreased secretion of the thyroid hormones in children. The conditions are stunted growth, mental defect, lack of skeletal development and deformed bones. They are called as cretins.

Myxoedema

- It is caused by deficiency of thyroid hormones in adults. They are mentally sluggish, increase in body weight, puffiness of the face and hand, oedematous appearance.

Hyperthyroidism

- It is caused due to the excess secretion of the thyroid hormones which leads to Grave's disease. The symptoms are protrusion of the eyeballs (Exopthalmia), increased metabolic rate, high body temperature, profuse sweating, loss of body weight and nervousness.

Parathyroid Gland

- The parathyroid glands are four small oval bodies that are situated on the posterior surface of the thyroid lobes. The chief cells of the gland are mainly concerned with secretion of parathormone.

Functions of Parathormone

- The parathormone regulates calcium and phosphorus metabolism in the body.They act on bone, kidney and intestine to maintain blood calcium levels.

Parthyroid Dysfunction

- The secretion of parathyroid hormone can be altered due to the following conditions.

- Removal of parathyroid glands during thyroidectomy (removal of thyroid) causes decreased secretion of parathormone. The conditions are

1. Muscle spasm known as **Tetany**(sustained contraction of muscles in face, larynx, hands and feet).
2. Painful cramps of the limb muscles

Pancreas (Islets of Langerhans)

- Pancreas is an elongated, yellowish gland situated in the loop of stomach and duodenum. It is exocrine and endocrine in nature. The exocrine pancreas secretes pancreatic juice which plays a role in digestion while, the endocrine portion is made up of Islets of Langerhans.

- The Islets of Langerhans consists of two types of cells namely alpha cells and beta cells. The alpha cells secrete glucagon and beta cells secrete insulin.

Human insulin was first discovered by Fredrick Banting, Charles Best and MacLeod in 1921. Insulin was first used in treatment of diabetes on 11th January 1922.

Functions of Pancreatic hormones

- A balance between insulin and glucagon production is necessary to maintain blood glucose concentration.

Insulin

- ❖ Insulin helps in the conversion of glucose into glycogen which is stored in liver and skeletal muscles.
- ❖ It promotes the transport of glucose into the cells.
- ❖ It decreases the concentration of glucose in blood.

Glucagon

- ❖ Glucagon helps in the breakdown of glycogen to glucose in the liver.

- ❖ It increases blood glucose levels.

Diabetes mellitus

- The deficiency of insulin causes Diabetes mellitus. It is characterised by

- ❖ Increase in blood sugar level (Hyperglycemia).
- ❖ Excretion of excess glucose in the urine (Glycosuria).
- ❖ Frequent urination (Polyuria).
- ❖ Increased thirst (Polydipsia).

- ❖ Increase in appetite (Polyphagia).

Adrenal Gland

- The adrenal glands are located above each kidney. They are also called supra renal glands.

- The outer part is the adrenal cortex and the inner part is the adrenal medulla. The two distinct parts are structurally and functionally different.

Adrenal Cortex

- The adrenal cortex consists of three layers of cells. They are zonaglomerulosa, zonafasciculata and zonareticularis

Hormones of Adrenal Cortex

- The hormones secreted by the adrenal cortex are corticosteroids. They are classified into

1. Glucocorticoids
2. Mineralocorticoids

Functions of adrenocortical hormones
Glucocorticoids

- The glucocorticoids secreted by the zonafasciculata are cortisol and corticosterone

- ❖ They regulate cell metabolism.
- ❖ It stimulates the formation of glucose from glycogen in the liver.
- ❖ It is an anti-inflammatory and anti-allergic agent.

Mineralocorticoids

- The mineralocorticoids secreted by zonaglomerulosa is aldosterone

1. It helps to reabsorb sodium ions from the renal tubules.
2. It causes increased excretion of potassium ions.

- It regulates electrolyte balance, body fluid volume, osmotic pressure and blood pressure.

Adrenal Medulla

- The adrenal medulla is composed of chromaffin cells.They are richly supplied with sympathetic and parasympathetic nerves.

Hormones of Adrenal Medulla

- It secretes two hormones namely

1. Epinephrine (Adrenaline)
2. Norepinephrine (Noradrenaline)

- They are together called as "Emergency hormones". It is produced during conditions of stress and emotion. Hence it is also referred as "flight, fright and fight hormone".

Functions of adrenal medullary hormones
Epinephrine (Adrenaline)

- It promotes the conversion of glycogen to glucose in liver and muscles.
- It increases heart beat and blood pressure.
- It increases the rate of respiration by dilation of bronchi and trachea.
- It causes dilation of the pupil in eye.
- It decreases blood flow through the skin.

Norepinephrine (Noradrenalin)

- Most of its actions are similar to those of epinephrine.

Reproductive Glands (Gonads)

- The sex glands are of two types the **testes** and the **ovaries**. The testes are present in male, while the ovaries are present in female.

Testes

- Testes are the reproductive glands of the males. They are composed of seminiferous tubules, Leydig cells and Sertoli cells. Leydig cells form the endocrine part of the testes. They secrete the male sex hormone called testosterone.

Functions of testosterone

- ❖ It influences the process of spermatogenesis.
- ❖ It stimulates protein synthesis and controls muscular growth.
- ❖ It is responsible for the development of secondary sexual characters (distribution of hair on body and face, deep voice pattern, etc).

Ovary

- The ovaries are the female gonads located in the pelvic cavity of the abdomen. They secrete the female sex hormones

1. Estrogen
2. Progesterone

- Estrogen is produced by the Graafian follicles of the ovary and progesterone from the corpus luteum that is formed in the ovary from the ruptured follicle during ovulation.

Functions of estrogens

- ❖ It brings about the changes that occur during puberty.
- ❖ It initiates the process of oogenesis.
- ❖ It stimulates the maturation of ovarian follicles in the ovary.
- ❖ It promotes the development of secondary sexual characters (breast development, high pitched voice etc).

Functions of progesterone

- It is responsible for the premenstrual changes of the uterus.
- It prepares the uterus for the implantation of the embryo.
- It maintains pregnancy.
- It is essential for the formation of placenta.

Thymus Gland

- Thymus is partly an endocrine gland and partly a lymphoid gland. It is located in the upper part of the chest covering the lower end of trachea. Thymusin is the hormone secreted by thymus.

Functions of Thymosin

- It has a stimulatory effect on the immune function.
- It stimulates the production and differentiation of lymphocytes.

11th STD (Term I)
Unit – 6 Cell: The Unit of Life

- The word 'cell' comes from the Latin word 'Celle" which means 'a small compartment'. The world cell was first used by Robert Hooke (1662) therefore the term 'cell'is as old as 300 years.

Discovery

- **Aristotle** (384-322BC), was the one who first recognised that animals and plants consists of organised structural units but unable to explain what it was. In 1660's Robert Hooke observed something which looks like 'honeycomb with a great little boxes' which was later called as 'cell' from the cork tissue in 1665. He compiled his work as Micrographia. Later, Antonie von Leeuwenhoek observed unicellular particles which he named as 'animacules'. Robert Brown (1831-39) described the spherical body in the plant cells as nucleus. **H. J. Dutrochet**(1824), a French scientist, was the first to give idea on cell theory. Later, **Matthias Schleiden**(German Botanist) and **Theodor Schwann** (German Zoologist) (1833) outlined the basic features of the cell theory. **Rudolf Virchow** (1858) explained the cell theory by adding a feature stating that all living cells arise from pre-existing living cells by 'cell division'.

Microscopy

- Microscope is an inevitable instrument in studying the cell and subcellular structures. It offers scope in studying microscopic organisms therefore it is named as microscope (mikros – small; skipein – to see) in Greek terminology. Compound microscope was invented by Z. Jansen.

- Microscope works on the lens system which basically relies on properties of light and lens such as reflection, magnification and numerical aperture. The common light microscope which has many lenses are called as compound microscope. The microscope transmits visible light from sources to eye or camera through sample, where interaction takes place.

Bright field Microscope

- Bright field microscope is routinely used microscope in studying various aspects of cells. It allows light to pass directly through specimen and shows a well distinguished image from different portions of the specimen depending upon the contrast from absorption of visible light. The contrast can be increased by staining the specimen with reagent that reacts with cells and tissue components of the object.

- The light rays are focused by condenser on to the specimen on a microslide placed upon the adjustable platform called as stage. The light comes from the Compact Flourescent Lamp (CFL) or Light Emitting Diode (LED) light system. Then it passes through two lens

systems namely objective lens (closer to the object) and the eye piece (closer to eye). There are four objective lenses (5X, 10X, 45X and 100X) which can be rotated and fixed at certain point to get required magnification. It works on the principle of numerical aperture value and its ownresolving power.

- The first magnification of the microscope is done by the objective lens which is called primary magnification and it is real, inverted image. The second magnification of the microscope is obtained through eye piece lens called as secondary magnification and it is virtual and inverted image (Figure 6.2 a, b and c).

Dark Field Microscope

- The dark field microscope was discovered by Z. Sigmondy (1905). Here the field will be dark but object will be glistening so the appearance will be bright. A special effect in an ordinary microscope is brought about by means of a special component called 'Patch Stop Carrier'. It is fixed in metal ring of the condenser component. Patch stop is a small glass device which has a dark patch at centre of the disc leaving a small area along the margin through which the light passes. The light passing through the margin will travel oblique like a hollow cone and strikes the object in the periphery, therefore the specimen appears glistening in a dark background. (Figure 6.2 d and e).

Phase contrast microscope

- This was invented by Zernike (1935). It is a modification of light microscope with all its basic principle. The objects observed by increasing the contrast by bringing about change in amplitude of the light waves. The contrast can be increased by introducing the 'PhasePlate' in the condenser lens. Phase plate is a circular component with circular annular etching.

- Light passes with different velocity after coming out of the thinnest and thickest areas of the phase plate thereby increasing the contrast of the specimen. A hollow cone of light passes through the condenser. Direct light pass through thin area of phase plate, whereas light passing from the specimen reaches thick area of phase plate. The light passing through thicker area travel at low speed, on the other hand the light passing through thin area reach fast therefore contrast is increased in the specimen. Phase contrast microscope is used to observe living cells, tissues and the cells cultured invitro during mitosis (Figure 6.2 f and g).

Electron Microscope

- Electron Microscope was first introduced by Ernest Ruska (1931) and developed by G Binning and H Roher (1981). It is used to analyse the fine details of the cell and organelles called ultrastructure. It uses beam of accelerated electrons as source of illumination and therefore the resolving power is 1,00,000 times than that of light microscope.

- The specimen to be viewed under electron microscope is dehydrated and impregnated with electron opaque chemicals like gold or palladium. This is essential for withstanding electrons andalso for contrast of the image.

There are two kinds of electron microscopes namely

1. Transmission Electron Microscope (TEM)
2. Scanning Electron Microscope (SEM)

Transmission electron microscope:

- This is the most commonly usedelectron microscope which provides two dimensional image. The components of the microscope are as follows:

    a. Electron Generating System
    b. Electron Condensor
    c. Specimen Objective
    d. Tube Lens
    e. Projector

- A beam of electron passes through the specimen to form an image on fluorescent screen. The magnification is 1–3 lakhs times and resolving power is 2–10 Å. Itis used for studying detailed structrue of viruses, mycoplasma, cellular organelles, etc

Scanning Electron Microscope:

- This is used to obtain three dimensional image and has a lower resolving power than TEM. In this, electrons are focused by means of lenses into a very fine point. The interaction of electrons with the specimen results in the release of different forms of radiation (such as auger electrons, secondary electrons, back scattered electrons) from the surface of the specimen. These radiations are then captured by an appropriate detector, amplified and then imaged on fluorescent screen. The magnification is 2,00,000 times and resolution is 5–20 nm (Figure 6.5 a and b).

Cell Theory

- In 1833, German botanist Matthias Schleiden and German zoologist Theodor Schwann proposed that all plants and animals are composed of cells and that cells were the basic building blocks of life.

These observations led to the formulation of modern cell theory.

- All organisms are made up of cells.
- New cells are formed by the division of pre-existing cells.
- Cells contains genetic material, which is passed on from parents to daughter cells.
- All metabolic reactions take place inside the cells.

Exception to Cell Theory

- Viruses are puzzle in biology. Viruses, viroids and prions are the exception to cell theory. They lack protoplasm, the essential part of the cell and exists as obligate parasites which are sub-cellular in nature.

Cell Doctrine (Cell Principle)

The features of cell doctrine are as follows:

- All organisms are made up of cells.
- New cells are produced from the pre-existing cells.
- Cell is a structural and functional unit of all living organisms.
- A cell contains hereditary information which is passed on from cell to cell during cell division.
- All the cells are basically the same in chemical composition and metabolic activities.
- The structure and function of cell is controlled by DNA.
- Sometimes the dead cells may remain functional as tracheids and vessels in plants and horny cells in animals.

Protoplasm Theory

- Corti first observed protoplasm. Felix Dujardin (1835) observed a living juice in animal cell and called it "Sarcode" . Purkinje (1839) coined the term protoplasm for sap inside a plant cell. Hugo Va n Mo h l (1846) indicated importance of protoplasm.

- Max Schultze (1861) established similarity between Protoplasm and Sarcode and proposed a theory which later on called "Protoplasm Theory" by O. Hertwig (1892). Huxley (1868) proposed Protoplasm as a "physical basis of life".

Protoplasm as a Colloidal System

- Protoplasm is a complex colloidal system which was suggested by Fisher in 1894 and Hardy in 1899. It is primarily made of water contents and various other solutes of biological importance such as glucose, fatty acids, amino acids, minerals, vitamins, hormones and enzymes.

- These solutes may be homogeneous (soluble in water) or heterogeneous mass (insoluble in water) which forms the basis for its colloidal nature.

Physical Properties of Protoplasm

- The protoplasm exist either in semisolid (jelly-like) state called 'gel' due to suspended particles and various chemical bonds or may be liquid state called 'sol'. The colloidal protoplasm which is in gel form can change into sol form by solation and the sol can

change into gel by gelation. These gel-sol conditions of colloidal system are prime basis for mechanical behaviour of cytoplasm.

1. Protoplasm is translucent, odourless and polyphasic fluid.
2. It is a crystal colloid solution which is a mixture of chemical substances forming crystalloid i.e. true solution (sugars, salts, acids, bases) and others forming colloidal solution (Proteins and lipids)
3. It is the most important property of the protoplasm by which it exhibits three main phenomena namely Brownian movement, amoeboid movement and cytoplasmic streaming or cyclosis. Viscosity of protoplasm is 2–20 centipoises. The Refractive index of the protoplasm is 1.4.
4. The pH of the protoplasm is around 6.8, contain 90% water (10% in dormant seeds)
5. Approximately 34 elements are present in protoplasm but only 13 elements are main or universal elements i.e. C, H, O, N, Cl, Ca, P, Na, K, S, Mg, I and Fe. Carbon, Hydrogen, Oxygen and Nitrogen form the 96% of protoplasm.
6. Protoplasm is neither a good nor a bad conductor of electricity. It forms a delimiting membrane on coming in contact with water and solidifies when heated.
7. Cohesiveness: Particles or molecules of protoplasm are adhered with each other by forces, such as Van der Waal's bonds, that hold long chains of molecules together. This property varies with the strength of these forces.
8. Contractility: The contractility of protoplasm is important for the absorption and removal of water especially stomatal operations.
9. Surface tension: The proteins and lipids of the protoplasm have less surface tension, hence they are found at the surface forming the membrane. On the other hand the chemical substances (NaCl) have high surface tension, so they occur in deeper parts of the cell protoplasm.

Cell sizes and shapes

- Cell greatly vary in size, shape and also in function. Group of cells with similar structures are called tissue they integrate together to perform similar function, group of tissue join together to perform similar function called organ, group of organs with related function called organ system, organ system coordinating together to form an organism.

Shape

- The shape of cell vary greatly from organism to organism and within the organism itself. In bacteria cell shape vary from round (cocci) to rectangular (rod). In Vorus. shapepf the envelope varies from round to hexagonal or'T' shaped. In fungi, globular to elongatedcylindric cells and the spores of fungi vary greatly in shape. In plants and animals cells vary in shape according to cell types such as parenchyma, mesophyll, palisadem tracheid, fiber, epithelium and others

Types of cells

- On the basis of the cellular organization and the nuclear characteristics, the cell can be divided into

❖ Prokaryotes
❖ Mesokaryotes and
❖ Eukaryotes

Prokaryotes

- Those organisms with primitive nucleus are called as prokaryotes (pro – primitive; karyon – nucleus). The DNA lies in the 'nucleoid' which is not bound by the nuclear membrane and therefore it is not a true nucleus and is also a primitive type of nuclear material. The DNA is without histone proteins. Example: Bacteria, blue green algae, Mycoplasma, Rickettsiae and Spirochaetae.

Mesokaryotes

- In the year 1966, scientist Dodge and his coworkers proposed another kind of organisms called mesokaryotes. These organisms which shares some of the characters of both prokaryotes and eukaryotes. In other words these are organisms intermediate between pro and eukaryotes. These contains well organized nucleus with nuclear membrane and the DNA is organized into chromosomes but without histone protein components divides through amitosis similar withprokaryotes.CertainProtozoa like Noctiluca, some phytoplanktons like Gymnodinium,Peridinium and Dinoflagellates are representatives of mesokaryotes.

Eukaryotes

- Those organisms which have true nucleus are called Eukaryotes (Eu – True; karyon – nucleus). The DNA is associated with protein bound histones forming the chromosomes. Membrane bound organelles are present. Few organelles may be arisen by endosymbiosis which is a cell living inside another cell. The organelles like mitochondria and chloroplast well support this theory.

- The first cell might have evolved approximately 3.8 billion years ago. The primitive cell would have been similar to present day protists (Figure 6.7).

Plant and Animal cell
Ultra Structure of Eukaryotic Cell

- The eukaryotic cell is highly distinct in its organisation. It shows several variations in different organisms. For instance, the eukaryotic cellsin plants and animals vary greatly (Figure 6.8).

Animal Cell

- Animal cells are surrounded by cell membrane or plasma membrane. Inside this membrane the gelatinous matrix called protoplasm is seen to contain nucleus and other organelles which include the endoplasmic reticulum, mitochondria, golgi bodies, centrioles, lysosomes, ribosomes and cytoskeleton.

Plant cell

- A typical plant cell has prominent cell wall, a large central vacuole and plastids in addition to other organelles present in animal cell (Figure 6.9 and 6.10).

Protoplasm

- Protoplasm is the living content of the cell that is surrounded by plasma membrane. It is a colourless material that exists throughout the cell together with the cytoplasm, nucleus and other organelles. Protoplasm is composed of a mixture of small particles, such as ions, amino acids, monosaccharides, water, macromolecules like nucleic acids, proteins, lipids andpolysaccharides. It appears colourless, jelly like gelatinous, viscous elastic and granular. It appears foamy due to the presence of large number of vacuoles. It responds to the stimuli like heat, electric shock, chemicals and so on.

Cell Wall

- Cell wall is the outermost protective cover of the cell. It is present in bacteria, fungi and plants whereas it is absent in animal cell. It was first observed by Robert Hooke. It is an actively growing portion. It is made up of different complex material in various organism. In bacteria it is composed of peptidoglycan, in fungi chitin and fungal cellulose, in algae cellulose, galactans and mannans. In plants it is made up of cellulose, hemicellulose, pectin, lignin, cutin, suberin and silica.

- In plant, cell wall shows three distinct regions (a) Primary wall (b) Secondary wall (c) Middle lamellae (Figure 6.11).

Primary wall

- It is the first layer inner to middle lamellae, primarily consisting of loose network of cellulose microfibrils in a gel matrix. It is thin, elastic and extensible. In most plants the microfibrils are made up of cellulose oriented differently based on shape and thickness of the wall. The matrix of the primary wall is composed of hemicellulose, pectin, glycoprotein and water. Hemicellulose binds the microfibrils with matrix and glycoproteins control the orientation of microfibrils while pectin serves as filling material of the matrix. Cells such as parenchyma and meristems have only primary wall.

Secondary wall

- Secondary wall is laid during maturation. It plays a key role in determining the shape of a cell. It is thick, inelastic and is made up of cellulose and lignin. The secondary wall is divided into three sublayerstermed as S1, S2 and S3 where the cellulose microfibrils are compactly arranged with different orientation forming a laminated structure and the cell wall strength is increased.

Middle lamellae

- It is the outermost layer made up of calcium and magnesium pectate, deposited at the time of cytokinesis. It is a thin amorphous layer which cements two adjacent cells. It is optically inactive (isotropic).

Plasmodesmata and Pits

- Plasmodesmata act as a channel between the protoplasm of adjacent cells through which many substances pass through. Moreover, at few regions the secondary wall layer is laid unevenly whereas the primary wall and middle lamellae are laid continuously such regions are called pits. The pits of adjacent cells are opposite to each other. Each pit has a pit chamber and a pit membrane. The pit membrane has many minute pores and thus they are permeable. The pits are of two types namely simple and bordered pit.

Functions of cell wall

- The cell wall plays a vital role in holding several important functions given below

1. Offers definite shape and rigidity to the cell.
2. Serves as barrier for several molecules to enter the cells.
3. Provides protection to the internal protoplasm against mechanical injury.
4. Prevents the bursting of cells by maintaining the osmotic pressure.
5. Plays a major role by acting as a mechanism of defense for the cells.

Cell Membrane

- The cell membrane is also called cell surface (or) plasma membrane. It is a thin structure which holds the cytoplasmic content called 'cytosol'. It is extremely thin (less than 10nm).

Fluid Mosaic Model

- Jonathan Singer and Garth Nicolson (1972) proposed fluid mosaic model.It is made up of lipids and proteins together with a little amount of carbohydrate. The lipid membrane is made up of phospholipid. The phospholipid molecule has a hydrophobic tail and hydrophilic head. The hydrophobic tailrepels water and

hydrophilic head attracts water. The proteins of the membrane are globular proteins which are found intermingled between the lipid bilayer most of which are projecting beyond the lipid bilayer. These proteins are called as integral proteins. Few are superficially attached on either surface of the lipid bilayer which are called as peripheral proteins. The proteins are involved in transport of molecules across the membranes and also act as enzymes, receptors (or) antigens.

- The Carbohydrate moleculesof cell membrane are short chain polysaccharides. These are either bound with 'glycoproteins' or 'glycolipids' and form a 'glyocalyx'

- The movement of membrane lipids from one side of the membrane to the other side by vertical movement is called flip flopping or flip flop movement. This movement takes place more slowly than lateral diffusion of lipid molecule. The phospholipids can have flip flop movement because the phospholipids have smaller polar regions, whereas the proteins cannot flip flop because the polar region is extensive.

Function of Cell Membrane

- The functions of the cell membrane is enormous which includes cell signalling, transporting nutrients and water, preventing unwanted substances entering into the cell, and so on.

Cell Transport

- Cell membrane act as a channel of transport for molecules. The membrane is selectively permeable to molecules. It transports molecules through energy dependant process and energy independent process. The membrane proteins (channel andcarrier) are involved in movement of ions and molecules across the membrane (Figure 6.13).

Endocytosis and Exocytosis

- Cell surface membrane are able to transport individual molecules and ions. There are processes in which a cell can transport a large quantity of solids and liquids into cell (endocytosis) or out of cell (exocytosis) (Figure 6.14).

- **Endocytosis:** During endocytosis the cell wraps the cell surface membrane around the material and brings it into cytoplasm inside a vesicle. There are two types of endocytosis:

1. Phagocytosis – Particle is engulfed by membrane, which folds around it and forms a vesicle. The enzymes digest the material and products are absorbed by cytoplasm.
2. Pinocytosis – Fluid droplets are engulfed by membrane, which forms vesicles around them.

- **Exocytosis:** Vesicles fuse with plasma membrane and eject contents. This passage of material out of the cell is known as exocytosis. This material may be a secretion in the case of digestive enzymes, hormones or mucus.

Signal Transduction

- The process by which the cell receive information from outside and respond is called signal transduction. Plants, fungi and animal cell use nitric oxide as one of the many signalling molecules. The cell membrane is the site of chemical interactions of signal transduction. Receptors receives the information from first messenger and transmit the message through series of membrane proteins. It activates second messenger which stimulates the cell to carry out specific function.

Cytoplasm

- Cytoplasm is the main arena of various activities of a cell. It is the semifluid gelatinous substance that fills the cell. It is made up of eighty percent water and is usually clear and colourless. The cytoplasm is sometimes described as non nuclear content of protoplasm. The cytoplasm serves as a molecular soup where all thecellular organelles are suspended and bound together by a lipid bilayer plasma membrane. It constitutes dissolved nutrients, numerous salts and acids to dissolve waste products. It is a very good conductor of electricity. It gives support and protection to the cell organelles. It helps movement of the cellular materials around the cell through a process called cytoplasmic streaming. Further, most cellular activities such as many metabolic pathways including glycolysis and cell division occur in cytoplasm.

Cell Organelles
Endomembrane System

- The system of membranes in a eukaryotic cell, comprising the plasma membrane, nuclear membrane, endoplasmic reticulum, golgi apparatus, lysosomes and vacuolar membranes (tonoplast). Endomembranes are made up of phospholipids with embedded proteins that are similar to cell membrane which occur within the cytoplasm. The endomembrane system is evolved from the inward growth of cell membrane in the ancestors of the first eukaryotes.

Endoplasmic Reticulum

- The largest of the internal membranes is called the endoplasmic reticulum (ER). The name endoplasmic reticulum was given by K.R. Porter (1948). It consists of double membrane. Morphologically the structure of endoplasmic reticulum consists of:

1. Cisternae are long, broad, flat, sac like structures arranged in parallel bundles or stacks to form lamella. The space between membranes of cisternae is filled with fluid.
2. Vesicles are oval membrane bound vacuolar structure.
3. Tubules are irregular shape, branched, smooth walled, enclosing a space

- Endoplasmic reticulum is associated with nuclear membrane and cell surface membrane. It forms a network in cytoplasm and gives mechanical support to the cell. Its chemical environment enables protein folding and undergo modification necessary for their function. Misfolded proteins are pulled out and are degraded in endoplasmic reticulum. When ribosomes are present in the outer surface of the membrane it is called as rough endoplasmic reticulum(RER), when the ribosomes are absent in the endoplasmic reticulum it is called as smooth Endoplasmic reticulum(SER). Rough endoplasmic reticulum is involved in protein synthesis and smooth endoplasmic reticulum are the sites of lipid synthesis. The smooth endoplasmic reticulum contains enzymes that detoxify lipid soluble drugs, certain chemicals and other harmful compounds.

Golgi Body (Dictyosomes)

- In 1898, Camillo Golgi visualized a netlike reticulum of fibrils near the nucleus, were named as Golgi bodies. In plant cells theyare found as smaller vesicles termed as dictyosomes. Golgi apparatus is a stack of flat membrane enclosed sacs. It consist of cisternae, tubules, vesicles and golgi vacuoles. In plants the cisternae are 10-20 in number placed in piles separated from each other by a thin layer of inter cisternal cytoplasm often flat or curved. Peripheral edge of cisternae forms a network of tubules and vesicles. Tubules interconnect cisternae and are 30-50nm in dimension. Vesicles are large round or concave sac. They are pinched off from the tubules.They are smooth/secretary or coated type. Golgi vacuoles are large spherical filled with granular or amorphous substance, some function like lysosomes. The Golgi apparatus compartmentalises a series of steps leading to the production of functional protein.Small pieces of rough endoplasmic reticulum are pinched off at the ends to form small vesicles. A number of these vesicles then join up and fuse together to make a Golgi body. Golgi complex plays a major role in post translational modification of proteins and glycosidation of lipids (Figure 6.16 and 6.17).

Functions:

- Glycoproteins and glycolipids are produced
- Transporting and storing lipids.
- Formation of lysosomes.
- Production of digestive enzymes.
- Cell plate and cell wall formation
- Secretion of Carbohydrates for the formation of plant cell walls and insect cuticles.
- Zymogen granules (proenzyme/pre-cursor of all enzyme) are synthesised.

Mitochondria

- It was first observed by A. Kolliker (1880). Altmann (1894) named it as Bioplasts. Later Benda (1897, 1898), named as mitochondria. They are ovoid, rounded, rod shape and pleomorphic structures. Mitochondrion consists of double membrane, the outer and

inner membrane. The outer membrane is smooth, highly permeable to small molecules and it contains proteins called Porins, which form channels that allows free diffusion of molecules smaller than about 1000 daltons and the inner membrane divides the mitochondrion into two compartments, outer chamber between two membranes and the inner chamber filled with matrix.

- The inner membrane is convoluted (infoldings), called crista (plural: cristae). Cristae contain most of the enzymes for electron transport system. Inner chamber ofthe mitochondrion is filled with proteinaceous material called mitochondrial matrix. The inner membrane consists of stalked particles called elementary particles or Fernandez Moran particles, F1 particles or Oxysomes. Each particle consists of a base, stem and a round head. In the head ATP synthase is present for oxidative phosphorylation. Inner membrane is impermeable to most ions, small molecules and maintains the proton gradient that drives oxidative phosphorylation

- Mitochondria contain 73% of proteins, 25-30% of lipids, 5-7 % of RNA, DNA (in traces) and enzymes (about 60 types). Mitochondria are called Power house of a cell, as they produce energy rich ATP.

- All the enzymes of Kreb's cycle are found in the matrix except succinate dehydrogenase. Mitochondria consist of circular DNA and 70S ribosome. They multiply by fission and replicates by strand displacement model. Because of the presence of DNA it is semi-autonomous organelle. Unique characteristic of mitochondria is that they are inherited from female parent only. Mitochondrial DNA comparisons are used to trace human origins. Mitochondrial DNA is used to track and date recent evolutionary time because it mutates 5 to 10 time faster than DNA in the nucleus.

Plastids

- The term plastid is derived from the Greek word Platikas (formed/moulded) and used by A.F.U. Schimper in 1885. He classified plastids into following types according to their structure, pigments and function. Plastids multiply by fission.

- According to Schimper, different kind of plastids can transform into one another.

Chloroplast

- Chloroplasts are vital organelle found in green plants. Chloroplast has a double membrane the outer membrane and the inner membrane separated by a space called periplastidial space. The space enclosed by the inner membrane of chloroplast is filled with gelatinous matrix, lipo-proteinaceous fluid called stroma. Inside the stroma there is flat interconnected sacs called thylakoid. The membrane of thylakoid enclose a space called thylakoid lumen.

- Grana (singular: Granum) are formed when many of these thylakoids are stacked together like pile of coins. Light is absorbed and converted into chemical energy in the granum, which is used in stroma to prepare carbohydrates. Thylakoid contain chlorophyll pigments. The chloroplast contains osmophilic granules, 70s ribosomes, DNA (circular and non histone) and RNA. These chloroplast genome encodes approximately 30 proteins involved in photosynthesis including the components of photosystem I & II, cytochrome bf complex and ATP synthase. One of the subunits of Rubisco is encoded by chloroplast DNA. It is the major protein component of chloroplast stroma, single most abundant protein on earth. The thylakoid contain small, rounded photosynthetic units called quantosomes. It is a semi-autonomous organelle and divides by fission

Functions:

- Photosynthesis
- Light reactions takes place in granum,
- Dark reactions take place in stroma,
- Chloroplast is involved in photo-respiration.

Ribosome

- Ribosomes were first observed by George Palade (1953) as dense particles or granules in the electron microscope. Electron microscopic observation reveals that ribosomes are composed of two rounded sub units, united together to form a complete unit. Mg2+ is required for structural cohesion of ribosomes. Biogenesis of ribosome are denova formation, auto replication and nucleolar origin. Each ribosome is made up of one small and one large sub-unit Ribosomes are the sites of protein synthesis in the cell. Ribosome is not a membrane bound organelle.

- Ribosome consists of RNA and protein: RNA 60 % and Protein 40%. During protein synthesis many ribosomes are attached to the single mRNA is called polysomes or polyribosomes. The function of polysomes is the formation of several copies of a particular polypeptide during protein synthesis. They are free in non-protein synthesising cells. In protein synthesising cells they are linked together with the help of Mg2+ ions.

Lysosomes (Suicidal Bags of Cell)

- Lysosomes were discovered by Christian de Duve (1953), these are known as suicidal bags. They are spherical bodies enclosed by a single unit membrane. They are found in eukaryotic cell. Lysosomes are small vacuoles formed when small pieces of golgi body are pinched off from its tubules.

- They contain a variety of hydrolytic enzymes, that can digest material within the cell. The membrane around lysosome prevent these enzymes from digesting the cell itself.

Functions:

- Intracellular digestion: They digest carbohydrates, proteins and lipids present in cytoplasm.
- Autophagy: During adverse condition they digest their own cell organelleslike mitochondria and endoplasmic reticulum
- Autolysis: Lysosome causes self destruction of cell on insight of disease they destroy the cells.
- Ageing: Lysosomes have autolytic enzymes that disrupts intracellular molecules.
- Phagocytosis: Large cells or contents are engulfed and digested by macrophages, thus forming a phagosome in cytoplasm. These phagosome fuse with lysosome for further digestion.
- Exocytosis: Lysosomes release their enzymes outside the cell to digest other cells.

Peroxisomes

- Peroxisomes were identified as organelles by Christian de Duve (1967). Peroxisomes are small spherical bodies and single membrane bound organelle. It takes part in photorespiration and associated with glycolate metabolism. In plants, leaf cells have many peroxisomes. It is also commonly found in liver and kidney of mammals. These are also found in cells of protozoa and yeast (Figure 6.23).

Glyoxysomes

- Glyoxysome was discovered by Harry Beevers (1961). Glyoxysome is a single membrane bound organelle. It is a sub cellular organelle and contains enzymes of glyoxylate pathway. β-oxidation of fatty acid occurs in glyoxysomes of germinating seeds Example: Castor seeds.

Microbodies

- Eukaryotic cells contain many enzyme bearing membrane enclosed vesicles called microbodies. They are single unit membrane bound cell organelles: Example: peroxisomes and glyoxysomes.

Sphaerosomes

- It is spherical in shape and enclosed by single unit membrane. Example: Storage of fat in the endosperm cells of oil seeds.

Centrioles

- Centriole consist of nine triplet peripheral fibrils made up of tubulin. The central part of the centriole is called hub, is connected to the tubules of the peripheral triplets by radial spokes (9+0 pattern). The centriole form the basal body of cilia or flagella and

spindle fibers which forms the spindle apparatus in animal cells. The membrane is absentin centriole (non-membranous organelle)

Vacuoles

- In plant cells vacuoles are large, bounded by a single unit membrane called Tonoplast. The vacuoles contain cell sap, which is a solution of sugars, amino acids, mineral salts, waste chemical and anthocyanin pigments. Beetroot cells contains anthocyanin pigments in their vacuoles. Vacuoles accumulate products like tannins. The osmotic expansion of a cell kept in water is chiefly regulated by vacuole and the water enters the vacuoles by osmosis.

- The major function of plant vacuole is to maintain water pressure known as turgor pressure, which maintains the plant structure. Vacuoles organises itself into a storage/sequestration compartment. Example: Vacuoles store, most of the sucrose of the cell.

1. Sugar in Sugar beet and Sugar cane.
2. Malic acid in Apple.
3. Acids in Citrus fruits.
4. Flavonoid pigment Cyanidin 3 rutinoside in the petals of Antirrhinum.
5. Tannins in Mimosa pudica.
6. Raphide crystals in Dieffenbachia.
7. Heavy metals in Mustard (Brassica).
8. Latex in Rubber tree and Dandelion stem.

Cell Inclusions

- The cell inclusions are the non-living materials present in the cytoplasm. They are organic and inorganic compounds

Inclusions in prokaryotes

- In prokaryotes, reserve materials such as phosphate granules, cyanophycean granules, glycogen granules, poly β-hydroxy butyrate granules, sulphur granules, carboxysomes and gas vacuoles are present. Inorganic inclusions in bacteria are polyphosphate granules (volutin granules) and sulphur granules. These granules are also known asmetachromatic granules.

Inclusions in Eukaryotes

- Reserve food materials: Starch grains, glycogen granules, aleurone grains, fat droplets
- Secretions in plant cells are essential oil, resins, gums, latex and tannins
- Inorganic crystals – plant cell have calcium carbonate, calcium oxalate and silica
- Cystolith – hypodermal leaf cells of Ficusbengalensis, calcium carbonate
- Sphaeraphides – star shaped calcium oxalate, Colocasia
- Raphides – calcium oxalate, Eichhornia

- Prismatic crystals – calcium oxalate, dry scales of Allium cepa

Nucleus

- Nucleus is an important unit of cell which control all activities of the cell. Nucleus holds the hereditary information. It is the largest among all cell organelles. It may be spherical, cuboidal, ellipsoidal or discoidal.

- It is surroundedby a double membrane structure called nuclearenvelope, which has the inner and outer membrane. The inner membrane is smooth without ribosomes and the outer membrane is rough by the presence of ribosomes and is continues with irregular and infrequent intervals with the endoplasmic reticulum. The membrane is perforated by pores known as nuclear pores which allows materials such as mRNA, ribosomal units, proteins and other macromolecules to pass in and out of the nucleus. The pores enclosed by circular structures called annuli. The pore and annuli forms the pore complex. The space between two membranes is called perinuclear space.

- Nuclear space is filled with nucleoplasm, a gelatinous matrix has uncondensedchromatin network and a conspicuous nucleoli. The chromatin network is the uncoiled, indistinct and remain thread like during the interphase. It has little amount of RNA and DNA bound to histone proteins in eukaryotic cells.

- During cell division chromatin is condensed into an organized form called chromosome. The portion of Eukaryotic chromosome which is transcribed into mRNA contains active genes that are not tightly condensed during interphase is called Euchromatin. The portion of a Eukaryotic chromosome that is not transcribed into mRNA which remains condensed during interphase and stains intensely is called Heterochromatin. I Nucleolus is a small, dense, spherical structure either present singly or in multiples inside nucleus and it's not membrane bound. Nucleoli possesses genes for rRNA and tRNA.

Functions of the nucleus

- Controlling all the cellular activities
- Storing the genetic or hereditary information.
- Coding the information in the DNA for the production of enzymes and proteins.
- DNA duplication and transcription takes place in the nucleus.
- In nucleolus ribosomal biogenesis takes place.

Chromosomes

- Strasburger (1875) first reported its present in eukaryotic cell and the term 'chromosome' was introduced by Waldeyer in 1888. Bridges (1916) first proved that chromosomes are the physical carriers of genes. It is made up of DNA and associated proteins.

Structure of chromosome

- The chromosomes are composed of thread like strands called chromatin which is made up of DNA, protein and RNA. Each chromosome consists of two symmetrical structures called chromatids. During cell division the chromatids forms well organized chromosomes with definite size and shape. They are identical and are called sister chromatids. A typical chromosome has narrow zones called constrictions. There are two types of constrictions namely primary constriction and secondary constriction. The primary constriction is made up of centromere and kinetochore. Both the chromatids are united at centromere, whose number varies. The monocentric chromosome has one centromere and the polycentric chromosome has many centromeres. The centromere contains a complex system of protein fibres called kinetochore. Kinetochore is the region of chromosome which is attached to the spindle fibre during mitosis.

- Besides primary there are secondary constrictions, represented with few occurrence. Nucleoli develop fromthesesecondary constrictions are called nucleolar organizers. Secondary constrictions contains the genes for ribosomal RNA which induce the formation of nucleoli and are called nucleolar organizer regions

- A satellite or SAT Chromosome are short chromosomal segment or rounded body separated from main chromosome by a relatively elongated secondary constriction. It is a morphological entity in certain chromosomes.

- Based on the position of centromere, chromosomes are called telocentric (terminal centromere), Acrocentric (terminal centromere capped by telomere), Sub metacentric (centromere subterminal) and Metacentric (centromere median). The eukaryotic chromosomes may be rodshaped (telocentric and acrocentric), L-shaped (sub-metacentric) and V-shaped (metacentric).

- Telomere is the terminal part of chromosome. It offers stability to the chromosome. DNA of the telomere has specific sequence of nucleotides. Telomere in all eukaryotes are composed of many repeats of short DNA sequences (5'TTAGGG3' sequence in Neurosporacrassa and human beings). Maintenance of telomeres appears to be an important factor in determining the life span and reproductive capacity of cells so studies of telomeres and telomerase have the promise of providing newinsights into conditions such as ageing and cancer. Telomeres prevents the fusion of chromosomal ends with one another.

- Holocentric chromosomes have centromere activity distributed along the whole surface of the chromosome during mitosis. Holocentric condition can be seen in Caenorhabditiselegans (nematode) and many insects. There are three types of centromere in eukaryotes. They are as follows:

- **Point centromere:** the type of centromere in which the kinetochore is assembled as a result of protein recognition of specific DNA sequences. Kinetochores assembled on point centromere bind a single microtubule. It is also called as localized centromere. It occurs in budding yeasts

- **Regional centromere:** In regional centromere where the kinetochore is assembled on a variable array of repeated DNA sequences. Kinetochore assembled on regional centromeres bind multiple microtubules. It occurs in fission yeast cell, humans and so on.

- **Holocentromere-** The microtubules bind all along the mitotic chromosome. Example: Caenorhabditiselegans (nematode) and many insects.

- Based on the functions of chromosome it can be divided into autosomes and sex chromosomes.

- Autosomes are present in all cells controlling somatic characteristics of an organism. In human diploid cell, 44 chromosomes are autosomes whereas two are sex chromosomes. Sex chromosomes are involved in the determination of sex.

- Special types of chromosomes are found only in certain special tissues. These chromosomes are larger in size and are called giant chromosomes in certainplants and they are found in the suspensors of the embryo. The polytene chromosome and lamp brush chromosome occur in animals and are also called as giant chromosomes.

- Polytene chromosomes observed in the salivary glands of Drosophila (fruit fly) by C.G. Balbiani in 1881. In larvae of many flies, midges (Dipthera) and some insects the interphase chromosomes duplicates and reduplicates without nuclear division. A single chromosome which is present in multiple copies form a structure called polytene chromosome which can be seen in light microscope. They are genetically active. There is a distinct alternating dark bands and light inter-bands. About 95% of DNA are present in bands and 5% in inter-bands. The polytene chromosome has extremely large puff called Balbiani rings which is seen in Chironomous larvae. It is also known as chromosomal puff. Puffing of bands are the sites of intense RNA synthesis. As this chromosome occurs in the salivary gland it is known as salivary gland chromosomes. Polyteny is achieved by repeated replication of chromosomal DNA several times without nuclear division and the daughter chromatids aligned side by side and do not separate (called endomitosis). Gene expression, transcription of genes and RNA synthesis occurs in the bands along the polytene chromosomes. Maternal and paternal homologues remain associated side by side is called somatic pairing.

- Lampbrush chromosomes occur at the diplotene stage of first meiotic prophase in oocytes of an animal Salamandar and in giant nucleus of the unicellular alga Acetabularia. It was first observed by Flemming in 1882. The highly condensedchromosome forms the chromosomal axis, from which lateral loops of DNA extend as a result of intense RNA synthesis.

Flagella
Prokaryotic Flagellum

- Bacterial flagella are helical appendages helps in motility. They are much thinner than flagella or cilia of eukaryotes. The filament contains a protein called flagellin. The structure consists of a basal body associated with cytoplasmic membrane and cell wall with short hook and helical filament. Bacteria rotates their helical flagella and propels rings present in the basal body which are involved in the rotary motor that spins the flagellum.

Structure of flagella in Bacteria

- The gram positive bacteria contain only two basal rings. S-ring is attached to the inside of peptidoglycan and M-ring is attached to the cell membrane. In Gram negative bacteria two pairs of rings proximal and distal ring are connected by a central rod. They are L-Lipopolysaccharide ring P-Peptidoglycan ring, S-Super membrane ring and M-membrane ring. The outer pair L and P rings is attached to cell wall and the inner pair S and M rings attached to cell membrane (Figure 6.28).

Mechanism of flagellar movement – proton motive force

- In flagellar rotation only proton movements are involved and not ATP. Protons flowing back into the cell through the basal body rings of each flagellum drives it to rotate. These rings constitute the rotary motor. The proton motive force (The force derived from the electrical potential and the hydrogen ion gradient across the cytoplasmic membrane) drives the flagellar motor. For the rotation of flagellum the energy is derived from proton gradient across the plasma membrane generated by oxidative phosphorylation. In bacteria flagellar motor is located in the plasma membrane where the oxidative phosphorylation takes place. Therefore, plasma membrane is a site of generation of proton motive force.

Eukaryotic Flagellum– Cell Motility
Structure

- Eukaryotic Flagella are enclosed by unit membrane and it arises from a basal body.Flagella is composed of outer nine pairs of microtubules with two microtubules in its centre (9+2 arrangement). Flagella are microtubule projection of the plasma membrane. Flagellum is longer than cilium (as long as 200μm). The structure of flagellum has an axoneme made up microtubules and protein tubulin (Figure 6.29).

Movement

- Outer microtubule doublet is associated with axonemal dynein which generates force for movement. The movement is ATP driven. The interaction between tubulin and dynein is the mechanism for the contraction of cilia and flagella. Dynein molecules uses energy from ATP to shift the adjacent microtubules. This movement bends the cilium or flagellum.

Cilia

- Cilia (plural) are short cellular, numerous microtubule bound projections of plasma membrane. Cilium (singular) ismembrane bound structure made up of basal body, rootlets, basal plate and shaft. The shaft or axoneme consists of nine pairs of microtubule doublets, arranged in a circle along the periphery with a two central tubules, (9+2) arrangement of microtubules is present. Microtubules are made up of tubulin. The motor protein dynein connects the outer microtubule pair and links them to the central pair. Nexin links the peripheral doublets of microtubules

Cytological Techniques
Prearation of Slides

- There are different types of mounting based on the portion of a specimen to be observed

a. **Whole mount:** The whole organism or smaller structure ismounted over a slide and observed.
b. **Squash:** Is a preparation where the material to be observed is crushed/ squashed on to a slide so as to reveal their contents. Example: Pollen grains, mitosis and meiosis in root tips and flower buds to observe chromosomes.
c. Smears: Here the specimen is in the fluid (blood, microbial cultures etc.,) are scraped, brushed or aspirated from surface of organ. Example: Epithelial cells.
d. Sections: Free hand sections from a specimen and thin sections are selected, stained and mounted on a slide. Example: Leaf and stem of plants.

Recording the Observations

- The observations made through a microscope can be recorded by hand diagrams or through microphotographs.
- **Hand diagrams:** Hand diagrams are drawn using ordinary pencil by observing the slide and drawing manually.

- **Microphotograph:** Images of structures observed through microscopes can be further magnified, projected and saved by attaching a camera to the microscope by a microscope coupler or eyepiece adaptor. Picture taken using a inbuilt camera in a microscope is called microphotography or microphotograph.

Staining Techniques

- Staining is very important to observe different components of the cell. Each component of the cell has different affinity towards different stains. The technique of staining the cells and tissue is called 'histochemical staining' or 'histochemistry'.

- One of the most important features of the living cells is their power to grow and divide. New cells are formed by the division of pre-existing cells. Cells increase in number by cell division. The parent cell divides and passes on genetic material to the daughter cells.

The Role of the Nucleus

- As studied earlier, the nucleus is the organising centre of the cell. The information in the nucleus is contained within structures called chromosomes. These uniquely:
- Control activities of the cell.
- Genetic information copied from cell to cell while the cell divides.
- Hereditary characters are passed on to new individuals when gametic cells fuse together in sexual reproduction.

Chromosomes

- At the time when a nucleus divides, the chromosomes become compact and multicoiled structure. Only in this condensed state do the chromosomes become clearly visible in cells. All othertimes, the chromosomes are very long, thin, uncoiled threads. In this condition they give the stained nucleus the granular appearance. The granules are called chromatin.

The four important features of the chromosome are:

- The shape of the chromosome is specific: The long, thin, lengthy structured chromosome contains a short, constricted region called centromere. A centromere may occur anywhere along the chromosome, but it is always in the same position on any given chromosome.
- The number of chromosomes per species is fixed: for example the mouse has 40 chromosomes, the onion has 16 and humans have 46.
- Chromosomes occur in pairs: The chromosomes of a cell occur in pairs, called homologous pairs. One of each pair come originally from each parent. Example, human has 46 chromosomes, 23 coming originally from each parent in the process of sexual reproduction.
- Chromosomes are copied: Between nuclear divisions, whilst the chromosomes are uncoiled and cannot be seen, each chromosome is copied. The two identical structures formed are called chromatids.

Nuclear Divisions

- There are two types of nuclear division, as mitosis and meiosis. In mitosis, the daughter cells formed will have the same number of chromosomes as the parent cell,

typically diploid (2n) state. Mitosis is the nuclear division that occurs when cells grow or when cells need to be replaced and when organism reproduces asexually.

- In meiosis, the daughter cells contain half the number of chromosomes of the parent cell and is known as haploid state (n).

- Whichever division takes place, it is normally followed by division of the cytoplasm to form separate cells, called as cytokinesis.

Cell Cycle

- Definition: A series of events leading to the formation of new cell is known as cell cycle. The phenomenonal changes leading to formation of new population take place in the cell cycle. It was discovered by Prevost and Dumans (1824). The series of events include several phases.

Duration of Cell Cycle

- Different kinds of cells have varied duration for cell cycle phases. Eukaryotic cell divides every 24 hours. The cell cycle is divided into mitosis and interphase. In cell cycle 95% is spent for interphase whereas the mitosis and cytokinesis last only for an hour.The different phases of cell cycle are as follows.

Interphase

- Longest part of the cell cycle, but it is of extremely variable length. At first glance the nucleus appears to be resting but this is not the case at all. The chromosomes previously visible as thread like structure, have dispersed. Now they are actively involved in protein synthesis, at least for most of the interphase.

G_1 Phase

- The first gap phase – 2C amount of DNA in cells of G_1. The cells become metabolically active and grows by producing proteins, lipids, carbohydrates and cell organelles including mitochondria and endoplasmic reticulum. Many checkpoints controlthe cell cycle. The checkpoint called the restriction point at the end of G_1, determines a cells fate whether it will continue in the cell cycle and divide or enter a stage called G_0 as a quiescent stage and probably as specified cell or die. Cells are arrested in G_1 due to
- Nutrient deprivation
- Lack of growth factors or density dependant inhibition
- Undergo metabolic changes and enter into G_0 state.

- Biochemicals inside cells activates the cell division. The proteins called kinases and cyclins activate genes and their proteins to perform cell division. Cyclins act as major checkpoint which operates in G_1 to determine whether or not a cell divides.

G_0Phase

- Some cells exit G_1 and enters a quiescent stage called G_0, where the cells remain metabolically active without proliferation. Cells can exist for long periods in G_0 phase. In G_0 cells cease growth with reduced rate of RNA and protein synthesis. The G_0 phase isnot permanent. Mature neuron and skeletal muscle cell remain permanently in G_0. Many cells in animals remains in G_0 unless called on to proliferate by appropriate growth factors or other extracellular signals. G_0 cells are not dormant.

S phase – Synthesis phase – cells with intermediate amounts of DNA.

- Growth of the cell continues as replication of DNA occur, protein molecules called histones are synthesised and attach to the DNA. The centrioles duplicate in the cytoplasm. DNA content increases from 2C to 4C.

G2 – Te second Gap phase – 4C amount of DNA in cells of G2 and mitosis

- Cell growth continues by protein and cell organelle synthesis, mitochondria and chloroplasts divide. DNA content remains as 4C. Tubulin is synthesised and microtubules are formed. Microtubles organise to form spindle fbre. Te spindle begins to form and nuclear division follows.

- One of the proteins synthesized only in the G2 period is known as Maturation Promoting Factor (MPF). It brings about condensation of interphase chromosomes into the mitotic form.

- DNA damage checkpoints operates in G1 S and G2 phases of the cell cycle.

Cell Division
Amitosis (Direct Cell Division)

- Amitosis is also calleddirect or incipient cell division. Here there is no spindle formation and chromatin material does not condense. It consist of two steps: (Figure 7.2).

Karyokinesis:

- Involves division of nucleus.
- Nucleus develops a constriction at the center and becomes dumbell shaped.
- Constriction deepens and divides the nucleus into two.

Cytokinesis:

- Involves division of cytoplasm.
- Plasma membrane develops a constriction along nuclear constriction.

- It deepens centripetally and finally divides the cell into two cells.

- Example: Cells of mammalian cartilage, macronucleus of Paramecium and old degenerating cells of higher plants.

Drawbacks of Amitosis

- Causes unequal distribution of chromosomes.
- Can lead to abnormalities in metabolism and reproduction.

Mitosis

- The most important part of cell division concerns events inside the nucleus. Mitosis occurs in shoot and root tips and other meristematic tissues of plants associated with growth. The number of chromosomes in the parent and the daughter (Progeny) cells remain the same so it is also called as equational division.

Closed and Open Mitosis

- In closed mitosis, the nuclear envelope remains intact and chromosomes migrate to opposite poles of a spindle within the nucleus (Figure 7.3).Example: Many single celled eukaryotes including yeast and slime molds.

- open mitosis, the nuclear envelope breaks down and then reforms around the 2 sets of separated chromosome.
- Example: Most plants and animalsMitosis is divided into four stages prophase, metaphase, anaphase and telophase (Figure 7.6).

Prophase

- Prophase is the longest phase in mitosis. Chromosomes become visible as long thin thread like structure, condenses to form compact mitotic chromosomes. In plant cells initiation of spindle fibres takes place, nucleolus disappears. Nuclear envelope breaks down. Golgi apparatus and endoplasmic reticulum are not seen.

- In animal cell the centrioles extend a radial array of microtubules towards the plasma membrane when they reach the poles of the cell. T is arrangement of microtubules is called an aster. Plant cells do not form asters.

Metaphase

- Chromosomes (two sister chromatids) are attached to the spindle fibres by kinetochore of the centromere. The spindle fibres is made up of tubulin. The alignment of chromosome into compact group at the equator of the cell is known as metaphase plate. This is the stagewhere the chromosome morphology can be easily studied.

- Kinetochore is a DNA–Protein complex present in the centromere DNA where the microtubules are attached. It is a trilaminar disc like plate.The spindle assembly checkpoint which decides the cell to enter anaphase.

Anaphase

- Each chromosome split simultaneously and two daughter chromatids begins to migrate towards two opposite poles of a cell. Each centromere splits longitudinally into two, freeing the two sister chromatids from each other. Shortening of spindle f bre and longitudinal splitting of centromere creates a pull which divides chromosome into two halves. Each half receive two chromatids (that is sister chromatids are separated). When the sister chromatids separate the actual partitioning of the replicated genome is complete.

- Aubiquitine ligase is activated called as the anaphase-promoting complex cyclosome (APC/C) leads to degradation ofthe key regulatory proteins at the transition of metaphase to anaphase. APC is a cluster of proteins that induces the breaking down of cohesion proteins which leads to the separation of chromatids during mitosis (Figure 7.5).

Telophase

- Two sets of daughter chromosomes reach opposite poles of the cell, mitotic spindle disappears. Division of genetic material is completed afer this karyokinesis, cytokinesis (division of cytoplasm) is completed, nucleolus and nuclear membranes reforms. Nuclear membranes form around each set of sister chromatids now called chromosomes, each has its own centromere. Now the chromosomes decondense. In plants, phragmoplast are formed between the daughter cells. Cell plate is formed between the two daughter cells, reconstruction of cell wall takes place. Finally the cells are separated by the distribution of organelles, macromolecules into two newly formed daughter cells.

