BUREAU'S

HIGHER SECONDARY

BIOLOGY

Class-XII

Prescribed by Council of Higher Secondary Education, Odisha, Bhubaneswar

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FOREWORD

In our continuous effort to evolve and to keep pace with this fast changing world, this Biology book for Class-XII students has been written. The book comprises of 5 units and there are 17 chapters wherein certain important aspects of Biological Science have been incorporated. As I find, the chapters include day to day affairs of the modern man. It will not only benefit our students to face the challenges of different types of examination but in the long run, help them to become complete citizens, also.

The Council of Higher Secondary Education (CHSE), Odisha has implemented the Biology syllabus in line with National Council of Education, Research and Training, New Delhi from the session 2016-17, so as to integrate our students with national mainstream. This part, meant for Class-XII students are again, very important since our students have to appear at the Council Examination with the help of this textbook.

This unique work has been done by the board of writers in Biology and were reviewed rigorously by the board of reviewers. Finally, the proofs have been meticulously read and recast by Dr. Basanta Kumar Choudhury and Dr. Pradip Kumar Mohanty. I sincerely thank all of them. This book could see light of the day because of their efforts only.

My sincere thanks to Government of Odisha for its interest in the publication of this textbook. I also appreciate CHSE, Odisha for selecting us for the preparation and publication of the book. The Bureau will humbly appreciate the constructive comments and suggestions to improve the standard of the book.

Sri Umakanta Tripathy
Director
Odisha State Bureau of Textbook
Preparation and Production,
Bhubaneswar

PREFACE

Bioengineering expert, Catherine J. Paul, of Lund University, Sweden has the following observation on Biological Science, "a previously completely unknown ecosystem has revealed itself to us. Formerly, you could hardly see any bacteria at all and now, thanks to such as massive DNA sequencing and flow cytometry, we suddenly see 80 thousand bacteria per ml of drinking water". We know that the modern biological science which had a humble beginning at the turn of last century by rediscovery of Mendelism made a breakthrough with deciphering the structure of the wonder molecule, DNA. After that landmark event, the science of biology has made giant strides in many spheres last six or seven decades.

We tamed the nature and exploited its natural resources beyond its replenishment level. We knew more and more about our anatomy, physiology etc. in order to live a sophisticated life. In the process, we got detached from nature. Our evolution and improvement in the quality of life had share of ill effects.

A student of Biology should know all these so as live a life in harmony with nature. Class-XII Biology syllabus has been framed in such a way by National Council of Education, Research and Training (NCERT), New Delhi and corroborated by Council of Higher Secondary Education (CHSE), Odisha that all the discussed ingredients have been incorporated in it. The writers and reviewers in Biology here made an humble attempt to deal all the chapters so that it can be clearly understood. They hope it will cater to the needs of our students as well as teaching community.

Keeping an eye on the requirements of our students certain chapters have been conveniently elaborated. It is now open to the students and teachers in Biology to go through all the chapters and provide their valuable constructive feedback. The boards never claim the write up is original one. It has been taken from certain primary and secondary sources.

The writers and reviewers acknowledge their deep sense of gratitude to Odisha State Bureau of Textbook Preparation and Production, CHSE, Odisha and Department of School and Mass Education, Government of Odisha for relying on the same team to write the Class-XII Biology book. All the members of both the boards unequivocally thank Sri Biraj Bhushan Mohanty, Deputy Director of the Bureau for his unceasing efforts and inspiration. Lastly, they thank Sri Ashok Kumar Ojha, DTP Operator, M/s. Print-tech Offset Pvt. Ltd., Bhubaneswar, Odisha for undertaking the arduous task of typing and designing all text materials of the book.

Srigundicha,
25.06.2017

Writers & Reviewers

BIOLOGY SYLLABUS

CLASS - XII

(Science)

(Theory)

I. REPRODUCTION (Periods - 22)

(a) **Reproduction in organisms :** Reproduction, a characteristic feature of all organisms for continuation of species; Modes of reproduction - Asexual and sexual; Asexual reproduction; Modes- Binary fission, sporulation, budding, gemmule formation, fragmentation; vegetative propagation in plants.

Sexual reproduction in flowering plants : Flower structure; Development of male and female gametophytes; Pollination-types, agencies and examples; Outbreeding devices; Pollen-Pistil interaction; Double fertilization; Post fertilization events- Development of endosperm and embryo, Development of seed and formation of fruit; Special modes- apomixis, parthenocarpy, polyembryony; Significance of seed and fruit formation.

(b) Human Reproduction : Male and female reproductive systems; Microscopic anatomy of testis and ovary; Gametogenesis- spermatogenesis and oogenesis; Menstrual cycle; Fertilization, embryo development upto blastocyst formation, implantation; Pregnancy and placenta formation (Elementary idea); Parturition (Elementary idea); Lactation (Elemntary idea).

Reproductive health : Need for reproductive health and prevention of sexually transmitted diseases (STD); Birth control- Need and Methods, Contraception and Medical Termination of Pregnancy (MTP); Amniocentesis; Infertility and assisted reproductive technologies - IVF, ZIFT, GIFT (Elementary idea for general awareness).

II. GENETICS AND EVOLUTION (Periods - 20)

(a) Heredity and Variation : Mendelian Inheritance; Deviations from Mendelism-Incomplete dominane, Co-dominance, Multiple alleles and Inheritance of blood groups, Pleiotropy; Elementary idea of polygenic inheritance; Chromosome theory of inheritance; Chromosomes and genes; Linkage and crossing over.

(b) Sex determination : In humans, birds, honey bee; Sex linked inheritance- Haemophilia, Colour blindness; Mendelian disorders in humans- Thalasemia; Chromosomal disorders in humans- Down's syndrome, Turner's and Klinefelter's syndromes.

(c) **Molecular Basis of Inheritance :** Search for genetic material and DNA as genetic material; Structure of DNA and RNA; DNA packaging; DNA replication; Central dogma; Transcription, Genetic code, Translation; Gene expression and regulation- Lac Operon; Genome and human genome project; DNA finger printing.

(d) **Evolution :** Origin of life; Biological evolution and evidences for biological evolution (Paleontological, comparative anatomy, embryology and molecular evidence); Darwinism, Modern Synthetic theory of Evolution; Mechanism of evolution- Variation (Mutation and Recombination) and Natural Selection with examples, types of natural selection; Gene flow and genetic drift; Hardy-Weinberg's principle; Adaptive Radiation; Human evolution (in brief).

III. BIOLOGY AND HUMAN WELFARE (Periods - 08)

(a) Health and Disease: Pathogens; parasites causing human diseases (Malaria, Filariasis, Ascariasis, Typhoid, Pneumonia, common cold, amoebiasis, ring worm); Basic concepts of immunology- vaccines; Cancer, HIV and AIDS; Adolescence, drug and alcohol abuse.

(b) Improvement in food production:

(i) Plant breeding, tissue culture, single cell protein, Biofortification;

(ii) Apiculture and Animal husbandry.

(c) Microbes in human welfare : In household food processing, industrial production, sewage treatment, energy generation and as biocontrol agents and biofertilizers.

IV. BIOTECHNOLOGY AND ITS APPLICATIONS (Periods - 08)

(a) Principles and process of Biotechnology : Genetic engineering (Recombinant DNA technology).