Cytokinesis
Cytokinesis in Animal Cells

- It is a contractile process. The contractile mechanism contained in contractile ring located inside the plasma membrane. The ring consists of a bundle of microfilaments assembled from actin and myosin. This fibril helps for the generation of a contractile force. This force draws the contractile ring inward forming a cleavage furrow in the cell surface dividing the cell into two.

Cytokinesis in Plant Cell

- Division of the cytoplasm ofen starts during telophase. In plants, cytokinesis cell plate grows from centre towards lateral walls -centrifugal manner of cell plate formation.

- Phragmoplast contains microtubules, actin filaments and vesicles from golgi apparatus and ER. The golgi vesicles contains carbohydrates such as pectin, hemicellulose which move along the microtubule of the pharagmoplast to the equator fuse, forming a new plasma membrane and the materials which are placed their becomes new cell wall. The first stage of cell wall construction is a line dividing the newly forming cells called a cell plate. The cell plate eventually stretches right across the cell forming the middle lamella. Cellulose builds up on each side of the middle lamella to form the cell walls of two new plant cells.

Significance of Mitosis

- Exact copy of the parent cell is produced by mitosis (genetically identical).

1. Genetic stability – daughter cells are genetically identical to parent cells.
2. Growth – as multicellular organisms grow, the number of cells making up their tissue increases. The new cells must be identical to the existing ones.
3. Repair of tissues - damaged cells must be replaced by identical new cells by mitosis.
4. Asexual reproduction – asexual reproduction results in offspring that are identical to the parent. Example Yeast and Amoeba.
5. In flowering plants, structure such as bulbs, corms, tubers, rhizomes and runners are produced by mitotic division. When they separate from the parent, they form a new individual.The production of large numbers of ofsprings in a short period of time, is possible only by mitosis. In genetic engineering and biotechnology, tissues are grown by mitosis (i.e. in tissue culture).
6. Regeneration – Arms of star fish

Meiosis

- In Greek meioum means to reduce. Meiosis is unique because of synapsis, homologous recombination and reduction division. Meiosis takes place in the reproductiveorgans. It results in the formation of gametes with half the normal chromosome number.Haploid sperms are made in testes; haploid eggs are made in ovaries of animals.

1. In flowering plants meiosis occurs during microsporogenesis in anthers and megasporogenesis in ovule. In contrast to mitosis, meiosis produces cells that are not genetically identical. So meiosis has a key role in producing new genetic types which results in genetic variation.

Stages in Meiosis

- Meiosis can be studied under two divisions i.e., meiosis I and meiosis II. As with mitosis, the cell is said to be in interphase when it is not dividing.

Meiosis I-Reduction Division

- **Prophase I** – Prophase I is of longer duration and it is div ided into 5 substages – Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis**Leptotene** – Chromosomes are visible under light microscope. Condensation of chromosomes takes place. Paired sister chromatids begin to condense.

- Zygotene – Pairing of homologous chromosomes takes place and it is known as synapsis. Chromosome synapsis is made by the formation of synaptonemal complex. The complex formed by the homologous chromosomes are called as bivalent (tetrads).

- **Pachytene**– At this stage bivalent chromosomes are clearly visible as tetrads. Bivalent of meiosis I consists of 4chromatids and 2 centromeres. Synapsis is completed and recombination nodules appear at a site where crossing over takes place between non-sister chromatids of homologous chromosome. Recombination of homologous chromosomes is completed by the end of the stage but the chromosomes are linked at the sites of crossing over. This is mediated by the enzyme recombinase.

- **Diplotene**– Synaptonemal complex disassembled and dissolves. The homologous chromosomes remain attached at one or more points where crossing over has taken place. These points of attachment where 'X' shaped structures occur at the sites of crossing over is called Chiasmata. Chiasmata are chromatin structures at sites where recombination has been taken place. They are specialised chromosomal structures that hold the homologous chromosomes together. Sister chromatids remain closely associated whereas the homologous chromosomes tend to separate from each other but are held together by chiasmata. This substage may last for days or years depending on the sex and organism. The chromosomes are very actively transcribed in females as the egg stores up materials for use during embryonic development. In animals, the chromosomes have prominent loops called lampbrush chromosome.

- **Diakinesis** – Terminalisation of chiasmata. Spindle fbres assemble. Nuclear envelope breaks down. Homologous chromosomes become short and condensed. Nucleolus disappears.

Metaphase I

- Spindle fibres are attached to the centromeres of the two homologouschromosomes. Bivalent (pairs of homologous chromosomes) aligned at theequator of the cell known as metaphase plate. Each bivalent consists of two centromeres and four chromatids.
- The random distribution of homologous chromosomes in a cell in Metaphase I is called independent assortment.

Anaphase I

- Homologous chromosomes are separated from each other. Shortening of spindle fibers takes place. Each homologous chromosomes with its two chromatids and

undivided centromere move towards the opposite poles of the cells. The actual reduction in the number of chromosomes takes place at this stage. Homologous chromosomes which move to the opposite poles are either paternal or maternal in origin. Sister chromatids remain attached with their centromeres.

Telophase I

- Haploid set of chromosomes are present at each pole. The formation of two daughter cells, each with haploid number of chromosomes. Nuclei are reassembled. Nuclear envelope forms around the chromosome and the chromosomes becomes uncoiled. Nucleolus reappears.

- In plants, after karyokinesis cytokinesis takes place by which two daughter cells are formed by the cell plate between 2 groupsof chromosomes known as dyad of cells (haploid).The stage between the two meiotic divisions is called interkinesis which is short-lived.

Meiosis II – Equational division.

- This division is otherwise called mitotic meiosis. Since it includes all the stages of mitotic divisions.

Prophase II

- chromosome with 2 chromatids becomes short, condensed, thick and becomes visible. New spindle develops at right angles to the cell axis. Nuclear membrane and nucleolus disappear.

Metaphase II

- Chromosome arranged at the equatorial plane of the spindle. Microtubules of spindle gets attached to the centromere of sister chromatids.

Anaphase II

- Sister chromatids separate. The daughter chromosomes move to the opposite poles due to shortening of microtubules. Centromere of each chromosome split, allowing to move towards opposite poles of the cells holding the sister chromatids.

Telophase II

- Four groups of chromosomes are orgamnised into dour haploid nuclei. The spindle disappears. Nuclear envelope, nucleolus reappears.

- After karyokinesis, cytokinesis follows and four haploid daughter cells are formed, called tetrads.

Significance of Meiosis

- This maintains a definite constant mumber of chromosomes is organisms.
- Crossing over takes place and exchange of genetic material leads to variations amog species. These variations are the raw materials to evolution. Meiosis leads to genetic variability by partitioning different combinations of genes into gametes through independent assortment.
- Adaptation of organisms to various environmental stress.

Mitogens

- The factors which promote cell cycle proliferation is called mitogens. Plant mitogens include gibberellin, ethylene, Indole acetic acid, kinetin. These increase mitotic rate.

Mitotic Poisons (Mitotic Inhibitors)

- Certain chemical components act as inhibitors of the mitotic cell division and they are called mitotic poisons.

Endomitosis

- The replication of chromosomes in the absence of nuclear division and cytoplasmic division resulting in numerous copies within each cell is called endomitosis. Chromonema do not separate to form chromosomes, but remain closely associated with each other. Nuclear membrane does not rupture. So no spindle formation. It occurs notably in the salivary glands of Drosophila and other flies. Cells in these tissues contain giant chromosomes (polyteny), each consisting of over thousands of intimately associated, or synapsed, chromatids. Example: Polytene chromosome.

Anastral

- This is present only in plant cells. No asters or centrioles are formed only spindle fibres are formed during cell division.

Amphiastral

- Aster and centrioles are formed at each pole of the spindle during cell division. This is found in animal cells.

Unit -8 Biomolecules

Water

- Water is the most abundant component in living organisms. Life on earth is inevitablu linked to water. Water makes up 70 % of human cell and upto 95% of mass of aplanet cell

- Water is a tiny polar molecule and can reasily pass through membranes. Two electronegative atoms of oxygen share a hydrogen bonds of two water molecule. Thus, they can stick together by cohesion and results in lattice formation (Figure 8.4).

Properties of Water

- Adhesion and cohesion property
- High latent heat of vaporisation
- High melting and boiling point
- Universal solvent
- Specific heat capacity

Primary and Secondary Metabolites

- Most plants, fungi and other microbes synthesizes a number of organic compounds. These components are called as metabolites which are intermediates and products of metabolism. The term metabolite is usually restricted to small molecules. It can be catergorized into two types namely primary and secondary metabolites based on their role in metabolic process (Figure 8.5).

- **Primary metabolites** are those that are required for the basic metabolic processes like photosynthesis, respiration, protein and lipid metabolism of living organisms.

- **Secondary metabolites** does not show any direct function in growth and development of organisms.

Organic Molecules

- Organic molecules may be small and simple. These simple molecules assemble and form large and complex molecules called macromolecules. These include four main classes – carbohydrates, lipids, proteins and nucleic acids. All macromolecules except lipids are formed by the process of polymerisation, a process in which repeating subunits termed monomers are bound into chains of different lengths. These chains of monomers are called polymers.

Carbohydrates

- Carbohydrates are organic compounds made of carbon and water. Thus one molecule of water combines with a carbon atom to form CH2O and is repeated several (n) times to form (CH2O)n where n is an integer ranging from 3–7. These are also called as saccharides. The common term sugar refers to a simple carbohydrate such as a monosaccharide or disaccharide that tastes sweet are soluble in water (Figure 8.7).

Monosaccharides – The Simple Sugars

- Monosaccharides are relatively small molecules constituting single sugar unit. Glucose has a chemical formula of C6H12O6. It is a six carbon molecule and hence is called as hexose
- All monosaccharides contain one of two functional groups. Some are aldehydes, like glucose and are referred as aldoses; other are ketones, like fructose and are referred as ketoses.

Disaccharides

- Disaccharides are formed when two monosaccharides join together. An example is sucrose. Sucrose is formed from a molecule of α-glucose and a molecule of fructose.

- In the reverse process, a disaccharide is digested to the component monosaccharide in a hydrolysis reaction. This reaction involves addition of a water (hydro) molecule and splitting (lysis) of the glycosidic bond.

Polysaccharides

- These are made of hundreds of monosaccharide units. Polysaccharides also called "Glycans". Long chain of branched or unbranchedmonosaccharides are held together by glycosidic bonds. Polysaccharide is an example of giant molecule, a macromolecule and consists of only one type of monomer.

- This is a condensation reaction releasing water. The bond formed between the glucose and fructose molecule by removal of water is called glycosidic bond. This is another example of strong, covalent bond.aresweetless. Cellulose is an example built from repeated units of glucose monomer.Depending on the function, polysaccharides are of two types -storage polysaccharide and structural polysaccharide (Figure 8.8).

Starch

- Starch is a storage polysaccharides made up of repeated units of amylose and amylopectin. Starch grains are made up of successive layers of amylose and amylopectin, which can be seen as growth rings. Amylose is a linear, unbranched

polymer which makes up 80% of starch. Amylopectin is a polymer with some 1, 6 linkages that gives it a branched structure.

Test for Strach

- We test the presence of starch by adding a solution of iosin in potassium iodide. Iodine molecules fit nearly into the starch helix, creating a blue-black colocu

- Test on potato; b. test on starch –iodine reaction c. starch – iodine reaction

Chitin

- Chitin is a homo polysaccharide with amino acids added to form mucopolysaccharide. The basic unit is anitrogen containing glucosederivative known as N-acetyl glucoseamine.It forms the exoskeleton of insects and other arthropods. It is also present in the cell walls of fungi.

Test for Reducing Sugars

- Aldoses and ketoses are reducing sugars. This means that, when heated with an alkaline solution of copper (II) sulphate (a blue solution called benedict's solution), the aldehyde or ketone group reduces Cu2+ ions to Cu+ ions forming brick red precipitate of copper(I) oxide. In the process, the aldehyde or ketone group is oxidised to a carboxyl group (–COOH). This reaction isused as test for reducing sugar and is known as Benedict's test. The results of benedict's test depends on concentration of the sugar. If there is no reducing sugar it remains blue.

- Sucrose is not a reducing sugar

- The greater the concentration of reducing sugar, the more is the precipitate formed and greater is the colour change.

Lipids

- The term lipid is derived from greek word lipos, meaning fat. These substances are not soluble in polar solvent such as water but dissolve in non-polar solvents such as benzene, ether, chloroform. This is because they contain long hydrocarbon chains that are non-polar and thus hydrophobic. The main groups of compounds classifiedas lipids are triglycerides, phospholipids, steroids and waxes.

Triglycerides

- Triglycerides are composed of single molecule of glycerol bound to 3 fatty acids. These include fats and oils. Fatty acids are long chain hydrocarbons with a carboxyl group at one end which binds to one of the hydroxyl groups of glycerol, thus forming an ester bond. Fatty acids are structural unit of lipids and are carboxylic acid of long chain

hydrocarbons. The hydrocarbon can vary in length from 4 – 24 carbons and the fat may be saturated or unsaturated. In saturated fatty acids the hydrocarbon chain is single bonded (Eg. palmitic acid, stearic acid) and in unsaturated fatty acids (Eg. Oleic acid,linoleic acid) the hydrocarbon chain is double bonded (one/two/three). In general solid fats are saturated and oils are unsaturated, in which most are globules.

Membrane Lipids

- A class of lipids that serves as major structural component of cell membrane is phospholipids. These contain only 2 fatty acids attached to the glycerol, while the third glycerol binding site holds a phosphate group. This phosphate group is in turn bonded to an alcohol. These lipids have both hydrophobicand hydrophilic regions. The structure of lipid bilayer helps the membrane in function such as selective permeability and fluid nature (Figure 8.15).

Steroids

- These are complex compounds commonly found in cell membrane and animal hormones. Eg. Cholesterol which reinforces the structure of the cell membrane in animal cells and in an unusual group of cell wall deficient bacteria – Mycoplasma.

Waxes

- These are esters formed between a long chain alcohol and saturated fatty acids.Lecithin is a food additive and dietery supplement

- Fur, feathers, fruits, leaves, skin and insect exoskeleton are naturally waterproofed with a coating of wax.

Proteins

- Proteins are the most diverse of all macromolecule. Proteins make up 2/3 of total dry mass of a cell. The term protein was coined by Gerardus Johannes Mulder and is derived form a greek word proteos which means of the first rank.

- Amino acids are building blocks of proteins. There are about 20 different amino acids exist naturally. All amino acids have a basic skeleton consisting of a carbon (a-carbon) linked to a basic amino group.(NH_2), an acidic carboxylic group (COOH) and a hydrogen atom (H) and side chain or variable R group. The amino acid is both an acid and a base and is called amphoteric.

- A zwitterion also called as dipolar ion, is a molecule with two or more functional groups, of which at least one has a positive and other has a negative electrical charge and the net charge of the entire molecule is zero. The pH at which this happens is known as the isoelectric point (Figure 8.19).

Classification of Amino acids

- Based on the R group amino acids are classified as acidic, basic, polar, non-polar.The amino group of one amino acid reacts with carboxyl group of other amino acid, forming a peptide bond. Tw o amino acids can react together with the loss of water to form a dipeptide. Long strings of amino acids linked by peptide bonds are called polypeptides. In 1953 Fred Sanger first sequenced the Insulin protein (Figure 8.18 and 8.20 a and b).

Structure of Protein

- Protein is synthesised on the ribosome as a linear sequence of amino acids which are held together by peptide bonds. After synthesis,the protein attains conformational change into a specific 3D form for proper functioning. According to the mode of folding, four levels of protein organisation have been recognised namely primary, secondary, tertiary and quaternary.
- The primary structure is linear arrangement of amino acids in a polypeptide chain.
- Secondary structure arises when various functional groups are exposed on outer surface of the molecular interaction by forming hydrogen bonds. This causes the aminoacid chain to twist into coiled configuration called α-helix or to fold into a flat β-pleated sheets.
- Tertiary protein structure arises when the secondary level proteins fold into globular structure called domains.
- Quaternary protein structure may be assumed by some complex proteins in which more than one polypeptide forms a large multiunit protein. The individual polypeptide chains of the protein are called subunits and the active protein itself is called amultimer.

- For example: Enzymes serve as catalyst for chemical reactions in cell and are non-specific. Antibodies are complex glycoproteins with specific regions of attachment for various organisms.

Protein Denaturation

- Denaturation is the loss of 3D structure of protein. Exposure to heat causes atoms to vibrate violently, and this disrupts the hydrogen and ionic bonds. Under these conditions, protein molecules become elongated, disorganised strands. Agents such as soap, detergents, acid, alcohol and some disinfectants disrupt the interchain bond and cause the molecule to be nonfunctional (Figure 8.25).

Protein Bonding

- There are three types of chemical bonding

Hydrogen Bond

- It is formed between some hydrogen atoms of oxygen and nitrogen in polypeptide chain. The hydrogen atoms have a smallpositive charge and oxygen and nitrogen have small negative charge. Opposite charges attract to form hydrogen bonds.

- Though these bonds are weak, large number of them maintains the molecule in 3D shape

Ionic Bond

- It is formed between any charged groups that are not joined together by peptide bond. It is stronger than hydrogen bond and can be broken by changes in pH and temperature.

Disulfide Bond

- Some amino acids like cysteine and methionine have sulphur. These form disulphide bridge between sulphur atoms and amino acids.

Hydrophobic Bond

- This bond helps some protein to maintain structure. When globular proteins are in solution, their hydrophobic groups point inwards away from water.

Test for Proteins

- The biuret test is used as an indicator of the presence of protein because it gives a purple colour in the presence of peptide bonds (–C– N–). To a protein solution an equal quantity of sodium hydroxide solution is added and mixed. Then a few drops of 0.5% copper (II) sulphate is added with gentle mixing. A distinct purple colour develops without heating.

Enzymes

- Enzymes are globular proteins that catalyse the many thousands of metabolic reactions taking place within cells and organism. The molecules involved in such reactions are metabolites. Metabolism consists of chains and cycles of enzyme-catalysed reactions, such as respiration, photosynthesis, protein synthesis and other pathways. These reactions are classified as

    - **anabolic** (building up of organic molecules). Synthesis of proteins from amino acids and synthesis of polysaccharides from simple sugars are examples of anabolic reactions.
    - **catabolic** (breaking down of larger molecules). Digestion of complexfoods and the breaking down of sugar in respiration are examples of catabolic reactions.

- Enzymes can be extracellular enzyme as secreted and work externally exported from cells. Eg. digestive enzymes; or intracellular enzymes that remain within cells and work there. These are found inside organelles or within cells. Eg. insulin

Properties of Enzyme

- All are globular proteins.
- They act as catalysts and effective even in small quantity.
- They remain unchanged at the end of the reaction.
- They are highly specific.
- They have an active site where the reaction takes place.
- Enzymes lower activation energy of the reaction they catalyse.

- As molecules react they become unstable, high energy intermediates, but they are in this transition state only momentarily. Energy is required to raise molecules to this transitionstate and this minimum energy needed is called the activation energy. This could be explained schematically by 'boulder on hillside' model of activation energy.

Lock and Key Mechanism of Enzyme

- In a enzyme catalysed reaction, the starting substance is the substrate. It is converted to the product. The substrate binds to the specially formed pocket in the enzyme – the active site, this is called lockand key mechanism of enzyme action. As the enzyme and substrate form a ES complex, the substrate is raised in energy to a transition state and then breaks down into products plus unchanged enzyme.

Factors Affecting the Rate of Enzyme Reactions

- Enzymes are sensitive to environmental condition. It could be affected by temperature, pH, substrate concentration and enzyme concentration.

- The rate of enzyme reaction is measured by the amount of substrate changed or amount of product formed, during a period of time.

Temperature

- Heating increases molecular motion. Thus the molecules of the substrate and enzyme move more quickly resulting in a greater probability of occurence of the reaction. The temperature that promotes maximum activity is referred to as optimum temperature.

- The optimum pH is that at which the maximum rate of reaction occurs. Thus the pH change leads to an alteration of enzyme shape, including the active site. If extremes of pH are encountered by an enzyme, then it will be denatured.

Substrate Concentration

- For a given enzyme concentration, the rate of an enzyme reaction increases with increasing substrate concentration (Figure 8.32).

Enzyme Concentration

- The rate of reaction is directly proportional to the enzyme concentration.

Introducing the Michaelis-Menton Constant (Km) and ItsSignificance

- When the initial rate of reaction of an enzyme is measured over a range of substrate concentrations (with a fixed amount of enzyme) and the results plotted on a graph. With increasing substrate concentration, the velocity increases – rapidly at lower substrate concentration.

- However the rate increases progressively, above a certain concentration of the substrate the curve flattened out. No further increase in rate occurs.

- This shows that the enzyme is working at maximum velocity at this point. On the graph, this point of maximum velocity is shown as Vmax.

Inhibitors of Enzyme

- Certain substances present in the cells may react with the enzyme and lower the rate of reaction. These substances are called inhibitors. It is of two types competitive and non-competitive

Competitive Inhibitor

- Molecules that resemble the shape of the substrate and may compete to occupy the active site of enzyme are known as competitive inhibitors. For Example: the enzyme that catalyses the reaction between carbon di oxide and the CO2 acceptor molecule in photosynthesis, known as ribulosebiphosphate carboxylase oxygenase (RUBISCO) is competitively inhibited by oxygen/carbon-di-oxide in the chloroplast. The competitive inhibitor is malonate for succinic dehydrogenase.

Non-competitive Inhibitors

- There are certain inhibitors which may be unlike the substrate molecule but still combines with the enzyme. This either blocks the attachment of the substrate to active site or change the shape so that it is unable to accept the substrate. For example the effect of the amino acids alanine on the enzyme pyruvate kinase in the final step of glycolysis.

- Certain non-reversible/irreversible inhibitors bind tightly and permanently to an enzyme and destroy its catalytic properties entirely. These could also be termed as poisons. Example – cyanide ions which blocks cytochrome oxidase in terminal oxidation in cell aerobic respiration, the nerve gas sarin blocks a neurotransmitter in synapse transmission.

Allosteric Enzymes

- They modify enzyme activity by causing a reversible change in the structure of the enzyme active site. This in turn affects the ability of the substrate to bind to the enzyme. Such compounds are calledallosteric inhibitors. Eg. The enzyme hexokinase which catalysis glucose to glucose-6 phosphate in glycolysis is inhibited by glucose 6 phosphate. This is an example for feedback allosteric inhibitor.

End Product Inhibition (Negative Feedback Inhibition)

- When the end product of a metabolicpathway begins to accumulate, it may act as an allosteric inhibitor of the enzyme controlling the first step of the pathway. Thus the product starts to switch off its own production as it builds up. The process is self – regulatory. As the product is used up, its production is switched on once again. This is called end-product inhibition (Figure 8.35).

Enzyme Cofactors

- Many enzymes require non-protein components called cofactors for their efficient activity. Cofactors may vary from simple inorganic ions to complex organic molecules. They are of three types: inorganic ions, prosthetic groups and coenzymes.

- Holoenzyme – active enzyme with its non protein component.

- Apoenzyme – the inactive enzyme without its non protein component.
- Inorganic ions help to increase the rate of reaction catalysed by enzymes. Example: Salivary amylase activity is increased in the presence of chloride ions.

- Prosthetic groups are organic molecules that assist in catalytic function of an enzyme. Flavin adenine dinucleotide (FAD) contains riboflavin (vitB2), the function of which is to accept hydrogen. 'Haem' is an iron-containing prosthetic group with an iron atom at its centre.

- Coenzymes are organic compounds which act as cofactors but do not remain attached to the enzyme. The essential chemical components of many coenzymes are vitamins. Eg. NAD, NADP, Coenzyme A, ATP

Nomenclature of Enzymes

- Most of the enzymes have a name based on their substrate with the ending –ase. For example lactase hydrolyses lactose and amylase hydrolyses amylose. Other enzymes like renin, trypsin do not depict any relation with their function.

Nucleic Acids

- As we know DNA and RNA are the two kinds of nucleic acids. These were originally isolated from cell nucleus. They are present in all known cells and viruses with special coded genetic programme with detailed and specific instructions for each organism heredity.DNA and RNA are polymers of monomers called nucleotides, each of which is composed of a nitrogen base, a pentose sugar and a phosphate. A purine or a pyrimidine and a ribose or deoxyribose sugar is called nucleoside. A nitrogenous base is linked to pentose sugar through n-glycosidic linkage and forms a nucleoside. When a phosphate group is attached to a nucleoside it is called a nucleotide. The nitrogen base is aheterocyclic compound that can be either a purine (two rings) or a pyrimidine (one ring). There are 2 types of purines –adenine (A) and guanine (G) and 3 types of pyrimidines – cytosine (C), thymine (T) and uracil (U) (Figure 8.38).

- A characteristic feature that differentiates DNA from RNA is that DNA contains nitrogen bases such as Adenine, guanine, thymine (5-methyl uracil) and cytosine and the RNA contains nitrogen bases such as adenine, guanine, cytosine and uracil instead of thymine. The nitrogen base is covalently bonded to the sugar ribose in RNA and to deoxyribose (ribose with one oxygen removed from C2) in DNA. Phosphate group is a derivative of (PO43-) phosphoric acid, and forms phosphodiester linkages with sugar molecule (Figure 8.39).

Formation of Dinucleotide and Polynucleotide

- Two nucleotides join to form dinucleotide that are linked through 3′-5′ phosphodiester linkage by condensation between phosphate groups of one with sugar of other. This is repeated many times to make polynucleotide.

Structure of DNA

- Watson and Crick shared the Nobel Prize in 1962 for their discovery, along with Maurice Wilkins, who had produced the crystallographic data supporting the model. Rosalind Franklin (1920–1958) had earlier produced the first clear crystallographic evidence for a helical structure. James Watson and Francis Crick (Figure 8.40) of Cavendish laboratory in Cambridge built a scale model of double helical structure of DNA which is the most prevalent form of DNA, the B-DNA. This is the secondary structure of DNA.

- As proposed by James Watson and Francis Crick, DNA consists of right handed double helix with 2 helical polynucleotide chains that are coiled around a common

axis to form righthanded B form of DNA. The coils are held together by hydrogen bonds which occur between complementary pairs of nitrogenous bases. The sugar is called 2′-deoxyribose because there is no hydroxyl at position 2′. Adenine and thiamine base pairs has two hydrogen bonds while guanine and cytosine base pairs have three hydrogen bonds.

- As published by Erwin Chargaff in 1949, a purine pairs with pyrimidine and vice versa. Adenine (A) always pairs with Thymine (T) by double bond and Guanine (G) always pairs with Cytosine (C) by triple bond.

Features of DNA

- If one strand runs in the 5'-3' direction, the other runs in 3'-5' direction and thus are antiparallel (they run in opposite direction). The 5' end has the phosphate group and 3end has the OH group.

- The angle at which the two sugars protrude from the base pairs is about 120°, for the narrow angle and 240° for the wide angle. The narrow angle between the sugars generates a minor groove and the large angle on the other edge generates major groove.
- Each base is 0.34 nm apart and a complete turn of the helix comprises 3.4 nm or 10 base pairs per turn in the predominant B form of DNA.

- DNA helical structure has a diameter of 20 A° and a pitch of about 34 A°. X-ray crystal study of DNA takes a stack of about 10 bp to go completely around the helix (360°).

- Thermodynamic stability of the helix and specificity of base pairing includes (i) the hydrogen bonds between the complementary bases of the double helix (ii) stacking interaction between bases tend to stack about each other perpendicular to the direction of helical axis. Electron cloud interactions (Ft - n) between the bases in the helical stacks contribute to the stability of the double helix.

- The phosphodiesterlinkages gives an inherent polarity to the DNA helix. They form strong covalent bonds, gives the strength and stability to the polynucleotide chain.

- Plectonemic coiling - the two strands of the DNA are wrapped around each other in a helix, making it impossible to simply move them apart withoutbreaking the entire structure. Whereas in paranemic coiling the two strands simply lie alongside one another, making them easier to pull apart.

- Based on the helix and the distance between each turns, the DNA is of three forms – A DNA, B DNA and Z DNA.

Ribonucleic Acid (RNA)

- Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA is single stranded and is unstable when compared to DNA.

Types of RNA

- mRNA (messenger RNA): Single stranded, carries a copy of instructions for assembling amino acids into proteins. It is very unstable and comprises 5% of total RNA polymer. Prokaryotic mRNA (Polycistronic) carry coding sequences for many polypeptides. Eukaryotic mRNA (Monocistronic) contains information for only one polypeptide.

- tRNA (transfer RNA): Translates the code from mRNA and transfers aminoacids to the ribosome to build proteins. It is highly folded into an elaborate 3D structure and comprises about 15% of total RNA. It is also called as soluble RNA.

- rRNA (ribosomal RNA): Single stranded, metabolically stable, make up the two subunits of ribosomes. It constitutes 80% of the total RNA. It is a polymer with varied length from 120–3000 nucleotides and gives ribosomes their shape. Genes for rRNA are highly conserved and employed for phylogenetic studies.

Genitics

10th book
Unit – 18 -Heredity

Gregor Johann Mendel - Father of Genetics

- Mendel (1822-1884) was an Austrian monk who discovered the basic principles of heredity through his experiments. His experiments are the foundation for modern genetics. He was born in 1822 to a family of farmers in Silesian of Czechoslovakia. After finishing his high school at the age of 18, he entered the Augustinian Monastery at Brunn as a priest. From there he went to the University of Vienna for training in physics, mathematics and natural science. Mendel returned to the monastery in 1854 and continued to work as a priest and teach in high school. In his leisure time he started his famous experiments on the garden pea plant. He conducted his experiments in the monastery for about nine years from 1856 to 1865. He had worked on nearly 10000 pea plants of 34 different varieties. Mendel noted that they diff er from one another in many ways.

Mono hybrid Cross – Inheritance of One Gene

- crosses involving inheritance of only one pair of contrasting characters are called monohybrid crosses. For example it is a cross between two forms of a single trait like cross between tall and dwarf plant

Mendel 's Explanation of Monohybrid Cross:

- Parental generation: Pure breeding tall plant and a purebreeding dwarf plant **F1 generation:** Plant raise from the seeds of pure breeding parental cross in F1 generation were tall and monohybrids.

- **F2 generation:**

  Selfing of th F1 monohybrids resulted in talland dwarf plants respectively in the ratio of 3:1.The actual number of talland dwarf plants obtained by Mendel was 787 tall and 277 dwarf External expression of a particular trait is known as phenotype. So the phenotypic ratio is 3:1.

  - In the F2 generation 3 different types were obtained:
  - Tall Homozygous – TT(Pure) – 1
  - Tall Heterozygous – Tt -2
  - Dwarf Homozygous – tt– 1
  - So the genotypic ratio 1:2:1.A genotype is the genetic expression of an organism.

Mendal 's Interpretation on Monohybrid cross

- Based on these observations it was confirmed by Mendel that 'factors' are passed on from one generation to another, now refered to as genes. Tallness and Dwarfnessare determined by a pair for contrasting factors , tall plant possess a pair of factors (represented by T- taking the first letter of the dominant character) and a plant is dwarf because it possess factors for dwarfness (represented as t- recessive character). These factors occur in pairs and may be alike as in pure breeding tall plants (TT) and dwarf plants (tt). This is referred to as homozygous. If they are unlike (Tt) they are referred to as heterozygous.

- Two factors making up a pair of contrasting characters are called allelesor allelomorphs. One member of each pair is contributed by one parent

- When two factors for alternative expression of a trait are brought together by fertilization only one expresses itself, (tallness) masking the expression of the other (dwarfness). The character which expresses itself is called dominant condition and that which is masked is called recessive condition.

- The factors are always pure and when gametes are formed, the unit factors segregate so that each gamete gets one of the two alternative factors. It means that factors for tallness(T) and dwarfness(t) are separate entities and in a gamete either T or t is present. When F1 hybrids are self crossed the two entities separate and then unite independently, forming tall and dwarf plants.

Dihybrid Cross- Inheritance Two Genes and Law of Independent Assortment:

- Dihybrid cross involves the inheritance of two pairs of contrasting characteristics (or contrasting traits) at the same time. The two pairs of contrasting characteristics chosen by Mendel were shape and colour of seeds: round-yellow seeds and wrinkled-green seeds.

- Mendel crossed pea plants having round-yellow seeds with pea plants having wrinkled-green seeds. Mendel made the following observations:

- Mendel first crossed pure breeding pea plants having round-yellow seeds with pure breeding pea plants having wrinkled-green seeds and found that only round-yellow seeds were produced in the first generation (F1). No wrinkled-green seeds were obtained in the F1 generation. From this it was concluded that round shape and yellow colour of the seeds were dominant traits over the wrinkled shape and green color of the seeds.

- When the hybrids of F1 generation pea plants having round-yellow seeds were cross-bred by self pollination, then four types of seeds having different combinations of shape and color were obtained in second generation or F2 generation. They were round yellow, round-green, wrinkled yellow and wrinkled-green seeds.

- The ratio of each phenotype (or appearance) of seeds in the F2 generation is 9:3:3:1. This is known as the Dihybrid ratio.

- From the above results it can be concluded that the factors for each character or trait remain independent and maintain their identity in the gametes. The factors are independent to each other and pass to the offsprings (through gametes).

Results of a Dihybrid Cross:

Mendel got the following results from his dihybrid cross

- **Four Types of Plants:**
  A dihybrid cross produced four types of F2 offsprings in the ratio of 9 with two dominant traits, 3 with one dominant trait and one recessive trait, 3 with another dominant trait and another recessive trait and 1 with two recessive traits.

- **New Combination:**
  Two new combinations of traits with round green and wrinkled yellow had appeared in the dihybrid cross (F2 generation).

Mendel's Laws

- Based on his experiments of monohybrid and dihybrid cross, Mendel proposed three important laws which are now called as Mendel's Laws of Heredity.

Law of Dominance:

- "When two homozygous individuals with one or more sets of contrasting characters are crossed, the characters that appear in the F1 hybrid are dominant and those that do not appear in F1 are recessive characters".

Law of Segregation or Law of purity of gametes:

- "When a pair of contrasting factors or genes or allelomorphs are brought together in a heterozygote or hybrid, the two members of the allelic pair remain together without mixing and when gametes are formed, the two separate out, so that only one enters each gamete."

Law of independent assortment:

- "In case of inheritance of two or more pairs of characters simultaneously, the factors or genes of one pair assort out independently of the other pair."

Down's syndrome

- This condition was first identified by a doctor named Langdon Down in1866.

- It is a genetic condition in which there is an extra copy of chromosome 21 (Trisomy 21). It is associated with mental retardation, delayed development, behavioural problems, weak muscle tone, vision and hearing disability are some of the conditions seen in these children.

Gene or point mutation

- Gene mutation is the changes occurring in nucleotide sequence of a gene. It involves substitution, deletion, insertion or inversion of a single or more than one nitrogenous base. Gene alteration results in abnormal protein formation in an organism.

Sickle cell anaemia is caused by the mutation of a single gene. Alteration in the gene brings a change in the structure of the protein part of haemoglobin molecule. Due to the change in the protein molecule, the red blood cell (RBC) that carries the haemoglobin is sickle shaped.

Unit – 19 -Origin & Evolution of Life

Theories on Origin of Life:

- Many theories have been postulated to explain the origin of life. The views on the origin of life has been putforth as

Special creation:

- This idea embodies that life on Earth is a divine creation and also attributes to supernatural event at a particular time in the past.It also emphasizes that life has not changed ever since its origin.

Spontaneous generation (Abiogenesis):

- According to this theory life originated spontaneously from lifeless matter. It was believed that fishes originated from mud, frogs from moist soil and insects from decaying matter.

Biogenesis:

- It was speculated by Louis Pasteur (1862) that life originates from pre-existing life. He showed that pre-sterilised flasks kept closed airtight, with killed yeast, did not give rise to any life form, while in another flask kept open to air living organisms arose from killed yeast.

Extraterresterial or Cosmic origin:

- Some scientists still believe that life came from outer space. This states that units of life called spores (Panspermia) were transferred to different planets including earth. This is still an idea of some astronomers.

Chemical Evolution of Life:

- This idea was developed by Oparin (1922) and Haldane (1929). They proposed that with the conditions prevailing on earth, life arose by a series of sequential chemical reactions. The first form of life could have come from pre-existing non-living inorganic molecules which gave rise to formation of diverse organic molecules which are transformed into colloid system to produce life. The modern concept on chemical evolution regarding origin of life was accepted.

Evidences from Morphology and Anatomy

- The comparative study of morphology and anatomy of animals, reveal that they possess common set of characteristics.

Homologous organs:

- The homologous organs are those which have inherited from common ancestors with similar developmental pattern in embryos. The fore limbs of mammals are homologous structures. A human hand, a front leg of a cat, flipper of a whale and a bat's wing look dissimilar and adapted for different functions. Their mode of development and basic structure of bone are similar.

Analogous organs:

- The analogous organs look similar and perform similar functions but they have different originand developmental pattern. The function of the wings of a bat, the wings of a bird and wings of an insect are similar, but their basic structures are different.

Vestigial organs:

- The degenerated and non-functional organsof animals are called vestigial organs. The same organs are found to be well-developed and functional, in some of the related forms. Some of the vestigial organs in man are vermiform appendix, nictitating membrane, caudal vertebra, coccyx etc.

Atavism:

- The reappearance of ancestral characters in some individuals is called atavism. e.g. Presence of rudimentary tail in new born babies, presence of thick hair on the human body.

Evidences from Embryology

- The study of comparative embryology of different animals, supports the concept of evolution. The embryos from fish to mammals are similar in their early stages of development. The differentiation of their special characters appear in the later stages of development.

- **Biogenetic law** or **Recapitulation theory** was given by Ernst Haeckel. According to this theory, Ontogeny recapitulates Phylogeny. The stages of development of the individualanimal repeats the evolutionary history of the entire race of the animal.

Evidences from Palaeontology:

- Palaeontology deals with the study of fossils. Leonardo da Vinci is called the Father of Palaeontology. The study of fossils helps us to understand the line of evolution of many invertebrates and vertebrates. Fossil records show that the evolution has taken a gradual process from simple to complex organisms. The origin of modern birds is supported by the evidences from palaeontology.

Archaeopteryx:

- Archaeopteryx is the oldest known fossil bird. It was an early bird-like form found in the Jurassic period.It is considered to be a connecting link between reptiles and birds. It had wings with feathers, like a bird. It had long tail, clawed digits and conical teeth, like a reptile.

Theories of Evolution

- Life had evolved along with evolution of earth towards the end of 18th century. Evolution is the gradual change occurring in living organisms over a period of time. Formation of new species due to changes in specific characters over several generations as response to natural selection, is called evolution. The natural changes occuring is explained through the theories of evolution as proposed by Lamarck and Darwin.

Lamarckism:

- Jean Baptiste Lamarck (1744-1829) was a French naturalist, well known for his theory of evolution. Lamarck's theory of evolution was published in 'Philosophic Zoologique' in the year 1809. It is popularly known as 'Theory of inheritance of Acquired Characters" or "Use and Disuse theory" or Lamarckism.

Principles of Lamarckism

I. Internal vital force

- Living organisms or their component parts tend to increase in size continuously. This increase in size is due to the inherent ability of the organisms

II. Environment and new needs

- A change in the environment brings about changes in the need of the organisms. In response to the changing environment, the organisms develop certain adaptive characters. The adaptations of the organisms may be in the form of development of new parts of the body.

III. Use and disuse theory

- Lamarck's use and disuse theory states that if an organ is used constantly, the organ develops well and gets strengthened. When an organ is not used for a long time, it gradually degenerates.

- The ancestors of giraffe were provided with short neck and short forelimbs. Due to shortage of grass, they were forced to feed onleaves from trees. The continuous stretching of their neck and forelimbs resulted in the development of long neck and long forelimbs which is an example for constant use of an organ. The degenerated wing of Kiwi is an example for organ of disuse.

IV. Theory of Inheritance of acquired characters

- When there is a change in the environment, the animals respond to the change. They develop adaptive structures. The characters developed by the animals during their life time, in response to the environmental changes are called acquired characters. According to Lamarck, the acquired characters are transmitted to the offspring by the process of inheritance.

Unit - 20 - Breeding & Biotechnology

Mutation Breeding

- Mutation is defined as the sudden heritable change in the nucleotide sequence of DNA in an organism. It is a process by which genetic variations are created which in turn brings about changes in the organism. The organism which undergoes mutation is called a mutant.

- The factors which induce mutations are known as mutagens or mutagenic agents. Mutagens are of two types namely physical mutagens and chemical mutagens.

Physical mutagens

- Radiations like X-rays, α, β and γ-rays, UV rays, temperature etc. which induce mutations are called physical mutagens

Chemical mutagens

- Chemical substances that induce mutations are called chemical mutagens. e.g. Mustard gas and nitrous acid. The utilisation of induced mutation in crop improvement is called mutation breeding.

Hybridization

- Hybridization may be defined as the process of crossing two or more types of plants for bringing their desired characters together into one progeny called hybrid. Hybrid is superior in one or more characters to both parents. Hybridization is the common method of creating genetic variation to get improved varieties.

Hybridization Experiment: Triticale (The first man – made cereal)

- Triticale is the first man- made cereal hybrid. It is obtained by crossing wheat (Triticum durum, 2n = 28) and rye (Secale cereal, 2n = 14). The F1 hybrid is sterile (2n = 21). Then the chromosome number is doubled using colchicine and it becomes a hexaploid Triticale (2n = 42).

- The cycle of crop raising and selection continues till the plants with the desired characters are finally obtained. The development of new varieties is a long-drawn process. Two main aspects of hybridization are to combine the characters of two plants in one plant and to utilize hybrid vigour.

Genetic Engineering

- Genetic engineering is the manipulation and transfer of genes from one organism to another organisms to create a new DNA called as recombinant DNA(rDNA). The term recombinant is used because DNA from two diff erent sources can be joined together. Hence, genetic engineering is also called as recombinant DNA technology.

Techniques of Genetic Engineering – Basic Requirements

Important discoveries that led to the stepping stone of rDNA technology were

- Presence of plasmid in bacteria that can undergo replication independently along with chromosomal DNA.
- Restriction enzymes cuts or break DNA at specifi c sites and are also called as molecular scissors.
- DNA ligases are the enzymes which help in ligating (joining) the broken DNA fragments.

Gene Cloning

- What reminds to your mind when you hear the word clone? Of course, 'DOLLY' the cloned sheep. The carbon copy of an individual is oft en called a clone. However, more appropriately, a clone means to make a genetically exact copy of an organism.
- In gene cloning, a gene or a piece of DNA fragment is inserted into a bacterial cell where DNA will be multiplied (copied) as the cell divides. A brief outline of the basic steps involved in gene cloning are:
  - Isolation of desired DNA fragment by using restriction enzymes
  - Insertion of the DNA fragment into a suitable vector (Plasmid) to make rDNA Transfer of rDNA into bacterial host cell (Transformation)
  - Selection and multiplication of recombinant host cell to get a clone
  - Expression of cloned gene in host cell.

Using this strategy several enzymes, hormones and vaccines can be produced

Biotechnology in Medicine

- Using genetic engineering techniques medicinally important valuable proteins or polypeptides that form the potential pharmaceutical products for treatment of various diseases have been developed on a commercial scale.

Pharmaceutical Products developed by rDNA technique

- Insulin used in the treatment of diabetes.
- Human growth hormone used for treating children with growth deficiencies
- Blood clotting factors are developed to treat haemophilia.
- Tissue plasminogen activator is used to dissolve blood clots and prevent heart attack.
- Development of vaccines against various diseases like Hepatitis B and rabies.

Gene Therapy

- Gene therapy refers to the replacement of defective gene by the direct transfer of functional genes into human to treat genetic disease or disorder. The genetic makeup of the patient' cell is altered using recombinant DNA technology. It was first successfully implemented in 1990.

Somatic gene therapy is the replacement of defective gene in Somatic cells.

- **Germ line gene therapy** replacement of defective gene in germ cell (Egg and Sperm)targeted only somatic (non –reproductive) cells. Correction of genetic defects in Somatic cells may be beneficial to the patient but the Corrected gene may not be carried to the next generation.

Stem Cells

- Our body is composed of over 200 specialised cell types, that can carry out specific functions. eg. neurons or nerve cell that can transmit signals, or heart cells which contract to pump blood or pancreatic cells to secrete insulin. These specialised cells are called as differentiated cell.In contrast to differentiated cells.
- In Contrast to differentiated cells, stem cells are undifferentiated or unspecialised mass of cells. The stem cells are the cells of variable potency. Potency refers to the number of possible fates that a cell can acquire. The two important properties of stem cells that differentiate them from other cells are:
  - Its ability to divide and give rise to more stem cells by self-renewal
  - Its ability to give rise to specialised cells with specific functions by the process of differentiation.

Types of stem cells

- **Embryonic stem cells** can be extracted and cultured from the early embryos. These cells are derived from the inner cell mass of blastocyst. These cells can be developed into any cell in the body.

- **Adult stem cell** or somatic stem cell are found in the neonatal (new born) and adults. They have the ability to divide and give rise to specific cell types. Sources of adult stem cells are amniotic fluid, umbilical cord and bone marrow.

Stem-cell therapy

- Sometimes cells, tissues and organs in the body may be permanently damaged or lost due to genetic condition or disease or injury. In such situations stem cells are used for the treatment of diseases which is called stem-cell therapy. In treating neurodegenerative disorders like Parkinson's disease and Alzheimer's disease neuronal stem cells can be used to replace the damaged or lost neurons.

DNA Finger Printing Technology

- The human genome has 3 billion base pairs. Did you know that the DNA pattern of two individuals cannot be same except for identical twins. Each person's DNA sequence is unique due to the small difference in the base pairs. Therefore, if we want to compare the genetic difference among the two individuals, DNA fingerprinting is the easier and quicker method. This technique was developed by Alec Jeffrey.

- The technique analyses each individual's unique DNA sequences and provides distinctive characteristics of individual which helps in identification. Variable number of tandem repeat sequences (VNTRs) serve as molecular markers for identification.

- In human beings, 99 % of the DNA base sequences are the same and this is called as bulk genomic DNA. The remaining 1 % DNA sequence differs from one individual to another. This 1 % DNA sequence is present as small stretch of repeated sequences which is known as satellite DNA. The number of copies of the repeat sequence also called as VNTRs differs from one individual to another, and results in variation in the size of the DNA segment.

VNTRs illustration of three persons

- As shown in the illustration, the sequence AGCT is repeated six times in fi rst person, fi ve times in second person and seven times in thirdperson. Because of this, DNA segment of third person will be larger in size followed by DNA segment of first person and then the second person. Thus, it is clear that satellite DNA bring about variation within the population. Variation in DNA banding pattern reveals differences among the individuals.

Applications of DNA Fingerprinting

- DNA fingerprinting technique is widely used in forensic applications like crime investigation such as identifying the culprit. It is also used for paternity testing in case of disputes.
- It also helps in the study of genetic diversity of population, evolution and speciation.

Genetically Modified Organisms (GMOs)

- One of the most tremendous development of genetic engineering is the production of genetically modified (GM) plants and animals. Genetic modification refers to the alteration or manipulation of genes in the organisms using rDNA techniques in order to produce the desired characteristics. The DNA fragment inserted is called transgene. Plants or animals expressing a modified endogenous gene or a foregin gene are also known as transgenic organisms.

- The transgenic plants are much stable, with improved nutritional quality, resistant to diseases and tolerant to various environment conditions. Similarly transgenic animalsare used to produce proteins of medicinal importance at low cost and improve livestock quality.

Genetically Modified Plants

Objective	Gene inserted	Achievement
Improved nutritional quality in Rice	Beta carotene gene (In humans, Beta carotene is required for the synthesis of Vitamin A)	Golden Rice (Genetically modified rice can produce beta carotene, that can prevent Vitamin A deficiency)
Increased crop production	Bt gene from bacteria Bacillus thuringiensis. (Bt gene produces a protein that is toxic to insects)	Insect resistant plants (These plants can produce the toxin protein that kills the insects which attack them)

Genetically Modified Animals

Objective	Gene inserted	Achievement
Improved wool quality and production	Genes for synthesis of amino acid, cysteine	Transgenic sheep (gene expressed)
Increased growth in fishes	Salmon or Rainbow trout or Tilapia growth hormone gene	Transgenic fish (gene expressed)

Principles of Inheritance and Variation

- Genetics is a branch of biology that dealswith the study of heredity and variations. It describes how characteristics and features pass on from the parents to their offsprings in each successive generation. The unit of heredity is known as the gene. Gene is the inherited factor that determines the biological character of an organism. A variation is the degree by which the progeny differs from their parents.

Multiple alleles

- The genetic segregations in Mendelian inheritance reveal that all genes have two alternative forms – dominant and recessive alleles e.g. tall versus dwarf (T and t). The former is the normal allele or wild allele and the latter the mutant allele. A gene can mutate several times producing several alternative forms. When three or more alleles of a gene that control a particular trait occupy the same locus on the homologous chromosome of an organism, they are called multiple alleles and their inheritance is called multiple allelism.

ABO blood types
Multiple allele inheritance of ABO blood groups

- Blood differs chemically from person to person. When two different incompatible blood types are mixed, agglutination (clumping together) of erythrocytes (RBC) occurs. The basis of these chemical differences is due to the presence of antigens (surface antigens) on the membrane of RBC and epithelial cells. Karl Landsteiner discovered two kinds of antigens called antigen 'A' and antigen 'B' on the surface of RBC's of human blood. Based on the presence or absence of these antigens three kinds of blood groups, type 'A', type 'B', and type 'O' (universal donor)were recognized. The fourth and the rarest blood group 'AB' (universal recipient) was discovered in 1902 by two of Landsteiner's students Von De Castelle and Sturli.

- Bernstein in 1925 discovered that the inheritance of different blood groups in human beings is determined by a number of multiple allelic series. The three autosomal alleles located on chromosome 9 are concerned with the determination of blood group in any person. The gene controlling blood type has been labeled as 'L' (after the name of the discoverer, Landsteiner) or I (from isoagglutination). The I gene exists in three allelic forms, IA, IB and IO. IA specifies A antigen. IB allele determines B antigen and IO allele specifies no antigen. Individuals who possess these antigens in their fluids such as the saliva are called secretors.

- Each allele (IA and IB) produces a transferase enzyme. IA allele produces N-acetyl galactose transferase and can add N-acetyl galactosamine (NAG) and IB allele encodes for the enzyme galactose transferase that adds galactose to the precursor (i.e. H substances)

In the case of IO/IO allele no terminal transferase enzyme is produced and therefore called "null" allele and hence cannotadd NAG or galactose to the precursor.

- From the phenotypic combinations it isevident that the alleles IA and IB are dominantto IO, but co-dominant to each other (IA=IB).Their dominance hierarchy can be givenas (IA=IB> IO). A child receives one of threealleles from each parent, giving rise to sixpossible genotypes and four possible bloodtypes (phenotypes). The genotypes are IAIA, IAIO, IBIB, IB IO, IAIB and IO IO.

Genetic basis of the human ABO blood groups

Genotype	ABO bloodgroupphenotype	Antigenspresent onred bloodcell	Antibodiespresent in blood plasma
IAIA	Type A	A	Anti -B
IAIo	Type A	A	Anti -B
IBIB	Type B	B	Anti -A
IBIo	Type B	B	Anti -A
IAIB	Type AB	A and B	Neither Anti -A nor Anti-B
IoIo	Type O	Neither A nor B	Anti –A and anti - B

Genetic control of Rh factor
Fisher and Race hypothesis:

- Rh factor involves three different pairs of alleles located on three different closely linked loci on the chromosome pair. This system is more commonly in use today, and uses the 'Cde' nomenclature.

Wiener Hypothesis

- Wiener proposed the existence of eight alleles (R1, R2, R0, Rz, r, r1, r11, ry) at a single Rh locus. All genotypes carrying a dominant 'R allele' (R1, R2 ,R0 ,Rz) will produce Rh+positivephenotype and double recessive genotypes (rr, rr1, rr11, rry) will give rise to Rh-negative phenotype.

Incompatibility of Rh – Factor – Erythroblastosis foetalis

- Rh incompatability has great significance in child birth. If a woman is Rh negative andthe man is Rh positive, the foetus may be Rh positive having inherited the factor fromits father. The Rh negative mother becomes sensitized by carrying Rh positive foetus within her body. Due to damage of blood vessels, during child birth, the mother's immune system recognizes the Rh antigens and gets sensitized. The sensitized mother produces Rh antibodies. The antibodies are IgG type which are small and can cross placenta and enter the foetal circulation. By the time the mother gets sensitized and produce anti 'D' antibodies, the child is delivered.

- Usually no effects are associated with exposure of the mother to Rh positive antigen during the first child birth, subsequent Rh positive children carried by the same mother, may be exposed to antibodies produced by the mother against Rh antigen, which are carried across the placenta into the foetal blood circulation. This causes haemolysis offoetal RBCs resulting in haemolytic jaundice and anaemia. This condition is known as Erythoblastosisfoetalis or Haemolyticdisease of the new born (HDN).

Inheritance of Y- linked genes

- Genes in the non-homologous region of the Y-chromosome are inherited directly from male to male. In humans, the Y-linked or holandric genes for hypertrichosis (excessive development of hairs on pinna of the ear) are transmitted directly from father to son, because males inherit the Y chromosome from the father. Female inherits only X chromosome from the father and are not affected.

Genetic Disorders:

- A genetic disorder is a disease or syndrome that is caused by an abnormalityin an individual DNA. Abnormalities can range from a small mutation in a single gene to the addition or subtraction of an entire chromosome or even a set ofchromosomes. Genetic disorders are of two types namely, Mendelian disorders and chromosomal disorders.

Mendelian disorders

- Alteration or mutation in a single gene causes Mendelian disorders. These disorders are transmitted to the offsprings on the same line as the Mendelian pattern of inheritance. Some examples for Mendelian disorders are Thalassemia, albinism, phenylketonuria, sickle cell anaemia, Huntington's chorea, etc., These disorders may be dominant or recessive and autosomal or sex linked.

Thalassemia

- Thalassemia is an autosomal recessive disorder. It is caused by gene mutation resultingin excessive destruction of RBC's due to the formation of abnormal haemoglobin molecules.

- Normally haemoglobin is composed of four polypeptide chains, two alpha and two betaglobin chains. Thalassemia patients have defects in either the alpha or beta globin chain causing the production of abnormal haemoglobin molecules resulting in anaemia.

- Thalassemia is classified into alpha and beta based on which chain of haemoglobin molecule is affected. It is controlled by two closely linked genes HBA1 and HBA2 on chromosome 16. Mutation or deletion of one or more of the four alpha gene alleles causes Alpha Thalassemia. In Beta Thalassemia, production of beta globin chain is affected. It is controlled by a single gene (HBB) on chromosome 11. It is the most common type of

Thalassemia and is also known as Cooley's anaemia. In this disorder the alphachain production is increased and damages the membranes of RBC.