(b) Application of Biotechnology in health and agriculture : Human insulin and vaccine production, gene therapy; Genetically modified organisms- Bt crops; Transgenic Animals; Biosafety issues- Biopiracy and patents.

V. ECOLOGY AND ENVIRONMENT (Periods 12)

(a) Organisms and environemnt : Habitat and niche; Population and ecological adaptations; population interactions-mutualism, competition, predation, parasitism; Population attributes-growth, birth rate and death rate, age distribution.

(b) Ecosystems : Patterns, components; productivity and decomposition; Energy flow; Pyramids of number, biomass, energy; Nutrient cycling (carbon and phosphorous); Ecological succession; Ecological Services-Carbon fixation; pollination, oxygen release.

(c) Biodiversity and its conservation : Concept of Biodiversity; Patterns of Biodiversity; Importance of Biodiversity; Loss of Biodiversity, conservation; Hotspots, endangered organisms, extinction, Red Data Book: Biosphere reserves, National parks and Sanctuaries.

(d) Environmental issues : Air pollution and its control; Water pollution and its control; agrochemicals and their effects; Solid waste management; Radioactive waste management; Greenhouse effect and global warming; Ozone depletion; Deforestation; Any three case studies as success stories addressing environmental issues.

N.B. : Ia, II a, c; III b (i), c and v units are to be taught by Botany Faculty. I b; II b; III a, b(ii); IV units are to be taught by Zoology Faculty.)

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QUESTION PATTERN AND DISTRIBUTION OF MARKS

BIOLOGY (Theory)

Class - XII (Science)

SECTION - A
(BOTANY)

Time : 1.5 hours — Full Marks : 35

Group A
(Objective Type - Compulsory)

Q1.	Multiple choice / one word answer : 1 mark each x 5	= 5 marks
Q2.	Correct the sentences / Fill up the blanks : 1 mark each x 5	= 5 marks

Group B
(Short Answer Type)

Q3.	Answer within three sentences : 2.5 marks each x 3	= 7.5 marks
Q4.	Difference between (3 important differences) (1 bit to be answered out of 3 bits) : 3.5 marks	= 3.5 marks

Group C
(Long Answer Type)

Answer two questions out of four : 7 marks x 2 = 14 marks

SECTION - B
(ZOOLOGY)

Time : 1.5 hours — Full Marks : 35

Group - A
(Objective Type-Compulsory)

Q1.	Multiple choice / one word answer : 1 mark each x 5	= 5 marks
Q2.	Correct the sentences / Fill up the blanks : 1 marks each x 5	= 5 marks

Group - B
(Short Answer Type)

Q3.	Answer within three sentences : 2.5 marks each x 3 (3 bits to be answered out of 6 bits)	= 7.5 marks
Q4.	Difference between (3 important differences) (1 bit to be answered out of 3 bits) : 3.5 marks	= 3.5 marks

Group - C
(Long Answer Type)

Answer two questions out of four : 7 marks x 2 = 14 marks

N.B : Long answer type questions are to be set **only from the portions underlined in the syllabus**.

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CONTENT

UNIT - I : REPRODUCTION

CHAPTER 1

REPRODUCTION IN ORGANISMS

Till date not other planet in the solar system except the Earth is known to sustain life. Hundreds and thousands of living organisms abound every conceivable space in this planet and they form the biospere. The organisms in their size, shape, structure, function, habit and habitat etc. vary extensively. Yet they consistently exhibit a unique character, i.e. from a living being another living organism is produced. As a result of this, the constancy and continuity of the said species is maintained. This is called the phenomenon of reproduction. In other words, reproduction is said to be a bilogical process of perennation and continuation of a species from generation to generation.

There is a great diversity in the living world in their method of reproduction. It is typical to the organism concerned. Factors like environment, physiology, habitat etc. of the organism influence simultaneously its mode of reproduction. Based on the type of reproductive unit, the reproduction process may be sexual or asexual. In the sexual reproduction, reproductive units called gametes which take part to produce zygote. The gametes when morphologically and physiologically similar are called isogametes. When differences occur between two fusing gametes, it may be called anisogametes or oogametes. The former one is the process of isogamy and the later, termed as heterogamy.

In the cases, where such gametes are not developed in offspring production, it is called asexual reproduction.

1.1 ASEXUAL REPRODUCTION :

In the asexual method of reproduction, the reproductive units are called spores which may be motile or nonmotile. Sometimes, the vegetative structures of the organisms may get segregated accidentally and grow into a new indivioluals. Later method may be called as vegetative propagation. Here, unlike to asexual or sexual method, no specialized reproductive units are formed for the production of progenies.

Asexual reproduction is very common among lower plants, animals and particularly in the kingdoms of Monera, Protista and Fungi. In these cases offsprings are produced by the processes called fragmentation, fission, budding, gemmule formation, sporulation etc.

1.1.1 Fragmentation (Fig. 1.1) :

This type of vegetative propagation occurs in most of the lower plants belonging to algae, fungi and bryophytes as well as many highly developed angiosperms. Among the animals

also such types of reproduction by fragmentation occur in lower animals like sponges, Hydra, etc. This is a process of accidental breakage of vegetative cells and development of the fragmented or broken parts into new organisms. The accidental breakage may occur due to wind, water current or injury caused by animals while feeding. In economically important horticultural or crop plants, human being uses this method of segregating plant parts for his own benefit.

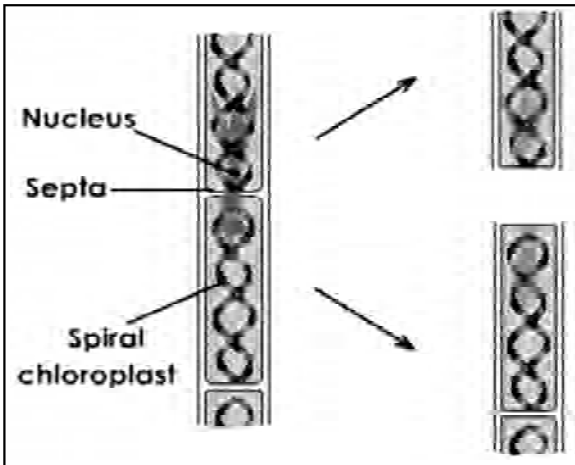

Fig.1.1 : Fragmentation in *Spirogyra* (an alga)

1.1.2 Fission :

Bacteria, certain unicellular algae and such types of organisms propagate by the method of cell division. In this process, the nucleus and cytoplasm grow and divide into two euql parts. This is called binary fission. It is the predominating form of reproduction in bacteria (Fig.1.2)

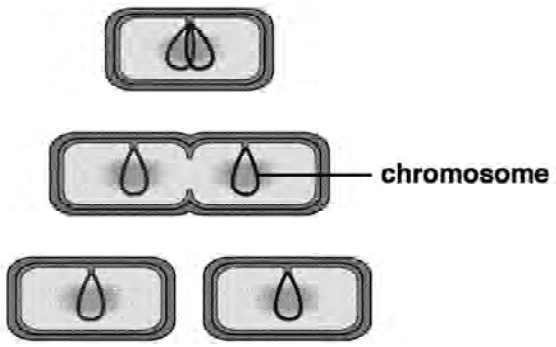

Fig.1.2 : Fission in Bacteria

Other organism like *Amoeba*, *Paramaecium* and *Euglena* also show binary fession. In favourable conditions an *Amoeba* enlarges in size and withdraws its pseudopodia. It gets ready for cell division (Fig. 1.3). As a result of mitosis, two cells are produced which are capable of growing into independent organisms.

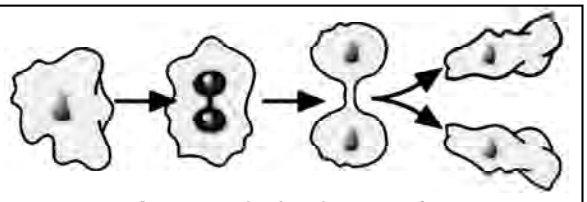

Fig.1.3 : Fission in *Amoeba*

Besides, binary fission, cells of certain algae like *Stanieria* divide into more than 2 cells and each part can develop to form new organisms (Fig. 1.4). This is called multiple fission.

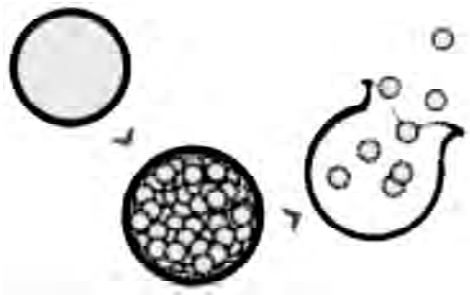

Fig.1.4 : Multiple fission in *Stanieria* (an alga)

Under unfavourable conditions like drying of water reservoir or condensation of water into ice, *Amoeba* shows a different type

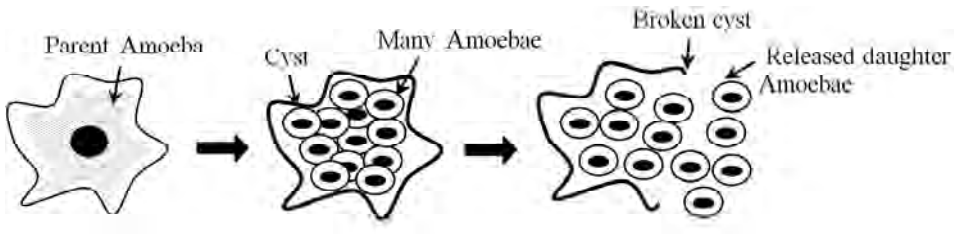

Fig.1.5 : Multiple fission in *Amoeba*

of fission. It withdraws its pseudopodia and encloses itself by 2 to 3 layered strong envelope. The process is called encystment. During the period, the metabolism of *Amoeba* is reduced to the minimum. But its nucleus divides repeatedly to form a large number of nuclei. Each nucleus remains surrounded by some amount of cytoplasm. When the suitable conditions sets in the cyst disintegrates and premature daughter Amoeba called Amoebula get released. This is an example of multiple fussion (Fig. 1.5). It is also called sporulation. Malaria causing *Plasmodium* also propagates by multiple fission.

1.1.3 Budding :

The unicellular fungus, yeast (*Saccharomyces*) generally propagates by this method. Outgrowths develop at the peripheral region of the mother cell which are called buds. Into this, nucleus along with cytoplasm from the mother cell migrate. This causes daughter cells to enlarge in size. It remains attached to the mother cell by a narrow neck. Gradually, it loses contact from mother cell and grows into a new organism (Fig. 1.6). In the process of fission, the nuclear material gets segregated in equal amounts in the daughter cell, but this may or may not happen in the case of budding.

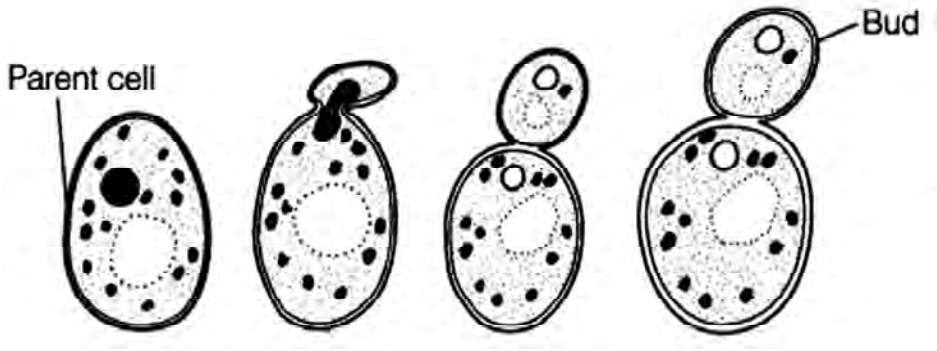

Fig.1.6 : Yeast Budding

Some other unicellular organisms and also muticellular ones like Sponge and *Hydra* reproduce by budding. These are of two types- (a) external buds, (b) internal buds.

(a) External budding : This occurs under favourable conditions when environmental conditions are suitable and enough nutirents are available. Initially, tiny swellings called buds develop in the median region of *Hydra* body. This gradually enlarges and coelenteron of *Hydra* migrates into it. At the terminal region of bud mouth tentacles develop. This is the *Hydra* in its infant stage which when separated from mother cell develop into new Hydra (Fig. 1.7).

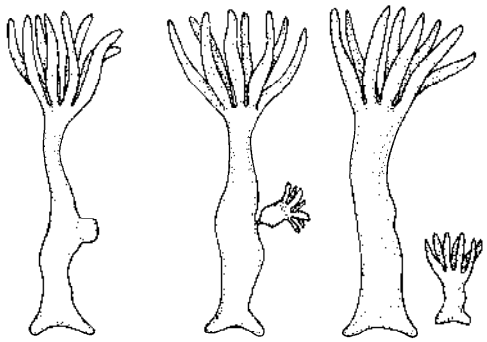

Fig.1.7 : External Budding in *Hydra.*

(b) Internal budding : When conditions become unsuitable and there is scarcity of the nutrient supply, sponge forms internal buds called gemmules. In the process, special types of cells called archaeocytes get aggregated. A hard coat is formed around it and the structure is now called gemmule (Fig. 1.8). It remains open outside by samll pore called micropyle. When suitable conditions again, sets in, the gemmules come out through micropyle and new sponges are formed.

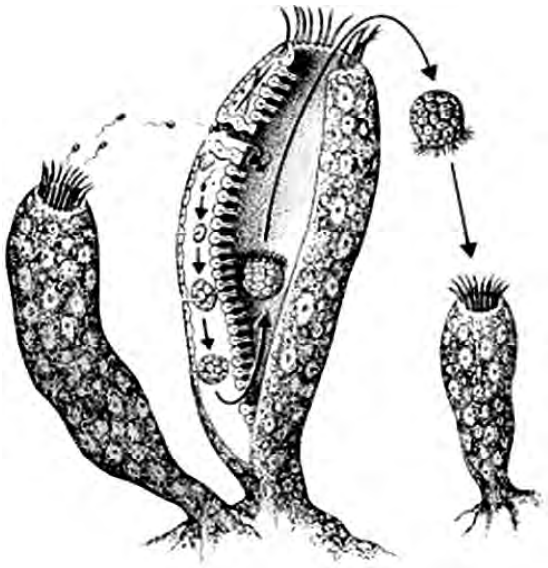

Fig.1.8 : Internal Budding in Sponges.