UNIT – 5 - Molecular Genetics

- Mendel's theory dispelled the mystery of why traits seemed to appear anddisappear magically from one generation to the next. Mendel's work reveals the patterns of heredity and reflect the transmission of evolved information from parents to offspring. This information is located on the chromosomes. One of the most advanced realizations of human knowledge was that our unique characteristics are encoded within molecules of DNA. The discovery that DNA is the genetic material left several questions unanswered.

- How is the information in DNA used? Scientists now know that DNA directs the construction of proteins. Proteins determine the shapes of cells and the rate of chemical reactions, such as those that occur during metabolism and photosynthesis. The hereditary nature of every living organism is defined by its genome, which consists of a long sequence of nucleic acids that provide the information needed to construct the organism. The genome contains the complete set of hereditary information for any organism. The genome may be divided into a number of different nucleic acid molecules. Each of the nucleic acid molecule may contain large number of genes. Each gene is a sequence within the nucleic acid that represents a single protein. In this chapter we will discuss the structure of DNA, its replication, the process of making RNA from DNA (transcription), the genetic code that determines the sequence of amino acid in protein synthesis (translation), regulation of gene expression and the essentials of human genome sequencing.

Gene as the functional unit of inheritance

- A gene is a basic physical and functional unit of heredity. The concept of the gene was first explained by Gregor Mendel in 1860's. He never used the term 'gene'. Hecalled it 'factor'. In 1909, the Danish biologist Wilhelm Johannsen, coined the term 'gene', that was referred to discrete determiners of inherited characteristics.

- According to the classical concept of gene introduced by Sutton in 1902, genes have been defined as discrete particles that follow Mendelian rules of inheritance, occupy a definite locus in the chromosome and are responsible for the expression of specific phenotypic character. They show the following properties:

    - Number of genes in each organism is more than the number of chromosomes; hence several genes are located on the same chromosome.

    - The genes are arranged in a single linear order like beads on a string.

    - Each gene occupies a specific position called locus.

    - Genes may exist in several alternate forms called alleles.

- Genes may undergo sudden change in positions and composition called mutations.
- Genes are capable of self-duplication producing their own copies.

One gene-one enzyme hypothesis:
The experiments of George Beadle and Edward Tatum in the early 1940's on Neurospora crassa (the red bread mould) led them to propose one geneone enzyme hypothesis, which states that one gene controls the production of one enzyme.

One gene-one polypeptide hypothesis:
It was observed that an enzyme may be composed of more than one polypeptide chain and a gene can code for only one polypeptide chain. Thus one gene-one polypeptide hypothesis states that one gene controls the production of only one polypeptide chain of an enzyme molecule.

In search of the genetic material

- As early as 1848, Wilhelm Hofmeister, a German botanist, had observed that cell nuclei organize themselves into small, rod like bodies during mitosis called chromosomes. In 1869, Friedrich Miescher, a Swiss physician, isolated a substance from the cell nuclei and called it as nuclein. It was renamed as nucleic acid by Altman (1889), and is now known as DNA. By 1920, it became clear that chromosomes are made up of proteins and DNA. Many experimentswere carried out to study the actual carriers of genetic information. Griffith's experiment proved that DNA is the genetic material which has been dealt in class XI. Bacterial transformation experiments provided the first proof that DNA is the genetic material.

- However, he could not understand the cause of bacterial transformation, and the biochemical nature of genetic material was not defined from his experiments.

- Later, Oswald Avery, Colin Macleod and Maclyn McCarty in 1944 repeated Griffith's experiments in an 'in vitro' system in order to identify the nature of the transforming substance responsible for converting a nonvirulent strain into virulent strain. They observed that the DNA, RNA and proteins isolated from the heat-killed S-strain when added to R-strain changed their surface character from rough to smooth and also made them pathogenic But when the extract was treated with DNase (an enzyme which destroys DNA) the transforming ability was lost. RNase (an enzyme which destroys RNA) and proteases(an enzyme which destroys protein) did not affect the transformation. Digestion with DNase inhibited transformation suggesting that the DNA caused the transformation. These experiments suggested that DNA and not proteins is the genetic material. The phenomenon, by which DNA isolated from one type of cell (R – strain), when introduced into another type (S-strain), is able to retain some of the properties of the R - strain is referred to as transformation.

DNA is the genetic material

- Many biologists despite the earlier experiments of Griffith, Avery and others, still believed that protein, not DNA, was the hereditary material in a cell. As eukaryotic chromosomes consist of roughly equal amounts of protein and DNA, it was said that only a protein had sufficient chemical diversity and complexity to encode the information required for genetic material. In 1952, however, the results of the Hershey-Chase experiment finally provided convincing evidence that DNA is the genetic material.

Properties of genetic material (DNA versus RNA)

- The experiment by Hershey and Chase clearly indicates that it is DNA that acts as a genetic material. However, in some viruses like Tobacco mosaic virus (TMV), bacteriophage θB, RNA acts as the genetic material. A molecule that can act as a genetic material should have the following properties:

Self Replication:

- It should be able to replicate. According to the rule of base pairing and complementarity, both nucleic acids (DNA and RNA) have the ability to direct duplications. Proteins fail to fulfil this criteria.

Stability:

- It should be stable structurally and chemically. The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of property of genetic material was clearly evident in Griffith's transforming principle. Heat which killed the bacteria did not destroy some of the properties of genetic material. In DNA the two strands being complementary, if separated (denatured) by heating can come together (renaturation) when appropriate condition is provided. Further 2' OH group present at every nucleotide in RNA is a reactive group that makes RNA liable and easily degradable. RNA is also known to be catalytic and reactive. Hence, DNA is chemically more stable and chemically less reactive when compared to RNA. Presence of thymine instead of uracil in DNA confers additional stability to DNA.

Information storage:

- It should be able to express itself in the form of 'Mendelian characters'. RNA can directly code for protein synthesis and can easily express the characters. DNA, howeverdepends on RNA for synthesis of proteins. Both DNA and RNA can act as a genetic material, but DNA being more stable stores the genetic information and RNA transfers the genetic information.

Variation through mutation:

- It should be able to mutate. Both DNA and RNA are able to mutate.RNA being unstable, mutates at a faster rate. Thus viruses having RNA genome with shorter life span can mutate and evolve faster.

- The above discussion indicates that both RNA and DNA can function as a genetic material. DNA is more stable, and is preferred for storage of genetic information.

- Chromosomes are carriers of genes which are responsible for various characters from generation to generation. Du Praw (1965) proposed a single stranded model (unineme), as a long coiled molecule which is associated with histone proteins in eukaryotes. Plants and animals have more DNA than bacteria and must fold this DNA to fit into the cell nucleus. In prokaryotes such as *E. coli* though they do not have defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region called the nucleoid. The DNA as a nucleoid is organized into large loops held by protein. DNA of prokaryotes is almost circular and lacks chromatin organization, hence termed genophore.

Transcription

- The process of copying genetic information from one strand of DNA into RNA is termed transcription. This process takes place in presence of DNA dependent RNA polymerase.

- In some retroviruses that contain RNA as the genetic material (e.g, HIV), the flow of information is reversed. RNA synthesizes DNA by reverse transcription, then transcribed into mRNA by transcription and then into proteins by translation.

- For a cell to operate, its genes must be expressed. This means that the gene products, whether proteins or RNA molecules must be made. The RNA that carries genetic information encoding a protein from genes into the cell is known as messenger RNA (mRNA). For a gene to be transcribed, the DNA which is a double helix must be pulled apart temporarily, and RNA is synthesized by RNA polymerase. This enzyme binds to DNA at the start of a gene and opens the double helix. Finally, RNA molecule is synthesized. The nucleotide sequence in the RNA is complementary to the DNA template strand from which it is synthesized.

- Both the strands of DNA are not copied during transcription for two reasons. If both the strands act as a template, they would code for RNA with different sequences. This in turn would code for proteins with different amino acid sequences. This would result in one segment of DNA coding for two different proteins, hence complicate the genetic information transfer machinery. If two RNA molecules were produced simultaneously, double stranded RNA complementary to each other would be formed. This would prevent RNA from being translated into proteins.

Transcription unit and gene

- A transcriptional unit in DNA is defined by three regions, a promoter, the structural gene and a terminator. The promoter is located towards the 5' end. It is a DNA sequence that provides binding site for RNA polymerase. The presence of promoter in a transcription unit, defines the template and coding strands. The terminator region located towards the 3' end of the coding strand contains a DNA sequence that causes the RNA polymerase to stop transcribing. In eukaryotes the promoter has AT rich regions called TATA box (Goldberg-Hogness box) and in prokaryotes this region is called Pribnow box. Besides promoter,eukaryotes also require an enhancer.

- The two strands of the DNA in the structural gene of a transcription unit have oppositepolarity. DNA dependent RNA polymerase catalyses the polymerization in only one direction, the strand that has the polarity 3' 5' acts as a template, and is called the template strand. The other strand which has the polarity 5' 3' has a sequence same as RNA (except thymine instead of uracil) and is displaced during transcription. This strand is called coding strand.

- The structural gene may be monocistronic (eukaryotes) or polycistronic (prokaryotes). In eukaryotes, each mRNA carries only a single gene and encodes information for only a singleprotein and is called monocistronic mRNA. In prokaryotes, clusters of related genes, known asoperon, often found next to each other on the chromosome are transcribed together to give a single mRNA and hence are polycistronic. Before starting transcription, RNA polymerase binds to the promoter, a recognition sequence in front of the gene. Bacterial (prokaryotic) RNA polymerase consists of two major components, the core enzyme and the sigma subunit. The core enzyme (β1, β, and α) is responsible for RNA synthesis whereas a sigma subunit isresponsible for recognition of the promoter. Promoter sequences vary in different organisms.

- RNA polymerase opens up the DNA to form the transcription bubble. The core enzyme moves ahead, manufacturing RNA leaving the sigma subunit behind at the promoter region. The end of a gene is marked by a terminator sequence that forms a hair pin structure in the RNA. The sub-class of terminators require a recognition protein, known as rho (ρ), to function.

Genetic Code

- DNA is the genetic material that carries genetic information in a cell and from generation to generation. At this stage, an attempt will be made to determine in what manner the genetic information exists in DNA molecule? Are they written in coded language on a DNA molecule? If they occur in the language of codes what is the nature of genetic code? The translation of proteins follows the triplet rule; a sequence of three mRNA base (a codon) designates one of the 20 different kinds of amino acids used in protein synthesis.

- Genetic code is the sequence relationship between nucleotide in genes (or mRNA) and the amino acids in the proteins they encode. There are 64 possible triplets, and 61 of them are used to represent amino acids. The remaining three triplet codons are termination signals for polypeptide chains. Since there are only 20 amino acids involved in protein synthesis, most of them are encoded by more than one triplet. Two things make this multiple (degenerate) coding possible. First, there is more than one tRNA for most amino acids. Each tRNA has a different anticodon. Second, this pairing is highly specific for the first two portions on the codon, permitting Watson and Crick base pairs (A – U and G - C) to be formed. But at the third position there is a great deal of flexibility as to which base pairs are acceptable. Most part of the genetic code is universal, being the same in prokaryotes and eukaryotes.

- The order of base pairs along DNA molecule controls the kind and order of amino acids found in the proteins of an organism. This specific order of base pairs is called genetic code, the blue print establishing the kinds of proteins to be synthesized which makes an organism unique.

- Marshall Nirenberg, Severo Ochoa (enzyme polynucleotide phosphorylase called Ochoa'senzyme), Hargobind Khorana, Francis Crick and many others have contributed significantlyto decipher the genetic code. The order in which bases are arranged in mRNA decides the order in which amino acids are arranged in proteins.

The salient features of genetic code are as follows:

- The genetic codon is a triplet code and 61 codons code for amino acids and 3 codons do not code for any amino acid and function as stop codon (Termination).

- The genetic code is universal. It means that all known living systems use nucleic acids and the same three base codons (triplet codon) direct the synthesis of protein from amino acids. For example, the mRNA (UUU) codon codes for phenylalanine in all cells of all organisms. Some exceptions are reported in prokaryotic, mitochondrial and chloroplast genomes. However similarities are more common than differences.

- A non-overlapping codon means that the same letter is not used for two different codons. For instance, the nucleotide sequence GUU GUC represents only two codons.

- It is comma less, which means that the message would be read directly from one end to the other i.e., no punctuation are needed between two codes.

- A degenerate code means that more than one triplet codon could code for a specific amino acid. For example, codons GUU, GUC, GUA and GUG code for valine.

- Non-ambiguous code means that one codon will code for one amino acid.

- The code is always read in a fixed direction i.e. from 5'→3' direction called polarity.

- AUG has dual functions. It acts as a initiator codon and also codes for the amino acid methionine.

- UAA, UAG (tyrosine) and UGA (tryptophan) codons are designated as termination (stop) codons and also are known as "non-sense" codons.

Mutation and genetic code

- Comparative studies of mutations (sudden change in a gene) and corresponding alteration in amino acid sequence of specific protein have confirmed the validity of the genetic code. The relationship between genes and DNA are best understood by mutation studies. The simplest type of mutation at the molecular level is a change in nucleotide that substitutes one base for another. Such changes are known as base substitutions which may occur spontaneously or due to the action of mutagens. A well studied example is sickle cell anaemia in humans which results from a point mutation of an allele of β-haemoglobin gene (βHb). A haemoglobin molecule consists of four polypeptide chains of two types, two α chains and two β-chains. Each chain has a heme group on its surface. The heme groups are involved in the binding of oxygen. The human blood disease, sickle cell anaemia is due to abnormal haemoglobin. This abnormality in haemoglobin is due to a single base substitution at the sixth codon of the beta globin gene from GAG to GTG in β -chain of haemoglobin. It results in a change of amino acid glutamic acid to valine at the 6th position of the β -chain. This is the classical example of point mutation that results in thechange of amino acid residue glutamic acid to valine. The mutant haemoglobinundergoes polymerisation under oxygen tension causing the change in the shape of the RBC from biconcave to a sickle shaped structure.

Regulation of gene expression

- We have previously established how DNA is organized into genes, how genes store genetic information, and how this information is expressed. We now consider the most fundamental issues in molecular genetics. How is genetic expression regulated? Evidence in support of the idea that genes can be turned on and off is very convincing. Regulation of gene expression has been extensively studied in prokaryotes, especially in E. coli. Gene expression can be controlled or regulated at transcriptional or post transcriptional or translational level. Here, we are going to discuss regulation of gene expression at transcriptional level. Usually, small extracellular or intracellular metabolites trigger initiation or inhibition of gene expression. The clusters of gene with related functions are called operons. They usually transcribe single mRNA molecules. In E.coli, nearly 260 genes are grouped into 75 different operons.

Structure of the operon:

- Each operon is a unit of gene expression and regulation and consistsof one or more structural genes and an adjacent operator gene that controls transcriptional activity of the structural gene.

- The structural gene codes for proteins, rRNA and tRNA required by the cell.

- Promoters are the signal sequences in DNA that initiate RNA synthesis. RNA polymerase binds to the promoter prior to the initiation of transcription.

- The operators are present between the promoters and structural genes. Therepressor protein binds to the operator region of the operon.

The Lac (Lactose) operon:

- The metabolism of lactose in E.coli requires three enzymes – permease, β-galactosidase (β-gal) and transacetylase. The enzyme permease is needed for entry of lactose into the cell, β-galactosidase brings about hydrolysis of lactose to glucose and galactose, while transacetylase transfers acetyl group from acetyl Co A to β-galactosidase.

- The lac operon consists of one regulator gene ('i' gene refers to inhibitor) promoter sites (p), and operator site (o). Besides these, it has three structural genes namely lac z,y and lac a. The lac 'z' gene codes for β-galactosidase, lac 'y' gene codes for permease and 'a' gene codes for transacetylase. Jacob and Monod proposed the classical model of Lac operon to explain gene expression andregulation in E.coli. In lac operon, a polycistronic structural gene is regulated by a common promoter and regulatory gene. When the cell is using its normal energy source as glucose, the 'i' gene transcribes a repressor mRNA and after its translation, a repressor protein is produced.

- It binds to the operator region of the operon and prevents translation, as a result, β-galactosidase is not produced. In the absence of preferred carbon source such as glucose, if lactose is available as an energy source for the bacteria then lactose enters the cell as a result of permease enzyme. Lactose acts as an inducer and interacts with the repressor to inactivate it.

- The repressor protein binds to the operator of the operon and prevents RNA polymerasefrom transcribing the operon. In the presence of inducer, such as lactose or allolactose, the repressor is inactivated by interaction with the inducer. This allows RNA polymerase to bind to the promotor site and transcribe the operon to produce lac mRNA which enables formation of all the required enzymes needed for lactose metabolism. This regulation of lac operon by the repressor is an example of negative control of transcription initiation. Lacoperon is also under the control of positive regulation as well.

Human GenomeProject (HGP)

- The international human genome project was launched in the year 1990. It was a mega project and took 13 years to complete. The human genome is about 25 times larger than the genome of any organism sequenced to date and is the first vertebrate genome to be completed. Human genome is said to have approximately 3×109 bp. HGP was closely associated with the rapid development of a new area in biology called bioinformatics.

Goals and methodologies of Human Genome Project

The main goals of Human Genome Project are as follows

- Identify all the genes (approximately 30000) in human DNA.
- Determine the sequence of the three billion chemical base pairs that makeup the human DNA.
- To store this information in databases.
- Improve tools for data analysis.
- Transfer related technologies to other sectors, such as industries.
- Address the ethical, legal and social issues (ELSI) that may arise from the project.

- The methodologies of the Human Genome Project involved two major approaches. One approach was focused on identifying all the genes that are expressed as RNA (ETSS – Expressed Sequence Tags). The other approach was sequence annotation. Here, sequencing the whole set of genome was taken, that contains all the coding and non-coding sequences and later assigning different regions in the sequences with functions. For sequencing, the total DNA froma cell is isolated and converted into random fragments of relatively smaller sizes and cloned in suitable hosts using specialized vectors. This cloning results in amplification of pieces of DNA fragments so that it could subsequently be sequenced with ease. Bacteria and yeast are two commonly used hosts and these vectors are called as BAC (Bacterial Artificial Chromosomes) and YAC (Yeast Artificial Chromosomes). The fragments are sequenced using automated DNA sequencers (developed by Frederick Sanger). The sequences are then arranged based on few overlapping regions, using specialized computer based programs. These sequences were subsequently annotated and are assigned to each chromosome. The genetic and physical maps on the genome are assigned using information on polymorphism of restriction endonuclease recognition sites and some repetitive DNA sequences, called microsatellites. The latest method of sequencing even longer fragments is by a method called Shotgun sequencing using super computers, which has replaced the traditional sequencing methods.

Salient features of Human Genome Project:

- Although human genome contains 3 billion nucleotide bases, the DNA sequences that encode proteins make up only about 5% of the genome.

- An average gene consists of 3000 bases, the largest known human gene being dystrophin with 2.4 million bases.

- The function of 50% of the genome is derived from transposable elements such as LINE and ALU sequence.

- Genes are distributed over 24 chromosomes. Chromosome 19 has the highest gene density. Chromosome 13 and Y chromosome have lowest gene densities

- The chromosomal organization of humangenes shows diversity.
- There may be 35000-40000 genes in thegenome and almost 99.9 nucleotide basesare exactly the same in all people.
- Functions for over 50 percent of thediscovered genes are unknown.
- Less than 2 percent of the genome codesfor proteins.
- Repeated sequences make up very largeportion of the human genome. Repetitivesequences have no direct coding functionsbut they shed light on chromosomestructure, dynamics and evolution(genetic diversity).
- Chromosome 1 has 2968 genes whereaschromosome 'Y' has 231 genes.
- Scientists have identified about 1.4million locations where single baseDNA differences (SNPs – Singlenucleotidepolymorphism – pronounce as'snips') occur in humans. Identification of'SNIPS' is helpful in finding chromosomallocations for disease associated sequencesand tracing human history

Applications and future challenges

- The mapping of human chromosomes is possible to examine a person's DNA and to identify genetic abnormalities. This is extremely useful in diagnosing diseases and to provide genetic counselling to those planning to have children. This kind of information would also create possibilities for new gene therapies. Besides providing clues to understand human biology, learning about non-human organisms, DNA sequences can lead to an understanding of their natural capabilities that can be applied towards solving challenges in healthcare, agriculture, energy production and environmental remediation. A new era of molecular medicine,characterized by looking into the most fundamental causes of disease than treating the symptoms will be an important advantage.
- Once genetic sequence becomes easierto determine, some people may attemptto use this information for profit or forpolitical power.
- Insurance companies may refuse to insurepeople at 'genetic risk' and this would savethe companies the expense of future medical bills incurred by 'less than perfect' people.
- Another fear is that attempts are beingmade to "breed out" certain genes ofpeople from the human population inorder to create a 'perfect race'.

Pharmacogenomics is the study of how genes affect a person's response to drugs. This relatively new field combines pharmacology (the science of drugs) and genomics (the study of genes and their functions) to develop effective, safe medications and doses that will be tailored to a person's genetic makeup.

DNA fingerprinting technique

- The DNA fingerprinting technique was first developed by Alec Jeffreys in 1985 (Recipient of the Royal Society's Copley Medal in 2014). Each of us have the same chemical structure of DNA. But there are millions of differences in the DNA sequence of base pairs. This makes the uniqueness among us so that each of us except identical twins is different from each other genetically. The DNA of a person and finger prints are unique. There are 23 pairs of human chromosomes with 1.5 million pairs of genes. It is a well known fact that genes are segments of DNA which differ in the sequence of their nucleotides. Not all segments of DNA code for proteins, some DNA segments have a regulatory function, while others are intervening sequences (introns) and still others are repeated DNA sequences. In DNA fingerprinting, short repetitive nucleotide sequences are specific for a person. These nucleotide sequences are called as variable number tandem repeats (VNTR).The VNTRs of two persons generally show variations and are useful as genetic markers.

- DNA finger printing involves identifying differences in some specific regions in DNA sequence called repetitive DNA, because in these sequences, a small stretch of DNA is repeated many times. These repetitive DNA are separated from bulk genomic DNA as different peaks during density gradient centrifugation. The bulk DNA forms a major peak and the other small peaks are referred to as satellite DNA. Depending on base composition (A : T rich or G : C rich), length of segment and number of repetitive units, the satellite DNA is classified into many sub categories such as micro-satellites, mini-satellites, etc., These sequences do not code for any proteins, but they form a large portion of human genome. These sequences show high degree of polymorphism and form the basis of DNA fingerprinting (Fig. 5.15). DNA isolated from blood, hair, skin cells, or other genetic evidences left at the scene of a crime can be compared through VNTR patterns, with the DNA of a criminal suspect to determine guilt or innocence. VNTR patterns are also useful in establishing the identity of a homicide victim, either from DNA found as evidence or from the body itself.

The Steps in DNA Fingerprinting technique

Extraction of DNA

- The process of DNA fingerprinting starts with obtaining a sample of DNA from blood, semen, vaginal fluids, hair roots, teeth, bones, etc.,

Polymerase chain reaction (PCR)

- In many situations, there is only a small amount of DNA available for DNA fingerprinting. If needed many copies of the DNA can be produced by PCR (DNA amplification).

Fragmenting DNA

- DNA is treated with restriction enzymes which cut the DNA into smaller fragments at specific sites.

Separation of DNA by electrophoresis

- During electrophoresis in an agarose gel, the DNA fragments are separated into bands of different sizes. The bands of separated DNA are sieved out of the gel using a nylon membrane (treated with chemicals that allow for it to break the hydrogen bonds of DNA so there are single strands).

Denaturing DNA

The DNA on gels is denatured by using alkaline chemicals or by heating.

Blotting

- The DNA band pattern in the gel istransferred to a thin nylon membraneplaced over the 'size fractionated DNAstrand' by Southern blotting.

Using probes to identify specific DNA

- A radioactive probe (DNA labeled witha radioactive substance) is added to theDNA bands. The probe attaches by basepairing to those restriction fragments thatare complementary to its sequence. Theprobes can also be prepared by using either'fluorescent substance' or 'radioactiveisotopes'.

Hybridization with probe

- After the probe hybridizes and the excessprobe washed off, a photographic filmis placed on the membrane containing'DNA hybrids'.

Exposure on film to make a genetic/DNA Fingerprint

- The radioactive label exposes the filmto form an image (image of bands)corresponding to specific DNA bands. The thick and thin dark bands form a pattern of bars which constitutes a genetic fingerprint.

Application of DNAfinger printing

- **Forensic analysis** - It can be used in theidentification of a person involved incriminal activities, for settling paternityor maternity disputes, and in determiningrelationships for immigration purposes.

- **Pedigree analysis –** inheritance patternof genes through generations and fordetecting inherited diseases.

- **Conservation of wild life –** protection ofendangered species. By maintaining DNArecords for identification of tissues of thedead endangered organisms.

- **Anthropological studies–**It is useful indetermining the origin and migration of human populations and genetic diversities.

UNIT – 6- Evolution:

Origin of life – Evolution of life forms

- Theory of special creation states that life was created by a supernatural power, respectfully referred to as "God". According to Hinduism, Lord Brahma created the Earth. Christianity, Islam and most religions believe that God created the universe, the plants and the animals.

- According to the theory of spontaneous generation or Abiogenesis, living organisms originated from non-living materials and occurred through stepwise chemical and molecular evolution over millions of years. Thomas Huxley coined the term abiogenesis.

- Big bang theory explains the origin of universe as a singular huge explosion in physical terms. The primitive earth had no proper atmosphere, but consisted of ammonia, methane, hydrogen and water vapour. The climate of the earth was extremely high. UV rays from the sun split up water molecules into hydrogen and oxygen. Gradually the temperature cooled and the water vapour condensed to form rain. Rain water filled all the depressions to form water bodies. Ammonia and methane in the atmosphere combined with oxygen to form carbon-dioxide and other gases.

Coacervates (large colloidal particles that precipitate out in aqueous medium) are the first pre-cells which gradually transformed into living cells.

- According to the theory of biogenesis life arose from pre-existing life. The term biogenesis also refers to the biochemical process of production of living organisms This term was coined by Henry Bastian.

- According to the theory of chemical evolution primitive organisms in the primordial environment of the earth evolved spontaneously from inorganic substances andphysical forces such, as lightning, UV radiations, volcanic activities, etc.,., Oparin (1924) suggested that the organic compounds could have undergone a series of reactions leading to more complex molecules. He proposed that the molecules formed colloidal aggregates or 'coacervates' in an aqueous environment. The coacervates were able to absorb and assimilate organic compounds from the environment. Haldane (1929) proposed that the primordial sea served as a vast chemical laboratory powered by solar energy. The atmosphere was oxygen free and the combination of CO2, NH3 and UV radiations gave rise to organic compounds. The sea became a 'hot' dilute soup containing large populations of organic monomers and polymers. They envisaged that groups of monomers and polymers acquired lipid membranes and further developed into the first living cell. Haldane coined the term prebiotic soup and this became the powerful symbol of the Oparin-Haldane view on the origin of life (1924-1929).

- Oparin and Haldane independently suggested that if the primitive atmosphere was reducing and if there was appropriate supply of energy such as lightning or UV light then a wide range of organic compounds can be synthesized.

Evidences for biological evolution
Paleontological evidences

- Paleontology is the study of prehistoric life through fossils. Fossils are described as the true witnesses of evolution or documents of various geological strata of evolution. Fossilization is the process by which plant and animal remains are preserved in sedimentary rocks. They fall under three main categories.

Actual remains :

- The original hard parts such as bones, teeth or shells are preserved as such in the earth's atmosphere. This is the most common method of fossilization. When marine animals die, their hard parts such as bones, shells, etc., are covered with sediments and are protected from further deterioration. They get preserved as such as they are preserved in vast ocean; the salinity in them prevents decay. The sediments become hardened to form definite layers or strata. For example, Woolly Mammoth that lived 22 thousand years ago were preserved in the frozen coast of Siberia as such. Several human beings and animals living in the ancient city of Pompeii were preserved intact by volcanic ash which gushed out from Mount Vesuvius.

Petrifaction

- When animals die the original portion of their body may be replaced molecule for molecule by minerals and the original substance being lost through disintegration. This method of fossilization is called petrifaction. The principle minerals involved in this type fossilization are iron pyrites, silica, calcium carbonate and bicarbonates of calcium and magnesium.

Natural moulds and casts

- Even after disintegration, the body of an animal might leave indelible impression on the soft mud which later becomes hardened into stones. Such impressions are called moulds. The cavities of the moulds may get filled up by hard minerals and get fossilized, which are called casts. Hardened faecal matter termed as coprolites occur as tiny pellets. Analysis of the coprolites enables us to understand the nature of diet the pre-historic animals thrived on.

Embryological evidences

- Embryology deals with the study of the development of individual from the egg to the adult stage. A detailed study of the embryonic development of different forms makes us to think that there is a close resemblance during development.

- The development of heart in all vertebrates follows the same pattern of development as a pair of tubular structures that later develop into two chambered heart in fishes, three chambered in amphibians and in most reptiles and four chambered in crocodiles, birds and mammals; indicating a common ancestry for all the vertebrates,

- Hence scientists in the 19th century concluded that higher animals during their embryonic development pass through stages of lower animals (ancestors). Ernst Von Haeckel, propounded the "biogenetic law ortheory of recapitulation" which states that the life history of an individual (ontogeny) briefly repeats or recapitulates the evolutionary history of the race (phylogeny). In other words "Ontogeny recapitulates Phylogeny". The embryonic stages of a higher animal resemble the adult stage of its ancestors. Appearance of pharyngeal gill slits, yolk sac and the appearance of tail in human embryos are some of the examples. The biogenetic law is not universal and it is now thought that animals do not recapitulate the adult stage of any ancestors. The human embryo recapitulates the embryonic history and not the adult history of the organisms.

- The comparative study of the embryo of different animals shows structural similarities among themselves. The embryos of fish, salamander, tortoise, chick and human start life as a single cell, the zygote, and undergo cleavage to produce the blastula, change to gastrula and are triploblastic. This indicates that all the above said animals have evolved from a common ancestor.

Molecular evidences

- Molecular evolution is the process of change in the sequence composition of molecules such as DNA, RNA and proteins across generations. It uses principles of evolutionary biology and population genetics to explain patterns in the changes of molecules.

- One of the most useful advancement in the development of molecular biology is proteins and other molecules that control life processes are conserved among species. A slight change that occurs over time in these conserved molecules (DNA, RNA and protein) are often called molecular clocks. Molecules that have been used to study evolution are cytochrome c (respiratory pathway) and rRNA (protein synthesis).

Theories of biological evolution
Lamarck's theory

- Jean Baptiste de Lamarck, was the first to postulate the theory of evolution in his famous book 'Philosophie Zoologique' in the year 1809. The two principles of Lamarckian theory are:

- **The theory of use and disuse–** Organsthat are used often will increase in size and those that are not used will degenerate. Neck in giraffe is an example of use and absence of limbs in snakes is an example for disuse theory.

- **The theory of inheritance of acquired characters** - Characters that are developed during the life time of an organism are called acquired characters and these are then inherited.

The main objection to Lamarckism

- Lamarck's "Theory of Acquired characters" was disproved by August Weismann who conducted experiments on mice for twenty generations by cutting their tails and breeding them. All mice born were with tail. Weismann proved that change in the somatoplasm will not be transferred to the next generation but changes in the germplasm will be inherited.

Neo-Lamarckism

- The followers of Lamarck (Neo-Lamarckists) like Cope, Osborn, Packard and Spencer tried to explain Lamarck's theory on a more scientific basis. They considered that adaptations are universal. Organisms acquire new structures due to their adaptations to the changed environmental conditions. They argued that external conditions stimulate the somatic cells to produce certain 'secretions' which reach the sex cells through the blood and bring about variations in the offspring.

Darwin's theory of Natural Selection

- Charles Darwin explained the theory of evolution in his book 'The Origin of Species by Natural Selection'. During his journey around the Earth, he made extensive observations of plants and animals. He noted a huge variety and remarkable similarities among organisms and their adaptive features to cope up to their environment. He proved that fittest organisms can survive and leave more progenies than the unfit ones through natural selection.

Darwin's theory was based on several facts, observations and influences. They are:

Over production (or) prodigality of production

- All living organisms increase their population in larger number. For example, Salmon fish produces about 28 million eggs during breeding season and if all of them hatch, the seas would be filled with salmon in few generations. Elephant, the slowest breeder that can produce six young ones in its life time can produce 6 million descendants at the end of 750 years in the absence of any check.

Struggle for existence

- Organisms struggle for food, space and mate. As these become a limiting factor, competition exists among the members of the population. Darwin denoted struggle for existence in three ways –

- Intra specific struggle between the same species for food, space and mate.

- Inter specific struggle with different species for food and space.
- Struggle with the environment to cope with the climatic variations, flood, earthquakes, drought, etc.,

Universal occurrence of variations

- No two individuals are alike. There are variations even in identical twins. Even the children born of the same parents differ in colour, height, behavior, etc., The useful variationsfound in an organism help them to overcome struggle and such variations are passed on to the next generation.

Origin of species by Natural Selection

- According to Darwin, nature is the most powerful selective force. He compared origin of species by natural selection to a small isolated group. Darwin believed that the struggle for existence resulted in the survival of the fittest. Such organisms become better adapted to the changed environment.

Objections to Darwinism

Some objections raised against Darwinism were –

- Darwin failed to explain the mechanism of variation.
- Darwinism explains the survival of the fittest but not the arrival of the fittest.
- He focused on small fluctuating variations that are mostly non-heritable.
- He did not distinguish between somatic and germinal variations.
- He could not explain the occurrence of vestigial organs, over specialization of some organs like large tusks in extinct mammoths, oversized antlers in the extinct Irish deer, etc.,

Neo Darwinism

- Neo Darwinism is the interpretation of Darwinian evolution through Natural Selection as it has been modified since it was proposed. New facts and discoveries about evolution have led to modifications of Darwinism and is supported by Wallace, Heinrich, Haeckel, Weismann and Mendel. This theory emphasizes the change in the frequency of genes in population arises due to mutation, variation, isolation and Natural selection.

Mutation theory

- Hugo de Vries put forth the Mutation theory. Mutations are sudden random changes that occur in an organism that is not heritable. De Vries carried out his experiments in the

Evening Primrose plant (Oenothera lamarckiana) and observed variations in them due to mutation.

- According to de Vries, sudden and large variations were responsible for the origin of new species whereas Lamarck and Darwin believed in gradual accumulation of all variations as the causative factors in the origin of new species.

Salient features of Mutation Theory

- Mutations or discontinuous variation are transmitted to other generations.
- In naturally breeding populations, mutations occur from time to time.
- There are no intermediate forms, as they are fully fledged.
- They are strictly subjected to natural selection.

Modern synthetic theory

- Sewell Wright, Fisher, Mayer, Huxley, Dobzhansky, Simpson and Haeckel explained Natural Selection in the light of Post-Darwinian discoveries. According to this theory gene mutations, chromosomal mutations, genetic recombinations, natural selection and reproductive isolation are the five basic factors involved in the process of organic evolution.

- **Gene mutation** refers to the changes in the structure of the gene. It is also called gene/ point mutation. It alters the phenotype of an organism and produces variations in their offspring.

- **Chromosomal mutation** refers to the changes in the structure of chromosomes due to deletion, addition, duplication, inversion or translocation. This too alters the phenotype of an organism and produces variations in their offspring.

- **Genetic recombination** is due to crossing over of genes during meiosis. This brings about genetic variations in the individuals of the same species and leads to heritable variations.

- **Natural selection** does not produce any genetic variations but once such variations occur it favours some genetic changes while rejecting others (driving force of evolution).

- **Reproductive isolation** helps in preventing interbreeding between related organisms.

Evolution by anthropogenic sources
Natural Selection (Industrial melanism)

- Natural selection can be explained clearly through industrial melanism. Industrial melanism is a classical case of Natural selection exhibited by the peppered moth, Bistonbetularia. These were available in two colours, white and black. Before industrialization peppered moth both white and black coloured were common in England. Pre-industrialization witnessed white coloured background of the wall of the buildings hence the white coloured moths escaped from their predators. Post industrialization, the tree trunks became dark due to smoke and soot let out from the industries. The black moths camouflaged on the dark bark of the trees and the white moths were easily identified by their predators. Hence the dark coloured moth population was selected and their number increased when compared to the white moths. Nature offered positive selection pressure to the black coloured moths. The above proof shows that in a population, organisms that can adapt will survive and produce more progenies resulting in increase in population through natural selection.

- Artificial selection is a byproduct of human exploitation of forests, oceans and fisheries or the use of pesticides, herbicides or drugs. For hundreds of years humans have selected various types of dogs, all of which are variants of the single species of dog. If human beings can produce new varieties in short period, then "nature" with its vast resources and long duration can easily produce new species by selection.

Adaptive Radiation

- The evolutionary process which produces new species diverged from a single ancestral form becomes adapted to newly invaded habitats is called adaptive radiation. Adaptive radiations are best exemplified in closely related groups that have evolved in relatively short time. Darwin's finches and Australian marsupials are best examples for adaptive radiation. When more than one adaptive radiation occurs in an isolated geographical area, having the same structural and functional similarity it is due to convergent evolution.

Darwin's finches

- Their common ancestor arrived on the Galapagos about 2 million years ago. During that time, Darwin's finches have evolved into 14 recognized species differing in body size, beak shape and feeding behavior. Changes in the size and form of the beak have enabled different species to utilize different food resources such as insects, seeds, nectar from cactus flowers and blood from iguanas, all driven by Natural selection. represents some of the finches observed by Darwin. Genetic variation in the ALX1 gene in the DNA of Darwin finches is associated with variation in the beak shape. Mild mutation in the ALX1 gene leads to phenotypic change in the shape of the beak of the Darwin finches.

- Marsupials in Australia and placental mammals in North America are two subclasses of mammals they have adapted in similar way to a particular food resource, locomotory skill or climate. They were separated from the common ancestor more than 100 million year ago and each lineage continued to evolve independently. Despite temporal and geographical separation, marsupials in Australia and placental mammals in North

America have produced varieties of species living in similar habitats with similar ways of life. Their overall resemblance in shape, locomotory mode, feeding and foraging are superimposed upon different modes of reproduction. This feature reflects their distinctive evolutionary relationships.

- Over 200 species of marsupials live in Australia along with many fewer species of placental mammals. The marsupials have undergone adaptive radiation to occupy the diverse habitats in Australia, just as the placental mammals have radiated across North America.

Mechanism of evolution

- Microevolution (evolution on a small scale) refers to the changes in allele frequencies within a population. Allele frequencies in a population may change due to four fundamental forces of evolution such as natural selection, genetic drift, mutation and gene flow.

Natural selection

- It occurs when one allele (or combination of alleles of differences) makes an organism more or less fit to survive and reproduce in a given environment. If an allele reduces fitness, its frequencies tend to drop from one generation to the next.

- The evolutionary path of a given gene i.e., how its allele's change in frequency in the population across generation, may result from several evolutionary mechanisms acting at once. For example, one gene's allele frequencies might be modified by both gene flow and genetic drift, for another gene, mutation may produce a new allele, that is favoured by natural selection.

Selection

- There are mainly three types of natural selection

Stabilising Selection (centipetal selection):

- This type of selection operates in a stable environment. The organisms with average phenotypes survive whereas the extreme individuals from both the ends are eliminated. There is no speciation but the phenotypic stability is maintained within the population over generation. For example, measurements of sparrows that survived the storm clustered around the mean, and the sparrows that failed to survive the storm clustered around the extremes of the variation showing stabilizing selection.

Directional Selection:

- The environment which undergoes gradual change is subjected to directional selection. This type of selection removes the individuals from one end towards the other end of phenotypic distribution. For example, size differences between male and female sparrows. Both male and female look alike externally but differ in body weight. Females show directional selection in relation to body weight.

Disruptive Selection (centrifugal selection):

- When homogenous environment changes into heterogenous environment this type of selection is operational. The organisms of both the extreme phenotypes are selected whereas individuals with average phenotype are eliminated. This results in splitting of the population into sub population/species. This is a rare form of selection but leads to formation of two or more different species. It is also called adaptive radiation. E.g. Darwin's finches-beak size in relation to seed size inhabiting Galapagos islands.

- Group selection and sexual selection are other types of selection. The two major group selections are Altrusim and Kin selection.

Gene flow

- Movement of genes through gametes or movement of individuals in (immigration) and out (emigration) of a population is referred to as gene flow. Organisms and gametes that enter the population may have new alleles or may bring in existing alleles but in different proportions than those already in the population. Gene flow can be a strong agent of evolution.

Genetic drift / Sewall Wright Effect

- Genetic drift is a mechanism of evolution in which allele frequencies of a population change over generation due to chance (sampling error). Genetic drift occurs in all population sizes, but its effects are strong in a small population . It may result in a loss of some alleles (including beneficial ones) and fixation of other alleles. Genetic drift can have major effects, when the population is reduced in size by natural disaster due to bottle neck effect or when a small group of population splits from the main population to form a new colony due to founder's effect.

Origin and Evolution of Man

- Mammals evolved in the early Jurassic period, about 210 million years ago. Hominid evolution occurred in Asia and Africa. Hominids proved that human beings are superior to other animals and efficient in making tools and culture. The earliest fossils of the prehistoric man like Ramapithecus and Sivapithecus lived some 14 mya and were derived from ape like Dryopithecus. Dryopithecus and Ramapithecus were hairy and walked like

gorillas and chimpanzees. Australopithecus lived in East African grasslands about 5 mya and was called the Australian ape man. He was about 1.5 meters tall with bipedal locomotion, omnivorous, semi erect, and lived in caves. Low forehead, brow ridges over the eyes, protruding face, lack of chin, low brain capacity of about 350 – 450 cc, human like dentition, lumbar curve in the vertebral column were his distinguishing features. Homo habilis lived about 2 mya. Their brain capacity was between 650 – 800cc, and was probably vegetarian. They had bipedal locomotion and used tools made of chipped stones.

- Homo erectus the first human like being was around 1.7 mya and was much closer to human in looks, skull was flatter and thicker than the modern man and had a large brain capacity of around 900 cc. Homo erectus probably ate meat.

- Homo ergaster and Homo erectus were the first to leave Africa. Neanderthal human was found in Neander Valley, Germany with a brain size of 1400 cc and lived between 34,000 - 1,00,000 years ago. They differ from the modern human in having semierect posture, flat cranium, sloping forehead, thin large orbits, heavy brow ridges, protruding jaws and no chin. They used animal hides to protect their bodies, knew the use of fire and buried their dead. They did not practice agriculture and animal domestication. Cro-Magnon was one of the most talked forms of modern human found from the rocks of Cro-Magnon, France and is considered as the ancestor of modern Europeans. They were not only adapted to various environmental conditions, but were also known for their cave paintings, figures on floors and walls.

- Homo sapiens or modern human arose in Africa some 25,000 years ago and moved to other continents and developed into distinct races. They had a brain capacity of 1300 – 1600 cc. They started cultivating crops and domesticating animals.

Isolating Mechanism

- Isolation is the separation of the members of a single population into sub populations so that genetic integrity of the subpopulation can be maintained. Closely related species living in the same area do not breed together; they are prevented by isolating barriers. An isolating barrier is any evolved character of the two species that stops them from interbreeding. Several kinds of isolating barriers are distinguished. The most important distinction is Prezygotic and post zygotic isolation. Prezygotic mechanisms include those which prevent two species from coming into contact. This includes ecological, seasonal, ethological and morphological. Post zygotic mechanisms are those which act after fertilization that include hybrid sterility, hybrid inviability and hybrid breakdown.

- **Ecological isolation or habitat isolation** – the members of the same population may be separated from one another by a differences in their habitat. For example Rana areolata occupies burrows dug by mammals and tortoises during the day and breeds in grassy shallow ponds whereas Rana grylio breeds in deep waters. Due to the difference in their habitat the two species are able to maintain their respective species identities.

- **Seasonal isolation** – In this type ofisolation, difference in the breedingseasons prevents interbreeding. E.g.Toad, Bufo americanus breeds muchearly in the spring; whereas Bufofowleri breeds very late in the season.They are able to maintain their speciesidentity because of the differences in thebreeding seasons

- **Sexual or ethological isolation/Behavioural isolation** – Preventsmating due to the difference in theirsexual behavior. The species are notseparated from one another either intime or in space. The mating calls oftwo closely related species of frogs,Hyla versicolor (grey tree frog) andHylafemoralis (pine wood tree frog) aredifferent which prevents interbreeding.

- **Morphological isolation or mechanical isolation** – This type of isolation isdue to the differences in their externalgenitalia that is seen in two differentspecies. The size difference between thetoad species Bufo quercicus and Bufovalliceps, prevents their interbreeding.

- **Physiological isolation –**Thoughmating may occur, the gametes areprevented from fertilization due tomechanical or physiological factors.E.g. The sperms of Drosophila virilissurvive only for about a day whenintroduced into the sperm receptacle ofDrosophila American a while the spermsof Drosophila American a live for a longertime.

- **Cytological isolation** – Fertilizationdoes not take place due to thedifferences in the chromosome numbersbetween the two species, the bull frogRana catesbiana and gopher frog Ranaareolata

- **Hybrid inviability** – In this type, thesperm enters the egg, fertilization occursand the embryo develops into the adultbut it dies before reaching maturity.In certain fishes, frogs, beetles, even iffertilization takes place between twospecies, due to genetic incompatibilitythey do not leave any surviving offspring.

- **Hybrid sterility** – In this type,hybrids are formed due to inter specificcrosses but they are sterile due to thefailure of the chromosomes to segregatenormally during meiosis, example Mule(inter specific cross between a horse anda donkey).

- **Hybrid breakdown** – F1 Hybrids areviable and fertile, but F2 hybrids may beinviable or sterile.

Speciation

- The process by which one species evolves into one or more different species is called speciation. A.E. Emerson defines species as a 'genetically distinctive, reproductively isolated natural population'. Speciation is a fundamental process in evolution. Evolution of a new species in a single lineage is called an agenesis / phyletic speciation. If one species diverges to become two or more species it is cladogenesisor divergent evolution.

Sympatric speciation/Reproductive isolation

- It is a mode of speciation through which new species form from a single ancestral species while both species continue to inhabit the same geographical region. Two or more species are involved. New species formed due to genetic modification in the ancestor that is naturally selected can no longer breed with the parent population. Sexual isolation is strongest. Phenotypic plasticity has emerged as potentially important first step in speciation initiated within an isolated population.

Phenotypic plasicity is the ability of single genotype to produce more than one phenotype. When this plasticity is expressed seasonally in planktons, it is referred to as cyclomorphosis.

Allopatric speciation/ Geographical speciation

- It is a mode of speciation that occurs when biological populations of similar species become isolated from each other that prevents gene flow. One species becomes two species due to geographical barriers hence new species is evolved e.g. Darwin's finches. The barriers are land separation, migration or mountain formation. When barriers occur between species, change in ecological conditions and environment leads to adaptations that produce differences. If there are no adaptations, they will not survive. Sexual isolation is weakest.

- A well studied example is the adaptation of Apple maggots that feed on apples in North America. When the apple trees were imported to North America, Apple maggot flies (Rhagoletispomonella) a parasitic insect that normally laid its eggs in the fruit of wild hawthorns until one subset of population began to lay its eggs in the fruit of domesticated apple trees (Malus domestica) that grew in the same area. This small group of apple maggot flies selected a different host species from the rest of the population and its offsprings became accustomed to domesticated apples.

Extinction of Animals
Extinction

- Extinction was common if not inevitable because species could not always adapt to large or rapid environmental changes. Theimpact of extinction can conveniently be considered at three levels.

- **Species extinction** eliminates an entire species, by an environmental event (flood etc.,) or by biological event (disease or non availability of food resource half or more).

- **Mass extinction** eliminates half or more species in a region or ecosystem, as might occur following a volcanic eruption. Five major mass extinction that occurred since the Cambrian period. This mass extinction is often referred to as K-T extinction

- **Global extinction** eliminates most of the species on a large scale or larger taxonomic groups in the continent or the Earth. Snow ball Earth and extinction following elevation in CO2 levels are example. Extinction events opens up new habitats and so can facilitate the radiation of organisms that survived the mass extinction.

UNIT- 10 - Application of Bio Technology

- Geneticengineering involves the manipulation of DNA and naturally occurring processes such as protein synthesis for a wide range of applications including the production of therapeutically important proteins. This also involves extracting a gene from one organism and transferring it to the DNA of another organism, of the same or another species. The DNA produced in this way is referred to as recombinant DNA (rDNA) and this technique asrecombinant DNA technology. All these are part of the broad field biotechnology which can be definedas the applications of scientific and engineering principles to the processing of material by biologicalagents to provide goods and services.

- Biotechnology is an umbrella term that covers various techniques for using the properties of living things to make products or provide services. The term biotechnology was first used before 20th century for such traditional activities as making idli, dosa, dairy products, bread or wine, but none of these would be considered biotechnology in the modern sense.

Gene Therapy

If a person is born with a hereditary disease, can a corrective therapy be given for such disease? Yes, this can be done by a process known as gene therapy. This process involves the transfer of a normal gene into a person's cells that carries one or more mutant alleles. Expression of normal gene in the person results in a functional gene product whose action produces a normal phenotype. Delivery of the normal gene is accomplished by using a vector. The main thrust of gene therapy has been directed at correcting single gene mutations as in cystic fibrosis and haemophilia. At present most genetic diseases have no effective treatment and so gene therapy could offer hope for many people. There are two strategies involved in gene therapy namely; Gene augmentationtherapy which involves insertion of DNA into the genome to replace the missing gene product and Gene inhibition therapy which involves insertion of the anti sense gene which inhibits the expression of the dominant gene The two approaches to achieve gene therapy are somatic cell and germ line gene therapy.

Diffentiation between somatic cell gene therapy and germ line gene therapy

Somatic Cell Gene Therapy	Germ Line Gene Therapy
Therapeutic genes transferred into the somatic cells.	Therapeutic genes transferred into the germ cells.
Introduction of genes into bone marrow cells, blood cells, skin cells etc.,	Genes introduced into eggs and sperms.
Will not be inherited in later generations.	Heritable and passed on to later generations.

The first clinical gene therapy was given in 1990 by French Anderson to a four year oldgirl with adenosine deaminase (ADA) deficiency. ADA deficiency or SCID (Severe combined immunodeficiency) is an autosomal recessive metabolic disorder. It is caused by the

deletion or dysfunction of the gene coding for ADA enzyme. In these patients the nonfunctioning T-Lymphocytes cannot elicit immune responses against invading pathogens.The right approach for SCID treatment would be to give the patient a functioning ADA which breaks down toxic biological products.

In some children ADA deficiency could be cured by bone marrow transplantation, where defective immune cells could be replaced with healthy immune cells from a donor. In some patients it can be treated by enzyme replacement therapy, in which functional ADA is injected into the patient.

During gene therapy the lymphocytes from the blood of the patient are removed and grown in a nutrient culture medium. A healthy and functional human gene, ADA cDNA encoding this enzyme is introduced into the lymphocytes using a retrovirus. The genetically engineered lymphocytes are
subsequently returned to the patient. Since these cells are not immortal, the patient requires periodic infusion of such genetically engineered lymphocytes. The disease could be cured permanently if the gene for ADA isolated from bone marrow cells are introduced into the cells of the early embryonic stages.

- Somatic cell therapy involves the insertion ofa fully functional and expressible gene into atarget somatic cell to correct a genetic diseasepermanently whereas Germline gene therapyinvolves the introduction of DNA into germ cellswhich is passed on to the successive generations.

- Gene therapy involves isolation of a specificgene and making its copies and inserting theminto target cells to make the desired proteins.It is absolutely essential for gene therapists toensure that the gene is harmless to the patientand it is appropriately expressed and that thebody's immune system does not react to theforeign proteins produced by the new genes.

Stem Cell Therapy

- Stem cells are undifferentiated cells found in most of the multi cellular animals. These cells maintain their undifferentiated state even after undergoing numerous mitotic divisions.

- Stem cell research has the potential to revolutionize the future of medicine with the ability to regenerate damaged and diseased organs. Stem cells are capable of self renewal and exhibit 'cellular potency'. Stem cells can differentiate into all types of cells that are derived from any of the three germ layers ectoderm, endoderm and mesoderm.

- In mammals there are two main types of stem cells – embryonic stem cells (ES cells) and adult stem cells. ES cells are pluripotent and can produce the three primary germ layers ectoderm, mesoderm and endoderm. Embryonic stem cells are multipotent stem cells that

can differentiate into a number of types of cells (Fig. 10.5). ES cells are isolated from the epiblast tissue of the inner cell mass of a blastocyst. When stimulated ES can develop into more than 200 cells types of the adult body. ES cells are immortal i.e., they can proliferate in a sterile culture medium and maintain their undifferentiated state.

- Adult stem cells are found in various tissues of children as well as adults. An adult stem cell or somatic stem cell can divide and create another cell similar to it. Most of the adult stem cells are multipotent and can act as a repair system of the body, replenishing adult tissues.The red bone marrow is a rich source of adult stem cells.

- The most important and potential application of human stem cells is the generation of cells and tissues that could be used for cell based therapies. Human stem cells could be used to test new drugs.

Totipotency (Toti-total) is the ability of a single cell to divide and produce all of the differentiated cells in an organism.

Pluripotency (Pluri-several) refers to a stem cell that has the potential to differentiate into any of the three germ layers-ectoderm, endoderm and mesoderm.
Multipotency (multi-Many) refers to the stem cells that can differentiate into various types of cells that are related. For example blood stem cells can differentiate into lymphocytes, monocytes , neutrophils etc.,

Oligopotency (Oligo-Few) refers to stem cells that can differentiate into few cell types. For example lymphoid or myeloid stem cells can differentiate into B and T cells but not RBC.

Unipotency (Uni- Single) refers to the ability of the stem cells to differentiate into only one cell type.

Stem Cell Banks

- Stem cell banking is the extraction, processing and storage of stem cells, so that they may be used for treatment in the future, when required. Amniotic cell bank is a facility that stores stem cells derived from amniotic fluid for future use. Stem cells are stored in banks specifically for use by the individual from whom such cells have been collected and the banking costs are paid. Cord Blood Banking is the extraction of stem cells from the umbilical cord during childbirth. While the umbilical cord and cord blood are the most popular sources of stem cells, the placenta, amniotic sac and amniotic fluid are also rich sources in terms of both quantity and quality.

Molecular Diagnostics

- Early diagnosis of infectious diseases or inherent genetic defects is essential for appropriate treatment. Early detection of the disease is not possible using conventional diagnostic methods like microscopic examinations, serum analysis and urine analysis.These laboratory techniques are indirect and not always specific. Scientists are

continuously searching for specific, sensitive and simple diagnostic techniques for diagnosis of diseases. Recombinant DNA technology, Polymerase Chain Reactions (PCR) and Enzyme Linked Immunosorbent Assay (ELISA) are some of the techniques that are reliable and help in early diagnosis. Presence of pathogens like virus, bacteria, etc., is detected only when the pathogen produces symptoms in the patient. By the time the symptoms appear concentration of pathogen becomes very high in the body. However very low concentration of a bacteria or a virus, even when the symptoms of the disease does not appear, can be detected by amplification of their nucleic acid.

ELISA [Enzyme Linked Immunosorbent Assay]

- ELISA is a biochemical procedure discovered by Eva Engvall and Peter Perlmanin (1971) to detect the presence of specific antibodies or antigens in a sample of serum, urine, etc., It is a very important diagnostic tool to determine if a person is HIV positive or negative. ELISA is a tool for determining serum antibody concentrations (such as the antibodies produced in a person infected by pathogens such as HIV) and also for detecting the presence of specific antigens and hormones such as human chorionic gonadotropins.