1.1.4. Sporulation :

This is a very general process of reproduction in organisms like algae and fungi. Almost all algae, produce motile asexual reproductive units called zoospores (Fig. 1.9). This can float freely in water with its cilia or

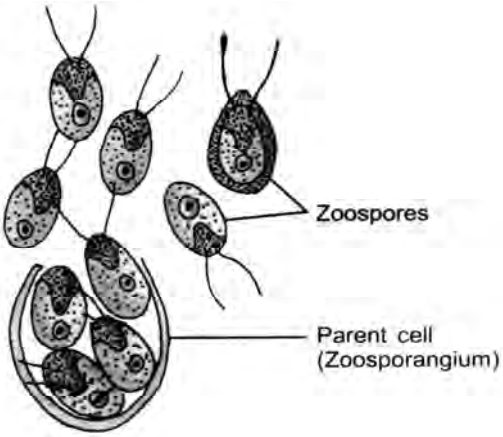

Fig.1.9 : Zoospore formation

flagella. Example: (*Ulothrix*). The zoospores endogenously borne inside the structures known as sporangia (Fig. 1.10) and are liberated from it when become mature. Lower fungi like Saprolegnia reproduce by zoospores (Fig. 1.11, a) but higher fungi like *Aspergillus*, *Penicillium* possess nonmotile structures called conidia (Fig. 1.11, b) These are exogenously borne and can be easily disseminated by external agent like air or water.

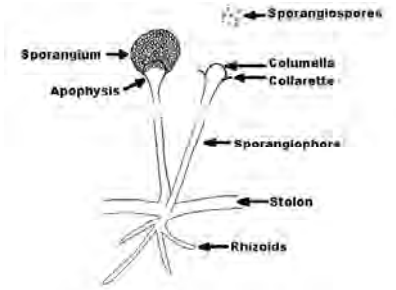

Fig.1.10 : Sporangium with sporangiophore

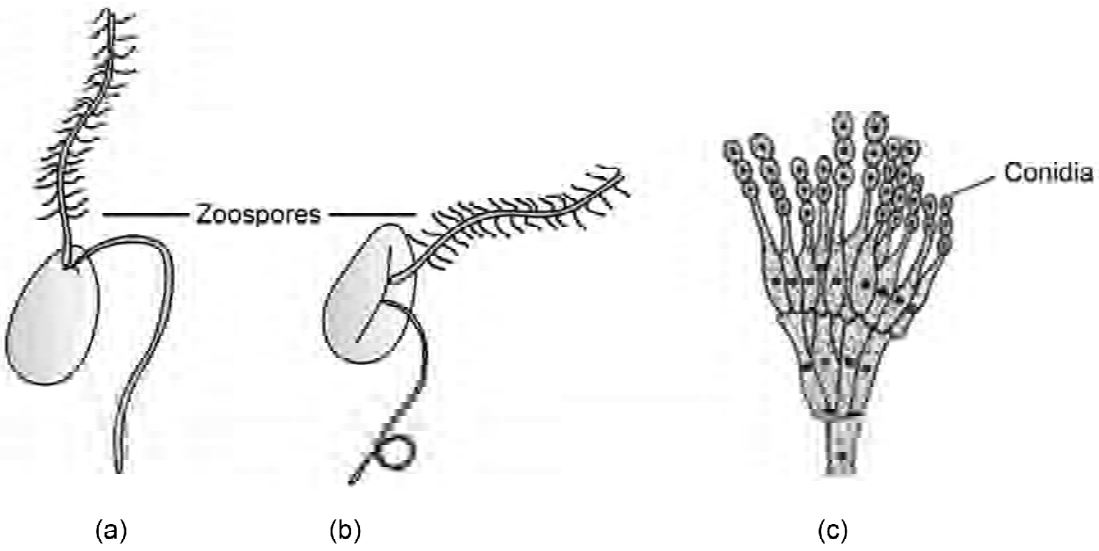

Fig.1.11 (a, b) : *Saprolegnia* spores, **(c)** *Penicillium* conidia

1.2. VEGETATIVE PROGAGATION IN PLANTS :

As described earlier no specialized reproductive units are produced in the process of vegetative propagation. When the plant is growing vegetatively, any part of it, say root, stem or leaf may get separated from this plant. If this separated part can grow into new individuals their it is called vegetative propagation.

Several types of vegetative propagation are seen among the flowering plants depending on part involved in the process. This may occur under normal conditions or may be induced artificially.

1.2.1. By roots :

Some tap or adventitious roots of sweet potato, *Dahlia* etc. become thick, swollen due to storage of food (Fig-1.12). The adventitious buds are borne on such structures. The buds produce leafy shoots, called slips. When such roots with adventitious buds are planted in the soil, they produce new plants and thus, vegetative propagation occurs.

1.2.2 By stems :

The stems are efficient means of vegetative propagation. This may be of the following two types.

(a) Subaerial stems : Subaerial stems may develop as lateral branches from the mother plant. This may break up from the parent plant and then, grow into new plants. (Fig.1.13) Example- Runners (*Oxalis*), sucker - (banana, *Chrysanthemum*), stolon (Jasmine), offset (*Eichhornia*)

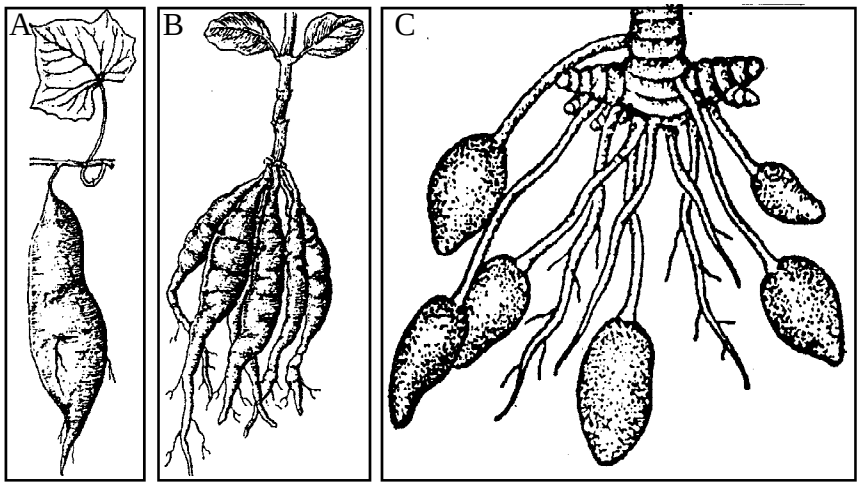

Fig.1.12 : A. Root Tuber of Sweet potato, **B.** Fasciculated root of *Dahlia*, **C.** Nodulose roots of mango ginger

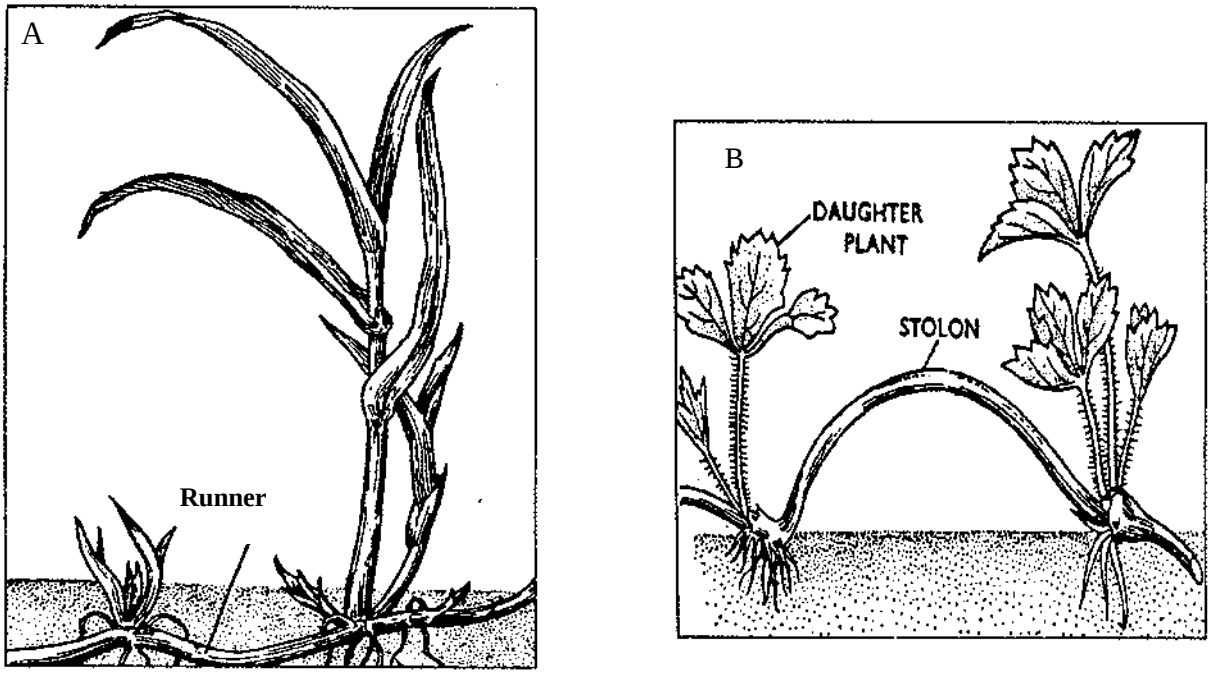

Fig.1.13 : Vegetative propagation by stem modifications : **A.** Runner, **B.** Stolon.

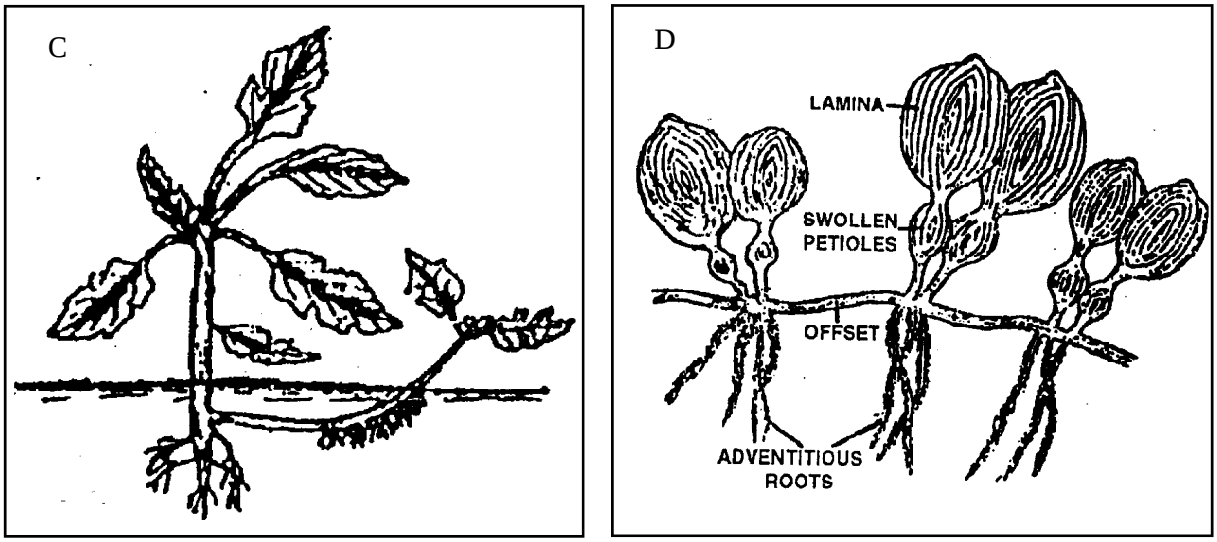

Fig.1.13 : Vegetative reproduction by stem modifications : C. Sucker, D. Offset

(b) Underground Stems : In certain plants (Fig. 1.14) the underground stems become modified for storage of food during the active phase of the growth. Examples- Rhizome (Ginger), tuber (Potato), bulb (Onion) and corm (colocasia)

1.2.3 By leaves :

The fleshy succulent leaves of *Bryophyllum* (Fig.1.15) bear adventitious buds in their notches located in the margins. When the leaves fall on moist soil, these buds develop into small plants completing the process of vegetative propagation.

1.3 ARTIFICIAL METHODS :

Farmers, gardeners, horticulturists have taken advantage of this type of propagation in plants. They have manipulated the process for their own benefit.

13.1. Cuttings :

This is a very common method. Here a piece of stem up to a suitable length is taken from the parent plant. This stem piece is called the cutting. It should have few nodes and internodes. The cutting is planted in moist soil with suitable nutrients. After sometime, roots emerge from the nodes of the basal portions of the cuttings and the upper buds give rise to the shoot. The plants of China rose, sugar cane, *Bougainvillea* etc are commonly grown by this method.

1.3.2. Grafting :

In this process, a detached part of one plant (i.e. twig or bud) is inserted into the stem or root system of another plant (Fig 1.16). The former is called **scion** (short piece of detached shoot containing several dormant buds) and the latter **stock** (lower portion of the plant which is fixed to the soil by its root system). The grafted portion is covered by grafting wax to avoid

infection. The scion becomes part of the plant into which it is grafted. The new plant developed bears flowers and fruits, characteristic of scion. Mango, rose, orange, guava etc. are generally propagated by graftings.

1.3.3 Layering :

Here, roots are artificially induced to grow on the branches before they are detached from the parent plant. There are three types of layering :

(i) **Serpentine layering :** Branches at the lower portions of the stem are put in the soil at many places to form new plants from them.