- During diagnosis the sample suspected to contain the antigen is immobilized on the surface of an ELISA plate. The antibody specific to this antigen is added and allowed to react with the immobilized antigen. The anti-antibody is linked to an appropriate enzyme like peroxidase. The unreacted anti-antbody is washed away and the substrate of the enzyme (hydrogen peroxidase) is added with certain reagents such as 4-chloronaphthol. The activity of the enzyme yields a coloured product indicating the presence of the antigen. The intensity of the colour is directly proportional to the amount of the antigen. ELISA is highly sensitive and can detect antigens in the range of a nanogram.

- There are four kinds of ELISA namely, Direct ELISA, Indirect ELISA, sandwich ELISA and competitive ELISA. It is a highly sensitive and specific method used for diagnosis. ELISA possesses the added advantages of not requiring radioisotopes or a radiation counting apparatus.

PCR (Polymerase Chain Reaction)

- The polymerase chain reaction (PCR) is an invitro amplification technique used for synthesising multiple identical copies (billions) of DNA of interest. The technique was developed by Kary Mullis (Nobel laureate, 1993) in the year 1983.

- Denaturation, renaturation or primer annealing and synthesis or primer extension, are the three steps involved in PCR. The double stranded DNA of interest is denatured to separate into two individual strands by high temperature . This is called denaturation. Each strand is allowed to hybridize with a primer (renaturation or primer annealing). The primer template is used to synthesize DNA by using Taq – DNA polymerase.

- During denaturation the reaction mixture is heated to 950 C for a short time to denature the target DNA into single strands that will act as a template for DNA synthesis. Annealing is done by rapid cooling of the mixture, allowing the primers to bind to the sequences on each of the two strands flanking the target DNA. During primer extension or synthesis the temperature of the mixture is increased to 750C for a sufficient period of time to allow Taq DNA polymerase to extend each primer by copying the single stranded template. At the end of incubation both single template strands will be made partially double stranded. The new strand of each double stranded DNA extends to a variable distance downstream. These steps are repeated again and again to generate multiple forms of the desired DNA. This process is also called DNA amplification.

- The PCR technique can also be used for amplifications of RNA in which case it is referred to as reverse transcription PCR (RT-PCR). In this process the RNA molecules (mRNA) must be converted to complementary DNA by the enzyme reverse transcriptase. The cDNA then serves as the template for PCR.

PCR In Clinical Diagnosis

- The specificity and sensitivity of PCR is useful for the diagnosis of inherited disorders (genetic diseases), viral diseases, bacterial diseases, etc., The diagnosis and treatment of a particular disease often requires identifying a particular pathogen. Traditional methods of identification involve culturing these organisms from clinical specimens and performing metabolic and other tests to identify them. The concept behind PCR based diagnosis of infectious diseases is simple – if the pathogen is present in a clinical specimen its DNA will be present.

Polymerase chain reaction

- Its DNA has unique sequences that can be detected by PCR, often using the clinical specimen (for example, blood, stool, spinal fluid, or sputum) in the PCR mixture. PCR is also employed in the prenatal diagnosis of inherited diseases by using chorionic villi samples or cells from amniocentesis. Diseases like sickle cell anemia, β-thalassemia and phenylketonuria can be detected by PCR in these samples. cDNA from PCR is a valuable tool for diagnosis and monitoring retroviral infections – e.g., Tuberculosis by Mycobacterium tuberculosis.

- Several virally induced cancers, like cervical cancer caused by Papilloma virus can be detected by PCR. Sex of human beings and live stocks, embryos fertilized invitro can be determined by PCR by using primers and DNA probes specific for sex chromosomes. PCR technique is also used to detect sex-linked disorders in fertilized embryos.

Applications of PCR

- The differences in the genomes of two different organisms can be studied by PCR. PCR is very important in the study of evolutions, more specifically phylogenetics.

- As a technique which can amplify even minute quantities of DNA from any source, like hair, mummified tissues, bones or any fossilized materials.

- PCR technique can also be used in the field of forensic medicine . A single molecule of DNA from blood stains, hair, semen of an individual is adequate for amplification by PCR. The amplified DNA is used to develop DNA fingerprint which is used as an important tool in forensic science.Thus, PCR is very useful for identification of criminals. PCR is also used in amplification of specific DNA segment to be used in gene therapy.

Transgenic Animals

- In early days selective breeding methods were carried out to improve the genetic characteristics of live stock and other domestic animals. With the advent of modern biotechnology it is possible to carry out manipulations at the genetic level to get the desired traits in animals. Transgenesis is the process of introduction of extra (foreign/exogenous) DNA into the genome of the animals to create and maintain stable heritable characters. The foreign DNA that is introduced is called the transgene and the animals that are produced by DNA manipulations are called transgenic animals or the genetically engineered or genetically modified organisms.

The various steps involved in the production of transgenic organisms are

- Identification and separation of desired gene.

- Selection of a vector (generally a virus) or direct transmission.

- Combining the desired gene with the vector.

- Introduction of transferred vector into cells, tissues, embryo or mature individual.

- Demonstration of integration and expression of foreign gene in transgenic tissue or animals. Transgenic animals such as mice, rat, rabbit, pig, cow, goat, sheep and fish have been produced.

Uses Of Transgenesis

- Transgenesis is a powerful tool to study gene expression and developmental processes in higher organisms.

- Transgenesis helps in the improvement of genetic characters in animals.Transgenic animals serve as good models for understanding human diseases which help in the investigation of new treatments for diseases.Transgenic models exist for many human diseases such as cancer, Alzheimer's, cystic fibrosis, rheumatoid arthritis and sickle cell anemia.

- Transgenic animals are used to produce proteins which are important for medical and pharmaceutical applications.

- Transgenic mice are used for testing the safety of vaccines.

- Transgenic animals are used for testing toxicity in animals that carry genes which make them sensitive to toxic substances than non-transgenic animals exposed to toxic substances and their effects are studied.

- Transgenesis is important for improving the quality and quantity of milk, meat, eggs and wool production in addition to testing drug resistance.

Biological products and their uses

- A biological product is a substance derived from a living organism and used for the prevention or treatment of disease. These products include antitoxins, bacterial and viral vaccines, blood products and hormone extracts. These products may be produced through biotechnology in a living system, such as a microorganism, plant cell or animal cell, and are often more difficult to characterize than small molecule drugs. Through recombinant DNA technology it is possible to produce these biological products on demand. There are many types of biological products approved for use -they are, therapeutic proteins, monoclonal antibodies and vaccines. Health care and pharmaceutical industries have been revolutionised by biotechnological proteins. Hormones and antibodies are produced commercially, primarily for the medical industry. Recombinant hormones like Insulin, Human growth hormone, Recombinant vaccines and recombinant proteins like human alpha lactalbumin are available today.

- Animals are used as bioreactors to produce desirable proteins. Antibodies are substances that react against the disease causing antigens and these can be produced using transgenic animals as bioreactors. Monoclonal antibodies, which are used to treat cancer, heart disease and transplant rejection are produced by this technology. Natural protein adhesives are non toxic, biodegradable and rarely trigger an immune response, hence could be used to reattach tendons and tissues, fill cavities in teeth, and repair broken bones.

Animal Cloning

- Cloning is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature many organisms produce clones through asexual reproduction.

- Cloning in biotechnology refers to the process of creating copies of organisms or copies of cells or DNA fragments (molecular cloning).

- Dolly was the first mammal (Sheep) clone developed by Ian Wilmut and Campbell in 1997. Dolly, the transgenic clone was developed by the nuclear transfer technique and the phenomenon of totipotency. Totipotency refers to the potential of a cell to develop different cells, tissues, organs and finally an organism.

- The mammary gland udder cells (somatic cells) from a donor sheep (ewe) were isolated and subjected to starvation for 5 days. The udder cells could not undergo normal growth cycle, entered a dormant stage and became totipotent. An ovum (egg cell) was taken from another sheep (ewe) and its nucleus was removed to form an enucleated ovum. The dormant mammary gland cell/udder cell and the enucleated ovum were fused. The outer membrane of the mammary cell was ruptured allowing the ovum to envelope the nucleus. The fused cell was implanted into another ewe which served as a surrogate mother. Five months later dolly was born. Dolly was the first animal to be cloned from a differentiated somatic cell taken from an adult animal without the process of fertilization

Advantages and Disadvantages Of Cloning Animals:

- Offers benefits for clinical trials and medical research. It can help in the production of proteins and drugs in the field of medicine.
- Aids stem cell research.
- Animal cloning could help to save endangered species.
- Animal and human activists see it as a threat to biodiversity saying that this alters evolution which will have an impact on populations and the ecosystem.
- The process is tedious and very expensive.
- It can cause animals to suffer.
- Reports show that animal surrogates were manifesting adverse outcomes and cloned animals were affected with disease and have high mortality rate.
- It might compromise human health through consumption of cloned animal meat.
- Cloned animals age faster than normal animals and are less healthy than the parent organism as discovered in Dolly.
- Cloning can lead to occurrence of genetic disorders in animals.
- More than 90% of cloning attempts fail to produce a viable offspring.

Ian Wilmut and Campbell removed 277 cells from the udder of an adult sheep and fused those cells with 277 unfertilised egg cells from which the nuclear material was removed.

After culturing the resulting embryos for 6 days , they implanted 29 embryos into the surrogate mother's womb and only one Dolly was produced.

A gene 'knock out' is a genetically engineered organism that carries one or more genes in its chromosomes that have been made inoperative.

Ethical Issues

- Biotechnology has given to the society cheap drugs, better friuts and vegetables, pest resistant crops, indigenious cure to diseases and lot of controversy. This is mainly because the major part of the modern biotechnology deals with genetic manipulations. People fear that these genetic manipulations may lead to unknown consequences. The major apprehension of recombinant DNA technology is that unique microorganisms either inadvertently or deliberately for the purpose of war may be developed that could cause epidemics or environmentalcatastrophies. Although many are concerned about the possible risk of genetic engineering, the risks are in fact slight and the potential benefits are substantial.

Regulations in Biotechnology

- Regulations apply to the production, sale and use of biotech products and genetically modified organisms. GMOs are carefully tested and documented before the products are available. GMOs should be labelled and used according to instructions. These regulations are designed to protect the people, living organisms and the environment. The Biotechnology Regulatory Authority of India (BRAI) is a proposed regulatory body in India for uses of biotechnology products including GMOs. The Genetic Engineering Approval Committee (GEAC), a body under the Ministry of Environment, forests and climate change (India) is responsible for approval of genetically engineered products in India. If the bill is passed the responsibility will be taken over by the Environmental Appraisal Panel, a subdivision of the BRAI. The bill also proposes setting up an inter ministerial governing body to oversee the performance of BRAI and a National Biotechnology Advisory Council of stakeholders to provide feedback on the use of, import and manufacture of biotechnology products and organisms in the society. The regulatory body is an autonomous and statutory agency to regulate the research, transport, import and manufacture of biotechnology products and organisms.

- GEAC is assisted by the State Biotechnology Co-ordination Committee (SBCC) and District Level committee (DLC). The most important committees are The Institutional Biosafety Committee (IBSC), responsible for the local implementation of guidelines; Review Committee on Genetic Manipulation (RCGM) is responsible for issuing permits and the GEAC is responsible for monitoring the large scale and commercial use of transgenic materials.

- The biotechnology industry is governed by different enactments depending on their relevance / applicability on a case to case basis. "Recombinant DNA safety guidelines, 1990" were released by the Department of Biotechnology (DBT) which cover areas of

research involving genetically engineered organisms and these guidelines were further revised in 1994.

- RCGM under the DBT comprises representatives of DBT, Indian Council for Medical Research, Indian Council for Agricultural research and Council for Scientific and Industrial Research.

Possible threats of Genetically Modified Organisms

- Genetically Modified Organisms (GMOs) also called Genetically Engineered organisms (GEOs) are created to play a role in agriculture, forestry, aquaculture, bioremediation and environmental management in developed and developing countries. However, deliberate or inadvertent release of GMOs into the environment could have negative ecological effects under certain circumstances.

The possible risks of GMOs

- Creating new or more vigorous pests and pathogens. Worsening the effects of existing pests through hybridization with related transgenic organisms.
    - Harming non-target species such as soil organisms, non-pest insects, birds and other animals.
    - Disrupting biotic communities including agro ecosystems.
    - Irreparable loss or changes in species diversity or genetic diversity within species.
    - Creating risks for human health.
- The release of GMOs into the environment could also have far reaching consequences. This is because the living GMOs proliferate, persist,disperse and sometimes may transfer their DNA into other organisms. GEOs could also displace the existing organism and create new species which may cause severe environmental damage. Due to these risks the regulatory authorities are very careful in permitting the field trials of GMOs into the environment.

Biosafety Guidelines

- Due to the growing concerns arising from Genetically Modified Organisms (GMOs) throughout the globe the WHO has built an informal working group on biosafety in 1991. This group prepared the 'voluntary code for the release of organisms into the environment'. ICGEB (International Centre for Genetic Engineering and Biotechnology) has played a significant role in issues related to biosafety and the environmentally sustainable use of biotechnology. The main 'topic of concern' related to the release of GMO's are risks for human health, environment, and agriculture which is found on the website of ICGEB.

- In India, DBT has evolved 'rDNA safety guidelines' to exercise powers conferred through the Environmental Protection Act 1986 for the manufacture, use, import, export and storage of hazardous micro organisms and genetically engineered organisms, cells etc., These guidelines are implemented and monitored by the Institutional Biosafety Committees (IBSCs), the Review Committee on Genetic Manipulation (RCGM) and the Genetic Engineering Approval Committee (GEAC) of the Ministry of Environment and Forest.

Intellectual Property Rights (IPR) and Protection (IPP)

- The physical objects like household goods or land or properties of a person and the ownership and rights on these properties is protected by certain laws operating in the country. This type of physical property is tangible; but the transformed microorganisms, plants, animals and technologies for the production of commercial products are exclusively the property of the intellectuals. The discoverer or inventor has complete rights on his property or invention. The rights of intellectuals are protected by laws framed by a country. The intellectual property is an intangible asset. Legal rights or patents provide an inventor only a temporary monopoly on the use of an invention, in return for disclosing the knowledge to the others who may use the knowledge to develop further inventions and innovations.

- The laws are formulated from time to time at national and international levels. Development of new crop varieties is also an intellectual property right. It is protected by 'plant breeders rights' (PBRs). PBRs recognize the fact that farmers and rural communities have contributed to the creation, conservation, exchange and knowledge of genetic and species utilization of genetic diversity. IPR and IPP are granted by the Government to plant breeders for producing a specific plant variety that is new and never existed before.

- IPR is protected by different ways like patents, copyrights and trade marks.

Patents

- The science of biotechnology involves the production of enormous number of commercial products of economic importance. The inventions include biotechnology products and processes. The products include living entities like micro organisms, animals, plants, cell lines, cell organelles, plasmids and genes and naturally occurring products like primary and secondary metabolites produced by living systems e.g. alcohol, antibiotics.

- The biotechnological processes involve isolation, purification, cultivation, bioconversion of novel, innovative, simple and cost effective processes, and creation of biotechnological products.

- A patent is a Government issued document that allows the person for an exclusive right to manufacture, use or sell aninvention for a defined period (usually 20 years). It is a legal

document safeguarding the rights and privileges of an inventor / invention. The purpose of patenting in biotechnology ensures fair financial returns for those who have invested finances, ideas, time and hard work for an invention.

The following criteria must be satisfied for patenting:

- The invention must be novel and useful;
- The product must be inventive and reproducible;
- The patent application should provide the full description of the invention and the invention must be patentable.

The first living organism that was patented was a genetically engineered species of bacteria - Pseudomonas putida in 1980 which was genetically engineered by Ananda Mohan Chakrabarty in 1971.

12th Botany
Classical Genetics

- Genetics is the study of how living things receive common traits from previous generations. No field of science has changed the world more, in the past 50 years than genetics. The scientific and technological advances in genetics have transformed agriculture, medicine and forensic science etc.

- Genetics – The Science of heredity (Inheritance) - "Genetics" is the branch of biological science which deals with the mechanism of transmission of characters from parents to off springs. Th e term Genetics was introduced by W. Bateson in 1906.

The four major subdisciplines of genetics are

- **Transmission Genetics / Classical Genetics** – Deals with the transmission of genes from parents to off springs. The foundation of classical genetics came from the study of hereditary behaviour of seven genes by Gregor Mendel.

- **Molecular Genetics** – Deals with the structure and function of a gene at molecular level.

- **Population Genetics** – Deals with heredity in groups of individuals for traits which is determined by a few genes.

- **Quantitative Genetics** – Deals with heredity of traits in groups of individuals where the traits are governed by many genes simultaneously.

What is the reason for similarities, diff erences of appearance and skipping of generations? Genes – Functional Units of inheritance: The basic unit of heredity (biological information) which transmits biochemical, anatomical and behavioural traits from parents to off springs.

Heredity and variation

- Genetics is often described as a science which deals with heredity and variation.

- **Heredity:** Heredity is the transmission of characters from parents to offsprings.

- **Variation:** The organisms belonging to the same natural population or species that shows a difference in the characteristics is called variation. Variation is of two types (i) Discontinuous variation and (ii) Continuous variation

1. **Discontinuous Variation:**

   Within a population there are some characteristics which show a limited form of variation. Example: Style length in Primula, plant height of garden pea. In discontinuous variation, the characteristics are controlled by one or two major genes which may have two or more allelic forms. These variations are genetically determined by inheritance

factors. Individuals produced by this variation show differences without any intermediate form between them and there is no overlapping between the two phenotypes. The phenotypic expression is unaffected by environmental conditions. This is also called as qualitative inheritance.

2. **Continuous Variation**:

This variation may be due to the combining effects of environmental and genetic factors. In a population most of the characteristics exhibit a complete gradation, from one extreme to the other without any break. Inheritance of phenotype is determined by the combined effects of many genes, (polygenes) and environmental factors. This is also known as quantitative inheritance. Example: Human height and skin color.

Importance of variations

- Variations make some individuals betterfitted in the struggle for existence.
- They help the individuals to adapt themselves to the changing environment.
- It provides the genetic material for natural selection
- Variations allow breeders to improve better yield, quicker growth, increased resistance and lesser input.
- They constitute the raw materials for evolution.

Mendelism

- The contribution of Mendel to Genetics is called Mendelism. It includes all concepts brought out by Mendel through his original research on plant hybridization. Mendelian genetic concepts are basic to modern genetics. Therefore, Mendel is called as Father of Genetics.

Father of Genetics – Gregor Johann Mendel (1822 – 1884)

- The first Geneticist, Gregor Johann Mendel unraveled the mystery of heredity. He was born on 22nd July 1822 in Heinzendorf Silesia (now Hyncice, Czechoslovakia), Austria. After school education, later he studied botany, physics and mathematics at the University of Vienna.He then entered a monastery of St.Thomas at Brunn in Austria and continued his interest in plant hybridization.In 1849 Mendel got a temporary position in a school as a teacher and he performed a series of elegant experiments with pea plants in his garden. In 1856, he started his historic studies on pea plants. 1856 to 1863 was the period of Mendel's hybridization experiments on pea plants. Mendel discovered the principles of heredity by studying the inheritance of seven pairs of contrasting traits of pea plant in his garden. Mendel crossed and catalogued 24,034plants through many generations. His paper entitled "Experiments on Plant Hybrids" was presented and

published in The Proceedings of the Brunn Society of Natural History in 1866. Mendel was the first systematic researcher in the field of genetics.

Mendel was successful because:

- He applied mathematics and statisticalmethods to biology and laws of probabilityto his breeding experiments.
- He followed scientific methods and keptaccurate and detailed records that includequantitative data of the outcome of hiscrosses.
- His experiments were carefully plannedand he used large samples.
- The pairs of contrasting characters whichwere controlled by factor (genes)werepresent on separate chromosomes.
- The parents selected by Mendel were purebreed lines and the purity was tested by selfcrossing the progeny for many generations.

Mendel's Experimental System – The Garden pea.
He chose pea plant because,

- It is an annual plant and has clearcontrasting characters that are controlledby a single gene separately.
- Self-fertilization occurred under normalconditions in garden pea plants. Mendelused both self-fertilization and cross-fertilization.
- The flowers are large hence emasculationand pollination are very easy forhybridization.

Mendel's experiments on pea plant

- Mendel's theory of inheritance, known as the Particulate theory, establishes the existence of minute particles or hereditary units or factors, which are now called as genes. He performed artificial pollination or cross pollination experiments with several true-breeding lines of pea plants. A true breeding lines (Pure-breeding strains) means it has undergone continuous self pollination having stable trait inheritance from parent to offspring. Matings within pure breeding lines produce offsprings having specific parental traits that are constant in inheritance and expression for many generations. Pure line breed refers to homozygosity only. Fusion of male and female gametes produced by the same individual i.e pollen and egg are derived from the same plant is known as self-fertilization. Self pollination takes place in Mendel's peas. The experimenter can remove the anthers (Emasculation) before fertilization and transfer the pollen from another variety of pea to the stigma of flowers where the anthers are removed. This results in cross-fertilization, which leads to the creation of hybrid varieties with different traits.

Mendel's work on the study of the pattern of inheritance and the principles or laws formulated, now constitute the Mendelian Genetics.

Can you identify Mendel's gene for regulating white colour in peas? Let us find the molecular answer to understand the gene function. Now the genetic mystery of Mendel's white flowers is solved.

It is quite fascinating to trace the Mendel's genes. In 2010, the gene responsible for regulating flower colour in peas were identified by an international team of researchers. It was called Pea Gene A which encodes a protein that functions as a transcription factor which is responsible for the production of anthocyanin pigment. So the flowers are purple. Pea plants with white flowers do not have anthocyanin, even though they have the gene that encodes the enzyme involved in anthocyanin synthesis.

Researchers delivered normal copies of gene A into the cells of the petals of white flowers by the gene gun method. When Gene A entered in a small percentage of cells of white flowers it is expressed in those particular cells, accumulated anthocyanin pigments and became purple.

In white flowers the gene A sequence showed a single-nucleotide change that makes the transcription factor inactive. So the mutant form of gene A do not accumulate anthocyanin and hence they are white.

- Mendel worked at the rules of inheritance and arrived at the correct mechanism before any knowledge of cellular mechanism, DNA, genes, chromosomes became available. Mendel insights and meticulous work into the mechanism of inheritance played an important role which led to the development of improved crop varieties and a revolution in crop hybridization.

- Mendel died in 1884. In 1900 the work of Mendel's experiments were rediscovered by three biologists, Hugo de Vries of Holland, Carl Correns of Germany and Erich von Tschermak of Austria.

Terminology related to Mendelism

- Mendel noticed two different expressions of a trait – Example: Tall and dwarf. Traits are expressed in different ways due to the fact that a gene can exist in alternate forms (versions) for the same trait is called alleles.

- If an individual has two identical alleles of a gene, it is called as homozygous(TT). An individual with two different alleles is called heterozygous(Tt). Mendels non-true breeding plants are heterozygous, called as hybrids

- When the gene has two alleles the dominant allele is symbolized with capital letter and the recessive with small letter. When both alleles are recessive the individual is called homozygous recessive (tt) dwarf pea plants. An individual with two dominant alleles is called homozygous dominant (TT) tall pea plants. One dominant allele and one recessive allele (Tt) denotes non-true breeding tall pea plants heterozygous tall.

Mendelian inheritance – Mendel's Laws of Heredity

- Mendel proposed two rules based on his observations on monohybrid cross, today these rules are called laws of inheritance The first law is The Law of Dominance and the second law is The Law of Segregation. These scientific laws play an important role in the history of evolution.

The Law of Dominance:

- The characters are controlled by discrete units called factors which occur in pairs. In a dissimilar pair of factors one member of the pair is dominant and the other is recessive. This law gives an explanation to the monohybrid cross (a) the expression of only one of the parental characters in F1 generation and (b) the expression of both in the F2 generation. It also explains the proportion of 3:1 obtained at the F2.

The Law of Segregation (Law of Purity of gametes):

- Alleles do not show any blending, both characters are seen as such in the F2 generation although one of the characters is not seen in the F1 generation. During the formation of gametes, the factors or alleles of a pair separate and segregate from each other such that each gamete receives only one of the two factors. A homozygous parent produces similar gametes and a heterozygous parent produces two kinds of gametes each having one allele with equal proportion. Gametes are never hybrid.

Monohybrid cross

- Monohybrid inheritance is the inheritance of a single character i.e. plant height. It involves the inheritance of two alleles of a single gene. When the F1 generation was selfed Mendel noticed that 787 of 1064 F2 plants were tall, while 277 of 1064 were dwarf. The dwarf trait disappeared in the F1 generation only to reappear in the F2 generation. The term genotype is the genetic constitution of an individual. The term phenotype refers to the observable characteristic of an organism. In a genetic cross the genotypes and phenotypes of offspring, resulting from combining gametes during fertilization can be easily understood with the help of a diagram called Punnett's Square named after a British Geneticist Reginald C.Punnett. It is a graphical representation to calculate the probability of all possible genotypes of offsprings in a genetic cross. The Law of Dominance and the Law of Segregation give suitable explanation to Mendel's monohybrid cross.

Reciprocal cross

- In one experiment, the tall pea plants were pollinated with the pollens from a true-breeding dwarf plants, the result was all tall plants. When the parental types were reversed, the pollen from a tall plant was used to pollinate a dwarf pea plant which gave only tall plants. The result was the same - All tall plants. Tall x Dwarf and Tall x Dwarf matings are done in both ways which are called reciprocal crosses.The results of the reciprocal crosses are the same. So it was concluded that the trait is not sex dependent. The results of Mendel's monohybrid crosses were not sex dependent.

- The gene for plant height has two alleles: Tall (T)x Dwarf (t). The phenotypic and genotypicanalysis of the crosses has been shown byChecker board method or by Forkline method.

Mendel's analytical and empirical approach

- Mendel chose two contrasting traits for each character. So it seemed logical that two distinct factors exist. In F1 the recessive trait and its factors do not disappear and they are hidden or masked only to reappear in ¼ of the F2 generation. He concluded that tall and dwarf alleles of F1 heterozygote segregate randomly into gametes. Mendel got 3:1 ratio in F2 between the dominant and recessive trait. He was the first scientist to use this type of quantitative analysis in a biological experiment. Mendel's data is concerned with the proportions of offspring.

- Mendel's analytical approach is truly an outstanding scientific achievement. His meticulous work and precisely executed breeding experiments proposed that discrete particulate units of heredity are present and they are transmitted from one generation to the other. Now they are called as genes. Mendel's experiments were well planned to determine the relationships which govern hereditary traits. This rationale is called an empirical approach. Laws that were arrived from an empirical approach is known as empirical laws.

Test cross

- Test cross is crossing an individual of unknowngenotype with a homozygous recessive.

- In Mendel's monohybrid cross all the plants are tall in F1 generation. In F2 tall and dwarf plants were in the ratio of 3:1.Mendel self pollinated dwarf F2 plants and got dwarf plants in F3 and F4 generations. So he concluded that the genotype of dwarf was homozygous (tt). The genotypes of tall plants TT or Tt from F1and F2 cannot be predicted. But how we can tell if a tall plant is homozygous or heterozygous? To determine the genotype of a tall plant Mendel crossed the plants from F2 with the homozygous recessive dwarf plant. This he called a test cross. The progenies of the test cross can be easily analysed to predict the genotype of the plant or the test organism. Thus in a typical test cross an organism (pea plants) showing dominant phenotype (whose genotype is to be determined) is crossed with the recessive parent instead of self crossing. Test cross is used to identify whether an individual is homozygous or heterozygous for dominant character.

Back Cross

- Back Cross is a cross of F1hybrid with any one of the parental genotypes . The back cross is f two type; they are dominant back cross and recessive back cross.

- It involves the cross between the F1 offspringwith either of the two parents.
- When the F1offsprings are crossed withthe dominant parents all the F2 developdominant character and no recessiveindividuals are obtained in the progeny.
- If the F1 hybrid is crossed with the recessive parent individuals of both the phenotypes appear in equal proportion and this cross is specified as test cross.
- The recessive back cross helps to identify the heterozygosity of the hybrid.

Dihybrid cross

- It is a genetic cross which involves individuals differing in two characters. Dihybrid inheritance is the inheritance of two separate genes each with two alleles.
- **Law of Independent Assortment** – When two pairs of traits are combined in a hybrid, segregation of one pair of characters is independent to the other pair of characters. Genes that are located in different chromosomes assort independently during meiosis. Many possible combinations of factors can occur in the gametes.
- Independent assortment leads to genetic diversity. If an individual produces genetically dissimilar gametes it is the consequence of independent assortment. Through independent assortment, the maternal and paternal members of all pairs were distributed to gametes, so all possible chromosomal combinations were produced leading to genetic variation. In sexually reproducing plants / organisms, due to independent assortment, genetic variation takes place which is important in the process of evolution. The Law of Segregation is concerned with alleles of one gene but the Law of Independent Assortment deals with the relationship between genes.
- The crossing of two plants differing in two pairs of contrasting traits is called dihybrid cross. In dihybrid cross, two characters (colour and shape) are considered at a time. Mendel considered the seed shape (round and wrinkled) and cotyledon colour (yellow & green) as the two characters. In seed shape round (R) is dominant over wrinkled (r) ; in cotyledon colour yellow (Y)is dominant over green (y). Hence the purebreeding round yellow parent is represented bythe genotype RRYY and the pure breeding greenwrinkled parent is represented by the genotyperryy. During gamete formation the paired genesof a character assort out independently of theother pair. During the F1 x F1 fertilization eachzygote with an equal probability receives oneof the four combinations from each parent.The resultant gametes thus will be geneticallydifferent and they are of the following four types:

Yellow round (YR)	9/16
Yellow wrinkled (Yr)	3/16

Green round (yR)	3/16
Green wrinkled (yr)	1/16

- These four types of gametes of F1 dihybridsunite randomly in the process of fertilization and produce sixteen types of individuals in F2 in the ratio of 9:3:3:1 as shown in the figure. Mendel's 9:3:3:1 dihybrid ratio is an ideal ratio based on the probability including segregation, independent assortment and randomfertilization. In sexually reproducing organism / plants from the garden peas to human beings, Mendel's findings laid the foundation for understanding inheritance and revolutionized the field of biology. The dihybrid cross and its result led Mendel to propose a second set of generalisations that we called Mendel's Law of independent assortment.

How does the wrinkled gene make Mendel's peas wrinkled? Find out the molecular explanation.

The protein called starch branching enzyme (SBEI) is encoded by the wild-type allele of the gene (RR) which is dominant. When the seed matures, this enzyme SBEI catalyzes the formation of highly branched starch molecules. Normal gene (R) has become interrupted by the insertion of extra piece of DNA (0.8 kb) into the gene, resulting in r allele. In the homozygous mutant form of the gene (rr) which is recessive, the activity of the enzyme SBEI is lost resulting in wrinkled peas. The wrinkled seed accumulates more sucrose and high water content. Hence the osmotic pressure inside the seed rises. As a result, the seed absorbs more water and when it matures it loses water as it dries. So it becomes wrinkled at maturation. When the seed has atleast one copy of normal dominant gene heterozygous, the dominant allele helps to synthesize starch, amylopectin an insoluble carbohydrate, with the osmotic balance which minimises the loss of water resulting in smooth structured round seed.

Trihybrid cross

- The trihybrid cross demonstrates that Mendel's laws are applicable to the inheritance of multiple traits. Mendel Laws of segregation and independent assortment are also applicable to three pairs of contrasting characteristic traits called trihybrid cross.

- A cross between homozygous parents that differ in three gene pairs (i.e. producing trihybrids) is called trihybrid cross. A self fertilizing trihybrid plant forms 8 different gametes and 64 different zygotes. In this a combination of three single pair crosses operating together.

Extensions of Mendelian Genetics

- Apart from monohybrid, dihybrid and trihybrid crosses, there are exceptions to Mendelian principles, i.e. the occurrence of different phenotypic ratios. The more complex patterns of inheritance are the extensions of Mendelian Genetics. There are examples where phenotype of the organism is the result of the interactions among genes.

- **Gene interaction** – A single phenotype is controlled by more than one set of genes, each of which has two or more alleles. This phenomenon is called Gene Interaction. Many characteristics of the organism including structural and chemical which constitute the phenotype are the result of interaction between two or more genes.

- Mendelian experiments prove that a single gene controls one character. But in the post Mendelian findings, various exception have been noticed, in which different types of interactions are possible between the genes. This gene interaction concept was introduced and explained by W. Bateson. This concept is otherwise known as Factor hypothesis or Bateson's factor hypothesis. According to Bateson's factor hypothesis, the gene interactions can be classified as

    - Intragenic gene interactions or Intra allelic or allelic interactions
    - Intergenic gene interactions or inter allelic or non-allelic interactions.

Intragenic gene interactions

- Interactions take place between the alleles of the same gene i.e., alleles at the same locus is called intragenic or intralocus gene interaction. It includes the following:

    - Incomplete dominance
    - Codominance
    - Multiple alleles
    - Pleiotropic genes are common examples for intragenic interaction.

Incomplete dominance – No blending of genes

- The German Botanist Carl Correns's (1905) Experiment - In 4 O' clock plant, Mirabilis jalapa when the pure breeding homozygous red (R1R1) parent is crossed with homozygous white (R2R2), the phenotype of the F1 hybrid is heterozygous pink (R1R2). The F1 heterozygous phenotype differs from both the parental homozygous phenotype. This cross did not exhibit the character of the dominant parent but an intermediate colour pink. When one allele is not completely dominant to another allele it shows incomplete dominance. Such allelic interaction is known as incomplete dominance. F1 generation produces intermediate phenotype pink coloured flower. When pink coloured plants of F1 generationwere interbred in F2 both phenotypic and genotypic ratios were found to be identical as 1 : 2 : 1(1 red : 2 pink : 1 white). Genotypic ratio is 1 R1R1 : 2 R1R2 : 1 R2R2.From this we conclude that the alleles themselves remain discrete and unaltered proving the Mendel's Law of Segregation. The phenotypic and genotypic ratios are the same. There is no blending of genes. In the F2 generation R1 and R2 genes segregate and recombine to produce red, pink and white in the ratio of 1 : 2 : 1. R1 allele codes for an enzyme responsible for the formation of red pigment. R2 allele codes for defective enzyme. R1 and R2 genotypes produce only enough red pigments to make the flower pink. Two R1R1 are needed for producing red flowers. Two R2R2 genes are needed for white flowers. If blending had taken place, the original pure traits would not have

appeared and all F2 plants would have pink flowers. It is very clear that Mendel's particulate inheritance takes place in this cross which is confirmed by the reappearanceof original phenotype in F2.

How will you explain incomplete dominance at the molecular level?
Gene expression is explained in a quantitative way. Wild-type allele which is a functional allele when present in two copies (R1 R1) produces an functional enzyme which synthesizes red pigments. The mutant allele which is a defective allele in two copies (R2 R2) produces an enzyme which cannot synthesize necessary red pigments. The white flower is due to the mutation causing complete loss of function. The F1 intermediate phenotype heterozygote (R1R2) has one copy of the allele R1. R1 produces 50% of the functional protein resulting in half of the pigment of red flowered plant and so it is pink. The intermediate phenotype pink heterogyzote with 50% of functional protein is not enough to create the red phenotype homozygous, which makes 100% of the functional protein.

Codominance (1 : 2 : 1)

- This pattern occurs due to simultaneous (joint) expression of both alleles in the heterozygote - The phenomenon in which two alleles are both expressed in the heterozygous individual is known as codominance. Example: Red and white flowers of Camellia, inheritance of sickle cell haemoglobin, ABO blood group system in humanbeings. In humanbeings, IA and IB alleles of I gene are codominant which follows Mendels law of segregation. The codominance was demonstrated in plants with the help of electrophoresis or chromatography for protein or flavonoid substance. Example: Gossypium hirsutum and Gossypium sturtianum, their F1 hybrid (amphiploid) was tested for seed proteins by electrophoresis. Both the parents havedifferent banding patterns for their seed proteins. In hybrids, additive banding pattern was noticed. Their hybrid shows the presence of both the types of proteins similar to their parents.

- The heterozygote genotype gives rise to a phenotype distinctly different from either of the homozygous genotypes. The F1 heterozygotes produce a F2 progeny in a phenotypic and genotypic ratios of 1 : 2 : 1.

Lethal genes

- An allele which has the potential to cause the death of an organism is called a "Lethal Allele". In 1907, E. Baur reported a lethal gene in snapdragon (Antirrhinum sp.). It is an example for recessive lethality. In snapdragon there are three kinds of plants.

- Green plants with chlorophyll. (CC)

- Yellowish green plants with carotenoids arereferred to as pale green, golden or aureaplants (Cc)

- White plants without any chlorophyll. (cc)

- The genotype of the homozygous greenplants is CC. The genotype of the homozygous white plant is cc.

- The aurea plants have the genotype Cc because they are heterozygous of green and white plants. When two such aurea plants are crossed the F1 progeny has identical phenotypic and genotypic ratio of 1 : 2 : 1 (viz. 1 Green (CC) : 2 Aurea (Cc) : 1 White (cc))

- Since the white plants lack chlorophyll pigment, they will not survive. So the F2 ratio is modified into 1 : 2. In this case the homozygous recessive genotype (cc) is lethal.

- The term "lethal" is applied to those changes in the genome of an organism which produces effects severe enough to cause death. Lethality is a condition in which the death of certain genotype occurs prematurely. The fully dominant or fully recessive lethal allele kills the carrier individual only in its homozygous condition. So the F2 genotypic ratio will be 2 : 1 or 1 : 2 respectively.

Pleiotropy – A single gene affects multiple traits

- In Pleiotropy, the single gene affects multiple traits and alter the phenotype of the organism. The Pleiotropic gene influences a number of characters simultaneously and such genes are called pleiotropic gene. Mendel noticed pleiotropy while performing breeding experiment with peas (Pisum sativum). Peas with purple flowers, brown seeds and dark spot on the axils of the leaves were crossed with a variety of peas having white flowers, light coloured seeds and no spot on the axils of the leaves, the three traits for flower colour, seed colour and a leaf axil spot all were inherited together as a single unit. This is due to the pattern of inheritance where the three traits were controlled by a single gene with dominant and recessive alleles. Example: sickle cell anemia.

Intergenic gene interactions

- Interlocus interactions take place between the alleles at different loci i.e between alleles of different genes.It includes the following:

- **Dominant Epistasis** – It is a gene interaction in which two alleles of a gene at one locus interfere and suppress or mask the phenotypic expression of a different pair of alleles of another gene at another locus. The gene that suppresses or masks the phenotypic expression of a gene at another locus is known as epistatic. The gene whose expression is interfered by non-allelic genes andprevents from exhibiting its character is known as hypostatic. When both the genes are present together, the phenotype is determined by the epistatic gene and not by the hypostatic gene.

- In the summer squash the fruit colour locus has a dominant allele 'W' for white colour and a recessive allele 'w' for coloured fruit. 'W' allele is dominant that masks the expression of any colour. In another locus hypostatic allele 'G' is for yellow fruit and its recessive allele 'g' for green fruit. In the first locus the white is dominant to colour where

as in the second locus yellow is dominant to green. When the white fruit with genotype WWgg is crossed with yellow fruit with genotype wwGG, the F1 plants have white fruit and are heterozygous (WwGg). When F1 heterozygous plants are crossed they give rise to F2 with the phenotypic ratio of 12 white : 3 yellow : 1 green.

Intra –genic or allelic interaction

Gene interaction	**Example**	**F2Phenotypic ratio**
Incomplete Dominance	Flower colour in Mirabilis jalapa. Flower colour in snapdragon(Antirrhinum spp.)	1 : 2 : 1 1 : 2 : 1
Codominance	ABO Blood group system in humans	1 : 2 : 1

Inter-genic or non-allelic interaction

Epistatic interaction	**Example**	**F2 Ratio Phenotypic ratio**
Dominant epistasis	Fruit colour in summer squash	12 : 3 : 1
Recessive epistasis	Flower colour of Antirrhinumspp.	9 : 3 : 4
Duplicate genes with cumulative effect	Fruit shape in summer squash	9 : 6 : 1
Complementary genes	Flower colour in sweet peas	9 : 7
Supplementary genes	Grain colour in Maize	9 : 3 : 4
Inhibitor genes	Leaf colour in rice plants	13 : 3
Duplicate genes	Seed capsule shape (fruit shape) in shepherd's purse Bursa bursa-pastoris	15 : 1

Polygenic Inheritance in Wheat (Kernel colour)

- Polygenic inheritance - Several genes combine to affect a single trait.
- A group of genes that together determine (contribute) a characteristic of an organism is called polygenic inheritance. It gives explanations to the inheritance of continuous traits which are compatible with Mendel's Law.
- The first experiment on polygenic inheritance was demonstrated by Swedish Geneticist H. Nilsson - Ehle (1909) in wheat kernels. Kernel colour is controlled by two genes each with two alleles, one with red kernel colour was dominant to white. He crossed the two

pure breeding wheat varieties dark red and a white. Dark red genotypes R1R1R2R2 and white genotypes are r1r1r2r2. In the F1 generation medium red were obtained with the genotype R1r1R2r2. F1 wheat plant produces four types of gametes R1R2, R1r2, r1R2, r1r2. The intensity of the red colour is determined by the number of R genes in the F2 generation.

- **Four R genes:** A dark red kernel colour is obtained.Three R genes: Medium - dark red kernel colour is obtained.Two R genes: Medium-red kernel colour is obtained.One R gene: Light red kernel colour is obtained.

- **Absence of R gene**:Results in White kernel colour.The R gene in an additive manner produces the red kernel colour. The number of each phenotype is plotted against the intensity of red kernel colour which produces a bell shaped curve. This represents the distribution of phenotype. Other example: Height and skin colour in humans are controlled by three pairs of genes.

Extra Chromosomal Inheritance or Extra Nuclear Inheritance (Cytoplasmic Inheritance)

- DNA is the universal genetic material. Genes located in nuclear chromosomes follow Mendelian inheritance. But certain traits are governed either by the chloroplast or mitochondrial genes. This phenomenon is known as extra nuclear inheritance. It is a kind of Non-Mendelian inheritance. Since it involves cytoplasmic organelles such as chloroplast and mitochondrion that act as inheritance vectors, it is also called Cytoplasmic inheritance. It is based on independent, self-replicating extra chromosomal unit called plasmogene located in the cytoplasmic organelles, chloroplast and mitochondrion.

Chloroplast Inheritance

- It is found in 4 O' Clock plant (Mirabilis jalapa). In this, there are two types of variegated leaves namely dark green leaved plants and pale green leaved plants. When the pollen of dark green leaved plant (male) is transferred to the stigma of pale green leaved plant (female) and pollen of pale green leaved plant is transferred to the stigma of dark green leaved plant, the F1 generation of both the crosses must be identical as per Mendelian inheritance. But in the reciprocal cross the F1 plant differs from each other. In each cross, the F1 plant reveals the character of the plant which is used as female plant.

- This inheritance is not through nuclear gene. It is due to the chloroplast gene found in the ovum of the female plant which contributes the cytoplasm during fertilization since the male gamete contribute only the nucleus but not cytoplasm.

Mitochondrial Inheritance

- Male sterility found in pearl maize (Sorgum vulgare) is the best example for mitochondrial cytoplasmic inheritance. So it is called cytoplasmic male sterility. In this,

male sterility is inherited maternally. The gene for cytoplasmic male sterility is found in the mitochondrial DNA.

- In this plant there are two types, one with normal cytoplasm (N) which is male fertile and the other one with aberrant cytoplasm (S) which is male sterile. These types also exhibit reciprocal differences as found in Mirabilis jalapa.

- Recently it has been discovered that cytoplasmic genetic male sterility is common in many plant species. This sterility is maintained by the influence of both nuclear and cytoplasmic genes. There are commonly two types of cytoplasm N (normal) and S (sterile). The genes for these are found in mitochondrion. There are also restores of fertility (Rf) genes. Even though these genes are nuclear genes, they are distinct from genetic male sterility genes of other plants. Because the Rf genes do not have any expression of their own, unless the sterile cytoplasm is present. Rf genes are required to restore fertility in S cytoplasm which is responsible for sterility.

- So the combination of N cytoplasm with rfrf and S cytoplasm with RfRf produces plants with fertile pollens, while S cytoplasm with rfrf produces only male sterile plants.

Atavism

- Atavism is a modification of a biological structure whereby an ancestral trait reappears after having been lost through evolutionary changes in the previous generations. Evolutionary traits that have disappeared phenotypically do not necessarily disappear from an organism's DNA. The gene sequence often remains, but is inactive. Such an unused gene may remain in the genome for many generations. As long as the gene remains intact, a fault in the genetic control suppressing the gene can lead to the reappearance of that character again. Reemergence of sexual reproduction in the flowering plant Hieracium pilosella is the best example for Atavism in plants.

UNIT – 3
Chromosomal Basis of Inheritance

Chromosomal Theory of Inheritance

- G. J. Mendel (1865) studied the inheritance of well-defined characters of pea plant but for several reasons it was unrecognized till 1900. Three scientists (de Vries, Correns and Tschermak) independently rediscovered Mendel's results on the inheritance of characters. Various cytologists also observed cell division due to advancements in microscopy. This led to the discovery of structures inside nucleus. In eukaryotic cells, worm-shaped structures formed during cell division are called chromosomes (colored bodies, visualized by staining). An organism which possesses two complete basic sets of chromosomes are known as diploid. A chromosome consists of long, continuous coiled piece of DNA in which genes are arranged in linear order. Each gene has a definite position (locus) on a chromosome. These genes are hereditary units. Chromosomal theory of inheritance states that Mendelian factors (genes) have specific locus (position) on chromosomes and they carry information from one generation to the next generation.

Historical development of chromosome theory

- The important cytological findings related to the chromosome theory of inheritance are given below.
    - **Wilhelm Roux (1883)** postulated that the chromosomes of a cell are responsible for transferring heredity.
    - **Montgomery (1901)** was first to suggest occurrence of distinct pairs of chromosomes and he also concluded that maternal chromosomes pair with paternal chromosomes only during meiosis.
    - **T.Boveri (1902)** supported theidea that the chromosomes containgenetic determiners, and he waslargely responsible for developing thechromosomal theory of inheritance.
    - **W.S. Sutton (1902)**, a young Americanstudent independently recognized aparallelism (similarity) between thebehaviour of chromosomes and Mendelian factors during gamete formation.
    - **Sutton and Boveri (1903)** independentlyproposed the chromosome theory of inheritance. Sutton united the knowledge of chromosomal segregation with Mendelian principles and called it chromosomal theory of inheritance.

Salient features of the Chromosomal theory of inheritance

- Somatic cells of organisms are derived from the zygote by repeated cell division (mitosis). These consist of two identical sets of chromosomes. One set is received from

female parent (maternal) and the other from male parent (paternal). These two chromosomes constitute the homologous pair.

- Chromosomes retain their structural uniqueness and individuality throughout the life cycle of an organism.

- Each chromosome carries specific determiners or Mendelian factors which are now termed as genes.

- The behaviour of chromosomes during the gamete formation (meiosis) provides evidence to the fact that genes or factors are located on chromosomes.

Support for chromosomal theory of heredity

- This theory was widely discussed and controversies by scientists around the world. However, this debate has been finally cleared by the works of Thomas Hunt Morgan (1910) on the fruit fly Drosophila melanogaster (2n=8). This fruit fly completed their life cycle within two weeks. The alleles for red or white eye colour are present on the X chromosome but there is no counterpart for this gene on the Y chromosome. Thus, females have two alleles for this gene, whereas males have only one. The genetic results were completely based on meiotic behaviour of the X and Y chromosomes. Similarly, the genes for yellow body colour and miniature wings are also carried on the X chromosome. This study strongly supports the idea that genes are located on chromosomes. The linked genes connected together on sex chromosome is called sex linkage.

Comparison between gene and chromosome behaviour

- Around twentieth century cytologists established that, generally the total number of chromosomes is constant in all cells of a species. A diploid eukaryotic cell has two haploid sets of chromosomes, one set from each parent. All somatic cells of an organism carry the same genetic complement. The behaviour of chromosomes during meiosis not only explains Mendel's principles but leads to new and different approaches to study about heredity.

Mendelian factors	**Chromosomes behaviour**
Alleles of a factor occur in pair	Chromosomes occur in pairs
Similar or dissimilar alleles of a factor separate during the gamete formation	The homologous chromosomes separate during meiosis
Mendelian factors can assort independently	The paired chromosomes can separate independently during meiosis but the linked genes in the same chromosome normally do not assort independently

The important aspects to be remembered about the chromosome behaviour during cell division (meiosis) are as follows.

- The alleles of a genotype are found in the same locus of a homologous chromosome (A/a).

- In the S phase of meiotic interphase each chromosome replicates forming two copies of each allele (AA/aa), one on each chromatid.

- The homologous chromosomes segregate in anaphase I, thereby separating two different alleles (AA) and (aa).

- In anaphase II of meiosis, separation of sister chromatids of homologous chromosomes takes place. Therefore, each daughter cell (gamete) carries only a single allele (gene) of a character (A), (A), (a) and (a).

Fossil Genes: Some of thejunk DNA is made up of pseudogenes, the sequences presence in that was once working genes. They lost their ability to make proteins. They tell the story of evolution through fossilized parts.

Linkage

- The genes which determine the character of an individual are carried by the chromosomes. The genes for different characters may be present either in the same chromosome or in different chromosomes. When the genes are present in different chromosomes, they assort independently according to Mendel's Law of Independent Assortment. Biologists came across certain genetic characteristics that did not assort out independently in other organisms after Mendel's work. One such case was reported in Sweet pea (Lathyrus odoratus) by Willium Bateson and Reginald C. Punnet in 1906. They crossed one homozygous strain of sweet peas having purple flowers and long pollen grains with another homozygous strain having red flowers and round pollen grains. All the F1 progenies had purple flower and long pollen grains indicating purple flower long pollen (PL/PL) was dominant over red flower round pollen (pl/pl). When they crossed the F1 with double recessive parent (test cross) in results, F2 progenies did not exhibit in 1:1:1:1 ratio as expected with independent assortment. A greater number of F2 plants had purple flowers and long pollen or red flowers and round pollen. So they concluded that genes for purple colour and long pollen grain and the genes for red colour and round pollen grain were found close together in the same homologous pair of chromosomes. These genes do not allow themselves to be separated. So they do not assort independently. This type of tendency of genes to stay together during separation of chromosomes is called Linkage.

- Genes located close together on the same chromosome and inherited together are called linked genes. But the two genes that are sufficiently far apart on the same chromosome are called unlinked genes or syntenic genes. Such condition is known as synteny. It is to be differentiated by the value of recombination frequency. If the recombination frequency value is more than 50 % the two genes show unlinked. when the recombination frequency

value is less than 50 %, they show linked. Closely located genes show strong linkage, while genes widely located show weak linkages.

Coupling and Repulsion theory

- The two dominant alleles or recessive alleles occur in the same homologous chromosomes, tend to inherit together into same gamete are called coupling or cis configuration. If dominant or recessive alleles are present on two different, but homologous chromosomes they inherit apart into different gamete are called repulsion or trans configuration.

Kinds of Linkage

- T.H. Morgan found two types of linkage. They are complete linkage and incomplete linkage depending upon the absence or presence of new combination of linked genes.

Complete Linkage

- If the chances of separation of two linked genes are not possible those genes always remain together as a result, only parental combinations are observed. The linked genes are located very close together on the same chromosome such genes do not exhibit crossing over. This phenomenon is called complete linkage. It is rare but has been reported in male Drosophila (Figure 3.7). C.B Bridges (1919) discovered that crossing over is completely absent in some species of male Drosophila.

Incomplete Linkage

- If two linked genes are sufficiently apart, the chances of their separation are possible. As a result, parental and non-parental combinations are observed. The linked genes exhibit some crossing over. This phenomenon is called incomplete linkage. This was observed in maize. (Figure 3.8) It was reported by Hutchinson.

Genetic Mapping

- Genes are present in a linear order along the chromosome. They are present in a specific location called locus (plural: loci). The diagrammatic representation of position of genes and related distances between the adjacent genes is called genetic mapping. It is directly proportional to the frequency of recombination between them. It is also called as linkage map. The concept of gene mapping was first developed by Morgan's student Alfred H Sturtevant in 1913. It provides clues about where the genes lies on that chromosome.

Map distance

- The unit of distance in a genetic map is called a map unit (m.u). One map unit is equivalent to one percent of crossing over (Figure 4.). One map unit is also called a centimorgan (cM) in honour of T.H. Morgan. 100 centimorgan is equal to one Morgan

(M). For example: A distance between A and B genes is estimated to be 3.5 map units. It is equal to 3.5 centimorgans or 3.5 % or 0.035 recombination frequency between the genes.

- Genetic maps can be constructed from a series of test crosses for pairs of genes called two point crosses. But this is not efficient because double cross over is missed.

Three point test cross

- A more efficient mapping technique is to construct based on the results of three-point test cross. It refers to analyzing the inheritance patterns of three alleles by test crossing a triple recessive heterozygote with a triple recessive homozygote. It enables to determine the distance between the three alleles and the order in which they are located on the chromosome. Double cross overs can be detected which will provide more accurate map distances.

- Three-point test cross can be best understood by considering following an example.

Uses of genetic mapping

- It is used to determine gene order, identify the locus of a gene and calculate the distances between genes.

- They are useful in predicting results of dihybrid and trihybrid crosses.

- It allows the geneticists to understand the overall genetic complexity of particular organism.

Multiple alleles

- A given phenotypic trait of an individual depends on a single pair of genes, each of which occupies a specific position called the locus on homologous chromosome. When any of the three or more allelic forms of a gene occupy the same locus in a given pair of homologous chromosomes, they are said to be called multiple alleles.

Characteristics of multiple alleles

- Multiple alleles of a series always occupythe same locus in the homologouschromosome. Therefore, no crossing overoccurs within the alleles of a series.

- Multiple alleles are always responsible forthe same character.

- The wild type alleles of a series exhibitdominant character whereas mutanttype will influence dominance or anintermediate phenotypic effect.

- When any two of the mutant multiplealleles are crossed the phenotype is alwaysmutant type and not the wild type.

Self-sterility in Nicotiana

- In plants, multiple alleles have been reported in association with self-sterility or self-incompatibility. Self-sterility means that thepollen from a plant is unable to germinate on its own stigma and will not be able to bring about fertilization in the ovules of the same plant. East (1925) observed multiple alleles in Nicotiana which are responsible for self-incompatibility or self-sterility. The gene for self-incompatibility can be designated as S, which has allelic series S1, S2, S3, S4 and S5.

- The cross-fertilizing tobacco plants were not always homozygous as S1S1 or S2S2, but all plants were heterozygous as S1S2, S3S4, S5S6. When crosses were made between different S1S2 plants, the pollen tube did not develop normally. But effective pollen tube development was observed when crossing was made with other than S1S2 for example S3S4.