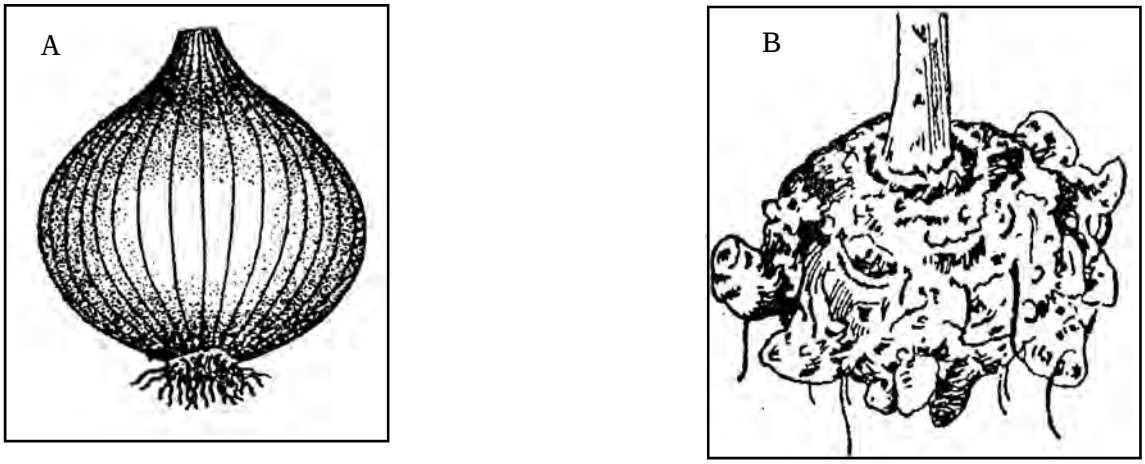

Fig.1.14 : Underground stem modifications : A. Bulb, B. Corm for vegetative propagation.

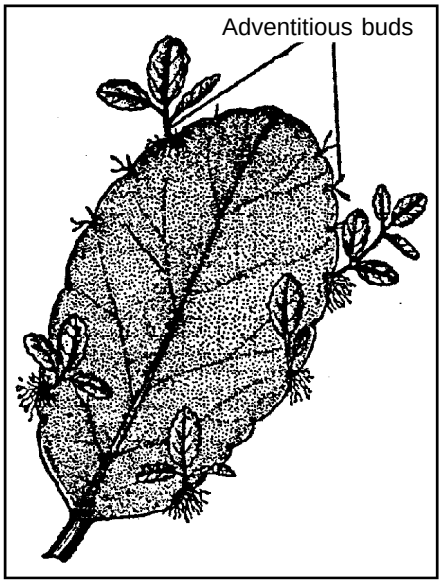

Fig.1.15 : Reproduction by leaf

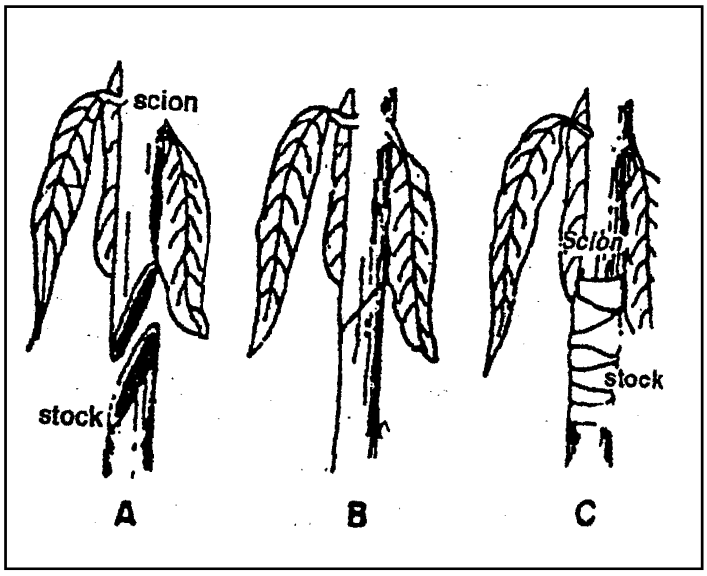

Fig.1.16 : Grafting by stem cutting (A-C) different stages.

(ii) **Mound layering** : Soft lower branches are selected and a ring of epidermal layer is removed. This part is then pegged in the soil with the apical portion remaining outside. After an interval of time, adventitions roots develop. Then the ringed portion is cut off to allow new plant grow independently.

(iii) **Air layering** : Here, a ring of bark is removed from the aerial branches. It is then covered by grafting clay (water, clay, cow dung) with a little amount of root inducing promoter. The entire portion is wrapped with polythene bandage. At a particular time interval, roots are developed and when separated it can grow into a new plant. Example - Pomegranate, orange, lemon etc.

Significance : This is a quick method of reproduction where survival rate of the progenies is very high. Endangered or threatened plant species can be saved by such propagations. Plants reproducing vegetatively take a short time to mature. Potato for example, takes three months to mature. Plants with desirable qualities may be developed by this method. The vegetative method of reproduction among angiosperms has a lot of agricultural and horticultural applications.

1.4 MICROPROPAGATION :

This process is similar to rooting of plant cuttings and is, in a way, another method of vegetative propagation of plants. However, it differs from the conventional procedure since it is carried out in aseptic condition and requires an artificial nutrient medium. A small plant cutting or explant (usually axillary bud) is sterilized and inoculated into culture vessel containing semi-solid nutrient medium. The inoculated culture vessel is incubated at room temperature. In a short span of time, a large number of shoots develop from the axillary buds through a process called axillary bud proliferation. Each growing point is then subcultured to give rise to shoot. This phenomenon is called adventitious shoot formation. Each shoot is stimulated by auxin to develop roots (Fig. 1.17 A,B). The new plantlet or propagule is then transferred to field.

This method is generally practised for ornamental, fruit and crop plants. This is useful because (i) the healthy propagules can only be obtained (pathogen free) (ii) rapid rates of multiplication can be ensured (iii) Development of plant materials with desired traits and their maintenance in a small space can be done.

Examples : Substantial benefits can be expected to occur in the production of crops such as tea, coffee, oil palm, date palm, coconut, fruit yielding plants like papaya, banana, Citrus and apples. Significant progress has also been achieved in developing protocols of micropropagation of tree species. Mass propagation, in vitro, of teak, Eucalyptus spp., sandal wood, different species of bamboo and many other trees has been successfully done.

Similarly, considerable progress has been made in commercial harvesting of medicinal plants such as *Dioscorea deltoidea, D. floribunda, Atropa belladona, Solanum Spp., Rauwolfia serpentina* etc. by microprogation techniques.