Sex determination in plants

- About 94% of all flowering plants have only one type of individual, which produces flowers with male organs (the stamens) and female organs (the carpels). Such plants are termed as sexually monomorphic. Some 6% of flowering plants which have two separate sexes are called dimorphic. Male plants produce flowers with stamens and female plants produce flowers with carpels only. Researchers are interested to study the mechanism of sex determination in plants.C.E. Allen (1917) discovered sex determination in plants. Sex determination is a complex process determined by genes, the environment and hormones.

- Sex determination in Silene latifolia (Melandrium album) is of controlled by three distinct regions in a sex chromosome.

    1. Y chromosome determines maleness
    2. X specifies femaleness
    3. X and Y show different segments (I II III IV and V)

Sex determination in papaya

- Recently researchers in Hawaii discovered sex chromosomes in Papaya (Carica papaya, 2n=36). Papaya has 17 pairs of autosomes and one pair of sex chromosomes. Male papaya plants have XY and female plants have XX. Unlike human sex chromosomes, papaya sex chromosomes look like autosomes and it is evolved from autosome. The sex chromosomes are functionally distinct because the Y chromosome carries the genes for male organ development and X bears the female organ developmental genes.

- In papaya sex determination is controlled by three alleles. They are m, M1 and M2 of a single gene.

Genotype	Dominant/ recessive	Modification	Sex
mm	Homozygous recessive	Restrict maleness	Female
M1m	Heterozygous	Induces maleness	Male
M2m	Heterozygous	Induces both the sex	Bisexual (rare)
M1M1 or M2M2 or M1M2	Homozygous/ Heterozygous dominant	Inviable plants	Sterile

Sex Determination in Sphaerocarpos

- Sex determination was first described in the bryophyte Sphaerocarposdonnellii which has heteromorphic chromosomes. The gametophyte is haploid and heteromorphic. The male gametophyte as well as the female gametophyte is an haploid organism with 8 chromosome (n=8). The diploid sporophyte is always heterogametic. Seven autosomes are similar in both male and female gametophyte. But the eighth chromosome of female is X which is larger than the seven autosomes. The eighth chromosome of male is Y which is comparatively smaller than autosomes. The sporophyte containing XY combination produces two types of meiospores, that is some with X and others with Y chromosomes. The meiospores with X chromosomes produce female gametophyte and those with Y chromosome produces male gametophyte.

Sex determination in maize

- Zea mays (maize) is an example for monoecious, which means male and female flowers are present on the same plant. There are two types of inflorescence. The terminal inflorescence which bears staminate florets develops from shoot apical meristem called tassel. The lateral inflorescence which develop pistillate florets from axillary bud is called ear or cob. Unisexuality in maize occurs through the selective abortion of stamens in ear florets and pistils in tassel florets. A substitution of two single gene pairs 'ba' for barren plant and 'ts' for tassel seed makes the difference between monoecious and dioecious (rare) maize plants. The allele for barren plant (ba) when homozygous makes the stalk staminate by eliminating silk and ears. The allele for tassel seed (ts) transforms tassel into a pistillate structure that produce no pollen. The table-3.7 is the resultant sex expression based on the combination of these alleles. Most of these mutations are shown to be defects in gibberellin biosynthesis. Gibberellins play an important role in the suppression of stamens in florets on the ears.

Mutation

- Genetic variation among individuals provides the raw material for the ultimate source of evolutionary changes. Mutation and recombination are the two major processes

responsible for genetic variation. A sudden change in the genetic material of an organisms is called mutation. The term mutation was introduced by Hugo de Vries (1901) while he has studying on the plant, evening primrose (Oenothera lamarkiana) and proposed 'Mutation theory'. There are two broad types of changes in genetic material. They are point mutation and chromosomal mutations.

- Mutational events that take place within individual genes are called gene mutations or point mutation, whereas the changes occur in structure and number of chromosomes is called chromosomal mutation. Agents which are responsible for mutation are called mutagens,that increase the rate of mutation. Mutations can occur either spontaneously or induced. The production of mutants through exposure of mutagens is called mutagenesis, and the organism is said to be mutagenized.

Types of mutation

Let us see the two general classes of gene mutation:

- Mutations affecting single base or basepair of DNA are called point mutation
- Mutations altering the number of copiesof a small repeated nucleotide sequence within a gene

Point mutation

- It refers to alterations of single base pairs of DNA or of a small number of adjacent base pairs

Types of point mutations

- Point mutation in DNA are categorised into two main types. They are base pair substitutions and base pair insertions or deletions. Base substitutions are mutations in which there is a change in the DNA such that one base pair is replaced by another. It can be divided into two subtypes: transitions and transversions. Addition or deletion mutations are actually additions or deletions of nucleotide pairs and also called base pair addition or deletions. Collectively, they are termed indel mutations (for insertion-deletion).

- Substitution mutations or indel mutations affect translation. Based on these different types of mutations are given below.

- The mutation that changes one codon for an amino acid into another codon for that same amino acid are called Synonymous or silent mutations. The mutation where the codon for one amino acid is changed into a codon for another amino acid is called Missense or non-synonymous mutations. The mutations where codon for one amino acid is changed into a termination or stop codon is called Nonsense mutation. Mutations that result in the addition or deletion of a single base pair of DNA that changes the reading frame for the

translation process as a result of which there is complete loss of normal protein structure and function are called Frameshift mutations.

Mutagenic agents

- The factors which cause genetic mutation are called mutagenic agents or mutagens. Mutagens are of two types, physical mutagen and chemical mutagen. Muller (1927) was the first to find out physical mutagen in Drosophila.

Physical mutagens:

- Scientists are using temperature and radiations such as X rays, gamma rays, alfa rays, beta rays, neutron, cosmic rays, radioactive isotopes, ultraviolet rays as physical mutagen to produce mutation in various plants and animals.

Temperature:

- Increase in temperature increases the rate of mutation. While rise in temperature, breaks the hydrogen bonds between two DNA nucleotides which affects the process of replication and transcription.

Radiation:

- The electromagnetic spectrum contains shorter and longer wave length rays than the visible spectrum. These are classified into ionizing and non-ionizing radiation. Ionizing radiation are short wave length and carry enough higher energy to ionize electrons from atom. X rays, gamma rays, alfa rays, beta rays and cosmic rays which breaks the chromosomes (chromosomal mutation) and chromatids in irradiated cells. Non-ionizing radiation, UV rays have longer wavelengths and carry lower energy, so they have lower penetrating power than the ionizing radiations. It is used to treat unicellular microorganisms, spores, pollen grains which possess nuclei located near surface membrane.

Sharbati Sonora

- Sharbati Sonora is a mutant variety of wheat, which is developed from Mexican variety (Sonora 64) by irradiating of gamma rays. It is the work of Dr. M.S.Swaminathan who is known as 'Father of Indian green revolution' and his team.

Castor Aruna

- Castor Aruna is mutant variety of castor which is developed by treatment of seeds with thermal neutrons in order to induce very early maturity (120 days instead of 270 days as original variety).

Chemical mutagens:

- Chemicals which induce mutation are called chemical mutagens. Some chemical mutagens are mustard gas, nitrous acid, ethyl and methyl methane sulphonate (EMS and MMS), ethyl urethane, magnous salt, formaldehyde, eosin and enthrosine. Example: Nitrous oxide alters the nitrogen bases of DNA and disturb the replication and transcription that leads to the formation of incomplete and defective polypeptide during translation.

Comutagens

- The compounds which are not having own mutagenic properties but can enhance the effects of known mutagens are called comutagens.
  Example: Ascorbic acid increase the damage caused by hydrogen peroxide.
  Caffeine increase the toxicity of methotrexate

Chromosomal mutations

- The genome can also be modified on a larger scale by altering the chromosome structure or by changing the number of chromosomes in a cell. These large-scale variations are termed as chromosomal mutations or chromosomal aberrations. Gene mutations are changes that take place within a gene, whereas chromosomal mutations are changes to a chromosome region consisting of many genes. It can be detected by microscopic examination, genetic analysis, or both. In contrast, gene mutations are never detectable microscopically. Chromosomal mutations are divided into two groups: changes in chromosome number and changes in chromosome structure.

Changes in chromosome number

- Each cell of living organisms possesses fixed number of chromosomes. It varies in different species. Even though some species of plants and animals are having identical number of chromosomes, they will not be similar in character. Hence the number of chromosomes will not differentiate the character of species from one another but the nature of hereditary material (gene) in chromosome that determines the character of species.

- Sometimes the chromosome number of somatic cells are changed due to addition or elimination of individual chromosome or basic set of chromosomes. This condition in known as numerical chromosomal aberration or ploidy. There are two types of ploidy.

I. Ploidy involving individual chromosomes within a diploid set (Aneuploidy)
II. (Ploidy involving entire sets of chromosomes (Euploidy)

Aneuploidy

- It is a condition in which diploid number is altered either by addition or deletion of one or more chromosomes. Organisms showing aneuploidy are known as aneuploids or heteroploids. They are of two types, Hyperploidy and Hypoploidy.

Hyperploidy

- Addition of one or more chromosomes to diploid sets are called hyperploidy. Diploid set of chromosomes represented as Disomy. Hyperploidy can be divided into three types. They are as follows,

Trisomy

- Addition of single chromosome to diploid setis called Simple trisomy(2n+1). Trisomics werefirst reported by Blackeslee (1910) in Daturastramonium (Jimson weed). But later it wasreported in Nicotiana, Pisum and Oenothera.Sometimes addition of two individualchromosome from different chromosomalpairs to normal diploid sets are called Doubletrisomy (2n+1+1).

Tetrasomy

- Addition of a pair or two individual pairs ofchromosomes to diploid set is called tetrasomy(2n+2) and Double tetrasomy (2n+2+2)respectively. All possible tetrasomics areavailable in Wheat.

Pentasomy

- Addition of three individual chromosome from different chromosomal pairs to normal diploid set are called pentasomy (2n+3).

2.Hypoploidy

- Loss of one or more chromosome from the diploid set in the cell is called hypoploidy. It can be divided into two types. They are

Monosomy

- Loss of a single chromosome from the diploid set are called monosomy(2n-1). However loss of two individual or three individual chromosomes are called double monosomy (2n-1-1) and triple monosomy (2n-1-1-1) respectively. Double monosomics are observed in maize.

Nullisomy

- Loss of a pair of homologous chromosomes or two pairs of homologous chromosomes from the diploid set are called Nullisomy (2n-2) and double Nullisomy (2n-2-2) respectively. Selfing of monosomic plants produce nullisomics. They are usually lethal.

Euploidy

- Euploidy is a condition where the organisms possess one or more basic sets of chromosomes. Euploidy is classified as monoploidy, diploidy and polyploidy. The condition where an organism or somatic cell has two sets of chromosomes are called diploid (2n). Half the number of somatic chromosomes is referred as gametic chromosome number called haploid(n). It should be noted that haploidy (n) is different from a monoploidy (x). For example, the common wheat plant is a polyploidy (hexaploidy) 2n=6x=72 chromosomes. Its haploid number (n) is 36, but its monoploidy (x) is 12. Therefore, the haploid and diploid condition came regularly one after another and the same number of chromosomes is maintained from generation to generation, but monoploidy condition occurs when an organism is under polyploidy condition. In a true diploid both the monoploid and haploid chromosome number are same. Thus a monoploid can be a haploid but all haploids cannot be a monoploid.

Polyploidy

- Polyploidy is the condition where an organism possesses more than two basic sets of chromosomes. When there are three, four, five or six basic sets of chromosomes, they are called triploidy (3x) tetraploidy (4x), pentaploidy (5x) and hexaploidy (6x) respectively. Generally, polyploidy is very common in plants but rarer in animals. An increase in the number of chromosome sets has been an important factor in the origin of new plant species. But higher ploidy level leads to death. Polyploidy is of two types. They are autopolyploidy and allopolyploidy

Autopolyploidy

- The organism which possesses more than twohaploid sets of chromosomes derived fromwithin the same species is called autopolyploid.They are divided into two types. Autotriploidsandautotetraploids.

- Autotriploids have three set of its own genomes. They can be produced artificially by crossing between autotetraploid and diploid species. They are highly sterile due to defective gamete formation. Example: The cultivated banana are usually triploids and are seedless having larger fruits than diploids. Triploid sugar beets have higher sugar content than diploids and are resistant to moulds. Common doob grass (Cyanodondactylon) is a natural autotriploid. Seedless watermelon, apple, sugar beet, tomato, banana are man madeautotriploids.

- Autotetraploids have four copies of its own genome. They may be induced by doubling the chromosomes of a diploid species. Example: rye, grapes, alfalfa, groundnut, potato and coffee.

2. Allopolyploidy

- An organism which possesses two or more basic sets of chromosomes derived from two different species is called allopolyploidy. It can be developed by interspecific crosses and fertility is restored by chromosome doubling with colchicine treatment. Allopolyploids are formed between closely related species only.

Significance of Ploidy

- Many polyploids are more vigorous andmore adaptable than diploids.
- Many ornamental plants are autotetraploidsand have larger flower and longer floweringduration than diploids.
- Autopolyploids usually have increase in freshweight due to more water content.
- Aneuploids are useful to determine thephenotypic effects of loss or gain of differentchromosomes.
- Many angiosperms are allopolyploids andthey play a role in an evolution of plants.

Structural changes in chromosome (Structural chromosomal aberration):

- Structural variations caused by addition or deletion of a part of chromosome leading to rearrangement of genes is called structural chromosomal aberration. It occurs due to ionizing radiation or chemical compounds. On the basis of breaks and reunion in chromosomes, there are four types of aberrations. They are classified under two groups.

Changes in the number of the gene loci

- Deletion or Deficiency
- Duplication or Repeat

Changes in the arrangement of gene loci

- Inversion
- Translocation

Deletion or Deficiency

- Loss of a portion of chromosome is calleddeletion. On the basis of location of breakageon chromosome, it is divided into terminaldeletion and intercalary deletion. It occursdue to chemicals, drugs and radiations. It isobserved in Drosophila and Maize.

There are two types of deletion:

Terminal deletion: Single break in any oneend of the chromosome.

- **Intercalary deletion or interstitial deletion**:It is caused by two breaks and reunion ofterminal parts leaving the middle.

- Both deletions are observable during meiotic pachytene stage and polytene chromosome. The unpaired loop formed in the normal chromosomal part at the time of chromosomal pairing. Such loops are called as deficiency loops and it can be seen in meiotic prophase. Larger deletions may lead to lethal effect.

Duplication or Repeat

- The process of arrangement of the same orderof genes repeated more than once in the same chromosome is known as duplication. Due to duplication some genes are present in more than two copies. It was first reported in Drosophila by Bridges (1919) and other examples are Maize and Pea. It is three types.

Tandem duplication

- The duplicated segment is located immediatelyafter the normal segment of the chromosome inthe same order.

Reverse tandem duplication

- The duplicated segment is located immediatelyafter the normal segment but the gene sequenceorder will be reversed.

Displaced duplication

- The duplicated segment is located in the samechromosome, but away from the normalsegment.

Duplications play a major role in evolution.

Inversion

- A rearrangement of order of genes in achromosome by reversed by an angle 1800. Thisinvolve two chromosomal breaks and reunion.During this process there is neither gain nor loss but the gene sequences is rearranged. Inversionwas first reported in Drosophila by Sturtevant(1926). There are two types of inversion,paracentric and pericentric (Figure 3.26).

I. **Paracentric inversion:** An inversion whichtakes place apart from the centromere

II. **Pericentric inversion:** An inversion that includes the centromere.
Inversions lead to evolution of a new species.

Translocation

- The transfer of a segment of chromosome to a non-homologous chromosome is called translocation. Translocation should not be confused with crossing over, in which an exchange of genetic material between homologous chromosome takes place. Translocation occurs as a result of interchange of chromosome segments in non-homologous chromosomes. There are three types

  - Simple translocation
  - Shift translocation
  - Reciprocal translocation

Simple translocation

- A single break is made in only one chromosome. The broken segment gets attached to one end of a non-homologous chromosome. It occurs very rarly in nature.

Shift translocation

- Broken segment of one chromosome gets inserted interstitially in a non-homologous chromosome.

Reciprocal translocations

- It involves mutual exchange of chromosomal segments between two non-homologous chromosomes. It is also called illegitimate crossing over. It is further divided into two types.

- **Homozygous translocation**: Both the chromosomes of two pairs are involved in translocation. Two homologous of each translocated chromosomes are identical.

- **Heterozygous translocation**: Only one of the chromosome from each pair of two homologous are involved in translocation, while the remaining chromosome is normal.

Translocations play a major role in the formation of species.

DNA Metabolism in Plants

- As the repository of genetic information, DNA occupies a unique and central place among biological macromolecules. The structure of DNA is a marvelous device for the storage of genetic information. The term "DNA Metabolism" can be used to describe process by which copies of DNA molecules are made (replication) along with repair and recombination.

- **DNA Replication:** In the double helix the two parental strands of DNA separate and each parental strand synthesizes a new complementary strand. DNA replication is semiconservative, i.e each new DNA molecule conserves one original strand.

DNA Repair: How is genomic stability maintained in all living organisms? How do organisms on earth survive? What is essential for their survival?

- DNA is unique because it is the only macromolecule where the repair system exists, which recognises and removes mutations. DNA is subjected to various types of damaging reactions such as spontaneous or environmental agents or natural endogenous threats. Such damages are corrected by repair enzymes and proteins, immediately after the damage has taken place. DNA repair system plays a major role in maintaining the genomic / genetic integrity of the organism. DNA repair systems protect the integrity of genomes from genotoxic stresses.

Plants are sessile. How do they protect themselves from the exposure of sunlight throughout the day? Plants have effective DNA repair mechanism to prevent UV damage from sunlight. They produce an enzyme called photolyase, which can repair the thymine dimers and restore the structure of DNA.

- **Recombination:** In cells the genetic information within and among DNA molecule are re-arranged by a process called genetic recombination. Recombination is the result of crossing over between the pairs of homologous chromosomes during meiosis. In earlier classes you have learnt chromosomal recombination. In molecular level it involves breakage and reunion of polynucleotides.

Eukaryotic DNA replication

- Replication starts at a specific site on a DNA sequence known as the Origin of replication. There are more than one origin of replication in eukaryotes. Saccharomyces cerevisiae (yeast) has approximately 400 origins of replication. DNA replication in eukaryotes starts with the assembly of a prereplication complex (preRC) consisting of 14 different proteins. Part of a preRC is a group of 6 proteins called the origin recognition complex (ORC) which acts as initiator in eukaryotic DNA replication. The origin of replication in yeast is called as ARS sites (Autonomously Replicating Sequences). In yeast, ORC was identified as a protein complex which binds directly to ARS elements.

- Replication fork is the site (point of unwinding) of separation of parental DNA strands where new daughter strands are formed. Multiple replication forks are found in eukaryotes. The enzyme helicases are involved in unwinding of DNA by breaking hydrogen bonds holding the two strands of DNA and replication protein A (RPA) prevents the separated polynucleotide strand from getting reattached.

- Topoisomerase is an enzyme which breaks DNAs covalent bonds and removes positive supercoiling ahead of replication fork. It eliminates the torsional stress caused by unwinding of DNA double helix.

- DNA replication is initiated by an enzyme DNA polymerase α / primase which synthesizes short stretch of RNA primers on both leading strand (continuous DNA

strand) and lagging strands (discontinuous DNA strand). Primers are needed because DNA polymerase requires a free 3′ OH to initiate synthesis. DNA polymerase covalently connects the nucleotides at the growing end of the new DNA strand.

- DNA Pol α (alpha), DNA Pol δ (delta) and DNA Pol ε (Epsilon) are the 3 enzymes involved in nuclear DNA replication.

  DNA Pol α – Synthesizes short primers of RNA
  DNA Pol δ – Main Replicating enzyme of cell nucleus
  DNA Pol ε – Extend the DNA Strands in replication fork

DNA Polymerase β does not play any role in the replication of normal DNA. Function - Removing incorrect bases from damagedDNA. It is involved in Base excision repair

Experimental evidence of DNA replication: Taylors Experiment

- J.Herbert Taylor, Philip Woods and WalterHughes demonstrated the semiconservativereplication of DNA in the root cells of Viciafaba. They labelled DNA with 3H Thymidine,a radioactive precursor of DNA and performedautoradiography. They grew root tips in amedium in the presence of radioactive labeled thymidine, so that the radioactivity wasincorporated into the DNA of these cells. Theoutline of this labelled chromosomes appears inthe form of scattered black dots of silver grainson a photographic film.

- In the chromosome of first generation theradioactivity was found to be distributed toboth the chromatids because in the originalstrand of DNA double helix was labeled with radioactivity and the new strand wasunlabelled.

- In the chromosome of the second generation only one of the two chromatids in eachchromosome was radioactive (labelled).
  The results proved the semiconservative method of DNA replication

Translation

- The genetic information in the DNA code is copied onto mRNA bound in ribosomes for making polypeptides. The mRNA nucleotide sequence is decoded into amino acid sequence of the protein which is catalyzed by the ribosome. This process is called translation.

Process of translation

The following are major steps in translation process

Initiation

- The translation begins with the AUG codon(start codon) of mRNA. Translation occurson the surface of the macromolecular arenacalled the ribosome. It is a non-membranousorganelle. During the process of translationthe two subunits of ribosomes unite (combine)together and hold mRNA between them. Theprotein synthesis begins with the reading ofcodons of mRNA. The tRNA brings amino acidto the ribosome, a molecular machine whichunites amino acids into a chain according to theinformation given by mRNA. rRNA plays thestructural and catalytic role during translation.

- A ribosome has one binding site for mRNA and two for tRNA. The two binding sites of tRNA are

  1. P-Site – The peptidyl – tRNA binding siteis one of the tRNA binding site. At this sitetRNA is held and linked to the growing endof the polypeptide chain.

  2. A-Site – The Aminoacyl – tRNA binding site. This is another tRNA binding site which holds the incoming amino acids called aminoacyl tRNA. The anticodons oftRNA pair with the codons of the mRNA inthese sites.

Elongation of polypeptide chain

- The P and A sites are nearby, so that two tRNAform base pairs with adjacent codon. Thepolypeptide chain is formed by the pairingof codons and anticodons according to thenucleotide sequence of the mRNA.

Translators of the genetic code - tRNA

- The tRNA translates the genetic code from the nucleic acid sequence to the amino acid sequence i.e from gene – Polypeptide. When an amino acid is attached to tRNA it is called aminoacylated or charged. This is an energy requiring process whichuses the ATP for its energy requirement. Protein synthesis takes place as the next aminoacyl tRNA binds to the A-Site.

- The translation begins with the AUG codon (start codon) of mRNA. The tRNA which carries first amino acid methionine attach itself to P-site of ribosome. The ribosome adds new amino acids to the growing polypeptides. The second tRNA molecules has anticodons which carries amino acid alanine pairs with the mRNA codon in the A-site of the ribosome. The aminoacids methionine and alanine are close enough so that a peptide bond is formed between them.

- The bond between the first tRNA and methionine now breaks. The first tRNA leaves the ribosome and the P-site is vacant. The ribosome now moves one codon along the mRNA strand. The second t-RNA molecule now occupies the P-site. The third t-RNA comes and fills the A site (serine).Now a peptide bond is formed betweenalanine and serine. The mRNA then movesthrough the ribosome by three bases. Thisexpels deacylated /

uncharged tRNA fromP-site and moves peptidyl tRNA into theP-site and empties the A-site.This movementof tRNA from A-site to P-site is said to betranslocation. The translocation requires thehydrolysis of GTP.

- The ribosome (ribozyme - peptidyl transferase) catalyses the formation of peptide bond by adding amino acid to the growing polypeptide chain.

- The ribosome moves from codon to codon along the mRNA in the 5′ to 3′ direction. Amino acids are added one by one translated into polypeptide as dictated by the mRNA. Translation is an energy intensive process. A cluster of ribosomes are linked together by a molecule of mRNA and forming the site of protein synthesis is called as polysomes or polyribosomes.

Termination of polypeptide synthesis

- Eukaryotes have cytosolic proteins called release factors which recognize the termination codon, UAA, UAG, or UGA when it is in the A site. When the ribosome reaches a stop codon the protein synthesis comes to an end. So ribosomes are the protein making factories of a cell. When the polypeptide is completed the ribosome releases the polypeptide and detaches from the mRNA molecule. Now the ribosome splits into small and large subunits after the release of mRNA.

Alternative Splicing in plants

- It is very useful in regulating gene expression to overcome the environmental stress in plants.

- Alternative splicing is an important mechanism / process by which multiple mRNA's and multiple proteins products can be generated from a single gene. The different proteins generated are called isoforms. There are various modes of alternative splicing. When multiple introns are present in a gene, they are removed separately or as a unit. In certain cases one or more exons which is present between the introns are also removed.

Significance of alternative splicing

- The proteins transcribed from alternatively spliced mRNA containing different aminoacid sequence lead to the generation of protein diversity and biological functions.

- Multiple protein isoforms are formed.

- It creates multiple mRNA transcriptsfrom a single gene. A process of producingrelated proteins from a single gene therebythe number of gene products are increased.

- It plays an important role in plant functions such as stress response and trait selection.The plant adapts or regulates itself to thechanging environment.

Jumping Genes

- This is the nick name of transposable genetic elements. Transposons are the DNA sequences which can move from one position to another position in a genome. This was first reported in 1948 by American Geneticist Barbara McClintock as "mobile controlling element" in Maize. One of the most significant scientists of 20th century was Barbara McClintock because she gave a shift in gene organization. McClintock was awarded Nobel prize in 1983 for her work on transposons. Barbara McClintock when studying aleurone of single maize kernels, noted the unstable inheritance of the mosaic pattern of blue, brown and red spots due to the differential production of vacuolar anthocyanins.

- In maize plant genome has AC / Ds transposon (AC = Activator, Ds = Dissociation). The activity of AC element is very distinct in maize plant. The transposition in somatic cells results in the changes in gene expression such as variegated pigmentation in maize kernels. Maize genome has transposable elements which regulated the different colour pattern of kernels.

- McClintock's findings concluded that Ds and AC genes were mobile controlling elements. We now call it as transposable elements, a term coined by maize geneticist, Alexander Brink. McClintock gave the first direct experimental evidence that genomes are not static but are highly plastic entities

Significance of transposons

- They contribute to many visible mutations and mutation rate in an Organism.

- In evolution, they contribute to genetic diversity.

- In genetic research transposons are valuable tools which are used as mutagens, as cloning tags, vehicles for inserting foreign DNA into model organism.

- **Plant genome** – The word genome is defined as the full complement of DNA (including all the genes and the intergenic regions) present in an organism It specifies the entire biological information of an organism. There are three distinct genomes in eukaryotic cells and they are

    - The nuclear genome
    - The mitochondrial genome and
    - The chloroplast genome present only in plants.

- It is a model plant for the study of genetic and molecular aspects of plant development.
- It belongs to mustard family and it is the first flowering plant, where its entire genome is sequenced.

- The two regions of the nucleolar organiser ribosomal DNA which codes for the ribosomal RNA are present at the extremity of chromosomes 2 and 4

- It is Diploid plant having small genome with 2n = 10 chromosomes. Several generations can be produced in one year. So it facilitates rapid genetic analysis. The genome has low repetitive DNA, over 60% of the nuclear DNA have protein coding functions.

- The plant is small, self fertilizes, annual long-day plant with short-life cycle (only 6 weeks), large numbers of seeds are produced and they are easy to be grown in laboratory. It is easy to induce mutations. It has many genomic resources and the transformation can be done easily.

- In 1982, Arabidopsis has successfully completed its life cycle in Microgravity i.e. space. This shows that Human Space Missions with plant companions may be possible.

UNIT – 4 -Principles and processes of Biotechnology

Advancements in Modern Biotechnology

- The modern biotechnology embraces all the genetic manipulations, protoplasmic fusion techniques and the improvements made in the old biotechnological processes. Some of the major advancements in modern biotechnology are described below.

Genetic Engineering

- Genetic engineering or recombinant DNA technology or gene cloning is a collective term that includes different experimental protocols resulting in the modification and transfer of DNA from one organism to another.

- The definition for conventional recombination was already given in Unit II. Conventional recombination involves exchange or recombination of genes between homologous chromosomes during meiosis. Recombination carried out artificially using modern technology is called recombinant DNA technology (r-DNA technology). It is also known as gene manipulation technique. This technique involves the transfer of DNA coding for a specific gene from one organism into another organism using specific agents like vectors or using instrumentslike electroporation, gene gun, liposome mediated, chemical mediated transfers and microinjection.

Steps involved in Recombinant DNA Technology
The steps involved in recombinant DNA technology are:

- Isolation of a DNA fragment containing a gene of interest that needs to be cloned. This is called an insert.

- Generation of recombinant DNA (rDNA) molecule by insertion of the DNA fragment into a carrier molecule called a vector that can self-replicate within the host cell.

- Selection of the transformed host cells that is carrying the rDNA and allowing them to multiply thereby multiplying the rDNA molecule.

Tools for Genetic Engineering

- Now we know from the foregoing discussion that in order to generate recombinant DNA molecule, certain basic tools are necessary for the process. The basic tools are enzymes, vectors and host organisms. The most important enzymes required for genetic engineering are the restriction enzymes, DNA ligase and alkaline phosphatase.

Restriction Enzymes

- The two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli were isolated in the year 1963. One was the enzyme which added methyl groups to DNA, while the other cut DNA. The later was called restriction endonuclease. A restriction enzyme or restriction endonuclease is an enzyme thatcleaves DNA into fragments at or near specific recognition sites within the molecule known as restrictionsites. Based on their mode of action restriction enzymes are classified into Exonucleases and Endonucleases.

- Exonucleases are enzymes which remove nucleotides one at a time from the end of a DNA molecule. e.g. Bal 31, Exonuclease III.

- Endonucleases are enzymes which break the internal phosphodiester bonds within a DNA molecule. e.g. Hind II, EcoRI, Pvul, BamHI, TaqI.

Vectors

- Another major component of a gene cloning experiment is a vector such as a plasmid. A Vector is a small DNA molecule capable of self-replication and is used as a carrier and transporter of DNA fragment which is inserted into it for cloning experiments. Vector is also called cloning vehicle or cloning DNA. Vectors are of two types:
    - Cloning Vector,
    - **Expression Vector.**

- Cloning vector is used for the cloning of DNA insert inside the suitable host cell. Expression vector is used to express the DNA insert for producing specific protein inside the host.

Properties of Vectors

- Vectors are able to replicate autonomously to produce multiple copies of them along with their DNA insert in the host cell.

- It should be small in size and of low molecular weight, less than 10 Kb (kilo base pair) in size so that entry/transfer into host cell is easy.

- Vector must contain an origin of replication so that it can independetly replicate within the host.

- It should contain a suitable marker such as antibiotic resistance, to permit its detection in transformed host cell.

- Vector should have unique target sites for integration with DNA insert and should have the ability to integrate with DNA insert it carries into the genome of the host cell. Most of

the commonly used cloning vectors have more than one restriction site. These are Multiple Cloning Site (MCS) or polylinker. Presence of MCS facilitates the use of restriction enzyme of choice.

- The following are the features that are required to facilitate cloning into a vector.

- **Origin of replication (ori):** This is a sequence from where replication starts and piece of DNA when linked to this sequence can be made to replicate within the host cells.

- **Selectable marker:** In addition to ori the vector requires a selectable marker, which helps in identifying and eliminating non transformants and selectively permitting the growth of the transformants.

- **Cloning sites:** In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes.

Types of vector

Few types of vectors are discussed in detail below:

Plasmid

- Plasmids are extra chromosomal, self replicating ds circular DNA molecules, found in the bacterial cells in addition to the bacterial chromosome. Plasmids contain Genetic information for their own replication.

pBR 322 Plasmid

- pBR 322 plasmid is a reconstructed plasmid and most widely used as cloning vector; it contains 4361 base pairs. In pBR, p denotes plasmid, Band R respectively the names of scientist Boliver and Rodriguez who developed this plasmid. The number 322 is the number of plasmid developed from their laboratory. It contains ampR and tetR two different antibiotic resistance genes and recognition sites for several restriction enzymes. (Hind III, EcoRI, BamH I, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes. Rop codes for the proteins involved in the replication of the plasmid.

Ti Plasmid

- Ti plasmid is found in Agrobacterium tumefaciens, a bacteria responsible for inducing tumours in several dicot plants. The plasmid carries transfer (tra) gene which help to transfer T- DNA from one bacterium to other bacterial or plant cell. It has Onco gene for oncogenecity,ori gene for origin for replication and inc gene for incompatibility. T-DNA of Ti-Plasmid is stably integrated with plant DNA. Agrobacterium plasmids have been used for introduction of genes of desirable traits into plants.

Transposon as Vector

- Transposons (Transposable elements or mobile elements) are DNA sequence able to insert itself at a new location in the genome without having any sequence relationship with the target locus and hence transposons are called walking genes or jumping genes. They are used as genetic tools for analysis of gene and protein functions, that produce new phenotype on host cell. The use of transposons is well studied in Arabidopsis thaliana and bacteria such as Escherichia coli.

Walking Genes - Gene walking involves the complete sequencing of large more than 1 kb stretches of DNA.

Expression vectors

- Vectors which are suitable for expressing foreign proteins are called expression vectors. This vector consists of signals necessary for transcription and translation of proteins in the host. This helps the host to produce foreign protein in large amounts. Example: pUC 19.

Competent Host (For Transformation with Recombinant DNA)

- The propagation of the recombinant DNA molecules must occur inside a living system or host. Many types of host cells are available for gene cloning which includes E.coli, yeast, animal or plant cells. The type of host cell depends upon the cloning experiment. E.coli is the most widely used organism as its genetic make-up has been extensively studied, it is easy to handle and grow, can accept a range of vectors and has also been studied for safety. One more important feature of E.coli to be preferred as a host cell is that under optimal growing conditions the cells divide every 20 minutes.

- Since the DNA is a hydrophilic molecule,it cannot pass through cell membranes, In order to force bacteria to take up the plasmid, the bacterial cells must first be made competent to take up DNA. This is done by treating them with a specific concentration of a divalent cation such as calcium. Recombinant DNA can then be forced into such cells by incubating the cells with recombinant DNA on ice, followed by placing them briefly at 420C (heatshock) and then putting them back on ice. This enables bacteria to take up the Recombinant DNA.

- For the expression of eukaryotic proteins, eukaryotic cells are preferred because to produce a functionally active protein it should fold properly and post translational modifications should also occur, which is not possible by prokaryotic cell (E.coli).

Methods of Gene Transfer

- The next step after a recombinant DNA molecule has been generated is to introduce it into a suitable host cell. There are many methods to introduce recombinant vectors and these are dependent on several factors such as the vector type and host cell.

- For achieving genetic transformation in plants, the basic pre-requisite is the construction of a vector which carries the gene of interestflanked by the necessary controlling sequences, i.e., the promoter and terminator, and deliver the genes into the host plant. There are two kinds of gene transfer methods in plants. It includes:

    - Direct or vectorless gene transfer
    - Indirect or vector – mediated gene transfer

Direct or Vectorless Gene Transfer

- In the direct gene transfer methods, the foreign gene of interest is delivered into the host plant without the help of a vector. The following are some of the common methods of direct gene transfer in plants.

- **Chemical mediated gene transfer:** Certain chemicals like polyethylene glycol (PEG) and dextran sulphate induce DNA uptake into plant protoplasts.

- **Microinjection:** The DNA is directly injected into the nucleus using fine tipped glass needle or micro pipette to transform plant cells. The protoplasts are immobilised on a solid support (agarose on a microscopic slide) or held with a holding pipette under suction.

- **Electroporation Methods of Gene Transfer:** A pulse of high voltage is applied to protoplasts, cells or tissues which makes transient pores in the plasma membrane through which uptake of foreign DNA occurs.

- **Liposome mediated method of Gene Transfer:** Liposomes the artificial phospholipid vesicles are useful in gene transfer. The gene or DNA is transferred from liposome into vacuole of plant cells. It is carried out by encapsulated DNA into the vacuole. This technique is advantageous because the liposome protects the introduced DNA from being damaged by the acidic pH and protease enzymes present in the vacuole. Liposome and tonoplast of vacuole fusion resulted in gene transfer. This process is called lipofection.

- **Biolistics:** The foreign DNA is coated onto the surface of minute gold or tungsten particles (1-3 μm) and bombarded onto the target tissue or cells using a particle gun (also called as gene gun/micro projectile gun/shotgun). Then the bombarded cells or tissues are cultured on selected medium to regenerate plants from the transformed cells.

Indirect or Vector-Mediated Gene Transfer

- Gene transfer is mediated with the help of a plasmid vector is known as indirect or vector mediated gene transfer. Among the various vectors used for plant transformation, the Ti-plasmid from Agrobacterium tumefaciens has been used extensively. This bacterium has a large size plasmid, known as Ti plasmid (Tumor inducing) and a portion of it referred as T-DNA (transfer DNA) is transferred to plant genome in the infected cells and cause plant tumors (crown gall). Since this bacterium has the natural ability to transfer T-DNA

region of its plasmid into plant genome, upon infection of cells at the wound site, it is also known as the natural genetic engineer of plants.

- The foreign gene (e.g.Bt gene for insect resistance) and plant selection marker gene, usually an antibiotic gene like npt II which confers resistance to antibiotic kanamycin are cloned in the T DNA region of Ti-plasmid in place of unwanted DNA sequences.

Screening for Recombinants

- After the introduction of r-DNA into a suitable host cell, it is essential to identify those cells which have received the r-DNA molecule. This process is called screening. The vector or foreign DNA present in recombinant cells expresses the characters, while the non-recombinants do not express the characters or traits. For this some of the methods are used and one such method is Blue-White Selection method.

Insertional Inactivation - Blue- White Colony Selection Method

- It is a powerful method used for screening of recombinant plasmid. In this method, a reporter gene lacZ is inserted in the vector. The lacZ encodes the enzyme β-galactosidase and contains several recognition sites for restriction enzyme.

- β-galactosidase breaks a synthetic substrates called X-gal (5-bromo-4-chloro-indolyl-β-D-galacto-pyranoside) into an insoluble blue coloured product. If a foreign gene is inserted into lacZ, this gene will be inactivated. Therefore, no-blue colour will develop (white) because β-galactosidase is not synthesized due to inactivation of lacZ. Therefore, the host cell containing r-DNA form white coloured colonies on themedium contain X-gal, whereas the other cells containing non-recombinant DNA will develop the blue coloured colonies. On the basis of colony colour, the recombinants can be selected.

Antibiotic resistant markers

- An antibiotic resistance marker is a gene that produces a protein that provides cells with resistance to an antibiotic. Bacteria with transformed DNA can be identified by growing on a medium containing an antibiotic. Recombinants will grow on these medium as they contain genes encoding resistance to antibiotics such as ampicillin, chloro amphenicol, tetracycline or kanamycin, etc., while others may not be able to grow in these media, hence it is considered useful selectable marker.

Replica plating technique

- A technique in which the pattern of colonies growing on a culture plate is copied. A sterile filter plate is pressed against the culture plate and then lifted. Then the filter is pressed against a second sterile culture plate. This results in the new plate being infected with cell in the same relative positions as the colonies in the original plate. Usually, the medium used in the second plate will differ from that used in the first. It may include an antibiotic or without a growth factor. In this way, transformed cells can be selected.

Molecular Techniques - Isolation of Genetic Material and Gel Electrophoresis

- Electrophoresis is a separating technique used to separate different biomolecules with positive and negative charges.

Principle

- By applying electricity (DC) the molecules migrate according to the type of charges they have. The electrical charges on different molecules are variable.

  + ve charged Cations will move towards -ve Cathode
  -ve charged Anions will move towards +ve Anode

Agarose GEL Electrophoresis

- It is used mainly for the purification of specific DNA fragments. Agarose is convenient for separating DNA fragments ranging in size from a few hundred to about 20000 base pairs. Polyacrylamide is preferred for the purification of smaller DNA fragments. The gel is complex network of polymeric molecules. DNA molecule is negatively charged molecule - under an electric field DNA molecule migrates through the gel. The electrophoresis is frequently performed with marker DNA fragments of known size which allow accurate size determination of an unknown DNA molecule by interpolation. The advantages of agarose gel electrophoresis are that the DNA bands can be readily detected at high sensitivity. The bands of DNA in the gel are stained with the dye Ethidium Bromide and DNA can be detected as visible fluorescence illuminated in UV light will give orange fluorescence, which can be photographed.

Nucleic Acid Hybridization - Blotting Techniques

- Blotting techniques are widely used analytical tools for the specific identification of desired DNA or RNA fragments from larger number of molecules. Blotting refers to the process of immobilization of sample nucleic acids or solid support (nitrocellulose or nylon membranes.) The blotted nucleic acids are then used as target in the hybridization experiments for their specific detection.

Types of Blotting Techniques

Southern Blotting: The transfer of DNA from agarose gels to nitrocellulose membrane.

Northern Blotting: The transfer of RNA to nitrocellulose membrane.

Western Blotting: Electrophoretic transfer of Proteins to nitrocellulose membrane.

Southern Blotting Techniques - DNA

- The transfer of denatured DNA from Agarose gel to Nitrocellulose Blotting or Filter Paper technique was introduced by Southern in 1975 and this technique is called Southern Blotting Technique.

Bioassay for Target Gene Effect

- Target gene is target DNA, foreign DNA, passenger DNA, exogenous DNA, gene of interest or insert DNA that is to be either cloned or specifically mutated. Gene targeting experiments have been targeting the nuclei and this leads to 'gene knock-out'. For this purpose, two types of targeting vectors are used. They are insertion vectors and replacement or transplacement vectors.

- Insertion vectors are entirely inserted into targeted locus as the vectors are linearized within the homology region. Initially, these vectors are circular but during insertion, become linear. It leads to duplication of sequences adjacent to selectable markers.

- The replacement vector has the homology region and it is co-linear with target. This vector is linearized prior to transfection outside the homology region and then consequently a crossing over occurs to replace the endogenous DNA with the incoming DNA.

Transfection: Introduction of foreign nucleic acids into cells by non-viral methods.

Genome Sequencing and Plant Genome Projects

- The whole complement of gene that determine all characteristic of an organism is called genome. The genome may be nuclear genome, mitochondrial genome or plastid genome. Genome of many plants contain both functional and non-expressive DNA proteins. Genome project refer to a project in which the whole genome of plant is analysed using sequence analysis and sequence homology with other plants. Such genome projects have so far been undertaken in Chlamydomonas(algae), Arabidopsis thaliana, rice and maize plants.

- Genome content of an organism is expressed in terms of number of base pairs or in terms of the content of DNA is expressed in c-value.

- **Genome sequencing:** The location of genes on the entire diploid chromosome of an organism.

Evolutionary pattern assessed using DNA.

- In recent years the evolutionary relationship between different plant taxa is assessed using DNA content as well as the similarities and differences in the DNA sequence (sequence homology). Based on such analysis the taxa and their relationship are indicated

in cladogram. Such cladogram will show the genetic distance between two taxa. It is also showed antiquity or modernity of any taxon with respect to one another.

Genome editing and CRISPR - Cas9

- Genome editing or gene editing is a group of technologies that has the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short form of Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

- Rice, was among the first plants to be used to demonstrate the feasibility of CRISPR-mediated targeted mutagenesis and gene replacement. The gene editing tool CRISPR can be used to make hybrid rice plants that can clone their seed. Imtiyaz Khand and Venkatesan Sundaresan and colleagues reported in a new study which clearly shows one can re-engineer rice to switch it from a sexual to an asexual mode.

RNA Interference (RNAi)

- All characters of organism are the result of expression of different genes which are regions of nuclear DNA. This expression involves transcription and translation. Transcription refers to the copying of genetic information from one strand of the DNA (called sense strand) by RNA. This RNA, as soon as it formed cannot be straight away sent to the cytoplasm to undertake the process of translation. It has to be edited and made suitable for translation which brings about protein synthesis. One of the main items removed from the RNA strand are the introns. All these changes before translation normally take place whereby certain regions of DNA are silence. However, there is an (RNAi) pathway. RNA interference is a biological process in which RNA molecules inhibit gene expression or translation. This is done by neutralisingtargetd mRNA molecules.

- A simplified model for the RNAi pathway is based on two steps, each involving ribonuclease enzyme. In the first step, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short interfering RNA (siRNA) by the RNase II enzymes called Dicer and Drosha. In the second step, siRNAs are loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target. This RNAi is seen in plant feeding nematodes.

Transgenic Plants / Genetically Modified Crops (Gm Crops)
Herbicide Tolerant – Glyphosate

- Weeds are a constant problem in crop fields. Weeds not only compete with crops for sunlight, water, nutrients and space but also a carrier for insects and diseases. If left uncontrolled, weeds can reduce crop yields significantly.

Transgenic plants contain a novel DNA introduced into its genome.

- Glyphosate herbicide produced by Monsanto, USA company under the trade name 'Round up' kills plants by blocking the 5-enopyruvate shikimate-3 phosphate synthase (EPSPS) enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant.

- One strategy is to incorporate a soil bacterium gene that produces a glyphosate tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

Advantages of Herbicide Tolerant Crops

- Weed control improves higher crop yields;
- Reduces spray of herbicide;
- Reduces competition between crop plant and weed;
- Use of low toxicity compounds which do not remain active in the soil; and
- The ability to conserve soil structure and microbes.

Herbicide Tolerant - Basta

- Trade name 'Basta' refers to a non-selective herbicide containing the chemical compound phosphinothricin. Basta herbicide tolerant gene PPT (L-phosphinothricin) was isolated from Medicago sativa plant. It inhibits the enzyme glutamine synthase which is involved in ammonia assimilation. The PPT gene was introduced into tobacco and transgenic tobacco produced was resistant to PPT. Similar enzyme was also isolated from Streptomyces hygroscopicus with bar gene encodes for PAT (Phosphinothricin acetyl transferase) and was introduced into crop plants like potato and sugar-beet and transgenic crops have been developed.

Insect resistance - Bt Crops:
Bt Cotton

- Bt cotton is a genetically modified organism (GMO) or genetically modified pest resistant plant cotton variety, which produces an insecticide activity to bollworm.

- Strains of the bacterium Bacillus thuringiensis produce over 200 different Bt toxins, each harmful to different insects. Most Bt toxins are insecticidal to the larvae of moths and butterflies, beetles, cotton bollworms and gatflies but are harmless to other forms of life.

- The genes are encoded for toxic crystals in the Cry group of endotoxin. When insects attack and eat the cotton plant the Cry toxins are dissolved in the insect's stomach.

- The epithelial membranes of the gut block certain vital nutrients thereby sufficient regulation of potassium ions are lost in the insects and results in the death of epithelial cells in the intestine membrane which leads to the death of the larvae.

Advantages

The advantages of Bt cotton are:
- Yield of cotton is increased due to effective control of bollworms.
- Reduction in insecticide use in the cultivation of Bt cotton
- Potential reduction in the cost of cultivation.

Disadvantages
Bt cotton has some limitations:

- Cost of Bt cotton seed is high.
- Effectiveness up to 120 days after that efficiency is reduced
- Ineffective against sucking pests like jassids, aphids and whitefly.
- Affects pollinating insects and thus yield

i. Bt Brinjal

- The Bt brinjal is another transgenic brinjal created by inserting a crystal protein gene (Cry1Ac) from the soil bacterium Bacillus thuringiensis into the genome of various brinjal cultivars. The insertion of the gene, along with other genetic elements such as promoters, terminators and an antibiotic resistance marker gene into the brinjal plant is accomplished using Agrobacterium-mediated genetic transformation. The Bt brinjal has been developed to give resistance against Lepidopteron insects, in particular the Brinjal Fruit and Shoot Borer (Leucinodesorbonalis).

ii. Dhara Mustard Hybrid (DMH)

- DMH -11 is transgenic mustard developed by a team of scientists Centre for Genetic Manipulation of Crop Plants at Delhi University under Government sponsored project. It is genetically modified variety of Herbicide Tolerant (HT) mustard. It was created by using "barnase/ barstar" technology for genetic modification by adding genes from soil bacterium that makes mustard, a self-pollinating plant. DMH -11 contains three genes viz. Bar gene, Barnase and Barstar sourced from soil bacterium. The bar gene had made plant resistant to herbicide named Basta.

Virus Resistance

- Many plants are affected by virus attack resulting in series loss in yield and even death. Biotechnological intervention is used to introduce viral resistant genes into the host plant so that they can resist the attack by virus. This is by introducing genes that produce resistant enzymes which can deactivate viral DNA.

FlavrSavr Tomato

- Agrobacterium mediated genetic engineering technique was followed to produce Flavr-Savr tomato, i.e., retaining the natural colour and flavor of tomato.

- Through genetic engineering, the ripening process of the tomato is slowed down and thus prevent it from softening and to increase the shelf life. The tomato was made more resistant to rotting by Agrobacterium mediated gene transfer mechanism of introducing an antisense gene which interferes with the production of the enzyme polygalacturonase, which help in delaying the ripening process of tomato during long storage and transportation.

Golden rice - Biofortification

- Golden rice is a variety of Oryza sativa (rice) produced through genetic engineering of biosynthesized beta-carotene, a precursor of Vitamin-A in the edible parts of rice developed by Ingo Potrykus and his group. The aim is to produce a fortified food to be grown and consumed in areas with a shortage of dietary Vitamin-A, which kills so many children under five year age. Golden rice differs from its parental strain by the addition of three beta-carotene biosynthesis genes namely 'psy' (phytoene synthase) from daffodil plant Narcissus pseudonarcissus and 'crt-1' gene from the soil bacterium Erwinia auredorora and 'lyc' (lycopene cyclase) gene from wild-type rice endosperm.

- The endosperm of normal rice, does not contain beta-carotene. Golden-rice has been genetically altered so that the endosperm now accumulates Beta-carotene. This has been done using Recombinant DNA technology. Golden rice can control childhood blindness - Xerophthalmia.

GM Food - Benefits

- High yield without pest
- 70% reduction of pesticide usage
- Reduce soil pollution problem
- Conserve microbial population in soil

Risks - believed to

- Affect liver, kidney function and cancer

- Hormonal imbalance and physical disorder
- Anaphylactic shock (sudden hypersensitive reaction) and allergies.
- Adverse effect in immune system because of bacterial protein.
- Loss of viability of seeds show in terminator seed technology of GM crops.

Polyhydroxybutyrate (PHB)

- Synthetic polymers are non-degradable and pollute the soil and when burnt add dioxin in the environment which cause cancer. So, efforts were taken to provide an alternative eco-friendly biopolymers. Polyhydroxyalkanoates (PHAs) and polyhydroxybutyrate (PHB) are group of degradable biopolymers which have several medical applications such as drug delivery, scaffold and heart valves. PHAs are biological macromolecules and thermoplastics which are biodegradable and biocompatible.

- Several microorganisms have been utilized to produce different types of PHAs including Gram-positive like Bacillus megaterium, Bacillussubtilis and Corynebacterium glutamicum, Gram-negative bacteria like group of Pseudomonas sp. and Alcaligenes eutrophus.

Polylactic acid (PLA)

- Polylactic acid or polylactide (PLA) is a biodegradable and bioactive thermoplastic. It is an aliphatic polyester derived from renewable resources, such as corn starch, cassava root, chips or starch or sugarcane. For the production of PLA, two main monomers are used: lactic acid, and the cyclic diester, lactide. The most common route is the ring-opening polymerization of lactide with metal catalysts like tin octoate in solution. The metal-catalyzed reaction results in equal amount of d and polylactic acid.

Green Fluorescent Protein (GFP)

- The green fluorescent protein (GFP) is a protein containing 238 amino acid residues of 26.9 kDa that exhibits bright green fluorescence when exposed to blue to ultraviolet range (395 nm). GFP refers to the protein first isolated from the jellyfish Aequorea victoria. GFP is an excellent tool in biology due to its ability to form internal chromophore without requiring any accessory cofactors, gene products, enzymes or substrates other than molecular oxygen. In cell and molecular biology, the GFP gene is frequently used as a reporter of expression. It has been used in modified forms to make biosensors.

Biopharming

- Biopharming also known as molecular pharming is the production and use of transgenic plants genetically engineered toproduce pharmaceutical substances for use of human beings. This is also called "molecular farming or pharming". These plants are different from medicinal plants which are naturally available. The use of plant systems as bioreactors is gaining more significance in modern biotechnology. Many pharmaceutical substances can be produced using transgenic plants. Example: Golden rice

Bioremediation

- It is defined as the use of microorganisms or plants to clean up environmental pollution. It is an approach used to treat wastes including wastewater, industrial waste and solid waste. Bioremediation process is applied to the removal of oil, petrochemical residues, pesticides or heavy metals from soil or ground water. In many cases, bioremediation is less expensive and more sustainable than other physical and chemical methods of remediation.Bioremediation process is a cheaper and eco-friendly approach and can deal with lower concentrations of contaminants more effectively. The strategies for bioremediation in soil and water can be as follows:

    - Use of indigenous microbial population as indicator species for bioremediation process.

    - Bioremediation with the addition of adapted or designed microbial inoculants.

    - Use of plants for bioremediation - green technology.

Some examples of bioremediation technologies are:

- **Phytoremediation** - use of plants to bring about remediation of environmental pollutants.

- **Mycoremediation** - use of fungi to bring about remediation of environmental pollutants.

- **Bioventing** is the process that increases the oxygen or air flow to accelerate the degradation of environmental pollutants.

- **Bioleaching** is the use of microorganisms in solution to recover metal pollutants from contaminated sites.

- **Bioaugmentation** is the addition of selected microbes to speed up degradation process.

- **Composting** is the process by which the solid waste is composted by the use of microbes into manure which acts as a nutrient for plant growth.

- **Rhizofiltration** is the uptake of metals or degradation of organic compounds by rhizosphere microorganisms.

- **Rhizostimulation** is the stimulation of plant growth by the rhizosphere by providing better growth condition or reduction in toxic materials.

Limitations

- Only biodegradable contaminants can be transformed using bioremediation processes.

- Bioremediation processes must be specifically made in accordance to the conditions at the contaminated site.

- Small-scale tests on a pilot scale must be performed before carrying out the procedure at the contaminated site.

- The use of genetic engineering technology to create genetically modified microorganism or a consortium of microbes for bioremediation process has great potential.

Biofuel: Algal Biofuel

- Algal fuel, also known as algal biofuel, or algal oil is an alternative to liquid fossil fuels, the petroleum products. This use algae as a source of energy-rich oils. Also, algal fuels are an alternative to commonly known biofuel sources obtained from corn and sugarcane. The energy crisis and the world food crisis have initiated interest in algal culture (farming algae) for making biodiesel and other biofuels using land unsuitable for agriculture. Botryococcusbraunii is normally used to produce algal biofuel.

Biological hydrogen production by algae

- The biological hydrogen production with algae is a method of photo biological water splitting. In normal photosynthesis the alga, Chlamydomonas reinhardtii releases oxygen. When it is deprived of sulfur, it switches to the production of hydrogen during photosynthesis and the electrons are transported to ferredoxins. [Fe]-hydrogenase enzymes combine them into the production of hydrogen gas.

Bioprospecting

- Bioprospecting is the process of discovery and commercialization of new products obtained from biological resources. Bioprospecting may involve biopiracy, in which indigenous knowledge of nature, originating with indigenous people, is used by others for profit, without authorization or compensation to the indigenous people themselves.

Biopiracy

- Biopiracy can be defined as the manipulation of intellectual property rights laws by corporations to gain exclusive control over national genetic resources, without giving adequate recognition or remuneration to the original possessors of those resources. Examples of biopiracy include recent patents granted by the U.S. Patent and Trademarks Office to American companies on turmeric, 'neem' and, most notably, 'basmati' rice. All three products are indigenous to the Indo-Pak subcontinent.

Biopiracy of Neem

- The people of India used neem and its oil in many ways to controlling fungal and bacterial skin infections. Indian's have shared the knowledge of the properties of the

neem with the entire world. Pirating this knowledge, the United States Department of Agriculture (USDA) and an American MNC (Multi Nation Corporation) W.R.Grace in the early 90's sought a patent from the European Patent Office (EPO) on the "method for controlling of diseases on plants by the aid of extracted hydrophobic neem oil". The patenting of the fungicidal and antibacterial properties of Neem was an example of biopiracy but the traditional knowledge of the Indians was protected in the end.

Biopiracy of Turmeric

- The United States Patent and Trademark Office, in the year 1995 granted patent to the method of use of turmeric as an antiseptic agent. Turmeric has been used by the Indians as a home remedy for the quick healing of the wounds and also for purpose of healing rashes. The journal article published by the Indian Medical Association, in the year 1953 wherein this remedy was mentioned. Therefore, in this way it was proved that the use of turmeric as an antiseptic is not new to the world and is not a new invention, but formed a part of the traditional knowledge of the Indians. The objection in this case US patent and trademark office was upheld and traditional knowledge of the Indians was protected. It is another example of Biopiracy.

Biopiracy of Basmati

- On September 2, 1997, the U.S. Patent and Trademarks Office granted Patent on "basmati rice lines and grains" to the Texas-based company RiceTec. This broad patent gives the company several rights, including exclusive use of the term 'basmati', as well proprietary rights on the seeds and grains from any crosses. The patent also covers the process of breeding RiceTec's novel rice lines and the method to determine the cooking properties and starch content of the rice grains.

- India had periled the United States to take the matter to the WTO as an infringement of the TRIPS agreement, which could have resulted in major embarrassment for the US. Hence voluntarily and due to few decisions take by the US patent office, Rice Tec had no choice but to lose most of the claims and most importantly the right to call the rice "Basmati". In the year 2002, the final decision was taken. Rice Tec dropped down 15 claims, resulting in clearing the path of Indian Basmati rice exports to the foreign countries. The Patent Office ordered the patent name to be changed to 'Rice lines 867'.

Applications of Biotechnology

- Biotechnology is one of the most important applied interdisciplinary sciences of the 21st century. It is the trusted area that enables us to find the beneficial way of life.

- Biotechnology has wide applications in various sectors like agriculture, medicine, environment and commercial industries.

- This science has an invaluable outcome like transgenic varieties of plants e.g. transgenic cotton (Bt-cotton), rice, tomato, tobacco, cauliflower, potato and banana.

- The development of transgenics as pesticide resistant, stress resistant and disease resistant varieties of agricultural crops is the immense outcome of biotechnology.

- The synthesis of human insulin and blood protein in E.coli and utilized forinsulin deficiency disorder in human is a breakthrough in biotech industries in medicine.

- The synthesis of vaccines, enzymes, antibiotics, dairy products and beverages are the products of biotech industries.

- Biochip based biological computer is one of the successes of biotechnology.

- Genetic engineering involves genetic manipulation, tissue culture involves aseptic cultivation of totipotent plant cell into plant clones under controlled atmospheric conditions.

- Single cell protein from Spirulina is utilized in food industries.

- Production of secondary metabolites, biofertilizers, biopesticides and enzymes.

- Biomass energy, biofuel, Bioremediation, phytoremediation for environmental biotechnology.

UNIT – 5- Plant Tissue Culture

- Growing plant protoplasts, cells, tissues or organs away from their natural or normal environment, under artificial condition, is known as Tissue Culture. It is also known as in vitro (In vitro is a Latin word, it means that - in glass or in test-tube) growth of plant protoplasts, cells, tissues and organs. A single explant can be multiplied into several thousand plants in short time period and space under controlled conditions. Tissue culture techniques are oft en used for commercial production of plants as well as for plant research. Plant tissue culture serves as an indispensable tool for regeneration of transgenic plants. Apart from this some of the main applications of Plant tissue culture are clonal propagation of elite varieties, conservation of endangered plants, production of virus-free plants, germplasm preservation, industrial production of secondary metabolites. etc., In this chapter let us discuss the history, techniques, types , applications of plant tissue culture and get aware on ethical issues. Gottlieb Haberlandt (1902) the German Botanist proposed the concept Totipotency and he was also the first person to culture plant cells in artificial conditions using the mesophyll cells of Lamium purpureum in culture medium and obtained cell proliferation. He is regarded as the father of tissue culture.

Basic concepts of Tissue Culture

- Basic concepts of plant tissue culture are totipotency, differentiation, dedifferentiation and redifferentiation.

Totipotency

- The property of live plant cells that they have the genetic potential when cultured in nutrient medium to give rise to a complete individual plant.

Differentiation

- The process of biochemical and structural changes by which cells become specialized in form and function.

Redifferentiation

- The further differentiation of already differentiated cell into another type of cell. For example, when the component cells of callus have the ability to form a whole plant in a nutrient medium, the phenomenon is called redifferentiation.

Dedifferentiation

- The phenomenon of the reversion of mature cells to the meristematic state leading to the formation of callus is called dedifferentiation. These two phenomena of redifferentiation and dedifferentiation are the inherent capacities of living plant cells or tissue. This is described as totipotency.

Plant Tissue Culture (PTC):

- Plant tissue culture is used to describe the in vitro and aseptic growth of any plant part on a tissue culture medium. This technology is based on three fundamental principles:
- The plant part or explant must be selectedand isolated from the rest of plant body.
- The explant must be maintained in controlled physically (environmental) and chemicallydefined (nutrient medium) conditions.

Explant: The tissue taken from a selected plant transferred to a culture medium often to establish a new plant.

Laboratory Facilities for PTC

For PTC, the laboratory must have the following facilities:

- Washing facility for glassware and ovens fordrying glassware.
- Medium preparation room with autoclave,electronic balance and pH meter.
- Transfer area sterile room with laminarair-flow bench and a positive pressureventilation unit called High EfficiencyParticulate Air (HEPA) filter to maintainaseptic condition.
- **Culture facility:** Growing the explantinoculated into culture tubes at 22-28° Cwith illumination of light 2400 lux, with aphotoperiod of 8-16 hours and a relativehumidity of about 60%.

Technique Involved in PTC
Sterilization:

- Sterilization is the technique employed to getrid of microbes such as bacteria and fungi in theculture medium, vessels and explants.
- **Maintenance of Aseptic Environment:**During in vitro tissue culture maintenance ofaseptic environmental condition should befollowed, i.e., sterilization of glassware, forceps,scalpels, and all accessories in wet steamsterilization by autoclaving at 15 psi (121°C)for 15 to 30 minutes or dipping in 70% ethanolfollowed by flaming and cooling.
- **Sterilization of culture room:** Floor andwalls are washed first with detergent and thenwith 2% sodium hypochlorite or 95% ethanol.The cabinet of laminar airflow is sterilized byclearing the work surface with 95% ethanol andthen exposure of UV radiation for 15 minutes.
- **Sterilization of Nutrient Media:** Culturemedia are dispensed in glass containers, plugged with non-absorbent cotton or sealed with plastic closures and then sterilized

using autoclave at15 psi (121°C) for 15 to 30 minutes. The plantextracts, vitamins, amino acids and hormonesare sterilized by passing through Millipore filterwith 0.2 mm pore diameter and then addedto sterilized culture medium inside LaminarAirflow Chamber under sterile condition.

- **Sterilization of Explants:** The plantmaterials to be used for tissue culture should besurface sterilized by first exposing the materialin running tap water and then treating it insurface sterilization agents like 0.1% mercuricchloride, 70% ethanol under aseptic conditioninside the Laminar Air Flow Chamber.

Media Preparation

- The success of tissue culture lies in thecomposition of the growth medium, plantgrowth regulators and culture conditionssuch as temperature, pH, light and humidity.No single medium is capable of maintainingoptimum growth of all plant tissues. Suitablenutrient medium as per the principle of tissueculture is prepared and used.

- MS nutrient medium (Murashige and Skoog 1962) is commonly used. It has carbon sources, with suitable vitamins and hormones. The media formulations available for plant tissue culture other than MS are B5 medium (Gamborg.et.al 1968), White medium (white 1943), Nitsch's medium (Nitsch & Nitsch 1969). A medium may be solid or semisolid or liquid. For solidification, a gelling agent such as agar is added.

Agar: A complex mucilaginous polysaccharide obtained from marine algae (sea weeds) used as solidifying agent in media preparation

Culture condition
Ph

- The pH of medium is normally adjusted between 5.6 to 6.0 for the best result.

Temperature

- The cultures should be incubated normally at constant temperature of 25°C ± 2°C for optimal growth.

Humidity and Light Intensity

- The cultures require 50-60% relative humidity and 16 hours of photoperiod by the illumination of cool white fluorescent tubes of approximately 1000 lux.

Aeration

- Aeration to the culture can be provided by shaking the flasks or tubes of liquid culture on automatic shaker or aeration of the medium by passing with filter-sterilized air.

Induction of Callus

- Explant of 1-2 cm sterile segment selected from leaf, stem, tuber or root is inoculated (transferring the explants to sterile glass tube containing nutrient medium) in the MS nutrient medium supplemented with auxins and incubated at 25°C ± 2°C in an alternate light and dark period of 12 hours to induce cell division and soon the upper surface of explant develops into callus. Callus is a mass of unorganized growth of plant cells or tissues in in vitro culture medium

Embryogenesis

- The callus cells undergoes differentiation and produces somatic embryos, known as Embryoids. The embryoids are sub-cultured to produce plantlets.

Hardening

- The plantlets developed in vitro require ahardening period and so are transferred togreenhouse or hardening chamber and then tonormal environmental conditions.

- Hardening is the gradual exposure of in vitrodeveloped plantlets in humid chambers indiffused light for acclimatization so as to enablethem to grow under normal field conditions.

Types of Plant tissue cultures

Based on the explants some other plant tissue culture types are

- Organ culture
- Meristem culture
- Protoplast culture
- Cell culture.

Organ culture

- The culture of embryos, anthers, ovaries, roots, shoots or other organs of plants on culture media.

Meristem Culture:

- The culture of any plant meristematic tissue onculture media.

Protoplast Culture:

- Protoplasts are cells without a cell wall, butbounded by a cell membrane or plasmamembrane. Using protoplasts, it is possible toregenerate whole plants from single cells andalso develop somatic hybrids. The steps involved in protoplast culture.

Isolation of protoplast:

- Small bits of planttissue like leaf tissue are used for isolation ofprotoplast. The leaf tissue is immersed in 0.5%Macrozyme and 2% Onozuka cellulase enzymesdissolved in 13% sorbitol or mannitol at pH 5.4.It is then incubated over-night at 25°C. After agentle teasing of cells, protoplasts are obtained,and these are then transferred to 20% sucrosesolution to retain their viability. They are thencentrifuged to get pure protoplasts as differentfrom debris of cell walls.

Fusion of protoplast:

- It is done through theuse of a suitable fusogen. This is normally PEG(Polyethylene Glycol). The isolated protoplastare incubated in 25 to 30% concentration ofPEG with Ca++ ions and the protoplast showsagglutination (the formation of clumps of cells)and fusion.

Culture of protoplast:

- MS liquid medium is used with some modification in droplet, platingor micro-drop array techniques. Protoplastviability is tested with fluorescein diacetatebefore the culture. The cultures are incubated incontinuous light 1000-2000 lux at 25°C. The cellwall formation occurs within 24-48 hours andthe first division of new cells occurs between2-7 days of culture.

Selection of somatic hybrid cells:

- Thefusion product of protoplasts without nucleus ofdifferent cells is called a cybrid. Following thisnuclear fusion happen. This process is calledsomatic hybridization.

Cell Suspension Culture

- The growing of cells including the cultureof single cells or small aggregates of cellsin vitroin liquid medium is known as cellsuspension culture. The cell suspension isprepared by transferring a portion of callus tothe liquid medium and agitated using rotary shaker instrument. The cells are separated from the callus tissue and used for cell suspension culture.

Production of Secondary Metabolites

- Cell suspension culture can be useful for the production of secondary metabolites like alkaloids, flavonoids, terpenoids, phenolic compounds and recombinant proteins. Secondary metabolites are chemical compounds that are not required by the plant for normal growth and development but are produced in the plant as 'byproducts' of cell metabolism. For Example: Biosynthesis and isolation of indole alkaloids from Catharanthus roseusplant cell culture.

- The process of production of secondary metabolites can be scaled up and automated using bio-reactors for commercial production. Many strategies such as biotransformation, elicitation and immobilization have been used to make cell suspension cultures more

efficient in the production of secondary metabolites. Few examples of industrially important plant secondary metabolites are listed below in the table

Secondary metabolites	Plant source	Uses
Digoxin	Digitalis purpuria	Cardiac tonic
Codeine	Papaver sominiferum	Analgesic
Capsaicin	Capsicum annum	Rheumatic pain treatment
Vincristine	Catharanthus roseus	Anti-carcinogenic
Quinine	Cinchona officinalis	Antimalarial

Somatic Embryogenesis

- Somatic embryogenesis is the formation of embryos from the callus tissue directly and these embryos are called Embryoids or from the in vitro cells directly form pre-embryonic cells which differentiate into embryoids.

Applications

- Somatic embryogenesis provides potentialplantlets which after hardening period can establish into plants.
- Somatic embryoids can be be used for theproduction of synthetic seeds.
- Somatic embryogenesis is now reportedin many plants such as Allium sativum, Hordeum vulgare,Oryza sativa, Zea mays and this possible in any plant.

Synthetic seeds are produced by encapsulation of embryoids in agarose gel or calcium alginate.

Applications of Plant Tissue Culture

Plant tissue culture techniques have several applications such as:

- Improved hybrids production throughsomatic hybridization.
- Somatic embryoids can be encapsulatedinto synthetic seeds (synseeds). Theseencapsulated seeds or synthetic seeds helpin conservation of plant biodiversity.
- Production of disease resistant plantsthrough meristem and shoot tip culture.
- Production of stress resistant plants likeherbicide tolerant, heat tolerant plants.
- Micropropagation technique to obtainlarge numbers of plantlets of both crop andtree species useful in forestry within a shortspan of time and all through the year.

- Production of secondary metabolites fromcell culture utilized in pharmaceutical,cosmetic and food industries.

Somaclonal variations: Somatic variations found in plants regenerated in vitro (i.e. variations found in leaf, stem, root, tuber or propagule)

Gametoclonal variations: Gametophytic variations found in plants regenerated in vitro gametic origin (i.e. variations found in gametes and gametophytes)

Artificial Seed

- Artificial seeds or synthetic seeds (synseeds) are produced by using embryoids (somatic embryos) obtained through in vitro culture. They may even be derived from single cells from any part of the plant that later divide to form cell mass containing dense cytoplasm, large nuclceus, starch grains, proteins, and oils etc., To prepare the artificial seeds different inert materials are used for coating the somatic embryoids like agrose and sodium alginate

Advantages of Artificial seeds

Artificial seeds have many advantages over the true seeds

- Millions of artificial seeds can be producedat any time at low cost.
- They provide an easy method to producegenetically engineered plants with desirable traits.
- It is easy to test the genotype of plants.
- They can potentially stored for long timeunder cryopreservation method.
- Artificial seeds produce identical plants
- The period of dormancy of artificial seeds isgreatly reduced, hence growth is faster witha shortened life cycle.

Virus-free plants

- The field grown plants like perennial crops, usually are infected by variety of pathogens like fungi, bacteria, mycoplasma, viruses which cause considerable economic losses. Chemical methods can be used to control fungal and bacterial pathogens, but not viruses generally.
- Shoot meristem tip culture is the method to produce virus-free plants, because the shoot meristem tip is always free from viruses.

Germplasm Conservation

- Germplasm conservation refers to the conservation of living genetic resources like pollen, seeds or tissue of plant material maintained for the purpose of selective plant breeding, preservation in live condition and used for many research works.

- Germplasm conservation resources is a part of collection of seeds and pollen that are stored in seed or pollen banks, so as to maintain their viability and fertility for any later use such as hybridization and crop improvement. Germplasm conservation may also involve a gene bank, DNA bank of elite breeding lines of plant resources for the maintenance of biological diversity and also for food security.

Cryopreservation (–195.C)

- Cryopreservation, also known asCryo-conservation, is a process by which protoplasts, cells, tissues, organelles, organs, extracellular matrix, enzymes or any other biological materials are subjected to preservation by cooling to very low temperature of –196°C using liquid nitrogen. At this extreme low temperature any enzymatic or chemical activity of the biological material will be totally stopped and this leads to preservation of material in dormant status. Later these materials can be activated by bringing to room temperature slowly for any experimental work.

- Protective agents like dimethyl sulphoxide, glycerol or sucrose are added before cryopreservation process. These protective agents are called cryoprotectants, since they protect the cells, or tissues from the stress of freezing temperature.

Intellectual Property Right (IPR)

- Intellectual property right (IPR) is a category of property that includes intangible creation of the human intellect, and primarily consists of copyrights, patents, and trademarks. It also includes other types of rights, such as trade secrets, publicity rights, moral rights, and rights against unfair competition.
- In biotechnology, the transformedmicroorganisms and plants and technologies for the production of commercial productsare exclusively the property of the discoverer.
- The discoverer has the full rights on hisproperty. It should not be neglected by theothers without legal permission.
- The right of discoverer must be protectedand it does by certain laws framed by acountry.
- The IPR is protected by different wayslike patents, copyrights, trade secrets and trademarks, designs and geographical indications.

Patents

- It is a special right to the discoverer/inventorthat has been granted by the governmentthrough legislation for trading new articles.

- A patent is a personal property whichcan be licensed or sold by the person ororganisation just like any other property.

- Patent terms give the inventor the rights toexclude others from making, using or sellinghis invention.

- It is difficult to keep secret certain inventionsand therefore, guidance should be obtainedfrom a qualified patent attorney.

- A patent consists of three parts: the grant,specifications and claims.

- The grant is filled at the patent office whichis not published. It is a signed document,actually the agreement that grants patentright to the inventor.

- The specification and claims are publishedas a single document which is made publicfrom the patent office. The specification partis narrative in which the subject matter ofinvention is described as how the inventionwas carried out.

- The claim specifically defines the scope ofthe invention to be protected by the patentwhich the others may not practice.

Biosafety and Bioethics

- Advances in biotechnology and their applications are mostly associated with controverisies. This is because the major part of the modern biotechnology deals with genetic manipulations. ELSI which represents ethical, legal and social implications of biotechnology broadly covers the relationship between biotechnology and society with particular reference to ethical and legal aspects.

Biosafety

- Biosafety is the prevention of large-scale loss of biological integrity, focusing both on ecology and human health. These prevention mechanisms include conduction of regular reviews of the biosafety in laboratory settings, as well as strict guidelines to follow. Biosafety is used to protect from harmful incidents. Many laboratories handling biohazards employ an ongoing risk management assessment and enforcement process for biosafety. Failures to follow such protocols can lead to increased risk of exposure to biohazards or pathogens. Human error and poor techniques contribute to unnecessary exposure to hazards and compromise the best safeguards set into place for protection.

Potential risks and consideration for safety aspects

- Pathogenicity of living organisms andviruses - natural and genetically modified-to infect humans, animals and plants tocause diseases.

- Toxicity of allergy associated with microbialproduction.

- Increasing number of antibiotic resistantpathogenic microorganisms.

- Problems associated with the disposal ofspent microbial biomass and purification ofeffluent from biotechnological process.

- Safety aspects associated with contamination, infection or mutation of process strains.

- Safety aspects associated with the industrialuse of microorganisms containing in vitrorecombinants.

Biosafety guidelines are being implemented by:

- The Institutional Bio-safety Committees(IBSCs) monitor the research activity atinstitutional level.

- The Review Committee on GeneticManipulation (RCGM) functioning inthe Department of Biotechnology (DBT)monitors the risky research activities in thelaboratories.

- The Genetic Engineering ApprovalCommittee (GEAC) of Ministry ofEnvironment and Forest has the powerto permit the use of Genetically ModifiedOrganism (GMO) at commercial level andopen field trials of transgenic materialsincluding agricultural crops, industrialproducts and health care products.

Bioethics - Ethical, Legal and Social Implications (ELSI)

- Bioethics refers to the study of ethical issues emerging from advances in biology and medicine. It is also a moral discernment as it relates to medical policy and practice. Bioethicists are concerned with the ethical questions that arise in the relationships amonglife sciences, biotechnology and medicine. It includes the study of values relating to primary care and other branches of medicine.The scope of bioethics is directly related to biotechnology, including cloning, gene therapy, life extension, human genetic engineering, astroethics life in space, and manipulation of basic biology through altered DNA, RNA and proteins. These developments in biotechnology will affect future evolution, and may require new principles, such as biotic ethics, that values life and its basic biological characters and structures.

- The Ethical, Legal, and Social Implications (ELSI) program was founded in 1990 as an integral part of the Human Genome Project. The mission of the ELSI program was to identify and address issues raised by genomic research that would affect individuals, families, and society. A percentage of the Human Genome Project budget at the National Institutes of Health and the U.S. Department of Energy was devoted to ELSI research.

Ethical issues in Genomic Research

- Privacy and fairness in the use of geneticinformation, including the potential forgenetic discrimination in employment andinsurance.

- The integration of new genetic technologies,such as genetic testing, into the practice ofclinical medicine.

- Ethical issues surrounding the design andconduct of genetic research with people,including the process of informed consent.

Genetic Engineering Appraisal Committee (GEAC)

- GEAC is an apex body under Ministry of Environment, Forests and Climate change for regulating manufacturing, use, import, export and storage of hazardous microbes or genetically modified organisms (GMOs) and cells in the country. It was established as an apex body to accord approval of activities involving large scale use of hazardous microorganismsand recombinants in research and industrial production. The GEAC is also responsible for approval of proposals relating to release of genetically engineered organisms and products into the environment including experimental field trials (Biosafety Research Level trial-I and II known as BRL-I and BRL-II).

Future of Biotechnology

- Biotechnology has become a comprehensive scientific venture from the point of academic and commercial angles, within a short time with the sequencing of human genome and genome of some important organisms. The future developments in biotechnology will be exciting. Thus the development in biotechnology will lead to a new scientific revolution that would change the lives and future of people. Like industrial and computer revolution, biotechnological revolution will also promise major changes in many aspects of modern life.
-

Human Diseases

6th Term I

Unit 6 – Health & Hygiene

- As defined by World Health Organization (WHO), it is a "State of complete physical, mental, and social well being, and not merely the absence of disease or infirmity." Health is a dynamic condition resulting from a body's constant adjustment and adaptation in response to stresses and changes in the environment for maintaining an inner equilibrium called homeostasis.

- Hygiene is a science of the establishment and maintenance of health conditions or practices (as of cleanliness) conducive to health has poor personal hygiene. Brushing your teeth regularly is an important part of good oral hygiene. Hygiene is the practice of keeping yourself and your surroundings clean, especially in order to prevent illness or the spread of diseases.

Components of Food

- The Chemical constituents of food which give us energy, help to build our body and protect us from diseases are called Nutrients.

- Carbohydrate
- Proteins
- Fats
- Vitamins
- Minerals
- Water

Carbohydrates

Carbohydrates are energy giving component of the food.

S.No	Form of Carbohydrates	Sources
1	Sugar	Fruits, Honey, Cane Sugar, Sugar Beet
2	Starch	Rice, Wheat, Maize, Potato, etc.
3	Dietary fibre	Whole grain, nuts,etc.

Fats

- Fat is also an energy-giving food and provides more energy than Carbohydrates. Some important sources of fats are butter, ghee, milk, cheese, paneer, nuts, meat, fish, egg yolk etc. Apart from giving energy, they insulate our body and protect the cells

PROTEIN

Body Building Foods

- Proteins are necessary for our growth and repair, as well as for regulating various body functions such as digestion. The sources of proteins are pulses, eggs, fish, milk, chicken, soya bean, nut, grams etc, Proteins are body building foods.

Soyabean is the highly rich source of protein

Vitamins

- Vitamins are required for carrying out various biochemical reactions in our body. Fruits, vegetables, grains, meat products are good sources of vitamins. Vitamins are called as protective food. There are six major vitamins A, B, C, D, E and K. Vitamins B and Vitamins C are water soluble, Vitamins A, D, E and K are fat soluble.

Vitamin	**Found abundantly in**	**Disease we get if deficient in this**	**Symptoms**
Vitamin A	Fish oil, egg, milk, ghee, carrot, corn, Yellow fruits, greens	Night blindness	Poor vision, difficulty in seeing in dim light
Vitamin B	Whole grain, unpolished rice, milk, fish, meat, peans, lentils Green Vegetables	Beriberi	Nervous weakness, fatigue.
Vitamin C	Oranges, Gooseberry, Greenchilly, Tomato	Scurvy	Bleeding gums
Vitamin D	Fish oil, milk and eggs. It is also made in our skin using sunlight	Rickets	Weak, flexible bones.
Vitamin E	Vegetable oils, Green Vegetables, whole wheat, Mango, apple, greens	Nervous weakness, dimming of eyesight	Childlessness, lack of resistance power to illnesses
Vitamin K	Green Vegetables, Tomato, cabbage, eggs, milk products.	Weakness of the bones, teeth etc.	Even a small cut bleeds profusely.

Gooseberries contains nearly 20 times the vitamin C than Orange

Minerals

- Mineralsare required for growth as well as for the regulation of normal body function.Green leafy vegetables like spinach, pulses, eggs, milk, fish and fruits are important sources of minerals in our diet. Minerals are also a protective foods.

Minerals	Functions
Calcium	Strong bones and teeth, clotting of blood
Phosphorus	Strong bones and teeth
Iodine	Synthesis of thyroid hor- mone
Iron	Formation of haemoglobin and brain development

80% of the world production of Moringa Leaves is in India. The Major countries which import Moringa Leaves are China, US, Germany, Canada, South Korea and European countries

Water

- Our body needs an adequate supply of water is order to maintain good health. Any human being should take minimum eight tumblers (2 Litres) of water every day.

Health

- Health is a state of complete physical, mental and social well-being and not merely absence of diseases. Eating a healthy diet keeps you physically and mentally fit. When you are physically healthy, you feel confident you are more outgoing and have a greater capacity for enjoying life.

- Unhealthy food choices lead to obesity and illness, preventing you from socializing with friends and family. So choose your diet carefully.

Balanced Diet

- A diet should contain adequate amount of all the necessary nutrients required for healthy growth and activity.

- An increased capacity to work
- Good physical and mental health
- Increased capacity to resist diseases.
- Help in proper growth of the body.

- A balanced diet contains sufficient amount of various nutrients to ensure good health. Food should also provide the appropriate amount of energy and adequate amount of water.

Malnutrition:

- Malnutrition occurs when all the nutrients that the body needs are not obtained in the proper proportions from the diet. The word malnutrition refers to the condition that results when a person does not take a balanced diet. Malnutrition leads to deficiency disease. The diseases that are caused due to lack of Nutrients in the diet are called Deficiency Diseases.

India has the second highest number of obese children in the world after China, according to a study that has found that 14.4 million children in the country have excess weight.

Protein and Mineral Deficiency Diseases

Protein Diseases	**Symptoms**
Kwashiorkar	Stunted growth, swelling of face and limbs, Diarrhoea.
Marasmus	Skinny appearance, slow body growth.

Mineral	**Deficiency Disease**
Calicum	Rickets
Phosphorus	Osteomalatia
Iodine	Cretinism (in child) Goitre (in adult)
Iron	Anaemia

Physical Exercise and Rest

- Physical exercise is any bodily activity that enhances or maintains physical fitness and overall health and wellness. It is performed for various reasons, including

- increase in growth and development,
- strengthening muscles and the cardiovascular system,
- developing athletic skills, weight loss or maintenance, and enjoyment.

- Physical exercise may help to decrease some of the effects of childhood and adult obesity.

Deep sleep seems to be one of the most critical time for body repair

REST

- Proper amount of rest is essential for physical and mental health. Rest is as important as nutrition and physical activity for growth and development and good health.

Personal Cleanliness

- Hygiene is a set of practices performed to preserve health. According to the World Health Organization (WHO), "Hygiene refers to conditions and practices that help to maintain health and prevent the spread of diseases."

- **Personal hygiene** involves those practices performed by an individual to care for one's bodily health and well being, through cleanliness. It includes such personal habit choices as how frequently to bathe, wash hands, trim fingernails, and change clothing. It also includes attention to keep surfaces in the home and workplace, including bathroom facilities, clean and pathogen-free.

Introduction of Microbes

- The diseases or conditions caused by microorganism due to the negligence of personal hygiene.

- Diarrhoea
- Tooth decay
- Athlete's foot(Madurai's foot)
- Dandruff.

Most of the microbes belong to four major groups:

- Bacteria
- Virus
- Protozoa
- Fungi

Bacteria

- Bacteria are very small prokaryotic microorganisms.
- Bacterial cells do not have nucleus and do not usually have membrane bound organelles.

- Bacteria can exist either as independent organisms or as parasites
- They invade tissues
- They produce pus or harmful wastes

Bacterial Diseases

S.No	Bacterial diseases	Mode of transmission
1	Cholera	Contaminated water
2	Pneumonia	Inhalation of airborne droplets from a sneeze or cough
3	Tetanus	Contamination of wounds with the bacteria.
4	Tuberculosis	Inhalation of airborne droplets from

		sneeze or cough
5	Typhoid	Contaminated food or water

Disease
Disease is a definite pathological process having a characteristic set of signs and symptoms.
Disorder
Disorder is a derangement or abnormality of function.

Virus

- Virus is an acellular agent that replicates only inside the cells of other living organisms. Virus can infect all types of life forms plant, animals and microorganisms. They invade living normal cells and use their cell machinery to multiply. They can kill damage or change the cells and make you sick.

Diseases Caused By Virus

- Common cold
- Influenza
- Hepatitis
- Polio
- Smallpox
- Chicken pox
- Measles

Microscopes help to study the structure of the microorganisms

7th Term I

Unit 6 – Health & Hygiene

- Health is the best wealth. If you have good health, you will have a sound mind and you will gain good knowledge and wealth also. To maintain good health, you should follow good hygiene, eat nutritious food, do exercise, take rest and have a sound sleep.

- It is also refers to a state of a sound mind and body free from any sickness or ailment, stress and problems. In simple words, health refers to the physical, emotional and psychological well-being of a person.

- Hygiene refers to the good habits and their practices which is followed to prevent diseases, maintain good health, especially through cleanliness, consumption of safe drinking water and proper disposal of sewage. It refers to all those activities that are done for improving and maintaining good health and sound mind.

Cleanliness

- Cleanliness refers to the maintenance of personal and environmental hygiene. In simple words, It refers to the state of being clean which is essential for good health. To protect us from diseases it is essential to maintain good health by taking regular bath, cleaning the clothes and surroundings and also avoiding unhygienic food consumption.

- Personal hygiene is defined as "the branch of health which is concerned with the individual's adjustment to the physiological needs of the body and mind for the attainment of the maximum level of health, it also refers to the cleaning and grooming of the body.

- Colds and the flu are common communicable diseases. It is caused not only by bacteria but also by virus. When you have cold and flu, you may also have running nose, cough, sore throat, and sometimes fever or pain in the joints. For some, this condition may also lead to mild diarrhoea.

- Secretions oozing out from the nose may contains the bacteria or virus. When the patient touch the nose and some other object or someone else the virus is transferred. When the patient sneezes or coughs the virus comes out with the droplets and become airborne. Hence it is a good practice for the patientwith cold and flu to use a handkerchief to blow the noses and also wash the hands often to ensure that they do not accidentally spread the virus to others.

Community Hygiene

- A community is formed by a group of people living together in a particular area. If the people in a community wish to lead a healthy life, they should maintain basic community hygiene. It can be done by adopting the following measures.

- The surroundings should be kept clean.
- Drains should be covered properly.
- The domestic wastes should be segregated and properly disposed off safely in separate dust bins provided by the Government (Green and Blue).
- Used water from houses should not be let out into open drains or open areas

Dengue is spread by mosquitoes of Aedesaegypti caused by DEN-1, 2 virus belonging to the type- flavivirus. It decrease counting of the blood platelets of human blood and it has a maximum flight range of 50–100 meters in and around the places.

Care of the body

- A human body is a massive miracle. It consists of organs and systems, which functions day in and out. Our body in compared to a machine. The human body systems work well with proper maintenance and guidance. For smooth functioning, all the parts of the body should work in unison. The digestive system, circulatory and muscular system is the core systems that should be in synchronization and functioning well. So keep them well by proper care.

Dental Care

- Dental care or broadly speaking oral hygiene is an important aspect of the personal health of an individual. Good oral hygiene implies sound teeth and healthy gums with healthy surrounding tissues. The physical act of chewing food promotes saliva and gastric secretions which helps digestion. The act of chewing and tasting is called 'mastication'. It gives pleasure and emotional satisfaction of eating food. Teeth is essential for good appearance and clear speech also.

- When you brush two times a day, it will prevent the formation of tartar and plaque on your teeth and gums.
- When you Floss, it will remove food particles, plaque, and bacteria which build up between your teeth. When you start flossing, your gums may bleed a little bit, but after few days that will be stopped. It should be started only with proper medical guidance.

Diseases affecting the teeth

- Diseases affecting the teeth and gums, their causative agents and remedial measure are given below:

S.No	Name of the Diseases	Causative Agents	Impacts/ Consequences	Remedial measures
1	Bleeding Gums	Vitamin C deficiency	Bleeding of the gums	Eating citrus fruits.
2	Tooth decay	Bacteria in plaque	Bacteria produce acids	Brushing and flossing the teeth can prevent decay.
3	Periodontitis	Tobacco chewing	Severe form of gum disease ruin the bones, gums, and other tissues.	Chewing type of tobacco should be avoided. Eat a well – balanced diet.

Eye Care

- Eyes are an important organ of our body. They are considered as "windows to the world". Eyesight is the most important sense. 80% of what we perceive comes through the sense of sight. The protection of the eyes, can reduce the odds of blindness and vision loss. we protection of our eye from the diseases, surroundings, climate condition.

Diseases affecting Eye

Disease affecting the eye and their remedial measure are given below:

Hair Care

- The condition of the hair reflects to some extent the nutritional status and general health of the body. Thin, sparse hair and the loss of hair indicates a poor nutritional status. The deficiencies in diet, physical and mental illness of various kinds may also leads to premature graying of hair.

- The hair follicles from which the hair grows produce oil which keeps the hair smooth. The sweat and the dead skin cells come off the scalp.

- The oil, sweat and dead cells all add together and can make the hair greasy and look dirty unless it is washed regularly.

S.No	Name of the Disease	Causative Agents	Impacts/ Consequences	Remedial measures
1	Night Blindness	A lack of vitamin A, a	Makes it hard to see well at night	Eat food rich in anti – oxidant,

		disorder of the cells in your retina	or in poor light.	vitamins and minerals.
2	Conjunctivitis (Pink eye)	Caused by a virus and bacteria	One or both eyes can be affected. Highly contagious; can be spread by contamination and sneezing	Antibiotic eye drops or ointments, home remedy.
3	Color blindness	Genetic condition	Difficulty distinguishing between colours Inability to see shades or tones of the same colour.	There is no known cure for colour blindness. contact lenses and glasses with filters

To keep the hair clean and healthy:

- The regular hair wash and massage of the scalp will remove the dead skin cells, excess oil and dust.

- Rinsing the hair well with clear water and using good toothed comb for hair dressing is highly essential for their maintenance.

Diseases

- A disease is the functional or physical change from a normal state that affects the health of a person by causing disability or discomfort. The following are reasons that could leads to the development of disease in an individual.

- Infection caused by disease-causing microbes
- Lack of balanced diet
- Poor lifestyle and unhealthy habits
- Malfunctioning of one or more body parts or organs.

Different kinds of sickness and their causes

- The prevention and treatment of sickness can be considered in two groups for their better understanding. They are, communicable and non-communicable disease.

Communicable Diseases

- Communicable diseases are those that spread from one person to another. Healthy persons must be protected from people with communicable diseases. Diseases spread through contaminated air, water, food or vectors (insects and other animals).

Diseases Caused by Bacteria

- Communicable disease (like tuberculosis, Cholera and Typhoid), which are caused by microbes and spread through air, water and some other organisms also.

Tuberculosis:

- TB is caused by Mycobacterium tuberculae and spreads from one person to another person through air by spitting and prolonged contact with sharing materials of the patient. The symptoms are fever, weight loss, chronic cough, bloody spitting and difficulty in breathing.

Prevention and treatment

- BCG vaccination,
- Giving special attention to the patient,
- Regular medication like DOT

Cholera:

- Cholera is caused by Vibrio cholera and spread through the consumption contaminated food or water. The symptoms of Cholera is Vomiting, severe diarrhea and cramps in legs

Prevention and treatment

- Good hygienic practices like, washing hands before eating.
- Avoid eating uncovered food from street vendoers.
- Drinking boiled water.
- Getting Vaccination against cholera

Typhoid:

- Typhoid is caused by Salmonella typhi and spreads by contaminated food and water. The symptoms are Anorexia, headache, rashes on abdomen, dysentery and high fever up to 1040F.

Prevention and treatment

- Drinking boiled clean water
- Proper disposal of sewage
- Vaccination

Disease Caused by Virus

- Viral diseases are extremely widespread infections caused by many type of viruses. In this lesson you will learn about some disease caused by viruses like, Hapatitis, Chickenpox and Rabies.

Hepatitis

- Hepatitis is one of the most dangerous and fatal diseases caused by Hepatitis virus- A, B, C, D, E. Its mode of transmission is Contaminated water, sharing of needles and blood transfusion. The symptoms of hepatitis is loss of appetite, (Anorexia), vomiting, eyes and urine in yellow color.

Prevention and treatment

- Drinking boiled water,
- Proper cleaning of hands

Chickenpox

- Chickenpox (chicken pox), also known as varicella, is a highly contagious infection caused by the varicella zoster virus. . This disease spreads through air and contact with an infected person. Its symptoms is appearance of rashes on the whole body, fever, headache and tiredness.

Prevention and treatment

- The chickenpox (varicella) vaccine is the best way to prevent chickenpox
- Special attention should be given to the infected persons

Rabies

- Rabies is a fatal disease. Which is transmitted by the bite of the infected dog, rabbit, monkey, cat etc. The virus present in the saliva of dog enters the brain via neurons. The symptoms of rabies is hydrophobia (extreme fear for water), fever for 2 – 12 weeks and exaggerations in behavior.

Prevention and treatment

- In early stages rabies is very difficult to detect
- After an animal is bitten it usually takes two to twelve weeks to shows any symptoms and it may take as long as two years also.
- Fatality can be prevented by timely vaccination before the onset of symptoms.

Vaccine
A vaccine is a biological preparation that provides active acquired immunity to a particular disease. Vaccines like (BCG, Polio, MMR) are given at early child wood to protect from

other diseases

Non-Communicable diseases

- Communicable diseases do not spread from person to person. They are caused by other factors. Therefore, it is important to know which sickness are communicable and which are not. They are never caused by germs, bacteria, or other living organisms that infect the body. Antibiotics, or medicines that fight against germs do not help to cure non-communicable diseases.

Problems caused by wearing out of body parts:

- Rheumatism, heart attack, epileptic seizures, stroke, migraine headach. Cataract and cancer

Problems caused by external harmful agents entering the body:

- Allergies, asthma, poisons, snakebite, cough from smoking, stomach ulcer, alcoholism.

Problems caused by a lack of trace elements in the body:

- Anemia, pellagra, night blindness and xerophthalmia, goiter and hypothyroidism.

Problems caused by Malnutrition.

- Nutritious food is needed for a person to grow well, work hard, and stay healthy. Many common sicknesses are caused by malnutrition.

Leucoderma is a non – communicable diseases caused by partial or total loss of pigmentation in the skin. (melanin pigment). This condition affects any age, gender and ethnicity. There is no cure. It does not spread by touching, sharing food and sitting together.

Specific health problems of children

Anaemia

- It is caused by eating food with less iron content and can also caused due to feeding some other foods instead of breast milk. Severe anemia in children may leads to hookworm infection, chronic diarrhoea and dysentery. In recent day school going children, especially the girls are affected by anemia. The Government of Tamil Nadu provides weekly iron folic tablets to all the girls in the schools of all areas.

The signs of anemia are:

- Pale or transparent skin, The inner surface of eye lids are pale, white fingernails, pale gums, weakness and fatigue.

- In severe cases, face and feet may be swollen, the heartbeat is rapid and with shortness of breath.

- Children and women who eats mud are usually anemic.

Consuming iron containing food Sources

- **Food** – Moringa leaves, Dates, Liver (Sheep and Chicken), Green, green leafy vegetables like beans, peas, lentils and Greed banana.

- **Pills** - Cod liver oil tablet, Ferrous sulfate

As a general rule, iron supplements should be given orally, not to be injected, because it leads to dangerous

Safety and First Aid

- First aid is the immediate treatment given to the victim of trauma or sudden illness before medical help is made available.

The first aid is

- To save the life
- To prevent further bleeding and determine the condition of the patient
- To relieve the pain
- To provide a medical care available at the earliest

Burns

- The tissue damage caused by heat, chemical, electricity, sunlight or nuclear radiation is known as Burns. Mostly burns are caused by scalds, building fires, flammable liquid and gases. There are three types of burns, according to degree of burning.

- First-degree burns affect only the outer layer (called the epidermis) of the skin
- Second-degree burns damage the epidermis and the layer beneath it (called the dermis)
- Third-degree burns involve damage or complete destruction of the skin to its full depth and damage to underlying tissues also. People who experience such burns often require skin grafting.

First Aid for Burning

- In case of minor burns, the affected area should be washed with cold water and an antiseptic cream should be applied. In case of severe burns, where deeper layers of tissues get destroyed and blisters appear, use of water should be avoided. The burnt area should be covered with a clean non- sticking cloth or bandages. Larger burns need immediate medical attention.

Cut and Scratches

- Cuts and scratches are areas of damage on the surface of the skin. A cut is a line of damage that can go through the skin and into the muscle tissues below, whereas a scratch is surface damage that does not penetrate the lower tissues.

- Cuts and scratches may bleed or turn red, become infected and leave scars.

First aid for cuts

- For minor cuts, the affected area should be washed with cold running water and cleaned with an antiseptic liquid. Then apply an antiseptic cream on the wound and sterilized bandage should be tied to prevent infection. If the cut is deep, a clean cotton pad should be placed on the cut and pressed, and the injured person should be taken to a doctor immediately.

Basic cleanliness and protection.

- The most important thing is to help anybody, but you must also protect yourself from HIV and other blood-borne diseases when you help someone who is bleeding. You should wear gloves or a clean plastic bag on your hands.

- Be careful not to prick yourself with needles or other sharp objects around the person you are helping.

9th Full Book

Unit 21- Nutrition & Health

Introduction

- Food is the basic necessity of life. Food is defined as any substance of either plant or animal origin consumed to provide nutritional support for an organism. It contains essential nutrients that provide energy, helps in normal growth and development, repair the worn out tissues and protect the body from diseases. Food contamination with microorganisms is a major source of illness either in the form of infections or poisoning. Food safety is becoming a major concern these days.

- Adulteration of foodstuffs is commonly practiced in India by traders. Food is contaminated or adulterated from production to consumption for financial gain. The physiological functions of a consumer are affected due to either addition of a deleterious substance or the removal of a vital component. Food laws have come into existence to maintain the quality of food produced in our country.

Classes of Nutrients

Nutrients are classified into the following major groups as given below.

- Carbohydrates
- Proteins
- Fats
- Vitamins
- Minerals

Carbohydrates

- Carbohydrates are organic compounds composed of carbon, hydrogen and oxygen. Carbohydrate is an essential nutrient which provides the chief source of energy to the body. Glucose, sucrose, lactose, starch, cellulose are examples for carbohydrates.

- Carbohydrates are classified as monosaccharide (Glucose), disaccharide (Sucrose) and polysaccharide (Cellulose). The classification is based on the number of sugar molecules present in each group.

Proteins

- Proteins are the essential nutrients and also the building blocks of the body. They are essential for growth and repair of body cells and tissues. Proteins are made of amino acids.

- Essential amino acids are those that cannot be biosynthesized by the body and must be obtained from the diet. The nine essential amino acids are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine.

Fats

- Fat in the diet provides energy. They maintain cell structures and are involved in metabolic functions.

- Essential fatty acids cannot be synthesized in the body and are provided through diet. Essential fatty acids required in human nutrition are omega fatty acids.

Vitamins

- Vitamins are the vital nutrients, required in minute quantities to perform specific physiological and biochemical functions.

Human skin can synthesize Vitamin D when exposed to sunlight (especially early morning). When the sun rays falls on the skin dehydrocholesterol is converted into Vitamin D. Hence, Vitamin D is called as Sunshine vitamin. Vitamin D improves bone strength by helping body to absorb calcium.

Minerals

- Minerals are inorganic substances required as an essential nutrient by organisms to perform various biological functions necessary for life. They are the constituents of teeth, bones, tissues, blood, muscle and nerve cells.

- The macrominerals required by the human body are calcium, phosphorus, potassium, sodium and magnesium. The microminerals required by the human body also called trace elements are sulfur, iron, chlorine, cobalt, copper, zinc, manganese, molybdenum, iodine and selenium.

Dietary sources of major foodstuffs

Major food Stuffs	Dietary sources	Daily requirements (grams)
Carbohydrates	Honey, Sugarcane, fruits, whole grains, starchy vegetables, rice	150-200
Proteins	Legumes, pulses , nuts, soya bean, green leafy vegetables, fish, poultry products, egg, milk and dairy products	40
Fats	Egg Yolk, saturated oil, meat	35

Vitamins-Dietary sources, Deficiency disorders and Symptoms

Vitamins	Dietary sources	Deficiency disorders	Symptoms
Fat Soluble Vitamins			
Vitamin A(Retinol)	Carrot, Papaya, leafy vegetables, fish liver oil, egg yolk, liver, dairy products	XerophthalmiaNyctalopia (Night blindness)	Dryness of Cornea unable to see in the night (dim light) scaly skin.
Vitamin D (Calciferol)	Egg, liver, dairy products, Fish, Synthesized by the skin in sunlight	Rickets (in children)	Bow legs, defective ribs, development of pigeon chest.
Vitamin E (Tocopherol)	Whole Wheat, meat, vegetable oil, milk.	Sterility in rats, Reproductive abnormalities	Sterility
Vitamin K (Derivative of Quinone)	Leafy vegetables, Soyabeans, milk	Blood Clotting is prevented	Excessive bleeding due to delayed blood clotting.
Water Soluble Vitamins			
Vitamin B1 (Thiamine)	Whole grains, Yeast, eggs, liver, sprouted pulses	Beriberi	Degenerative changes in the nerves, muscles become weak, Paralysis.
Vitamin B2 (Riboflavin)	Milk, eggs, liver, green vegetables, whole grains	Ariboflavinosis (Cheilosis)	Irritation in eyes, dry skin, inflammation of lips, fissures in the corners of the mouth.
Vitamin B3 (Niacin**)**	Milk, eggs, liver, lean meat, ground nuts, bran	Pellagra	Inflammation of skin, loss of memory, diarrhoea
Vitamin B6 (Pyridoxine)	Meat, fish, eggs, germs of grains and cereals, rice polishings.	Dermatitis	Scaly skin, nervous disorders.
Vitamin B12 (Cyanocobalamine)	Milk, meat, liver, pulses, cereals, fish	Pernicious anaemia	Decrease in red blood cell production, degeneration of spinal cord.
Vitamin C (Ascorbic acid)	Leafy vegetables, sprouts, citrus fruits like	Scurvy	Swollen and bleeding gums, delay in healing of

	gooseberry (Amala), lemon, orange		wounds, teeth and bones malformed.

Minerals - Dietary sources, Functions and Deficiency disorders

Minerals	Sources	Functions	Deficiency disorders
Macro nutrients			
Calcium	Dairy Products, beans, cabbage, eggs, fish	Constituent of bones and enamel of teeth, clotting of blood and controls muscle contraction.	Bone deformities, poor skeletal growth, Osteoporosis in adults.
Sodium	Common Salt	Maintains fluid balance and involved in neurotransmission	Muscular cramps, nerve impulses do not get transmitted.
Potassium	Banana, Sweet Potato, nuts, whole grains, citrus fruits	Regulates nerve and muscle activity	Muscular fatigue, nerve impulses do not get transmitted.
Micro nutrients			
Iron	Spinach, dates, greens, broccoli, whole cereals, nuts, fish, liver	Important component of haemoglobin	Anaemia
Iodine	Milk, Seafood, Iodised salt	Formation of thyroid hormones.	Goitre

Protein Energy Malnutrition (PEM)

- Absence of certain nutrients in our daily diet over a long period of time leads to deficiency diseases. This condition is referred as Malnutrition. Deficiency of proteins and energy leads to severe conditions like: Kwashiorkar and Marasmus.

- **Kwashiorkar:** It is a condition of severe protein deficiency. It affects children between 1-5 years of age, whose diet mainly consists of carbohydrates but lack in proteins.

- **Marasmus:** It usually affects infants below the age of one year when the diet is poor in carbohydrates, fats and proteins.

Food Hygiene

- Poor personal hygiene may allow pathogenic microorganisms to cause food spoilage Food spoilage is an undesirable change in the normal state of food and is not suitable for human consumption. Signs of food spoilage include a changes in appearance, colour, texture,odour and taste. Factors responsible for Food Spoilage are given below.

- Internal **factors:** It include enzymatic activities and moisture content of the food.

- **External factors:** It include adulterants in food, contaminated utensils and equipment, unhygienic cooking area and lack of storage facilities.

Food Preservation

- Food preservation is the process of prevention of food from decay or spoilage, by storing in a condition fit for future use. Food is preserved to:

- increase the shelf life of food
- retain the colour, texture, flavour and nutritive value
- increase food supply
- decrease wastage of food

Methods of Food Preservation
The various method of food preservation are explained below

- Drying**:** Drying is the process of preservation of food by removal of water/moisture content in the food. It can be done either by sun-drying, (e.g. cereals, fish) or vacuum drying (e.g. milk powder, cheese powder) or hot air drying (e.g. grapes, dry fruits, potato flakes). Drying inhibits the growth of microorganism such as bacteria, yeasts and moulds.

- Smoking**:** In this process, food products like meat and fish are exposed to smoke. The drying action of the smoke tends to preserve the food.

- Irradiation**:** Food irradiation is the process of exposing food to optimum levels of ionizing radiations like x-rays, gamma rays or UV rays to kill harmful bacteria and pests and to preserve its freshness.

- Cold **storage:** It is a process of storing the perishable foods such as vegetables, fruits and fruit products, milk and milk products etc. at low temperature. Preserving the food products at low temperature slows down the biological and chemical reactions and prevents its spoilage.

- Freezing**:** Freezing is one of the widely used methods of food preservation. This process involves storing the food below 00C at which microorganisms cannot grow, chemical reactions are reduced and metabolic reactions are also delayed.

Pasteurization: Pasteurization is a process of heat treatment of liquid food products. e.g. For preservation of milk and beverages. This process also involves boiling of milk to a temperature of 63°C for about 30 minutes and suddenly cooling to destroy the microbes present in the milk.

Bananas are best stored at room temperature. When it is kept in a refrigerator, the enzyme responsible for ripening becomes inactive. In addition, the enzyme responsible for browning and cell damage becomes more active thereby causing the skin colour change from yellow to dark brown

- Canning**:** In this method of food preservation, most vegetables, fruits, meat and dairy products, fruit juices and some ready-to-eat foods are processed and stored in a clean, steamed air tight containers under pressure and then sealed. It is then subjected to high temperature and cooled to destroy all microbes.

Addition of Preservatives

- Food can be preserved by adding natural and synthetic preservatives.

Natural preservatives

- Some naturally available materials like salt, sugar and oil are used as food preservatives.

- Addition **of salt:** It is one of the oldest methods of preserving food. Addition of salt removes the moisture content in the food by the process of osmosis. This prevents the growth of bacteria and reduces the activity of microbial enzymes. Meat, fish, gooseberry, lemon and raw mangoes are preserved by salting. Salt is also used as a preservative in pickles, canned foods etc.

- Addition **of sugar:** Sugar/Honey is added as a preservative to increase the shelf life of fruits and fruit products like jams, jellies, squash, etc. The hygroscopic nature of sugar/honey helps in reducing the water content of food and also minimizing the process of oxidation in fruits.

- **Addition of oil:** Addition of oil in pickles prevents the contact of air with food. Hence microorganisms cannot grow and spoil the food.

Synthetic preservatives

- Synthetic food preservatives like sodium benzoate, citric acid, vinegar, sodium meta bisulphate and potassium bisulphate are added to food products like sauces, jams, jellies, packed foods and ready- to- eat foods. These preservatives delay the microbial growth and keep the food safe for long duration.

Food Adulteration

- Adulteration is defined as the addition or subtraction of any substance to or from food, so that the natural composition and the quality of food substance is affected. Adulterant is any material which is used for the purpose of adulteration.

- Some of the common adulterated foods are milk and milk products, cereals, pulses, coffee powder, tea powder, turmeric powder, saffron, confectionary, non-alcoholic beverages, spices, edible oils, meat, poultry products etc. The adulterants in food can be classified in three categories:

- Natural adulterants
- Incidental/unintentionally added adulterants
- Intentionally added adulterants.

Natural adulterants

- Natural adulterants are those chemicals or organic compounds that are naturally present in food. e.g. toxic substances in certain poisonous mushrooms, Prussic acid in seeds of apples and cherry, marine toxins, fish oil poisoning, environmental contaminants.

Incidental/unintentionally added adulterants

- These types of adulterants are added unknowingly due to ignorance or carelessness during food handling and packaging. It includes:

- Pesticide residues
- Droppings of rodents, insects, rodent bites and larva in food during its storage
- Microbial contamination due to the presence of pathogens like Escherichia coli, Salmonella in fruits, vegetables, ready-to-eat meat and poultry products.

Intentionally added adulterants

- These adulterants are added intentionally for financial gain and have serious impact on the health of the consumers. These types of adulterants include:

- Additives and preservatives like vinegar, citric acid, sodium bicarbonate (baking soda), hydrogen peroxide in milk, modified food starch, food flavours, synthetic preservatives and artificial sweeteners.

- Chemicals like calcium carbide to ripen bananas and mangoes.

- Non certified food colours containing chemicals like metallic lead are used to give colours to vegetables like green leafy vegetables, bitter gourd, green peas etc. These colours are added to give a fresh look to the vegetables.

- Edible synthetic wax like shellac or carnauba wax is coated on fruits like apple, pear to give a shining appearance.

Health Effects of Adulterated Foods

- Consumption of these adulterated foods may lead to serious health effects like fever, diarrhoea, nausea, vomiting, gastrointestinal disorders, asthma, allergy, neurological disorder, skin allergies, immune suppression, kidney and liver failure, colon cancer and even birth defects.