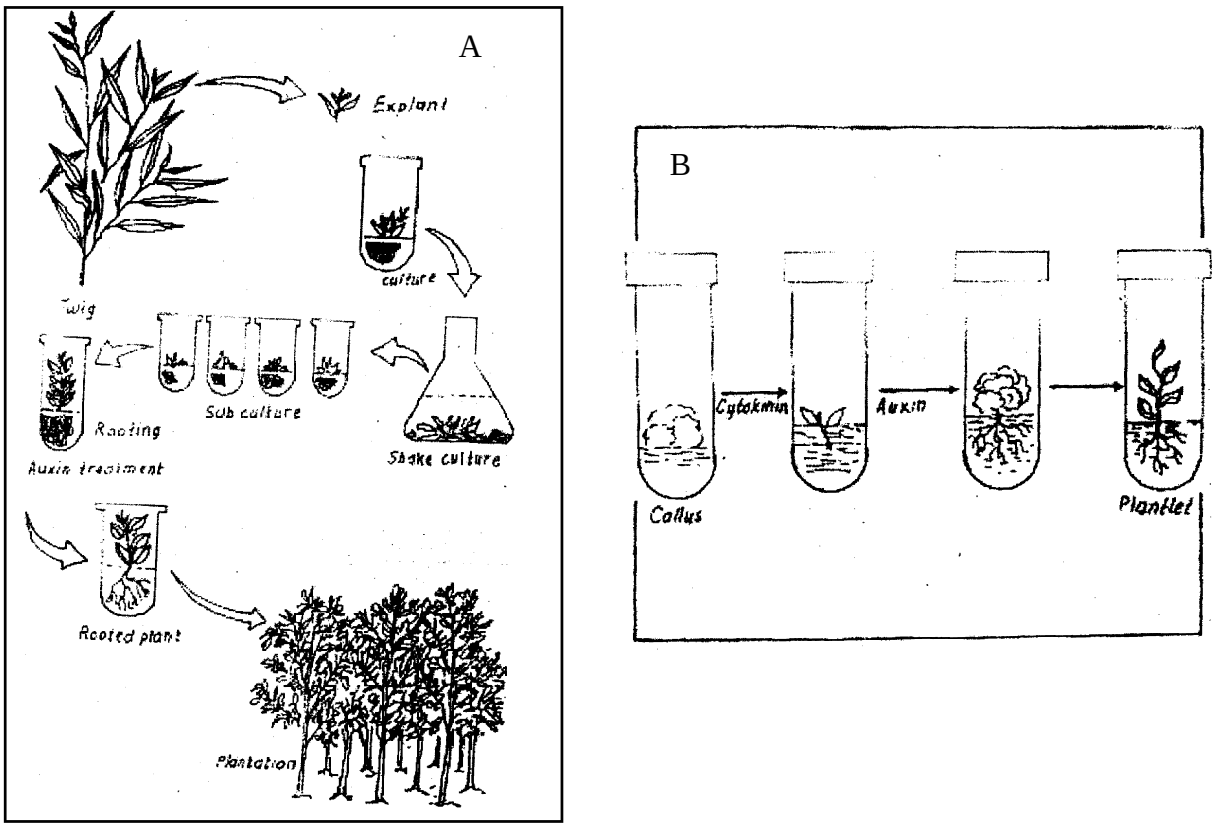

Fig.1.17 : A. Micropropagation of meristematic plant shoot (axillary bud) cutting through plant tissue culture technique; B. Development of shoot and root, one following the other, through plant tissue culture technique (organogenesis).

Some species of potato, Cassava, sugarcane and banana are severely and chronically affected by viruses. Yields of these crops can be increased significantly by planting disease free stocks.

Potato is of most important and widely grown food crop in the world. But it is susceptible to many viral pathogens, some of which may be present without perceptible symptoms. The pathogens cause gradual decrease in vigour and yield of potatoes. Eradication of viruses can be achieved by employing micropropagation techniques where healthy meristematic buds are cultured. More than 500 plants can be obtained in about three to four months starting from a single meristem. By manipulating the medium composition, light intensity and temperature, plantlets can be induced to produce microtubers. These disease free microtubers can be grown under controlled conditions in soil to form minitubers. The minitubers can be planted directly in the field to raise a disease free crop.

Apart from the application of micropropagation techniques for generating true-to-type planting material from elite genotypes, micropropagation holds special significance in situations where rapid bulking of extremely limited stock material is required. The desired genetic gains achieved through plant breeding can be multiplied several fold on an economic and rapid time scale.

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the balnks with correct answers from the choices given bracket :

(i) Nonmotile asexulal reproductive units are called _____.

(Zoospores, Buds, gametes, conidia)

(ii) In _____, a living organisms divide equationally.

(fragmentation, fission, budding, sporulation)

(iii) Yeast, generally, reproduces by _____.

(fission, budding, sporulation, gametangia)

(iv) *Dahlia* propagates by _____.

(roots, stem, leaf, seed)

(v) The process by which one plant part is inserted into another to grow a new individual plant is called _____.

(layering, cutting, grafting, micropropagation)

2. Anwser in one word only :

(i) What is called the motile asexual reproduction units?

(ii) In which asexual method do yeasts generally divide?

(iii) What can be called to sexual reproductive units?

(iv) What is the general asexual method of reproduction in Amoeba?

(v) In the process of grafting, what is called to detached part?

(vi) In which process can large number of adventitious buds be formed?

3. Correct the statements without changing underlined words only :

(i) In <u>mound</u> layering, branches at lower portion of the stem are put in the soil at many places.

(ii) <u>*Dahlia*</u> reproduces vegetatively by stems.

(iii) <u>*Aspergillus*</u> reproduces asexually by <u>zoospores</u>.

(iv) <u>Internal buds in sponges</u> are called gemma cups.

(v) In <u>binary fission</u>, many cells can be produced from one cell.

4. Fill in the balnks :

(i) The process of perennation of species takes place by _____.

(ii) Zoospores are borne inside _____.

(iii) Under unfavourable conditions when a number of tiny *Amoeba* are produced by multiple fission, it is called _____.

(iv) Internal buds in *Hydra* are called _____.

(v) In Bryophyllum, adeventitious buds are borned on _____

GROUP - B
(Short Answer-type Questions)

1. Write notes on the following with at least 2 valid points :

(i) Asexual reproduction

(ii) Micropropagation

(iii) Cutting

(iv) Layering

(v) Fission

(vi) Budding

(vii) Fragmentation

(viii) Micropropagation

2. Differentiate between the following with at least 3 valid points :

(i) Zoospores and condia.

(ii) Asexual reproduction and Sexual reproduction

(iii) Grafting and Layering

(iv) Budding and Fission.

(v) Internal budding and Enternal budding.

(vi) Fragmentation and budding.

GROUP - C
(Long Answer-type Questions)

1. Give and account of vegetative reproduction in angiosperms
2. Describe the process of micropropagation and its advantages.
3. Describe asexual reproduction process in lower animals.
4. Describe the asexual reproduction process in lower plants.

❑ ❑ ❑

SEXUAL REPRODUCTION IN FLOWERING PLANTS

CHAPTER 2

2.1 INTRODUCTION :

Sexual reproduction is a natural phenomenon among the angiosperms. The flowers bear sex organs (stamens and carpels) or reproductive organs which finally produce haploid male and female gametes. These haploid male and female gametes fuse to produce a diploid zygote that develops into a new diploid plant body, the sporophyte.

The flowering plants like other living organisms have two distinct phases in their life cycle, such as; a diploid sporophytic phase and a haploid gametophytic phase. Both the phases alternate with each other. The flowering plants, as we see them from outside are the sporophytes which bear reproductive structures in flowers. The flower performs very important role in the life cycle of Angiosperms as various steps of sexual reproduction occur in such structures.

2.2 STEPS IN SEXUAL REPRODUCTION :

2.2.1 Development that leads to formation of haploid male gametes :

1. Formation of *microsporangia* in the anther of stamen.
2. Formation of haploid **microspores** called **microsporogenesis** which involves meiosis in the microspore mother cells developed within the anther. The haploid microspore or pollen grain is the first cell of the **male gametophyte**.
3. Pollination - The microspores once released from the anther are dispersed in various ways to reach the stigma of the carpel.
4. Development of male gametophyte in the pollengrain that produces two male gametes (**sperms**).

2.2.2 Development that leads to formation of haploid female gamete :

1. Development of **ovule (megasporangium)** and female gametophyte within it.
2. Formation of **megaspores (megasporogensis)** within the female gametophyte (Embryo sac). The haploid megaspore is the first cell of the **female gametophyte**.
3. Development of female gamete into embryo sac with formation of egg apparatus, secondary or definite nuclei and antipodal cells.