Food Quality Control

- The government always ensures that pure and safe food is made available to the consumers. In 1954, the Indian Government enacted the Food Law known as Prevention of Food Adulteration Act and the Prevention of Food Adulteration Rules in 1955 with the objective of ensuring pure and wholesome food to the consumers and protect them from fraudulent trade practices.

- Minimum standards of quality for food and strict hygienic conditions for its sale are clearly outlined in the Act

A slogan From farm to plate, make food safe was raised on World Health Day (7th April 2015) to promote and improve food safety.

Food Quality Control Agencies

- ISI, AGMARK, FPO, FCI and other health departments enforce minimum standards for the consumer products. FCI (Food Corporation of India) was set up in the year 1965 with the following objectives:

- Effective price support operations for safeguarding the interest of farmers.
- Distributing food grains throughout the country.
- Maintaining satisfactory levels of operational and buffer stock of food grains to ensure national security.
- Regulate the market price to provide food grains to consumers at reliable price.

10th Full Book

Unit 21 – Health & Disease

Introduction

- Abuses occur in a variety of forms and are deeply rooted in cultural, social and economic practices. Solving this global problem however requires a much better understanding of its occurrence, causes and consequences with context to sexual and childhood abuse, this is followed by substance abuse. Are people leading healthier lives in today's modern world than their generations did in the past? For instance, smoking cigarettes, alcohol addiction, use of drugs, eating high fat and cholesterol rich diets, excessive intake of junk foods, reduced physical activity are some of the risk factors for illness and early death. The role of behaviour in health has been receiving increased attention in countries around the world. The health habits of the individuals and their behaviour influence the development of chronic and fatal diseases such as diabetes, obesity, heart disease, cancer and AIDS. These conditions can be substantially reduced by adopting lifestyles that promote wellness and protect their health by taking nutritious diet, regular exercise and by avoiding drugs, alcohol and smoking.

Abuse and Types of Abuse

- Abuse refers to cruel, violent, harmful or injurious treatment of another human being. It includes physical, emotional or psychological, verbal, child and sexual abuses. Abuse can occur within the family and with people who are not associated with the family.

- These days the use of drugs, alcohol and tobacco has been increasing especially among teenagers and adolescents for adventure, excitement, curiosity and experimentation

Child Abuse

- Child abuse constitutes all forms of physical or emotional ill treatment, sexual abuse, exploitation resulting in child's ill health, survival and development. Physical abuse of a child is defined as those acts that cause physical harm such as threatening, beating, kicking and hitting the child.

Sexual Abuse

- Sexual harassment is a form of power and dominance of one person over another, which can result in harmful consequence tothe victim. It refers to inappropriate or forced sexual contact. Adolescent girls and women encounter sexual harassment in different forms. Sexual abuse is more common at work places. Verbal remarks, comments, gestures and looks are the most common forms of abuse. This results in psychological distress, physical illness and eating disorders in the affected individuals.

Child Sexual Abuse

- Children are considered soft targets for sexual abuse because they may not realize that they are being abused. Commonly, abusers are persons well known to the child, may even be living in the same locality. Abusers also bribe (use chocolates and toys) to lure children and take advantage of the child's innocence.

- Sexually abused children show symptoms of genital injury, abdominal pain, frequent urinary infection and behavioural problems.

Approaches for Protection of an Abused Child

- Measures adopted for monitoring and assessment of abused child who have undergone signs and symptoms of distress are:

  - ✓ **Child Helpline:** The Child Helpline provides a social worker who can assist the child by providing food, shelter and protection.

  - ✓ **Counselling the child:** Psychologists and social workers should provide guidance, counselling and continous support to a victimized child.

  - ✓ **Family support:** The victimized child should be supported by the family members. They should be provided with proper care and attention to overcome their sufferings.

  - ✓ **Medical care:** A child victim of sexual offences should receive medical care and treatment from health care professionals to overcome mental stress and depression.

  - ✓ **Legal Counsel:**The family or the guardian of the child victim shall be entitled to free assistance of a legal counsel for such offence.

  - ✓ **Rehabilitation:** Enrolling in schools and resuming their education is an important step towards rehabilitation of the child. It is essential that the child's life is gradually returned to normal after the incidence of abuse.

  - ✓ **Community based efforts:** Conducting awareness campaign on child abuse and its prevention.

Prevention of child sexual abuse

- The most important social policy proclaimed universally is the prevention of child abuse. Taking steps to prevent childhood sexual abuse is parental and institutional responsibility. Instructions to be given by parents and teachers to the child are.

- ➢ Do not talk to any suspected person or strangers and to maintain a distance.

- ➢ Not to be alone with unknown person.

- To be careful while travelling alone in public or private transport.
- Not to receive money, toys, gift s or chocolates from known or unknown person to them without the knowledge of their parents.
- Not to allow known or unknown person to touch them.

- It is the responsibility of every individual living in a society to ensure a safe and protected environment for our children to enable them to live with dignity and free from any form of violence.

Drug, Alcohol and Tobacco Abuse

- The physical and mental dependency on alcohol, smoking and drugs is called addiction. The addictive potential of these substances pulls an individual into a vicious cycle leading to regular abuse and dependency. This is of serious concern because abuse of tobacco, alcohol or drugs produce many harmful effects in an individual, to the family and even to the society. This dangerous behavior pattern among youth can be prevented through proper guidance.

Drug Abuse

- Drugs are normally used for the treatment of disease on advice of a physician and withdrawn after recovery. A person who is habituated to a drug due to its prolonged use is called drug addict. This is called drug addiction or drug abuse.

- A drug that modifies the physical, biological, psychological or social behaviour of a person by stimulating, depressing or disturbing the functions of the body and the mind is called addictive drug. These drugs interact with the central nervous system and affect the individual physically and mentally.

Types of Drugs

- There are certain drugs called psychotropic drugs which acts on the brain and alter the behaviour, consciousness, power of thinking and perception. They are referred as mood altering drugs.

Drug Dependence

- Persons who consume these drugs become fully dependent on them, they cannot live without drugs. This condition is referred as drug dependence.
- **Physical and mental dependence**
- Dependence on the drug for normal condition of well being and to maintain physiological state.

- **Psychological dependence** is a feel that drugs help them to reduce stress.

- International Day against Drug Abuse and Illicit Trafficking - June 26.
- Narcotic Drugs and Psychotropic Substances Act was introduced in 1985.

Behavioural Changes of Drug Users

- Adverse effects of drug use among adolescents are

- Drop in academic performance, absence from school or college.

- Lack of interest in personal hygiene, isolation, depression, fatigue and aggressive behaviour.

- Deteriorating relationship with family and friends.

- Change in food and sleeping habits

- Fluctuation in body weight and appetite

- Always looking out for an easy way to get money for obtaining drugs.

- Prone to infections like AIDS and Hepatitis-B.

World Health Organization (WHO) 1984 suggested the use of the term drug dependence in place of drug addiction or drug abuse

Drug De-addiction

- Management of de-addiction is a complicated and difficult task. The path to recovery of drug addicts is long and often slow.

- Family members, friends and society on the whole have a very important role to play.

  - ✓ **Detoxification:** The first phase of treatment is detoxification. The drug is stopped gradually and the addict is helped to overcome the withdrawal symptoms. The addict undergoes severe physical and emotional disturbance. This is taken care by specific medication.

  - ✓ **Psychotherapy:** Individual and group counselling is given by psychologists and counsellors. The treatment includes efforts to reduce the addict's stress, taught new ways to solve everyday's problems, adequate diet, rest and relaxation.

- ✓ **Counselling to family members:** Social workers counsell family members in order to change the attitude of rejection so that the addict is accepted by the family and the society.

- ✓ **Rehabilitation:** They are given proper vocational training so that they can lead a healthy life and become useful members of the society.

Tobacco Abuse

- Tobacco is obtained from the tobacco plant Nicotianatobaccum and Nicotianarustica. The dried and cured leaves of its young branches make the commercial tobacco used worldwide. Addiction to tobacco is due to 'nicotine' an alkaloid present in it. Nicotine is a stimulant, highly harmful and poisonous substance.

Tobacco Use

- Tobacco is used for smoking, chewing and snuffing. Inhaling tobacco smoke from cigars, cigarettes, bidis, pipes, hukka is called smoking. Tobacco in powder form is chewed with pan. When powdered tobacco is taken through nose, it is called snuffing.

Smoking Hazards and Effects of Tobacco

- When smoke is inhaled, the chemicals get absorbed by the tissues and cause the following harmful effects

- ➢ **Benzopyrene** and **polycyclic hydrocarbons** present in tobacco smoke is carcinogenic causing lung cancer.

- ➢ Causes inflammation of throat and bronchi leading to conditions like bronchitis and pulmonary tuberculosis.

- ➢ Inflammation of lung alveoli, decrease surface area for gas exchange and cause emphysema.

- ➢ Carbon monoxide of tobacco smoke binds to haemoglobin of RBC and decreases its oxygen carrying capacity causing hypoxia in body tissues.

- ➢ Increased blood pressure caused by smoking leads to increased risk of heart disease.

- ➢ Causes increased gastric secretion which leads to gastric and duodenal ulcers.

- ➢ Tobacco chewing causes oral cancer (mouth cancer).

Prevention of Smoking

- Knowing the dangers of smoking and chewing tobacco adolescents and the old people need to avoid these habits. Proper counselling and medical assistance can help an addict to give up the habit of smoking.

Alcohol Abuse

- The consumption of alcohol is a social evil practiced by the wealthier and poorer sections of the society. The dependence of alcohol is called alcoholism and the addict is termed as alcoholic. It is called alcohol abuse. Drinking of alcohol impairs one's physical, physiological and psychological functions.

Harmful Effects of Alcohol to Health

- Prolonged use of alcohol depresses the nervous system, by acting as a sedative and analgesic substance. Some of the harmful effects are

➢ Nerve cell damage resulting in various mental and physical disturbances

➢ Lack of co-ordination of body organs

➢ Blurred or reduced vision, results in road accidents

➢ Dilation of blood vessels which may affect functioning of the heart

➢ Liver damage resulting in fatty liver which leads to cirrhosis and formation of fibrous tissues

➢ Body loses its control and consciousness eventually leading to health complications and ultimately to death.

Rehabilitation Measures for Alcoholics

✓ **Education and counselling:** Education and proper counselling will help the alcoholics to overcome their problems and stress, to accept failures in their life.

✓ **Physical activity:** Individuals undergoing rehabilitation should be channelized into healthy activities like reading, music, sports, yoga and meditation.

✓ **Seeking help from parents and peer groups:** When a problematic situation occurs, the affected individuals should seek help and guidance from parents and peers. This would help them to share their feeling of anxiety, wrong doing and get rid of the habit.

- ✓ **Medical assistance:** Individual should seek help from psychologists and psychiatrists to get relieved from this condition and to lead a relaxed and peaceful life.

- Alcohol de-addiction and rehabilitation programmes are helpful to the individual so that they could get rid of the problem completely and can lead a normal and healthy life.

Diseases and Disorders due to Lifestyle Modifications

- Diseases are prevalent in our society due to our improper way of living, conditions of stress and strain. These diseases are non-communicable and affect the person who are suffering from particular symptoms. It is an impairment of the body tissue or organ, disturbances in metabolic function which require modification of an individual's normal life.

Diabetes Mellitus

- Diabetes mellitus is a chronic metabolic disorder. In Greek (Diabetes – running through; mellitus- sweet). It is characterised by increased blood glucose level due to insufficient, deficient or failure of insulin secretion. This is the most common pancreatic endocrine disorder. The incidence of Type-1 and Type-2 diabetes is increasing worldwide.

Type-1 Insulin Dependent Diabetes Mellitus (IDDM)

- IDDM accounts for 10 to 20% of the known diabetics. The condition also occurs in children (juvenile onset diabetes) and young adults, the onset is usually sudden and can be life threatening. This is caused by the destruction of β-cells of the pancreas. It is characterized by abnormally elevated blood glucose levels (hyperglycemia) resulting from inadequate insulin secretion.

Causes: Genetic inheritance and environmental factors (infections due to virus, acute stress) are the cause for this condition.

Type-2 Non-Insulin Dependent Diabetes Mellitus (NIDDM)

- This is also called as adult onset diabetes and accounting for 80 to 90% of the diabetic population. It develops slowly, usually milder and more stable. Insulin production by the pancreas is normal but its action is impaired. The target cells do not respond to insulin. It does not allow the movement of glucose into cells.

Causes: The causes are multifactorial which include increasing age (affecting middle aged and older people), obesity, sedentary life style, overeating and physically inactive.

Symptoms: Diabetes mellitus is associated with several metabolic alterations.The most important symptoms are

- ➢ Increased blood glucose level (Hyperglycemia)

- Increased urine output (Polyuria) leading to dehydration
- Loss of water leads to thirst (Polydipsia) resulting in increased fluid intake
- Excessive glucose excreted in urine (Glycosuria)
- Excess hunger (Polyphagia) due to loss of glucose in urine.
- Fatigue and loss of weight

According to WHO recommendation, if the fasting blood glucose is greater than 140 mg/dl or the random blood glucose is greater than 200 mg /ml on more than two occasions, diagnosis for confirming diabetes is essential.

Prevention and Control of Diabetes

- Diet, hypoglycemic drugs, insulin injection and exercise are the management options based on the type and severity of the condition. The overall goal of diabetes management is to maintain normal blood glucose level.

Differences between Type-1 and Type-2 Diabetes Mellitus

Factors	**Type-1 Insulin Dependent Diabetes Mellitus (IDDM)**	**Type-2 Non-Insulin Dependent Diabetes Mellitus (NIDDM)**
Prevalence	10-20%	80 – 90%
Age of onset	Juvenile onset (<20 Years)	Maturity onset (>30 Years)
Body Weight	Normal or underweight	obese
Defect	Insulin deficiency due to destruction of β – cells.	Target cells do respond to insulin
Treatment	Insulin administration is necessary	Can be controlled by diet, exercise and medicine

- ✓ **Dietary management:** Low carbohydrate and fibre rich diets are more appropriate. Carbohydrates should be taken in the form of starch and complex sugars. Refined sugars (sucrose and glucose) should be avoided. Diet comprising whole grains, millets (jowar, bajra, ragi), green leafy vegetables, wheat and unpolished rice should be included in diet regularly. Carbohydrates is maintained to about 50- 55% of the total calories. High protein content of 10-15% of the total intake is required to supply essential amino acids. Fat content in the diet should be 15-25% of the total calories. Saturated fat intake should be reduced. Polyunsaturated fatty acid content should be higher.

- ✓ **Management with insulin:** Commercially available insulin preparations (short and long acting) are also used to maintain blood glucose levels.

- ✓ **Physical activity:** Exercise plays an important role in facilitating a good control of diabetes, in addition to strengthening and toning up the muscles.

- ✓ **Education and Awareness:** People with diabetics should be educated on the nature of disease they have and the possibility of complications of the disease, if blood sugar is not kept under control. Instructions regarding diet, exercise and drugs should be explained.

Obesity

- Obesity is the state in which there is an accumulation of excess body fat with an abnormal increase in body weight. Obesity is a complex multifactorial chronic disease developing from influence of social, behavioural, psychological, metabolic and cellular factors.

- Obesity occurs if intake of calories is more than the expenditure of energy. Over weight and obesity are conditions where the body weight is greater than the mean standard weight for age and height of an individual. Body mass index (BMI) is an estimate of body fat and health risk.

$$\textbf{BMI = Weight (kg) / Height (m)}^{2}$$

Causes and risk factors: Obesity is due to genetic factors, physical inactivity, eating habits (overeating) and endocrine factors. Obesity is a positive risk factor in development of hypertension, diabetes, gall bladder disease, coronary heart disease and arthritis.

Prevention and Control of Obesity

- ➢ **Diet Management:** Low calorie, normal protein, vitamins and mineral, restricted carbohydrate and fat, high fiber diet can prevent overweight. Calorie restriction for weight reduction is safe and most effective.

- ➢ **Physical exercise:** A low calorie diet accompanied by moderate exercise will be effective in causing weight loss. Meditation, yoga and physical activity can also reduce stress related to overeating.

Heart Disease

- Cardiovascular disease (CVD) is associated with diseases of the heart and blood vessels. Coronary heart disease (CHD) is the most common form and is caused by deposition of cholesterol in the blood vessels.

- It usually develops slowly over many years beginning from childhood, they may form a fatty streak to a fibrous complicated plaque. It leads to the narrowing of blood vessels

leading to atherosclerosis in the large and medium sized arteries that supply the heart muscle with oxygen. It leads to sudden ischemia (deficient blood supply to heart muscle) and myocardial infarction (death of the heart muscle tissue).

- **Risk factors:** Hypercholesterolemia (High blood cholesterol) and high blood pressure (Hypertension) are the major causes and contributing factors for heart disease and if untreated may cause severe damage to brain, kidney and eventually lead to stroke.

- **Causes:** Heredity (family history), diet rich in saturated fat and cholesterol, obesity, increasing age, cigarette smoking, emotional stress, sedentary lifestyle, excessive alcohol consumption and physical inactivity are some of the causes.

- **Symptoms:** Shortness of breath, headache, tiredness, dizziness, chest pain, swelling of leg, and gastrointestinal disturbances.

HDL (High Density Lipoprotein) or "good" cholesterol lowers risk of heart disease while LDL (Low Density Lipoprotein) or "bad" cholesterol increases risk of heart disease.

Prevention and Control of Heart Disease

- **Diet management:** Reduction in the intake of calories, low saturated fat and cholesterol rich food, low carbohydrates and common salt are some of the dietary modifications. Diet rich in polyunsaturated fatty acids (PUFA) is essential. Increase in the intake of fibre diet, fruits and vegetables, protein, minerals and vitamin are required.

- **Physical activity:** Regular exercise, walking and yoga are essential for body weight maintenance

- **Addictive substance avoidance:** Alcohol consumption and smoking are to be avoided.

Cancer

- Cancer causes about 4 million deaths annually throughout the world. In India more than one million people suffer from cancer. Cancer is derived from Latin word meaning crab. The study of cancer is called Oncology (Oncos- Tumor).

- Cancer is an abnormal and uncontrolled division of cells that invade and destroy surrounding tissue forming a tumor or neoplasm (new growth). It is a heterogenous group of cells that do not respond to the normal cell division.

- The cancerous cells migrate to distant parts of the body and affect new tissues. This process is called metastasis. The frequent sites of metastasis are lungs, bones, liver, skin and brain.

Types of Cancers

- Cancers are classified on the basis of the tissues from which they are formed.

➢ Carcinomas arise from epithelial and glandular tissues. They include cancers of skin, lung, stomach and brain. About 85% of the tumours are carcinomas

➢ Sarcomas are occur in the connective and muscular tissue. They include the cancer of bones, cartilage, tendons, adipose tissue and muscles. These form 1% of all tumours.

➢ Leukaemia are characterized by an increase in the formation of white blood cells in the bone marrow and lymph nodes. Leukaemia are called blood cancers. Most common type of cancer which also affect children below 15 years of age.

Carcinogenic Agents

- Cancer causing agents are called carcinogens. They are physical, chemical agents, ionizing radiations and biological agents.

  ✓ **Physical Irritant:** Heavy smoking causes lung cancer and cancers of oral cavity, pharynx (throat) and larynx. Betel and tobacco chewing causes oral cancer. Excessive exposure to sunlight may cause skin cancer.

  ✓ **Chemical agents:** Nicotine, caffeine, products of combustion of coal and oil, pesticides, asbestos, nickel, certain dyes and artificial sweetners induce cancer

  ✓ **Radiations:** Ionizing radiations like X-rays, gamma- rays, radioactive substances and non-ionising radiations like UV rays cause DNA damage leading to cancer.

  ✓ **Biological agents:** Cancer causing viruses are called oncogenic viruses.

Treatment of Cancer

The treatment of cancer involves the following methods:

➢ **Surgery:** Tumours are removed by surgery to prevent further spread of cancer cells.

➢ **Radiation therapy:** Tumour cells are irradiated by lethal doses of radiation while protecting the surrounding normal cells.

➢ **Chemotherapy:** It involves administration of anticancerous drugs which prevent cell division and are used to kill cancer cells.

➢ **Immunotherapy:** Biological response modifiers like interferons are used to activate the immune system and help in destroying the tumors.

Preventive measures for Cancer

- Cancer control programmes should focus on primary prevention and early detection.

- To prevent lung cancer tobacco smoking is to be avoided and protective measures to be taken against exposure to toxic pollutants of industries. Excessive exposure to radiation is to be avoided to prevent skin cancer.

AIDS (Acquired Immunedeficiency Syndrome)

- AIDS is a severe viral disease and caused by Human Immunodeficiency Virus (HIV). It is a condition in which immune system fails and suppress the body's disease fighting mechanism. They attack the lymphocytes and the affected individual is prone to infectious diseases.

Dr.Suniti Solomon, pioneered HIV research and treatment in India. She set up the first voluntary testing and counselling centre and an AIDS Research group in Chennai during 80's. Her team was the first to document evidence of HIV infection in India in 1985 (First Indian AIDS patient in Chennai).

Transmission of HIV

- AIDS virus has been found in urine, tears, saliva, breast milk and vaginal secretions. The virus is transmitted by an infected patient who comes in contact with blood of a healthy person. HIV/AIDS is not transmitted by touch or any physical contact. It spreads through contact of body fluids or blood.

HIV is transmitted generally by

- Sexual contact with infected person
- Use of contaminated needles or syringes especially in case of intravenous drug abusers
- By transfusion of contaminated / infected blood or blood products
- From infected mother to her child through placenta.

Symptoms and Treatment of AIDS

Symptoms: Infected individuals become immunodeficient. The person becomes more susceptible to viral, bacterial, protozoan and fungal infections. Swelling of lymph nodes, damage to brain, loss of memory, lack of appetite and weight loss, fever, chronic diarrhoea, cough, lethargy, pharyngitis, nausea and headache.

Diagnosis: The presence of HIV virus can be confirmed by Western Blot analysis or Enzyme Linked Immunosorbent Assay (ELISA)

Treatment: Anti-retroviral drugs and immunestimulative therapy can prolong the life of the infected person.

Prevention and Control of AIDS

- The following steps may help in controlling and prevent the spreading of HIV infection

- Screening of blood from blood banks for HIV before transfusion.
- Ensuring the use of disposable needles and syringes in hospitals and clinics.
- Advocating safe sex and advantages of using condoms.
- Creating awareness campaign and educating people on the consequences of AIDS.
- Persons with HIV/AIDS should not be isolated from the family and society.

11th Zoology

Chapter 5 – Digestion and Absorption

Nutrients, Vitamins and Minerals

- Food comprises of macronutrients and micronutrients. The nutrients required inlarger quantities are called macronutrients, whereas those required in small quantities are called micronutrients. Essential nutrients cannot be synthesized by the body; they have to be included in the diet. Macronutrients are lipids, carbohydrates, proteins and the micronutrients are vitamins and minerals. Water plays an important role in the metabolic processes and prevents dehydration of the body.

- Intake of too much of food or lesser amount of food than the basic requirement is called malnutrition. A diet which can provide all the metabolic requirements ofthe body in a right proportion is called balanced diet. That means it shouldcontain carbohydrates and fats for energy yielding, proteins for growth and replacement; and vitamins, minerals and water for physiological regulation.

Vitamins:

- Vitamins are naturally occurring organic substances regularly needed in minutequantities for maintaining normal health as metabolic regulators. The identified vitamins are classified as fat soluble (A,D,E and K) and vitamin B and vitamin C are water soluble. Vitamin A, D, E and K, if consumed beyond required level may cause defects, commonly referred to as hypervitaminosis.

Minerals:

- These are the inorganic chemical elements, i.e., Ca, Fe, I, K, Mg, Na, P, S, etc needed for regulation of various physiological functions. These can be classified into major minerals (Na, P, K, Ca, Mg, S, Cl) and others are trace minerals such as Fe,Cu, Zn, Co, Mn, I, and fluorine. Sodium ions are more abundant than any other cation in the body fluids.

N.I. Lunin discovered vitamins but the name vitamin was given by Dr. Funk (1912).The first vitamin isolated was B1 by Dr.Funk. The first vitamin produced by fermentation process using, Acetobacter bacteria is Vitamin C.

Fat soluble vitamins		
Vitamins	**Functions**	**Symptoms of Deficiency**
A (Retinol)/ Antixerophthalmic vitamin	Plays a vital role in visual perception. Maintenance and growth of epithelial tissue.	Night blindness (Nyctalopia), Xerophthalmia (drying of eyeballs), Bitot's spot in the cornea,

		Dermatosis(dry and scaly skin) and Keratomalacia Atrophy of lacrymal glands and reduction in tear secretion
D (Calciferol)/ Antirachiticvitamin	Promotes intestinal absorption of calcium and phosphorus. Formation of teeth and bones	Rickets in children (softness and deformities of bones and bow legs and pigeon chest) and Osteomalacia in adults (weak and fragile bones, bent, deformed pelvis).
E (Tocopherol) / Antisterility vitamin	Antioxidant It keeps the skin healthy by reduces the process of ageing	Sterility in animals, Ruptured red blood cells
K Anti haemorrahagic vitamin.	1. Required for the synthesis of prothrombin in the liver.	Defect in blood clotting called Haemorrhagic manifestations.

Water Soluble Vitamins

Vitamins	**Functions**	**Symptoms of Deficiency**
B1 (Thiamine)	Involved in carbohydrate metabolism. Act as a coenzyme	**Beriberi:**affects muscular, nervous and cardiovascular system
B2 (Riboflavin)	Acts as coenzyme in oxidation and reduction reactions	Inflammation, soreness and fissures in the corners of the mouth, lips and tongue. Loss of appetite. Skin and eye disorder
B3 (Pantothenic acid)	Acts as coenzyme A and is essential for the metabolism of fats and carbohydrates	Gastrointestinal disorders, anaemia, Burning feet syndrome, etc.
B4 (choline)	Precursor for acetylcholine	Fatty liver
B5 (Niacin / Nicotinic acid)	Derivatives of coenzymes	Pellagra (4D Syndrome) characterized by dermatitis, diarrhoea and dementia (mental deterioration) and death.
B6 (Pyridoxine)	Haemoglobin formation, brain, heart and liver activities	Dermatitis, convulsions, muscular twitching and anaemia
B7 (Biotin) / Vit.H	Acts as a coenzyme in synthesis of fat, glycogen and amino acids	Dermatitis

B9 (Folic acid)	It acts as a co-enzyme for synthesis of nucleic acid and essential for growth and formation of RBC	Megaloblastic anaemia (large, immature, nucleated RBC in blood)
B12 (Cobalamine)	Promotes DNA synthesis. Necessary for maturation of RBC and formation of myelin sheath.	Pernicious anaemia (immature nucleated RBC without haemoglobin). Causes nervous disorder
C (Ascorbic acid)	Acts as an antioxidant. Strengthens the immune system. Necessary for healthy gums and teeth.	Scurvy (Sailor's disease) characterized by spongy and bleeding gums, falling of teeth, fragile bones, delayed wound healing etc. - Infantile scurvy)

Food adulterants cause harmful effects in the form of headaches, palpitations, allergies, cancers and in addition reduces the quality of food. Common adulterants are addition of citric acid to lemon juice, papaya seeds to pepper, melamine to milk, vanillin for natural vanillin, red dyes to chillis, lead
chromate and lead tetraoxide to turmeric powder, etc.,

Caloric value of carbohydrates, proteins and fats

- We obtain 50% energy from carbohydrates 35% from fats and 15% from proteins. We require about 400 to 500 gm of carbohydrates, 60 to 70 gm of fats and 65 to 75 gm of proteins per day. Balanced diet of each individual will vary according to their age, gender, level of physical activity and others conditions such as pregnancy and lactation.

- Carbohydrates are sugar and starch. These are the major source of cellular fuel which provides energy. The caloric value of carbohydrate is 4.1 calories per gram and its physiological fuel value is 4 Kcal per gram.

- Lipids are fats and derivatives of fats, are also the best reserved food stored in our body which is used for production of energy. Fat has a caloric value of 9.45 Kcaland a physiological fuel value of 9 Kcal per gram.

Many research findings have proven that usage of chemical preservatives and artificial enhancers lead to highly harmful effects. It includes heart ailments, hypertension, infertility, gastrointestinal disorders, early puberty in girls, weakening of bones, damage in organs like kidney and liver, chronic obstructive pulmonary diseases, headache, allergies, asthma, skin rashes and even cancer. Remember that nothing will beat and overtake the taste and safety of homemade foods. "East or west home preparation is the best."

- Proteins are source of amino acids required for growth and repair of bodycells. They are stored in the body only to a certain extent; large quantities are excreted as nitrogenous waste. The caloric value and physiological fuel value of one gram of protein are 5.65 Kcal

and 4 Kcal respectively. According to ICMR (Indian Council of Medical Research and WHO (World Health Organization), the daily requirement of protein for an average Indian is 1gm per 1 kg body weight.

Nutritional and digestive disorders

- Intestinal tract is more prone to bacterial, viral and parasitic worm infections. Thisinfection may cause inflammation of the inner lining of colon called colitis. The most common symptoms of colitis are rectal bleeding, abdominal cramps, and diarrhoea.

Protein energy malnutrition: (PEM)

- Growing children require more amount of protein for their growth and development.Protein deficient diet during early stage of children may lead to protein energy malnutrition such as Marasmus and Kwashiorkor. Symptoms are dry skin, pot-belly, oedema in the legs and face, stunted growth, changes in hair colour, weakness and irritability. Marasmus is an acute form of protein malnutrition. This condition is due to a diet with inadequate carbohydrate and protein. Such children are suffer from diarrhoea, body becomes lean and weak (emaciated) with reduced fat and muscle tissue with thin and folded skin.

- **Indigestion:** It is a digestive disorder in which the food is not properly digestedleading to a feeling of fullness of stomach. It may be due to inadequate enzyme secretion, anxiety, food poisoning, over eating, and spicy food.

- **Constipation:** In this condition, the faeces are retained within the rectum because of irregular bowel movement due to poor intake of fibre in the diet and lack of physical activities.

- **Vomiting:** It is reverse peristalsis. Harmful substances and contaminated food from stomach are ejected through the mouth. This action is controlled by the vomit centre located in the medulla oblongata. A feeling of nausea precedes vomiting.

- **Jaundice:** It is the condition in which liver is affected and the defective liver fails to break down haemoglobin and to remove bile pigments from the blood. Deposition of these pigments changes the colour of eye and skin yellow. Sometimes, jaundice is caused due to hepatitis viral infections.

- **Liver cirrhosis:** Chronic disease of liver results in degeneration and destruction of liver cells resulting in abnormal blood vessel and bile duct leading to the formation of fibrosis. It is also called deserted liver or scarred liver. It is caused due to infection, consumption of poison, malnutrition and alcoholism.

- **Gall Stones:** Any alteration in the composition of the bile can cause theformation of stones in the gall bladder. The stones are mostly formed of crystallized cholesterol in the

bile. The gall stone causes obstruction in the cystic duct, hepatic duct and also hepato-pancreatic duct causing pain, jaundice and pancreatitis.

Appendicitis: It is the inflammation of the vermiform appendix, leading to severe abdominal pain. The treatment involves the removal of appendix by surgery. If treatment is delayed the appendix may rupture and results in infection of the abdomen, called peritonitis.

Hiatus hernia (Diaphragmatic hernia): It is a structural abnormality in which superior part of the stomach protrudes slightly above the diaphragm. The exact cause of hiatus hernias is not known. In some people, injury or other damage may weaken muscle tissue, by applying too much pressure (repeatedly) on the muscles around the stomach while coughing, vomiting, and straining during bowel movement and lifting heavy object. Heart burn is also common in those with a hiatus hernia. In this condition, stomach contents travel back into the oesophagus or even into oral cavity and causes pain in the centre of the chest due to the eroding natureof acidity.

Diarrhoea: It is the most common gastrointestinal disorder worldwide. It is sometimes caused by bacteria or viral infections through food or water. When the colon is infected, the lining of the intestine is damaged by the pathogens, thereby the colon is unable to absorb fluid. The abnormal frequency of bowel movement and increased liquidity of the faecal discharge is known as diarrhoea. Unless the condition is treated, dehydration can occur. Treatment is known as oral hydration therapy. This involves drinking plenty of fluids – sipping small amounts of water at a time to rehydrate the body.

Peptic ulcer: It refers to an eroded area of the tissue lining (mucosa) in the stomachor duodenum. Duodenal ulcer occurs in people in the age group of 25 - 45 years. Gastric ulcer is more common in persons above the age of 50 years. Ulcer is mostly due to infections caused by the bacterium Helicobacter pylori. It may also be caused due to uncontrolled usage of aspirin or certain antiinflammatory drugs. Ulcermay also be caused due to smoking, alcohol, caffeine and psychological stress.

Obesity: It is caused due to the storage of excess of body fat in adipose tissue. It may induce hypertension, atherosclerotic heart disease and diabetes. Obesity may be genetic or due to excess intake of food, endocrine and metabolic disorders. Degree of obesity is assessed by body mass index (BMI). A normal BMI range for adult is 19- 25; above 25 is considered as obese. BMI is calculated as body weight in Kg, divided by the square of body height in meters. For example, a 50 Kg person with a height of 160 cms would have a BMI of 19.5.
That is BMI = 50/1.62 = 19.5

Nobel Prize for the year 2005 was awarded to Robin Warren and Barry Marshall for the discovery of Helicobacter pylori which causes peptic ulcer

12th Zoology

Unit 7 – Human Health and Diseases

Common diseases in human beings

- Disease can be defined as a disorder or malfunction of the mind or body. It involves morphological, physiological and psychological disturbances which may be due to environmental factors or pathogens or genetic anomalies or life style changes. Diseases can be broadly grouped into infectious and non infectious types. Diseases which are transmitted from one person to another are called infectious diseases or communicable diseases. Such disease causing organisms are called pathogens and are transmitted through air, water, food, physical contact and vectors. The disease causing pathogen may be virus, bacteria, fungi, protozoan parasites, helminthic parasites, etc., Infectious diseases are common and everyone suffers from such diseases at some time or the other. Most of the bacterial diseases are curable but all viral diseases are not. Some infectious disease like AIDS may be fatal.

- Non-infectious diseases are not transmitted from an infected person to a healthy person. In origin they may be genetic (cystic fibrosis), nutritional (vitamin deficiency diseases) and degenerative (arthritis, heart attack, stroke). Among non - infectious diseases, cancer is one of the major causes of death.

Bacterial and viral diseases Bacterial diseases

- Though the number of bacterial species is very high, only a few bacteria are associated with human diseases and are called pathogenic bacteria. Such pathogens may emit toxins and affected the body.

- Bacteria spread through air, water or by inhaling the droplets/aerosols or even by sharing utensils, dresses with an infected person. Typhoid fever can be confirmed by Widal test

Viral diseases

- Viruses are the smallest intracellular obligate parasites, which multiply within living cells. Outside the living cells they cannot carry out the characteristics of a living organism. Viruses invade living cells, forcing the cells to create new viruses. The new viruses break out of the cell, killing it and invade other cells in the body, causing diseases in human beings. Rhino viruses cause one of the most infectious human ailment called the "Common cold".

- Viral diseases are generally grouped into four types on the basis of the symptoms produced in the body organs.

➢ Pneumotropic diseases (respiratory tract infected by influenza)

- Dermotropic diseases (skin and subcutaneous tissues affected by chicken pox and measles)

- Viscerotropic diseases (blood and visceral organs affected by yellow fever and dengue fever)

- Neurotropic diseases (central nervous system affected by rabies and polio).

Bacterial resistance

If an antibiotic is used too often to fight a specific bacterial infection, the bacteria may become resistant to the specific antibiotic. Hence the specific antibiotic can no longer be used to treat the bacterial infection. Some bacteria have developed resistance to many antibiotics. Therefore, infections caused by these bacteria are difficult to be cured.

Risk of bacterial resistance can be reduced by observing the following steps

- Avoid using antibiotics to treat minor infections that can be taken care by our immune system.
- Do not use an antibiotic to treat viral infections such as common cold or flu.
- Always follow the prescription. Skipping doses or failing to complete the prescription may allow antibiotic resistance to develop.

Bacterial diseases in human beings

S.No	Diseases	Causative agent	Site of infection	Mode of transmission	Symptoms
1	Shigellosis (Bacillary dysentery)	Shigella sp.	Intestine	Food and Water contaminated by faeces/ faecal oral route	Abdominal pain, dehydration, blood and mucus in the stools
2	Bubonic plague (Black death)	Yersinia pestis	Lymph nodes	Rat flea vector – Xenopsyllache opis	Fever, headache, and swollen lymph nodes.
3	Diphtheria	Corynebacterim diphtheriae	Larynx, skin, nasal and genital passage	Droplet infection	Fever, Sore throat, hoarseness and difficulty in breathing
4	Cholera	Vibrio cholerae	Intestine	Contaminated food and water/ faecal oral route	Severe diarrhoea and dehydration.
5	Tetanus (Lock Jaw)	Clostridium tetani	Spasm of muscles	Through wound	Rigidity of jaw muscle,

				infection	increased heart beat rate and spasm of the muscles of the jaw and face.
6	Typhoid(Enteric fever)	Salmonella typhi	Intestine	Through contaminated food and water	Headache, abdominal discomfort, fever and diarrhoea.
7	Pneumonia	Streptococcus pneumoniae	Lungs	Droplet infection	Fever, cough, Painful breathing and brown sputum
8	Tuberculosis	Mycobacterium tuberculosis	Lungs	Droplet infection	Thick mucopurulan t nasal discharge.

Viral diseases in human beings

S.No	Diseases	Causative Agent	Site of infection	Mode of transmission	Symptoms
1	Common Cold	Rhino Viruses	Respiratory tract	Droplet infection	Nasal congestion and discharge, sore throat, cough and headache.
2	Mumps	Mumps virus (RNA virus) Paramyxo virus	Salivary glands	Saliva and droplet infection	Enlargement of the parotid glands.
3	Measles	Rubella virus (RNA virus), Paramyxo virus	Skin and respiratory	Droplet infection	Sore throat, running nose, cough and fever. Reddish rashes on the skin, neck and ears.
4	Viral hepatitis	Hepatitis –	Liver	Parenterak	Liver damage,

		B Virus		route, blood transfusion	jaundice, nausea, Yellowish eyes, fever and pain in the abdomen
5	Chicken pox	Varicella Zoster virus (DNA Virus)	Respiratory tract, skin and nervous system	Droplet infection and direct contact	Mild fever with itchy skin, rash and blisters
6	Poliomyelitis	Polio virus (RNA virus)	Intestine, brain, spinal cord	Droplet infection through faecal oral route	Fever, muscular stiffness and weakness, paralysis and respiratory failure
7	Dengue fever (Break bone fever)	Dengue virus or flavi virus (DENV 1-4 virus)	Skin and blood	Mosquito vector Aedesaegypti	Severe flu like illness with a sudden onset of fever and painful headache, muscle and joint pain.
8	Chikungunya	Alpha virus (Toga virus)	Nervous system	Mosquito vector Aedesaegypti	Fever and joint pain, headache and joint swelling

Nipah virus is a zoonotic virus (transmitted from animals to humans) and also transmitted through contaminated food. In infected people, it causes a range of illness from asymptomatic infection to acute respiratory illness and fatal encephalitis.

Swine flu was first recognised in the 1919 pandemic and still circulates as a seasonal flu virus. Swine flu is caused by the H1N1 virus strain. Symptoms include fever, cough, sore throat, chills, weakness and body aches. Children, pregnant women and the elderly are at risk from severe infection

Protozoan diseases

- About 15 genera of protozoans live as parasites within the human body and cause diseases. Amoebiasis also called amoebic dysentery or amoebic colitis is caused by Entamoebahistolytica, which lives in the human large intestine and feeds on food

particles and bacteria. Infective stage of this parasite is the trophozoite, which penetrates the walls of the host intestine (colon) and secretes histolytic enzymes causing ulceration, bleeding, abdominal pain and stools with excess mucus. Symptoms of amoebiasis can range from diarrhoea to dysentery with blood and mucus in the stool. House flies (Muscadomestica) acts as a carrier for transmitting the parasite from contaminated faeces and water.

- African sleeping sickness is caused by Trypanosoma species. Trypanosoma is generally transmitted by the blood sucking Tsetse flies. Three species of Trypanosoma cause sleeping sickness in man.

- T. gambiense is transmitted by Glossinapalpalis (Tsetse fly) and causes Gambian or Central African sleeping sickness.

- T. rhodesiense is transmitted by Glossinamorsitans causing Rhodesian or East African sleeping sickness.

- T. cruzi is transmitted by a bug called Triatomamegista and causes Chagas disease or American trypanosomiasis.

- Kala – azar or visceral leishmaniasis is caused by Leishmaniadonovani, which is transmitted by the vector Phlebotomus (sand fly). Infection may occur in the endothelial cells, bone marrow, liver, lymph glands and blood vessels of the spleen. Symptoms of Kala azar are weight loss, anaemia, fever, enlargement of spleen and liver.

- Malaria is caused by different types of Plasmodium species such as P. vivax, P. ovale, P. malariae and P. falciparum . Plasmodium lives in the RBC of human in its mature condition it is called as trophozoite. It is transmited from one person to another by the bite of the infected female Anopheles mosquito.

Life cycle of Plasmodium

- Plasmodium vivax is a digenic parasite, involving two hosts, man as the secondary host and female Anopheles mosquito as the primary host. The life cycle of Plasmodium involves three phases namely schizogony, gamogony and sporogony

- The parasite first enters the human blood stream through the bite of an infected female Anopheles mosquito. As it feeds, the mosquito injects the saliva containing the sporozoites. The sporozoite within the blood stream immediately enters the hepatic cells of the liver. Further in the liver they undergo multiple asexual fission (schizogony) and produce merozoites. Afterbeing released from liver cells, the merozoites penetrate the RBC's.

- Inside the RBC, the merozoite begins to develop as unicellular trophozoites. The trophozoite grows in size and a central vacuole develops pushing them to one side of cytoplasm and becomes the signet ring stage. The trophozoite nucleus then divides asexually to produce the schizont. The large schizont shows yellowish - brown pigmented granules called Schuffners granules. The schizont divides and produces mononucleatedmerozoites. Eventually the erythrocyte lyses, releasing the merozoites and haemozoin toxin into the blood stream to infect other erythrocytes. Lysis of red blood cells results in cycles of fever and other symptoms. This erythrocytic stage is cyclic and repeats itself approximately every 48 to 72 hours or longer depending on the species of Plasmodium involved. The sudden release of merozoites triggers an attack on the RBCs. Occasionally,merozoites differentiate into macrogametocytes and microgametocytes. When these are ingested by a mosquito, they develop into male and female gametes respectively.

- In the mosquito's gut, the infected erythrocytes lyse and male and female gametes fertilize to form a diploid zygote called ookinete. The ookinete migrates to the mosquito's gut wall and develop into an oocyte. The oocyte undergoes meiosis by a process called sporogony to form sporozoites. These sporozoites migrate to the salivary glands of the mosquito. The cycle is now completed and when the mosquito bites another human host, the sporozoites are injected and the cycle begins a new.

- The pathological changes caused by malaria, affects not only the erythrocytes but also the spleen and other visceral organs. Incubation period of malaria is about 12 days. The early symptoms of malaria are headache, nausea and muscular pain. The classic symptoms first develop with the synchronized release of merozoites, haemozoin toxin and erythrocyte debris into the blood stream resulting in malarial paroxysms – shivering chills, high fever followed by sweating. Fever and chills are caused partly by malarial toxins that induce macrophages to release tumour necrosis factor (TNF-α) and interleukin.

Types of malaria

S.No	Types of Malaria	Causative agent	Duration of Erythrocytic Cycle.
1	Tertian, benign tertian or vivax malaria	P. Vivax	48 hours
2	Quartan malaria	P. malariae	72 hours
3	Mild tertian malaria	P. ovale	48 hours
4	Malignant tertian or quotidian malaria	P. falciparum	36-48 hours

- Ponds, drainage ditches and other permanent bodies of water can be stocked with fishes such as Gambusia which feed on mosquito larvae. Preparations containing Bacillus thuringiensis can be sprayed to kill the mosquito larvae since it is not toxic to other forms of life. The best protection against malaria is to avoid being bitten by mosquito. People are

advised to use mosquito nets, wire gauging of windows and doors to prevent mosquito bites.

- In the 1950's the World Health Organisation (WHO) introduced the Malaria eradication programme. This programme was not successful due to the resistance of Plasmodium to the drugs used to treat it and resistance of mosquito's to DDT and other insecticides.

Malaria vaccine is used to prevent malaria. The only approved vaccine as of 2015 is RTS,S(Mosquirix). It requires four injections and has relatively low efficacy (26–50%). Due to this low efficacy, WHO does not recommend the use of RTS,S vaccine in babies between 6 and 12 weeks of age.

Fungal diseases

- Fungi was recognized as a causative agent of human diseases much earlier than bacteria. Dermatomycosis is a cutaneous infection caused by fungi belonging to the genera Trichophyton, Microsporum and Epidermophyton.

- Ringworm is one of the most common fungal disease in humans. Appearance of dry, scaly lesions on the skin, nails and scalp are the main symptoms of the disease. Heat and moisture help these fungito grow and makes them to thrive in skin folds such as those in the groin or between the toes. Ringworms of the feet is known as Athlete's foot caused by Tineapedis. Ringworms are generally acquired from soil or by using clothes, towels and comb used by infected persons.

Helminthic diseases

- Helminthes are mostly endoparasitic in the gut and blood of human beings and cause diseases called helminthiasis. The two most prevalent helminthic diseases are Ascariasis and Filariasis.

- Ascaris is a monogenic parasite and exhibits sexual dimorphism. Ascariasis is a disease caused by the intestinal endoparasiteAscarislumbricoides commonly called the round worms. It is transmitted through ingestion of embryonated eggs through contaminated food and water. Children playing in contaminated soils are also prone to have a chance of transfer of eggs from hand to mouth. The symptoms of the disease are abdominal pain, vomiting, headache, anaemia, irritability and diarrhoea. A heavy infection can cause nutritional deficiency and severe abdominal pain and causes stunted growth in children. It may also cause enteritis, hepatitis and bronchitis.

- Filariasis is caused by Wuchereriabancrofti, commonly called filarial worm. It is found in the lymph vessels and lymph nodes of man. Wuchereriabancrofti is sexually dimorphic, viviparous and digenic. The life cycle is completed in two hosts, man and the female Culexmosquito The female filarial worm gives rise to juveniles called microfilariae larvae. In the lymph glands, the juveniles develop into adults. The accumulation of the worms block the lymphatic system resulting in inflammation of the lymph nodes.

- In some cases, the obstruction of lymph vessels causes elephantiasis or fi lariasis of the limbs, scrotum and mammary glands

Maintenance of personal and public hygiene

- Hygiene is a set of practices performed to conserve good health. According to the World Health Organization (WHO), hygiene refers to "conditions and practices that help to maintain health and prevent the spread of diseases." Personal hygiene refers to maintaining one's body clean by bathing, washing hands, trimming fingernails, wearing clean clothes and also includes attention to keeping surfaces in the home and workplace, including toilets, bathroom facilities, clean and pathogen-free.

- Our public places teem with infection, contamination and germs. It seems that every surface we touch and the air we breathe are with pollutants and microbes. It's not just the public places that are unclean, but we might be amazed at the number of people who do not wash their hands before taking food, after visiting the restroom, or who sneeze without covering their faces. Many infectious diseases such as typhoid, amoebiasis and ascariasis are transmitted through contaminated food and water.

- Advancement in science and technology provide effective controlling measures for many infectious and non-infectious diseases. The use of vaccines and adopted immunization programmes have helped to eradicate small pox in India. Moreover a large number of infectious diseases like polio, diphtheria, pneumonia and tetanus have been controlled by the use of vaccines and by creating awareness among the people.

Adolescence – drug and alcohol abuse

- Adolescence begins with a period of rapid physical and sexual development called puberty to maturity at 12 to 19 years of age. Adolescence is also a highly dynamic period of psychologicaland social changes in individuals. Adolescents are vulnerable to group (peer) pressure and many youngsters are pushed into experimenting with drugs and alcohol. Proper education and guidance would enable youth to say no to drugs and alcohol and to follow a healthy life style.

- Alcohol is a psychoactive drug, which acts on the brain, affecting a person's mind and behaviour. It is a depressant, which slows down the activity of the nervous system. The intake of certain drugs for a purpose other than their normal clinical use in an amount and frequency that impair one's physical, physiological and psychological functions is called drug abuse.

- The drugs which are commonly abused include opioids, cannabinoids, coca-alkaloids, barbiturates, amphetamines and LSD.

- Opioids are drugs which bind to specific opioid receptors present in the central nervous system and intestinal tract. Heroin (smack) is chemically diacetyl morphine, which is

white, odourless and bitter crystallinecompound. It is obtained by acetylation of morphine, which is extracted from flowers of the poppy plant. Morphine is one of the strongest pain killer and is used during surgery. It is the most widely abused narcotic drug which acts as a depressant and slows down body functions.

- Cannobinoids are a group of chemicals obtained from Cannabis sativa, the Indian hemp plant. Natural cannabinoids are the main source of marijuana, ganja, hashish and charas. It interferes in the transport of the neurotransmitter, dopamine and has a potent stimulating action on the CNS, producing increased energy and a sense of euphoria.

- Cocaine is a white powder that is obtained from the leaves of the coca plant, Erythroxylum coca. It is commonly called coke or crack. Cocaine causes serious physical and psychological problems including hallucinations and paranoia. The other plants with hallucinogenic properties are Atropa belladonna and Datura.

Classification of drugs

Group	Drugs	Effects
Stimulants	Amphetamines, cocaine, nicotine and tobacco	Accelerates the activity of the brain
Depressants	Alcohol, Barbiturates, Tranquilizers	Slows down the activity of the brain
Narcotic/ Analgesics	Opium, Morphine	Act as depressants on the Central Nervous System
Cannabis	Bhang (Marijuana), Ganja, Charas	Affects the cardiovascular system
Hallucinogens	Lysergic acid diethylamide (LSD), Phencyclidine	Distorts the way one sees, hears and feels.

- Drugs like methamphetamine, amphetamines, barbiturates, tranquilizers, Lysergic acid diethylamide (LSD) are normally used as medicine to treat patients with mental illness like depression and insomnia and are often abused.

- Tobacco is smoked, chewed and used as snuff. It increases the carbon monoxide content of blood and reduces the concentration of haem bound oxygen, thus causing oxygen deficiency in the body. Tobacco contains nicotine, carbon monoxide and tars, which cause problems in the heart, lung and nervous system. Adrenal glands are stimulated by nicotine to release adrenaline and nor adrenaline which increases blood pressure and heart beat.

Addiction and dependence

- Addiction is a physical or psychological need to do or take or use certain substance such as alcohol, to the point where it could be harmful to the individual. This addictive behaviour can be personally destructive to a person. Overtime addicts start to lose not only their jobs, homes and money, but also friendship, family relationships and contact

with the normal world. Addiction to drugs and alcohol can lead to a psychological attachment to certain effects such as euphoria and temporary feeling of well being.

- Repeated use of drugs and alcohol may affect the tolerance level of the receptors present in the body. These receptors then respond only to highest doses of drugs and alcohol leading to greater intake and addiction. Excessive use of drug and alcohol leads to physical and psychological dependence. When psychological dependence develops, the drug user gets mentally 'hooked on' to the drug. The drug user constantly thinks only about the drug and has continuous uncontrollable craving for it. This state called "euphoria" is characterized by mental and emotional preoccupation with the drug.

- Physical dependence is a state in which the user's body requires a continuous presence of the drug. If the intake of the drug or alcohol is abruptly stopped, he or she would develop withdrawal symptoms. In a sense, the body becomes confused and protests against the absence of the drug. The withdrawal symptoms may range from mild tremors to convulsions, severe agitation and fits, depressed mood, anxiety, nervousness, restlessness, irritability, insomnia, dryness of throat, etc, depending on the type of drug abuse.

Effects of drugs and alcohol

- Short-term effect appears only for a few minutes after the intake of drugs and alcohol. The abuser feels a false sense of well being and a pleasant drowsiness. Some short term effects are euphoria, pain, dullness of senses, alteration in behaviour, blood pressure, narcosis (deep sleep), nausea and vomiting.

- Drugs and alcohol have long-term effect that lead to serious damages, because of the constant and excessive use. The physical and mental disturbance makes the life of the user unbearable and torturous. For example heavy drinking permanently damages the liver and brain.

- The use of alcohol during adolescence may have long-term effects. Alcohol interferes with the ability of the liver to break down fat. Over time fat accumulation and high levels of alcohol destroy the liver cells and a scar tissue grows in the place of dead cells. This scarring of the liver is called "Liver cirrhosis". Alcohol irritates the stomach lining due to the production of excess acid leading to ulcers. Excessive alcohol use weakens the heart muscle, causing scar tissue to build up in the cardiac muscle fibers. As a result, heavy drinkers have an increased risk of high blood pressure, stroke, coronary artery disease and heart attack. Korsakoff syndrome, a chronic memory disorder is most commonly caused by alcohol misuse.

Alcoholism is the inability to control drinking due to physical and emotional dependence on alcohol. Treatment involves counseling by a healthcare professional. Detoxification programme in a hospital or medical facility is an option for those who need additional assistance. Medications are available to reduce the desire to drink and smoke.

Prevention and control

- It is practically possible to prevent some one from using drugs and alcohol. Here are some ways that help to prevent drug and alcohol abuse.

➢ **Effectively dealing with peer pressure**

The biggest reason for teens to start on drugs is due to their friends / peer groups imposing pressure on them. Hence, it is important to have a better group of friends to avoid such harmful drugs and alcohol.

➢ **Seeking help from parents and peers**

Help from parents and peer group should be sought immediately so that they can be guided appropriately. Help may even be sought from close and trusted friends. Getting proper advice to sort out their problems would help the young to vent their feelings of anxiety and guilt.

➢ **Education and counselling**

Education and counselling create positive attitude to deal with many problems and to accept disappointments in life.

➢ **Looking for danger signs**

Teachers and parents need to look for sign that indicate tendency to go in for addiction.

➢ **Seeking professional and medical assistance**

Assistance is available in the form of highly qualified psychologists, psychiatrists and de-addiction and rehabilitation programmes to help individuals to overcome their problems.

Mental health – Depression

- Mental health is a state of well being of the mind, with self esteem. Self esteem means liking yourself and being able to stand up for what you believe is right. Positive mental health is an important part of wellness. A mentally healthy person reflects a good personality. Activities of mentally healthy people are always appreciated and rewarded by the society as these persons are creative as well as cooperative with others. Mental health improves the quality of life.

- Depression is a common mental disorder that causes people to experience depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep poor appetite, low energy and poor concentration.

Alcoholic Anonymous

Alcoholic anonymous was started in 1935 by a businessman and a doctor who had been a "hopeless drunk" for many years. After the men helped each other to stop drinking and to stay sober, they then founded the alcoholic anonymous to help other alcoholics. Since that

time alcoholic anonymous has spread throughout the world.

Signs and symptoms of mental depression

- Loss of self confidence and self esteem
- Anxiety
- Not being able to enjoy things that are usually pleasurable or interesting.

- Lifestyle changes like exercise, meditation, yoga and healthy food habits can help to be relieved from depression. Exercise stimulates the body to produce serotonin and endorphins, which are neurotransmitters that suppress depression. Practicing exercise in daily life creates a positive attitude .

Participating in an exercise programme can:

- Increase self-esteem
- Boost self-confidence
- Create a sense of empowerment
- Enhance social connections and relationships

- Brain is one of the most metabolically active part of the body and needs a steady stream of nutrients to function. A poor diet may not provide the nutrients for a healthy body and may provoke symptoms of anxiety and depression.

Lifestyle disorders in human beings

- The old saying that "health is wealth" is truly applicable to human beings. With the changes in life style, there are many emerging medical conditions and diseases that are reducing human longevity. Life style disorder result due to activities involving smoking, alcohol and drug abuse, consuming high fat diet, lack of exercise or living a latent life.

- The World Health Organization (WHO) in its report cautions a slow moving public health disaster due to life style disorders in the form of non-communicable diseases like diabetes, cardiovascular and lung diseases. WHO believes that not thousands but millions of people die every year within the age groupof thirty to sixty due to life style related disorders.

- The following facts will help in better understanding of life style disorders.

- Life style disorder causes cardiovascular diseases resulting in 31 percent of global deaths.

- The sedentary life style also causes deficiency of vitamins such as vitamin D resulting in fatigue, tiredness, back pain, depression, bone loss, muscle pain, etc,

- Life style disorder also includes social isolation resulting in age related problems.

- Eating junk foods that have high caloric values, rich in carbohydrates and fat can lead to obesity and early health issues.
- Consumption of processed and packaged food, which lacks in fiber may result in constipation.
- Several people today complain of irritable bowel syndrome with stomach discomfort or pain and trouble with bowel movements, causing diarrhoea. The main cause of irritable bowel syndrome is stress and other illnesses.

Life style modifications

- Avoid eating junk food and foods that have preservatives and colouring agents.
- Physical exercises such as brisk walking and yoga can be done regularly.
- Following medical advice, if any health problems in addition to life style disorders.
- To avoid smoking drugs and drinking alcohol.
- To follow a healthy balanced diet rich in vitamins and proteins.
- 7 – 8 hours of sleep every day is required.

Chapter 8 – Immunology

8. Immunology

- In the previous chapter, we have studied in detail the various infections which cause diseases in human beings. In this chapter, we shall discuss how our body protects us from these infections by the effective mechanism of the immune system.

Basic concepts of immunology

- Immunology is the study of immune system. This system protects an individual from various infective agents. It refers to all the mechanisms used by the body for protection from environmental agents that are foreign to the body.

- When the immune system does not function efficiently in an individual, it leads to infection causing disease. The overall ability of body to fight against the disease causing pathogen is called immunity. It is also called disease resistance and the lack of immunity is known as susceptibility. Immunity is highly specific.

- Normally many of the responses of the immune system initiate the destruction and elimination of invading organisms and any toxic molecules produced by them. These immune reactions are destructive in nature and are made in response only to moleculesthat are foreign to the host and not to those of host itself. This ability to distinguish foreign molecules from self is another fundamental feature of the immune system. However, occasionally, it fails to make its distinction and reacts destructively against the host's own molecules; such autoimmune diseases can be fatal to the organism.

- Almost all the macromolecules e.g. proteins, polysaccharides, nucleic acids, etc., as long as they are foreign to recipient organism can induce immune response. Any substance capable of eliciting immune response is called an ANTIGEN (Antibody Generator). There are two broad classes of immunity responses namely, innate immunity and acquired immunity (Fig. 8.1).

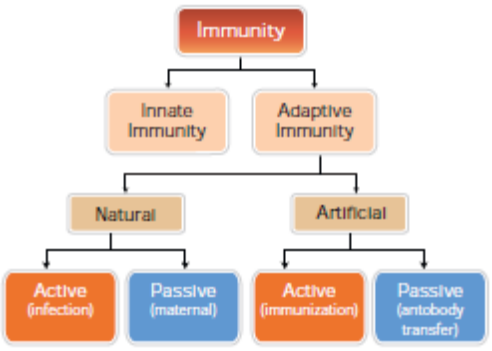

Fig. 8.1 Immune system

Type of innate Immunity	Mechanism
1. Anatomical barriers • **Skin** • **Mucus Membrane**	• Prevents the entry of microbes. Its acidic environment (pH 3-5) retards the growth of microbes. • Mucus entraps foreign microorganisms and competes with microbes for attachment.
2. Physiological barriers • **Temperature** • **Low pH** • **Chemical mediators**	• Normal body temperature inhibits the growth of pathogens. Fever also inhibits the growth of pathogens. • Acidity of gastric secretions (HCl) kills most ingested microbes. • Lysozyme acts as antibacterial agent and cleaves the bacterial cell wall. Interferon's induce antiviral state in the uninfected cells. Complementary substances produced from leucocytes lyse the pathogenic microbes or facilitate phagocytosis.
3. Phagocytic bazrriers	Specialized cells (Monocytes, neutrophils, tissue macrophages) phagocytose, and digest whole micro - organisms.
4. Inflammatory barriers	Tissue damage and infection induce leakage of vascular fluid, containing chemotactic signals like serotonin, histamine and prostaglandins. They influx the phagocytic cells into the affected area. This phenomenon is called diapedesis.

8.2 Innate immunity

- Innate immunity is the natural phenomenon of resistance to infection which an individual possesses right from the birth. The innate defense mechanisms are non-specific in the sense that they are effective against a wide range of potentially infectious agents. It is otherwise known as non-specific immunity or natural immunity. A number of innate defense mechanisms are operative non-specifically against a large number of microorganisms as shown in the Table 8.1 and Fig. 8.2.

Acquired immunity

- The immunity that an individual acquires after birth is known as acquired immunity. It is the body's resistance to a specific pathogen.

- The unique features of acquired immunity are antigenic specificity, diversity, recognition of self and non-self and immunological memory.

Components of acquired immunity

- Acquired immunity has two components – cell mediated immunity (CMI) and antibody mediated immunity or humeral immunity.

1. Cell mediated immunity

- When pathogens are destroyed by cells without producing antibodies, then it is knownas cell mediated immune response or cell mediated immunity. This is brought about by T cells, macrophages and natural killer cells.

2. Antibody mediated immunity or humoral immunity

- When pathogens are destroyed by the production of antibodies, then it is known as antibody mediated or humoral immunity. This is brought about by B cells with the help of antigen presenting cells and T helper cells. Antibody production is the characteristic feature of vertebrates only.

Types of acquired immunity

- Acquired immunity may be active immunity or passive immunity (Table 8.2). The immunological resistance developed by the organisms through the production of antibodies in their body is called active immunity. Active immunity is acquired through the use of a person's immune responses, which lead to the development of memory cells. Active immunity results from an infection or an immunization. Passive immunity does not require the body to produce antibodies to antigens. The antibodies are introduced from outside into the organism. Thus, passive immunity is acquired without the activation of a person's immune response, and therefore there is no memory.

The process of production of blood cells in the bone marrow is called haematopoiesis.

S. No	Active Immunity	Passive immunity
1.	Active immunity is produced actively by host's immune system.	Passive Immunity is received passively and there is no active host participation.
2.	It is produced due to contact with pathogen or bu its antigen.	It is produced due to antibodies obtained from outside.
3.	It is durable and effective in protection	It is transient and les effective.
4.	Immunological memory is present.	No memory.
5.	Booster effect on subsequent does is possible.	Subsequent dose is less effective.
6.	Immunity is effective only after a	Immunity develops immediately

	short period.	

Immune responses

- The immune responses may be primary or secondary (Table 8.3).

Primary immune response

- The primary immune response occurs when a pathogen comes in contact with the immune system for the first time. During this, the immune system has to learn to recognize the antigen, produce antibody against it and eventually produce memory lymphocytes. The primary immune response is slow and short – lived.

S. No	Primary Immune Response	Secondary Immune Response
1.	It occurs as a result of primary contact with an antigen.	It occurs as a result of second and subsequent contacts with the same antigen.
2.	Antibody level reaches peak in 7 to 10 days.	Antibody level reaches peak in 3 to 5 days.
3.	Prolonged periods is required	It establishes immunity in a short time.
4.	There is rapid decline in antibody level.	Antibody level remains high for longer period.
5.	It appears mainly in the lymph nodes and spleen.	It appears mainly in the bone marrow, followed by the spleen and lymph nodes.

Secondary immune response

- The secondary immune response occurs when a person is exposed to the same antigen again. During this, time immunological memory has been established and the immune system can start producing antibodies immediately. Within hours after recognition of the antigen, a new army of plasma cells are generated. Within 2 to 3 days, the antibody concentration in the blood rises steeply to reach much higher level than primary response. This is also called as "booster response".

Lymphoid organs

- Immune system of an organism consists of several structurally and functionally different organs and tissues that are widely dispersed in the body. The organs involved in the organ, maturation and proliferation of lymphocytes are called lymphoid organs (Fig.8.3). Based on their functions, they are classified into primary or central lymphoid organs and secondary or peripheral lymphoid organs trap antigens and make it available for mature lymphocytes, which can effectively fight against these antigens.

Primary lymphoid organs

- Bursa of Fabricius of birds, bone marrow and thymus gland of mammals constitute the primary lymphoid organs involved in the production and early selection of lymphocytes. These lymphocytes become dedicated to a particular antigenic specificity. Only when the lymphocytes mature in the primary lymphoidal organs, they become immunocompetent cells. In mammals, B cell maturation occurs in the bone marrow and T cells maturation occurs in the thymus.

Thymus

- The thymus is a flat and bilobed organ located behind the sternun, above the heart. Each lobe of the thymus contains numerous lobules, separated from each other by connective tissue called septa. Each lobule is differentiated into two compartments, the outer compartment or outer cortex, is densely packed with immature T cells called thymocytes, whereas the inner compartment or medulla is sparsely populated with thymocytes. One of its main secretions is the hormone thymosin. It stimulates the T cell to become mature and immune competent. By the early teens, the thymus begins to atrophy and is replaced by adipose tissue (Fig. 8.4). Thus thymus is most active during the neonatal and pre-adolescent periods.

Bone marrow

- Bone marrow is a lymphoid tissue found within the spongy portion of the bone. Bone marrow contains stem cells known as haematopoietic cells. These cells have the potential to multiply through cell division and either remain as stem cells or differentiate and mature into different kinds of blood cells.

Secondary or peripheral lymphoid organs

- In secondary or peripheral lymphoid organs, antigen is localized so that it can be effectively exposed to mature lymphocytes. The best examples are lymph nodes, appendix, Peyer's patches of gastrointestinal tract, tonsils, adenoids, spleen, MALT (Mucosal-Associated Lymphoid Tissue), GALT (Gut-Associated Lymphoid Tissue), BALT (Bronchial/Tracheal-Associated Lymphoid Tissue).

Peyer's patches are oval-shaped areas of thickened tissue that are embedded in the mucus-secreting lining of the small intestine of humans and other vertebrate animals. Peyer's patches contain a variety of immune cells, including macrophages, dendritic cells, T cells, and B cells.

The tonsils (palatine tonsils) are a pair of soft tissue masses located at the back of the throat (pharynx). The tonsils are part of the lymphatic system, which help to fight infections. They stop invading germs including bacteria and viruses.
Spleen is a secondary lymphoid organ located in the upper part of the abdominal cavity close to the diaphragm. Spleen contains B and T cells. It brings humoral and cell mediated

immunity.

- The adenoids are glands located in the roof of the mouth, behind the soft palate where the nose connects to the throat. The adenoids produce antibodies that help to fight infections. Typically, the adenoids shrink during adolescence and may disappear by adulthood.

Lymph node

- Lymph node is a small bean-shaped structure and is part of the body's immune system. It is the first one to encounter the antigen that enters the tissue spaces. Lymph nodes filter and trap substances that travel through the lymphatic fluid. They are packed tightly with white blood cells, namely lymphocytes and macrophages. There are hundreds of lymph nodes found throughout the body. They are connected to one another by lymph vessels. Lymph is a clear, transparent, colourless, mobile and extracellular fluid connective tissue. As the lymph percolates through the lymph node, the particulate antigen brought in by the lymph will be trapped by the phagocytic cells, follicular and interdigitating dendritic cells.

- Lymph node has three zones (Fig. 8.5). They are the cortex, paracortex and medulla. The outer most layer of the lymph node is called cortex, which consists of B-lymphocytes, macrophages, and follicular dendritic cells. The paracortex zone is beneath the cortex, which is richly populated by T lymphocytes and interdigitating dendritic cells. The inner most zone is called the medulla which is sparsely populated by lymphocytes, but many of them are plasma cells, which actively secrete antibody molecules. As the lymph enters, it slowly percolates through the cortex, paracortex and medulla, giving sufficient chance for the phagocytic cells and dendritic cells to trap the antigen brought by the lymph. The lymph leaving a node carries enriched antibodies secreted by the medullary plasma cells against the antigens that enter the lymph node. Sometimes visible swelling of lymph nodes occurs due to active immune response and increased concentration of lymphocytes. Thus swollen lymph nodes may signal an infection. There are several groups of lymph nodes are found in the neck, under the chin, in the armpits and in the groin.

The mucosa – associated lymphoid tissue (MALT) is a diffuse system of small concentrations of lymphoid tissue in the alimentary, respiratory and urino-genital tracts. MALT is populated by lymphocytes such as T and B cells, as well as plasma cells and macrophages, each of which is well situated to encounter antigens passing through the mucosal epithelium.

Gut-associated lymphoid tissue (GALT) is a component of the mucosa-associated lymphoid tissue (MALT) which worked in the immune system to protect the body from invasion in the gut.

Bronchus Associated Lymphoid Tissues **(BALT)** also a component of MALT is made of

lymphoid tissue (tonsils, lymph nodes, lymph follicles) is found in the respiratory mucosae from the nasal cavities to the lungs.

Cells of the immune system

- The immune system is composed of many interdependent cells that protect the body from microbial infections and the growth of tumour cells. The cellular composition of adult human blood is given in Table8. 4.

Cell type	Number of cells per μ l	Approximate percentage
Red blood	4200,000- 6500,000	-
White blood cells		
i **Agranulocytes**		
• Lymphocytes	1500 - 4000	20 – 30
• Monocytes	200 - 950	2 – 7
ii **Granulocytes**		
• Neutrophils	2000 – 7000	50- 70
• Basophils	50 - 100	<1
• Eosinophils	40 - 500	2 – 5
• Platelets	150,000 – 500,000	

- All these cells are derived from pluripotent haematopoetic stem cells. Each stem cell had the capacity to produce RBC, WBC and platelets. The only cells capable of specifically recognising and producing an immune response are the lymphocytes. The other types of white blood cells play an important role in non-specific immune response, antigen presentation and cytokine production.

Lymphocytes

- About 20-30% of the white blood cells are lymphocytes. They have a large nucleus filling most of the cell, surrounded by a little cytoplasm. The two main types of lymphocytes are B and T lymphocytes. Both these are produced in the bone marrow. B lymphocytes (B cells) stay in the bone marrow until they are mature. Then they circulate around the body. Some remain in the blood, while others accumulate in the lymph nodes and spleen. T lymphocytes leave the bone marrow and mature in the thymus gland. Once mature, T cells also accumulate in the same areas of the body as B cells. Lymphocytes have receptor proteins on their surface. When receptors on a B cell bind with an antigen, the B cell becomes activated and divides rapidly to produce plasma cells. The plasma cells produce antibodies. SomeB cells do not produce antibodies but become memory cells. These cells are responsible for secondary immune response. T lymphocytes do not produce antibodies. They recognize antigen-presenting cells and destroy them. The two important

types of T cells are Helper T cells and Killer T cells. Helper T cells release a chemical called cytokine which activates B cells. Killer cells move around the body and destroy cells which are damaged or infected (Fig. 8.6).

- Apart from these cells neutrophils and monocytes destroy foreign cells by phagocytosis. Monocytes when they mature into large cells, they are called macrophages which perform phagocytosis on any foreign organism.

Dendritic cells are called so because it's covered with long, thin membrane extensions that resemble dendrites of nerve cells. These cells present the antigen to T-helper cells. Four types of dendritic cells are known. They are Langerhans, interstitial cells, myeloid and lymphoid cells.

Antigens

- The term antigen (Ag) is used in two senses, the first to describe a molecule which generates an immune response and the second, a molecule which reacts with antibodies. In general antigens are large, complex molecular substances that can induce a detectable immune response. Thus an antigen is a substance that is specific to an antibody or a T-cell receptor and is often used as a synonym for immunogen.

The histocompatibility antigens are cell surface antigens that induce an immune response leading to rejection of allografts.

- An immunogen is a substance capable of initiating an immune response. Haptens are substance that are non-immunogenic but can react with the products of a specific immune response. Substances that can enhance the immune response to an antigen are called adjuvants. Epitope is an antigenic determinant and is the active part of an antigen. A paratope is the antigen – binding site and is a part of an antibody which recognizes and binds to an antigen.

Antigenicity is the property of a substance (antigen) that allows it to react with the products of the specific immune response.

Types of antigens

- On the basis of origin, antigens are classified into exogenous antigens and endogenous antigens. The antigens which enter the host from the outside in the form of microorganisms, pollens, drugs, or pollutants are called exogenous antigens. The antigens which are formed within the individual are endogenous antigens. The best examples are blood group antigens.

Antibodies

- Antibodies are immunoglobulin (Ig) protein molecules synthesized on exposure to antigen that can combine specifically with the antigen. Whenever pathogens enter our

body, the B-lymphocytes produce an army of proteins called antibodies to fight with them. Thus, they are secreted in response to an antigen (Ag) by the effect of B cells called plasma cells. The antibodies are classified into five major categories, based on their physiological and biochemical properties. They are IgG (gamma), IgM (mu), IgA (alpha), IgD (delta) and IgE (epsilon).

- In the 1950s, experiments by Porter and Edelman revealed the basic structure of the immunoglobulin. An antibody molecule is Y shaped structure that comprises of four polypeptide chains, two identical light chains (L) of molecular weight 25,000 Da (approximately 214 amino acids) and two identical heavy chains (H) of molecular weight 50,000 Da (approximately 450 amino acids). The polypeptide chains are linked together by di-sulphide (S-S) bonds. One light chain is attached to each heavy chain and two heavy chains are attached to each other to form a Y shaped (Fig. 8.7) structure. Hence, an antibody is represented by H2 L2. The heavy chains have a flexible hinge region at their approximate middles.

- Each chain (L and H) has two terminals. They are C - terminal (Carboxyl) and amino or N-terminal. Each chain (L and H) has two regions. They have variable (V) region at one end and a much larger constant (C) region at the other end. Antibodies responding to different antigens have very different (V) regions but their (C) regions are the same in all antibodies. In each arm of the monomer antibody, the (V) regions of the heavy and light chains combines to form an antigen – binding site shaped to 'fit' a specific antigenic determinant. Consequently each antibody monomer has two such antigen – binding regions. The (C) regions that forms the stem of the antibody monomer determine the antibody class and serve common functions in all antibodies. The functions of immunoglobulin are agglutination, precipitation, opsonisation, neutralization etc.,

Antigen and antibody interaction

- The reaction between an antigen and antibody is the basis for humoral immunity or antibody mediated immunity. The reaction between antigen and antibody occurs in three stages. During the first stage, the reaction involves the formation of antigen - antibody complex. The next stage leads to visible events like precipitation, agglutination, etc,.The final stage includes destruction of antigen or its neutralization (Fig. 8.8).

Binding force of antigen - antibody reaction

- The binding force between antigen and antibody is due to three factors. They are closeness between antigen and antibody, non-covalent bonds or intermolecular forces and affinity of antibody.When antigen and antibody are closely fitted, the strength of binding is great. When they are apart binding strength is low.The bonds that hold the antigen to the antibody combining site are all non-covalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds. Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.

- The chief application of antigen - antibody reactions are to determine blood groups for transfusion, to study serological ascertainment of exposure to infectious agents, to develop immunoassays for the quantification of various substances, to detect the presence or absence of protein in serum and to determine the characteristics of certain immunodeficiency diseases.

Different types of antigen and antibody reactions

- The reaction between soluble antigen and antibody leads to visible precipitate formation, which is called precipitin reaction. Antibodies that bring about precipitate formation on reacting with antigens are called as precipitins.
- Whenever a particulate antigen interacts with its antibody, it would result in clumping or agglutination of the particulate antigen, which is called agglutination reaction. The antibody involved in bringing about agglutination reaction is called agglutinin.

- Opsonisation or enhanced attachment is the process by which a pathogen is marked of ingestion and destruction by a phagocyte. Opsonisation involves the binding of an opsonin i.e., antibody, to a receptor on the pathogen's cell membrane. After opsonin binds to the membrane, phagocytes are attracted to the pathogen. So, opsonisation is a process in which pathogens are coated witha substance called an opsonin, marking the pathogen out for destruction by the immune system. This results in a much more efficient phagocytosis.

- The neutralization reactions are the reactions of antigen-antibody that involve the elimination of harmful effects of bacterial exotoxins or a virus by specific antibodies. These neutralizing substances i.e., antibodiesare known as antitoxins. This specific antibody is produced by a host cell in response to a bacterial exotoxin or corresponding toxoid (inactivated toxin).

Vaccines

- A vaccine is a biological preparation that provides active acquired immunity to a particular disease and resembles a microorganism and is often made from weakened of attenuated or killed forms of the microbes, their toxins, or one of its surface proteins. Vaccines "teach "our body how to defend itself when viruses or bacteria, invade it/ Vaccines deliver only very little amounts of inactivated or weakened viruses or bacteria, or parts of them. This allows the immune system to recognize the organism without actually experiencing the diseases. Some vaccines need to be given more than once (i.e., a 'booster' vaccination) to make sure the immune system can overcome a real infection in the future.

- First generation vaccine is further subdivided into live attenuated vaccine, killed vaccine and toxoids (Fig. 8.9). Live attenuated vaccines use the weakened (attenuated), aged, less virulent form of the virus. E.g. Measles, mumps and rubella (MMR) vaccine and the

Varicella (chickenpox) vaccine, Killed (inactivated) vaccines are killed or inactivated by heat and other methods. E.g. Salk's polio vaccine. Toxoid vaccines contain a toxin or chemical secreted by the bacteria or virus. They make us immune to the harmful effects of the infection, instead of to the infection itself. E.g. DPT vaccine (Diphtheria, Pertussis and Tetanus).

- Second generation vaccine contains the pure surface antigen of the pathogen. E.g.Hepatitis-B vaccine. Third generation vaccine contains the purest and the highest potency vaccines which are synthetic in generation. The latest revolution in vaccine is DNA vaccine or recombinant vaccine (Refer Chapter- 10 for details).

Vaccino therapy is the method of use of vaccine from treatment of disease. Dr. Edward Jenner prepared first vaccine for small pox in 1796. Polio vaccine was developed by Dr. Jonas Salk (vaccine consists of inactivated microorganism) and Dr. Albert Sabin (live attenuated oral poliacaccine). Louis Pasteur (1885) discovered vaccine against rabies, anthrax and cholera. BCG vaccine was developed by Calmette and Guerin against tuberculosis in France in the Year 1908.

Vaccination and immunization

- "Vaccination is the process of administrating a vaccine into the body or the act of introducing a vaccine into the body to produce immunity to a specific disease." Immunization is the process of the body building up immunity to a particular disease. Immunization describes the actual changes in the body after receiving a vaccine. Vaccines work by fighting the pathogen and then recording it in their memory system to ensure that the next time this pathogen enters the body, it is eliminated far quickly. Once, the body is able to fight against the disease, it is believed to have built the immunity for it, also known as the body being immunized against the disease.

Hypersentivity – Overactive Immune Response

- Some of the individuals are very sensitive to some particles present in the environment. The exaggerated response of the immune system to certain antigens present in the environment is called allergy (allo-altered, erg-reaction). The substances to which such an immune response is produced are called allergens. Anallergen is an antigen that causes an allergic reaction. Allergic reactions begin within few seconds after the contact with the allergen and last about half an hour. The common examples of allergens are mites in dust, pollens and some proteins in insect venom. Hay fever and asthma are some common examples of allergy. Symptoms of allergic reactions include sneezing, watery eyes, running nose and difficulty in breathing. Allergy is a form of over active immune response mediated by IgE and mast cells. It can also be due to the release of chemicals like histamine and serotonin from the mast cells. Anaphylaxis is the classical immediate hypersensitivity reaction. It is a sudden, systematic, severe and immediate hypersensitivity reaction occurring as a result of rapid generalized mast-cell degranulation.

Immunodeficiency disease –Ineffective immune response – AIDS

- Immunodeficiency results from the failure of one or more components of the immune system. Primary immune deficiencies are caused by genetic developmental defects. Secondary immune deficiencies arise due to various reasons like radiation, use of cytolytic and immunosuppressive drugs and infections. AIDS is an acronym for Acquired Immuno Deficiency Syndrome. It is the deficiency of immune system, acquired during the life time of an individual indicating that it is not a congenital disease. AIDS is caused by Human Immuno Deficiency Virus (HIV). It selectively infects helper T cells. The infected helper T cells will not stimulate antibody production by B-cells resulting in loss of natural defence against viral infection. On the basis of genetic characteristics and differences in the viral antigens, HIV is classified into the types 1 and 2 (HIV-1, HIV-2).

Structure of HIV

- The human immunodeficiency virus belongs to the genus Lentivirus. When observed under the electron microscope, HIV is seen as a spherical virus, 100-120 nm in diameter, containing a dense core surrounded by a lipoprotein envelope. The envelope has glycoprotein (gp) spikes termed gp 41 and gp 120. At the core, there are two large single stranded RNA. Attached to the RNA are molecules of reverse transcriptase. It also contains enzymes like protease and ribonuclease. The core is covered by a capsid made of proteins. This is followed by another layer of matrix proteins as shown in the Fig 8.10.

HIV Transmission

- The HIV is often located within the cells especially in macrophages. HIV can survive for 1.5 days inside a cell but only about 6 hours outside a cell. Routes of HIV transmission include unsafe sexual contact, blood-contaminated needles, organ transplants, blood transfusion and vertical transmission from HIV infected mother to child. HIV is not transmitted by insects or by casual contact.

- After getting into the body of the person, the virus enters into macrophages where RNA genome of the virus replicates to form viral DNA with the help of the enzyme reverse transcriptase. This viral DNA gets incorporated into the DNA of host cells and directs the infected cells to produce viral particles. The macrophages continue to produce virus and in this way acts like a HIV factory. Simultaneously, HIV enters into helper T-lymphocytes, replicates and produces progeny viruses. The progeny viruses released in the blood attack other helper T-lymphocytes. This is repeated, leading to a progressive decrease in the number of helper T lymphocytes in the body of the infected person. During this period, the person suffers from bouts of fever, diarrhoea and weight loss. Due to decrease in the number of helper T lymphocytes, the person starts suffering from infections and becomes immune deficient and unable to protect against any infection.

- A simple blood test is available that can determine whether the person has been infected with HIV. The ELISA test (Enzyme Linked ImmuneSorbent Assay) detects the presence of HIV antibodies. It is a preliminary test. Western blot test is more reliable and a

confirmatory test. It detects the viral core proteins. If both tests detect the presence of the antibodies, the person is considered to be HIV positive.

- AIDS has no cure. Prevention of AIDS is the best option. Advocating safe sex and promoting regular check-up, safe blood for transfusion, use of disposable needles, use of condoms during sexual contact, prevention of drug abuse, AIDS awareness programme by NACO (National AIDS Control Organisation), NGOs (Non-Governmental Organisations) and WHO are to prevent the spreading of AIDS.

Autoimmune diseases

- Autoimmunity is due to an abnormal immune response in which the immune system fails to properly distinguish between self andnon-self and attacks its own body. Our body produces antibodies (auto antibodies) and cytotoxic T cells that destroy our own tissues. If a disease-state results, it is referred to as auto-immune disease. Thus, autoimmunity is a misdirected immune response. Autoimmunity is evidenced by the presence of auto antibodies and T cells that are reactive with host antigens. When the cells act as antigens in the same body, they are called auto antigens.

- Autoimmune diseases in human can be divided into two broad categories, namely organ-specific and non-organ-specific (systemic) autoimmune diseases. In organ-specific disease, the autoimmune process is directed mostly against one organ. The autoantibodies may block the functions performed by the organs. Examples include Hashimoto's thyroiditis, Graves' disease (thyroid gland) and Addison's disease (adrenal glands). In non-organ specific (systemic) disorders, autoimmune activity is widely spread throughout the body. Rheumatoid arthritis and multiple sclerosis are example for systemic disorder.

Tumour immunology

- A tumour or neoplasm is a group of cells whose growth has gone unchecked. When a tumour continues to grow and invades healthy tissue, it is called cancer. They spread to other parts of the body from the tumour and give rise to secondary tumour. This is known as metastasis. Tumour may be benign or malignant depending on its characteristics. Benign or non-cancerous tissues are capable of indefinite growth and do not invade other body parts. In the malignant tumour, the cells grow indefinitely, detach and migrate into healthy surrounding tissues.

- In normal cells, cell growth and differentiation is highly controlled and regulated. But in cancer cells, there is breakdown of this regulatorymechanism. Normal cells show a property called contact inhibition, which inhibits uncontrolled growth. Cancer cells do not have this property. As a result, cancerous cells divide continuously giving rise to mass of tissues called tumours (Table 8.5).

- When a cell undergoes malignant transformation, it acquires new surface antigen and may also lose some normal antigens. These antigens are present on the membranes of

malignant cells and they induce an immune response. Both humoral and cellular responses can be observed in malignancy. Cancer cells can avoid immune detection as they are not foreign bodies but are abnormally functioning body cells. This makes them difficult to treat.

- The concept of immunological surveillance postulates that the primary function of the immune system is to "seek and destroy" malignant cells that arise by somatic mutation. The efficiency of the surveillance mechanism reduces either as a result of ageing or due to congenital or acquired immune deficiencies, leads to increased incidence of cancer. Thus, if immunological surveillance is effective, cancer should not occur. The development of tumour represents a lapse in surveillance.

Immunotherapy of cancer

- Immunotherapy also called biological therapy uses substances made by the body or in a laboratory (monoclonal antibodies) to improve or to resist the immune system function. Different approaches have been attempted in the immunotherapy of cancer. Immunotherapy appears to be important in getting rid of the residual malignant cells after the gross tumour has been removed. The best results in the treatment of cancer is to follow an integrated approach to therapy, combining surgery, radiotherapy, chemotherapy and immunotherapy.

Scope of Immunology

The younger graduates in this field can find number of employment opportunities in Government as well as private hospitals. The scope of the immunology is immunotherapy, microbial immunology, clinical immunology, cellularimmunology, allergy and immunology, translational immunology, transplantation immunology, neuro-inflammatory disorders, tumour immunology, vaccine immunology, inflammatory disorders, ocular immunology and inflammation.

Summary

- Immunology deals with a study of the immune system. The immune system recognises and eliminates the invaders, and the ability of the body to overcome the pathogen is called immunity. Immunity is classified into innate immunity and acquired immunity. Acquired immunity is further classified into cell mediated immunity and antibody mediated immunity as its components. Acquired immunity may be active or passive immunity. Immune response is the body's response to pathogens and it may be primary or secondary. The organs involved in the origin, maturation and proliferation of lymphocytes are called lymphoid organs. Thymus, bone marrow are primary lymphoid organs. The secondary lymphoid organs are lymph node, MALT, GALT and BALT.

- An antigen is a substance that is specific to an antibody. An immunogen is a substance capable of initiating an immune response. Haptens are substance that are non-immunogenic but can react with the product of a specific immune response. Substances

that can enhance the immune response to an antigen are called adjuvants. An epitope is also known as antigenic determinant and is the active part of the antigen. A paratope is the part of antibody. Precipitation, agglutination, neutralization, opsonisation etc. are the different types of antigen and antibody reaction. A vaccine is biological preparation that provides active acquired immunity. The malfunctioning of immune system leads to hypersensitivity, immunodeficiency or autoimmune diseases. A tumour or neoplasm is a group of cells whose growth has gone unchecked. The best results in the treatment of cancer is achieved by an integrated approach to therapy, surgery, radiotherapy, chemotherapy and immunotherapy.

12th zoology
Unit - 9. Microbes in Human Welfare

- Microbes such as bacteria, fungi, protozoa, certain algae, viruses, viroids and prions are some of the major components of the biological system on Earth. Several microorganisms are beneficial and contribute to human welfare. Microbes are presenteverywhere – in soil, water, air and within bodies of animals and plants. Microbes like bacteria and fungi can be grown on nutritive media to form colonies which can be visibly seen. Some of the microbes useful to human welfare are discussed here.

Microbes in household products

- In every-day life, microbes and their products are used in the preparation of idli, dosa, cheese, curd, yogurt, dough, bread, vinegar, etc., Bacteria like Lactobacillus acidophilus, L. lactis and Streptococcus lactis commonly called lactic acid bacteria (LAB) are probiotics which check the growth of pathogenic microbes in the stomach and other parts of the digestive tract.The LAB bacteria grows in milk and convert it into curd, thereby digesting the milk protein casein. A small amount of curd added to fresh milk as a starter or inoculum contains millions of Lactobacilli, which under suitabletemperature (≤40oC) multiply and convert milk into curd. Curd is more nutritious than milk as it contains a number of organic acids and vitamins.

- **Prebiotics** are compounds in food (fibers) that induce the growth or activity of beneficial microorganisms.
- **Probiotics** are live microorganisms intended to provide health benefits when consumed, generally by improving or restoring the gut flora.

- Yogurt is produced by bacterial fermentation of milk, and lactic acid is produced as a by-product. Microorganisms such as *Streptococcus thermophilus* and Lactobacillus bulgaricus coagulate the milk protein and convert the lactose in the milk to lactic acid. The flavour in yogurt is due to acetaldehyde.Cheese is a dairy product produced in a wide range of flavours, textures and is formed by coagulation of the milk protein, casein. During cheese production, milk is usually acidified and the enzyme rennet is added to cause coagulation. The solids are separated and pressed to form cheese. Most cheese are made with a starter bacteria, Lactococcus, Lactobacillus or Streptococcus.

- Paneer (cottage cheese) is fresh cheese common in South Asia, especially in India. It is made by curdling milk with lemon juice, vinegar and other edible acids. Large holes in Swiss cheese is due to the production of large amount of carbon-di-oxide by the bacterium Propionibacteriumshermanii.

- The dough used in the preparation of idlis and dosas are fermented by the bacteria Leuconostocmesenteroides whereas the dough used in bread making is fermented by Saccharomyces cerevisiae (Baker's Yeast). Fermentation of glucose mainly forms ethyl alcohol and carbon-di-oxide, which is responsible for leavening of dough. When leavened

dough is baked, both carbon-di-oxide and ethyl alcohol evaporate making the bread porous and soft.

Single cell protein (SCP)

- Single cell protein refers to edible unicellular microorganisms like Spirulina. Protein extracts from pure or mixed cultures of algae, yeasts, fungi or bacteria may be used as ingredient or as a substitute for protein rich foods and is suitable for human consumption or as animal feed.

Microbes in industrial products

- Microbes are used to synthesize a number of products valuable to human beings. Products like beverages, antibiotics, organic acids, amino acids, vitamins, biofuels, single cell protein, enzymes, steroids, vaccines, pharmaceutical drugs, etc., are produced in industries. Production on a large scale requires growing microbes in very large vessels called fermentors. A fermentor (bioreactor) is a closed vessel with adequate arrangement for aeration, agitation, temperature, pH control and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products.

Antibiotic production

- Antibiotics are chemical substances produced by microorganisms which can kill or retard the growth of other disease causing microbes even in low concentration. Antibiotic means "against life". Antibiotics are used to treat diseases such as plague, meningitis, diphtheria, syphilis, leprosy, tuberculosis etc., Selman Waksman discovered Streptomycin and was the first to use the term "antibiotic" in 1943.

- While working on Staphylococci bacteria, Alexander Fleming observed a green mould growing in one of his unwashed culture plates around which Staphylococci could not grow. He found that it was due to a chemical produced by the mould and he named it as penicillin, which was the first antibiotic discovered by Alexander Fleming in 1926 (Fig. 9.1). Penicillin is produced by the fungi Penicilliumnotatum and Penicilliumchrysogenum. It is bactericidal (antibiotics that kill bacteria) in action and inhibits the synthesis of the bacterial cell wall. Penicillin is also referred as the "queen of drugs" and its full potential as an effective antibiotic was established much later by Earnest Chain and Howard Florey when they treated the wounded soldiers in World War II with penicillin. Fleming, Chain and Florey were awarded the Nobel prize in 1945 for the discovery of penicillin.

- Antibiosis is the property of antibiotics to kill microorganisms.
- Broad-spectrum antibiotics act against a wide range of disease-causing bacteria.
- Narrow-spectrum antibiotics are active against a selected group of bacterial types.

Hypersensitivity reaction is a major problem with the use of penicillin, resulting in nausea, vomiting, wheezing and ultimately cardiovascular collapse. To check the sensitivity

reaction, doctors use a needle to prick the forearm of the patients to give a weak dose of penicillin. An itchy red region in the forearm is an indication that the patient is allergic to penicillin. This test is important before administration of penicillin to a patient.

- Tetracycline is a broad spectrum bacteriostatic antibiotic (antibiotics that limit the growth of bacteria) that inhibits microbial protein synthesis. Chlortetracycline is the first antibiotic of this group, isolated from the cultures of Streptomyces aureofaciens. Streptomycin is a broad spectrum antibiotic isolated from the actinomycetes, Streptomyces griseus. It is bactericidal against both gram positive and gram negative bacteria, especially against Mycobacterium tuberculosis. Antibiotics, such as erythromycin, chloromycetin, griseofulvin, neomycin, kenamycin, bacitracin, etc., are also isolated as microbial products.

Antibiotic resistance

- Antibiotic resistance occurs when bacteria develop the ability to defeat the drug designed to kill or inhibit their growth. It is one of the most acute threat to public health. Antibiotic resistance is accelerated by the misuse and over use of antibiotics, as well as poor infection prevention control. Antibiotics should be used only when prescribed by a certified health professional. When the bacteria become resistant, antibiotics cannot fight against them and the bacteria multiply. Narrow spectrum antibiotics are preferred over broad spectrum antibiotics. They effectively and accurately target specific pathogenic organisms and are less likely to cause resistance. "Superbug" is a term used todescribe strains of bacteria that are resistant to the majority of antibiotics commonly used today.

Fermented beverages

- Microbes especially yeast is being used from time immemorial for the production of beverages like wine, beer, whisky, brandy and rum. Wine is among the oldest alcoholic beverages known and is produced by fermentation of fruit juice by yeast. Zymology is an applied science which deals with the biochemical process of fermentation and its practical uses. Saccharomyces cerevisiae commonly called brewer's yeast is used for fermenting malted cereals and fruit juices to produce various alcoholic beverages. Wine and beer are produced without distillation, whereas whisky, brandy and rum are obtained by fermentation and distillation.
- Oenology is the science and study of wine and wine making. Wine is made from the fermentation of grape juice. Grape juice is fermented by various strains of Saccharomyces cerevisiae into alcohol. Grape wine is of two types, red wine and white wine. For red wine, black grapes are used including skins and sometimes the stems also are used. In contrast white wine is produced only from the juice of either white or red grapes without their skin and stems. Beer is produced from germinated barley malt grain by Saccharomyces carlsbergensis or Saccharomyces cerevisiae. Rum is made from fermented sugarcane or molasses or directly from sugarcane juice by Saccharomyces cerevisiae. Whisky is a type of distilled alcoholic beverage made from fermented grain mash by Saccharomyces cerevisiae.

Alcohol content in various beverages

- Beer contains 3 to 5 percent of alcohol.
- Wine contains 9 to 14 percent alcohol. Wine coolers are made of wine mixed with carbonated water and flavourings. Wine coolers have about 4 to 6 percent alcohol.
- Distilled spirits such as whiskey, gin, scotch and vodka usually contain 35 to 50 percent alcohol.

- In some parts of South India, a traditional drink called pathaneer is obtained from fermenting sap of palms and coconut trees. A common source is tapping of unopened spadices of coconut. It is a refreshing drink, which on boiling produces jaggery or palm sugar. When pathaneer is left undisturbed for few hours it gets fermented to form toddy with the help of naturally occurring yeast, to form a beverage that contains 4 percent alcohol. After 24 hours toddy becomes unpalatable and is used for the production of vinegar.

- Saccharomyces cerevisiae is the major producer of ethanol (C_2H_5OH). It is used for industrial, laboratory and fuel purposes. So ethanol is referred to as industrial alcohol. Bacteria such as Zymomonasmobilis and Sarcinaventriculi are also involved in ethanol production. The principal substrates for the commercial production of industrial alcohol include molasses or corn, potatoes and wood wastes. The process of ethanol production starts by milling a feed stock followed by the addition of dilute or fungal amylase (enzyme) from Aspergillus to break down the starch into fermentable sugars.

- Yeast is then added to convert the sugars to ethanol which is then distilled off to obtain ethanol which is upto 96 percent in concentration. The two most common type of biofuels in use today are ethanol and biodiesel, both of them represent the first generation of biofuel technology. Ethanol is often used as a fuel, mainly as a biofuel additive for gasoline.

Biodiesel is a fuel made from vegetable oils, fats or greases. Biodiesel fuel can be used in diesel engines without altering the engine. Pure biodiesel is non-toxic, biodegradable and produces lower level of air pollutants than petroleum-based diesel fuel. The Government of India approved the National Policy on Biofuels in December 2009 and identified Jatrophacurcas as the most suitable oilseed for biodiesel production. Pongamia species is also a suitable choice for production of biodiesel.

Chemicals, enzymes and other bioactive molecules

- Microbes are not only used for commercial and industrial production of alcohol, but also used for production of chemicals like organic acids and enzymes. Examples of organic acid producers are Aspergillusniger for citric acid, Acetobacteraceti for acetic acid, Rhizopusoryzae for fumaric acid, Clostridium butyricum for butyric acid and Lactobacillus for lactic acid.

- Yeast (Saccharomyces cerevisiae) and bacteria are used for commercial production of enzymes. Lipases are used in detergent formulations and are used for removing oily stains from the laundry. Bottled juices are clarified by the use of pectinase, protease and cellulase. Rennet can also be used to separate milk into solid curds for cheese making. Streptokinase produced by the bacterium Streptococcus and genetically engineered Streptococci are used as "clot buster" forremoving clots from the blood vessels of patients who have undergone myocardial infarction.

- Cyclosporin A, an immunosuppressant used in organ transplantation is produced from the fungus Trichodermapolysporum. It is also used for its anti-inflammatory, anti-fungal and anti-parasitic properties. Statins produced by the yeast Monascuspurpureus have been used to lower blood cholesterol levels. It acts by competitively inhibiting the enzyme responsible for the synthesis of cholesterol. Recombinant human insulin has been produced predominantly using E. coli and Saccharomyces cerevisiae for therapeutic use in human.

Microbes in sewage treatment and energy generation

- Sewage is the waste generated every day in cities and towns containing human excreta. It contains large amounts of organic matter and microbes, which are pathogenic to humans and are bio-degradable pollutants. Domestic waste consists of approximately 99 percent water, suspended solids and other soluble organic and inorganic substances. Sewage should not be discharged directly into natural water bodies like rivers and streams. Before disposal, sewage should be treated in sewage treatment plants to make it less polluting (Fig. 9.2).

Wastewater treatment

- The main objective of a wastewater treatment process is to reduce organic and inorganic components in wastewater to a level that it nolonger supports microbial growth and to eliminate other potentially toxic materials. Microorganisms mainly bacteria and some protozoa play an essential part in the treatment of sewage to make it harmless. Sewage contains pathogenic bacteria. These bacteria must be destroyed in order to prevent the spread of diseases. Sewage treatment is usually performed in the following three stages.

Primary treatment

- Primary treatment involves the physical removal of solid and particulate organic and inorganic materials from the sewage through filtration and sedimentation. Floating debris is removed by sequential filtration. Then the grit (soil and small pebbles) are removed by sedimentation. All solids that settle form the primary sludge and the supernatant forms the effluent. The effluent from the primary settling tank is taken for secondary treatment.

Secondary treatment or biological treatment

- The primary effluent is passed into large aeration tanks where it is constantly agitated mechanically and air is pumped into it. This allows vigorous growth of useful aerobic microbes into floc (masses of bacteria associated with fungal filaments to form mesh like structures). While growing, these microbes consume themajor part of the organic matter in the effluent. This significantly reduces the BOD (Biochemical oxygen demand or Biological oxygen demand). BOD refers to the amount of the oxygen that would be consumed, if all the organic matter in one litre of water were oxidized by bacteria. The sewage water is treated till the BOD is reduced. The greater the BOD of the waste water more is its polluting potential.

- Once the BOD of sewage water is reduced significantly, the effluent is then passed into a settling tank where the bacterial "**flocs**" are allowed to sediment. This sediment is called **activated sludge**. A small part of activated sludge is pumped back into the **aeration tank** to serve as the inoculum. The remaining major part of the sludge is pumped into large tanks called **anaerobic sludge digesters**. Here, the bacteria which grow anaerobically, digest the bacteria and the fungi in the sludge. During this digestion, bacteria produce a mixture of gases such as methane, hydrogen sulphide and CO2. These gases form biogas and can be used as a source of energy.

Tertiary treatment

- Tertiary treatment is the final process that improves the quality of the waste water before it is reused, recycled or released into natural water bodies. This treatment removes the remaining inorganic compounds andsubstances, such as nitrogen and phosphorus. UV is an ideal disinfectant for wastewater since it does not alter the water quality – except for inactivating microorganisms. UV is a chemical-free process that can completely replace the existing chlorination system and also inactivates chlorine-resistant microorganisms like Cryptosporidium and Giardia.

Act enforced by Government to conserve water bodies

- National river conservation plan (NRCP) was enacted in 1995 to improve the water quality of the rivers, which are the major fresh water resources in our country. This important assignment taken up under the NRCP includes,

  - ✓ To capture the raw sewage flowing into the river through open drains and divert them for treatment.
  - ✓ Setting up sewage treatment plants for treating the diverted sewage.
  - ✓ Construction of low cost sanitation toilets to prevent open defecation on river banks.

- The ministry for environment, forest and climate change has initiated the Ganga action plan and the Yamuna action plan to save the major rivers of the country. The Ganga action plan was launched on 14th January 1986. The main objective of the programme is

to improve the water quality of River Ganges by interception, diversion and treatment of domestic sewage and to identify grossly polluting units to prevent pollution. The Yamuna Action Plan is a bilateral project between the Government of India and Japan. It was formally launched in April 1993. It was proposed to build large number of sewage treatment plants to discharge treated wastewater into the rivers.

Microbial fuel cell(MFC)

- A microbial fuel cell is a bio-electrochemical system that drives an electric current by usingbacteria and mimicking bacterial interaction found in nature (Fig. 9.3). Microbial fuel cells work by allowing bacteria to oxidize and reduce organic molecules. Bacterial respiration is basically one big redox reaction in which electrons are being moved around. A MFC consists of an anode and a cathode separated by a proton exchange membrane. Microbes at the anode oxidize the organic fuel generating protons which pass through the membrane to the cathode and the electrons pass through the anode to the external circuit to generate current.

Microbes in the production of biogas (Gobar gas)

- Biogas is a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen. Biogas can be produced from raw materials such as agricultural wastes, manure, municipal wastes, plant material, sewage, food waste, etc., Biogas is produced under anaerobic condition, when the organic materials are converted through microbiological reactions into gas and organic fertilizer. Biogas primarily consists of methane (63 percent), along with CO2 and hydrogen. Methane producing bacteria are called methanogens and one such common bacterium is Methanobacterium. Biogas is devoid of smell and burns with a blue flame without smoke. The Methanogens are also present in anaerobic sludge and rumen of cattle.

- In rumen, these bacteria help in the breakdown of cellulose. The excreta of cattle called dung is commonly called "Gobar". Gobar gas is generated by the anaerobic decomposition of cattle dung. It consists of methane, CO2 with some hydrogen, nitrogen and other gases in trace amounts.

- In a biogas plant, anaerobic digestion is carried out in an air tight cylindrical tank known as digester (Fig. 9.4). It is made up of concrete bricks and cement or steel. Bio-wastes are collected and slurry of dung is fed into this digester. It has a side opening into which organic materials for digestion are incorporated for microbial activity. Anaerobic digestion is accomplished in three stages: solubilisation, acidogenesis and methanogenisis. The outlet is connected to a pipe to supply biogas. The slurry is drained through another outlet and is used as fertilizer. Biogas is used for cooking and lighting. The technology of biogas production was developed in India mainly due to the efforts of Indian Agricultural Research Institute (IARI) and Khadi and Village Industries Commission (KVIC).

Microbes as bio control agents and bio fertilisers

- Large scale application of chemical insecticides and pesticides have a deleterious effect on the health of human beings and pollute our environment. Biocontrol is a method of controlling pest by use of microbessuch as fungi, bacteria, viruses or by naturally occurring substances derived from plants and animals. The use of a microbes or other biological agents to control a specific pest is called a biopesticide. Biopesticides are used to control insect pests. The lady bird beetle and dragonflies are useful to control aphids and mosquito larvae respectively. Bacillus thuringiensis is a soil dwelling bacterium which is commonly used as a biopesticide and contains a toxin called cry toxin (Fig. 9.5). Scientists have introduced this toxin producing genes into plants and have raisedgenetically engineered insect resistant plants. E.g.Bt-cotton.

- During sporulation Bacillus thuringiensis produces crystal proteins called Delta-endotoxin which is encoded by cry genes. Delta-endotoxins have specific activities against the insects of the orders Lepidoptera, Diptera, Coleoptera and Hymenoptera. When the insects ingest the toxin crystals their alkaline digestive tract denatures the insoluble crystals making them soluble. The cry toxin then gets inserted into the gut cell membrance and paralyzes the digestive tract. The insect then stops eating and starves to death.

- Weedicides are substances, which destroy weeds without harming the useful plants. Bioweedicides are compounds and secondary metabolites derived from microbes such as fungi, bacteria or protozoa. The first bio herbicide developed in 1981 was a Mycoheribicide derived from the fungus Phytophthorapalmivora. It controls the growth of strangler vine in citrus crops. Trichoderma species are free living fungi that are very common in the root ecosystem. They are effective biocontrol agents for several plant pathogens. Buculoviruses are pathogens thatattack insects and other arthropods. The genus Nucleopolyhedrovirus is used as a biocontrol agent. These viruses are species specific and have narrow spectrum insecticidal applications.

Biofertilisers

- Biofertilisers are formulation of living microorganisms that enrich the nutrient quality of the soil. They increase physico – chemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter. The main sources of biofertilisers are bacteria, fungi and cyanobacteria. Rhizobium is a classical example for symbiotic nitrogen fixing bacteria. This bacterium infects the root nodules of leguminous plants and fixes atmospheric nitrogen into organic forms. Azospirillum and Azotobacter are free living bacteria that fix atmospheric nitrogen and enrich the nitrogen content of soil.

- A symbiotic association between a fungus and the roots of the plants is called mycorrhiza. The fungal symbiont in these associations absorbs the phosphorus from soil and transfers to the plant. Plants having such association show other benefits such as resistance to root-borne pathogens, tolerance to salinity, drought, enhances plant growth and

developments. For example, many members of the genus Glomusform mycorrhiza. Cyanobacteria (or) blue green algae (BGA) are prokaryotic free-living organisms which can fix nitrogen. Oscillatoria, Nostoc, Anabaena, Tolypothrix are well known nitrogen fixing cyanobacteria. Their importance is realized in the water logged paddy fields where Cyanobacteria multiply and fix molecular nitrogen. Cyanobacteria secrete growth promoting substances like indole-3-acetic acid, indole-3- butyric acid, naphthalene acetic acid, amino acids, proteins, vitamins which promotes plant growth and production.

- Biofertilisers are commonly used in organic farming methods. Organic farming is a technique, which involves cultivation of plants and rearing of animals in natural ways. This process involves the use of biological materials, avoiding synthetic substances to maintain soil fertility and ecological balance thereby minimizing pollution and wastage.

Key features of organic farming

- ❖ Protecting soil quality using organic materials and encouraging biological activity.
- ❖ Indirect provision of crop nutrients using soil microorganisms.
- ❖ Nitrogen fixation in soils using legumes.
- ❖ Weed and pest control based on methods like crop rotation, biological diversity, natural predators, organic manures and suitable chemical, thermal and biological interventions.

Bioremediation

- The use of naturally occurring or genetically engineered microorganisms to reduce or degrade pollutants is called bioremediation. Bioremediation is less expensive and more sustainable than other remediation's available. It is grouped into in situ bioremediation(treatment of contaminated soil or water in the site) and ex situ bioremediation (treatment of contaminated soil or water that is removed from the site and treated).

Microorganisms involved in bioremediation

- Aerobic microbes degrade the pollutants in the presence of oxygen. They mainly degrade pesticides and hydrocarbons. Pseudomonas putida is a genetically engineered microorganism (GEM). Ananda Mohan Chakrabarty obtained patent for this recombinant bacterial strain. It is multi-plasmid hydrocarbon-degrading bacterium which can digest the hydrocarbons in the oil spills (Fig. 9.6).

- Nitrosomonaseuropaea is also capable of degrading benzene and a variety of halogenated organic compounds including trichloroethylene and vinyl chloride. Ideonellasakaiensis is currently tried for recycling of PET plastics (Fig. 9.7). These bacteria use PETase and MHETase enzymes to breakdown PET plastic into terephthalic acid and ethylene glycol.

- Anaerobic microbes degrade the pollutants in the absence of oxygen. Dechloromonasaromatica has the ability to degrade benzene anaerobically and to oxidize toluene and xylene. Phanerochaetechrysosporium an anaerobic fungus exhibits strong potential for bioremediation of pesticides, polyaromatic hydrocarbons, dyes, trinitrotoluene, cyanides, carbon tetrachloride, etc., Dehalococcoides species are responsible for anaerobic bioremediation of toxic trichloroethene to non-toxic ethane. Pestalotiopsismicrospora is a species of endophytic fungus capable of breaking down and digesting polyurethane. This makes the fungus a potential candidate for bioremediation projects involving large quantities of plastics.

- Ideonellasakaiensis adhere to PET film PET Bottle Eats the terephthalic acid & ethylene glycol Breaks down PET into terephthalic acid & ethylene glycol Fig. 9.7 Actions of Ideonellasakaiensis

Summary

- All microbes are not pathogenic, many of them are beneficial to human beings. We use microbes and their derived products almost every day. Lactic acid bacteria convert milk into curd. Saccharomyces cerevisiae (yeast) is used in bread making. Idly and dosa are made from dough fermented by microbes. Bacteria and fungi are used in cheese making. Industrial products like lactic acid, acetic acid and alcohol are produced by microbes. Antibiotics are produced from useful microbes to kill the disease causing harmful microbes. For more than a hundred years, microbes are beingused to treat sewage by the process of activated sludge formation. Bio-gas produced by microbes is used as a source of energy in rural areas. Microbes are also used as bio-control agents to avoid the use of toxic pesticides. Now a days chemical fertilisers are gradually replaced by bio-fertilisers. In bio-remediation naturally occurring or genetically engineered microorganisms are used to reduce or degrade pollutants.