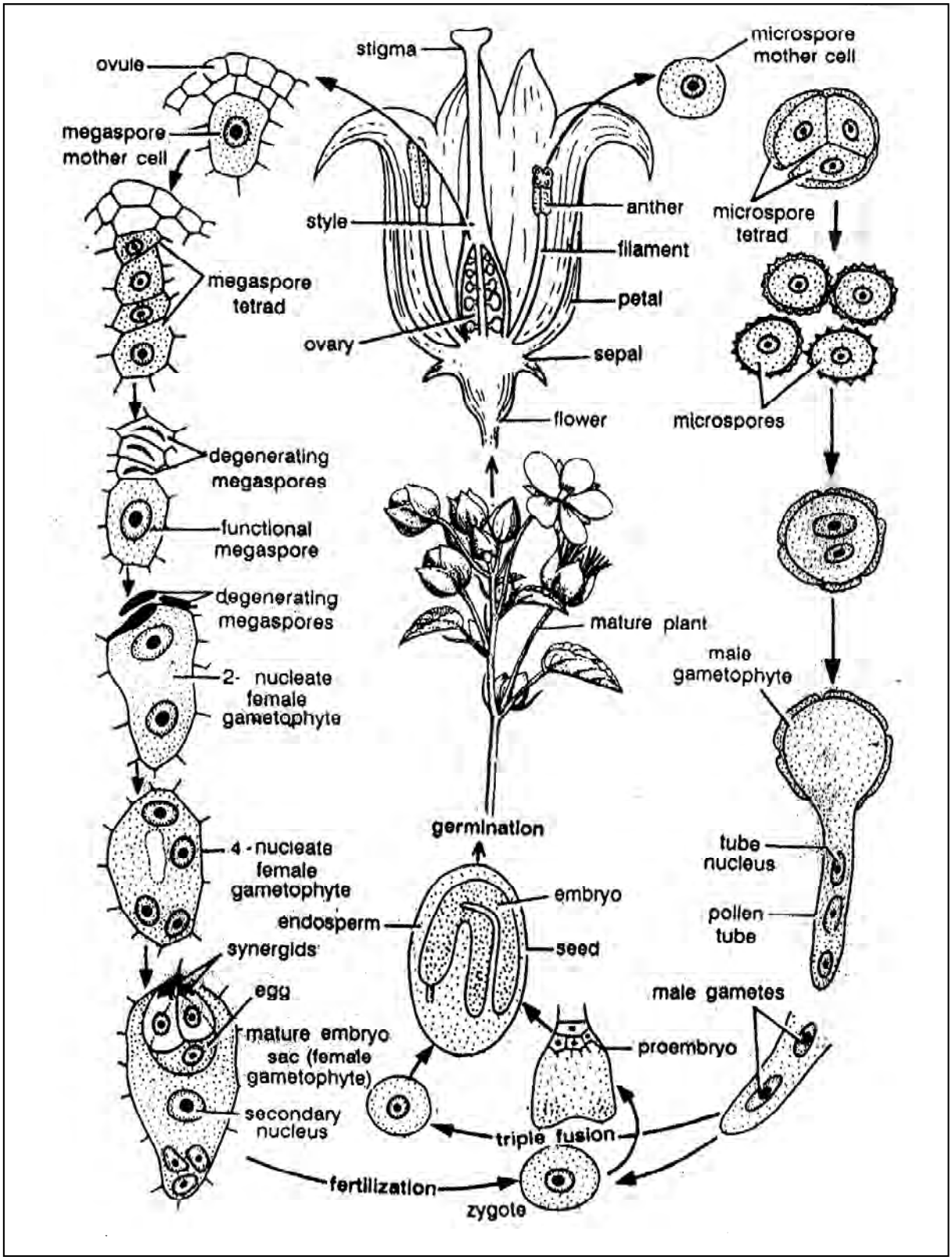

Fig. 2.1 : Life-Cycle of an Angiospermic plant

2.2.3 Fertilization :

The fusion of the two haploid gametes, i.e. one male gamete and the female gamete results in the formation of diploid **zygote**. By this way the sporophytic phase is restored. By this, the angiosperms exhibit the phenomenon of double fertilization to form the zygotes. The second male gamete fuses with definite nuclei and forms endosperm. This is called triple fusion.

2.2.4 Development of embryo (Embryogeny) and Endosperm :

Zygote develops into an embryo. Formation of endosperm is due to triple fusion (secondary nucleus - 2 nuclei + one male nucleous). Endosperm provides nutrition to the developing embryo.

2.2.5 Development of seed and fruit

The ovule devlops into the seed and the ovary develops in to the fuit. Therefore while studying various embryological processes (steps of sexual reproduction) it is essential to acquire familiarity with the organisation of the flower.

2.3 THE STRUCTURE OF THE FLOWER (Fig. 2.2) :

The flower is the branch of the shoot (stem) specially modified for sexual reproduction. Plant morphologists regard flower as a shoot of determinate growth with highly condensed or supressed internodes in between nodes and the leaves. It is also specialized variously to suit the functions of different floral organs. During floral initiation, the shoot apex gets transformed into a floral apex of the flower which is formed during onset of reprodctive phase of growth of the plant. The flower may occur singly or as a part of an inflorescence. During floral initiation, the apex of the flowering axis develops floral whorls in acropetal succession. The floral axis bearing floral organs is known as the receptacle. Since there is no significant elongagation of the internodes on the receptacle, the various floral whorls differentiate close to each other in a flower.

Typically, a flower has four sets of appendages or floral parts, arranged in whorls called floral whorls such as; **Calyx** (outermost whorl), **Corolla, Androecium** and **Gynoecium** (innermost whorl). The calyx and corolla whorls also called **accessory whorls** consist of sterile appendages called sepals and petals respectively. The sepals and petals differ in form, size and other characters. But in some families, particularly monocotyledons, they are alike and collectively called perianth. The calyx, corolla (or perianth) do not take part in sexual reproduction, thus represent the non-essential parts of the flower. But they are protective in function and attract insects etc. during pollination. The androecium and gynoecium both are called **essential whorls** which bear the sexual reproductive organs in angiosperms. The individual members (organs) constituting androecium whorl are called the stamens or the male sex organs. Similarly, the carpels constituting the gynoecium whorl are the female sex organs.

Flowers exhibit a great variation in size, colour, shape and insertion of various floral whorls. The flower of Duckweed, *(Wolffia microscopica)* has a size of 0.1 mm across and is the

smallest flower among the angiosperms. The flower of certain species of *Rafflesia* (a root parasite found in forests of Malaysia) is the largest known flower measuring a diameter upto one meter. The colour of flower is mostly due to the colour of petals and ranges from dead white through ivory, yellow, orange, red, violet and blue. The flowers exhibit nearly the whole range of visible spectrum of colours.

A flower is regarded as **complete** if it has all the floral whorls. If any one whorl is absent it is called an **incomplete flower**. An incomplete flower is said to the perfect (bisexual or hermaphrodite) when both the androecium (bearing male sex organs) and gynoecium (bearing female sex organs) are present. A flower is said to be **imperfect** (or unisexual) when either of the two sexes are missing.

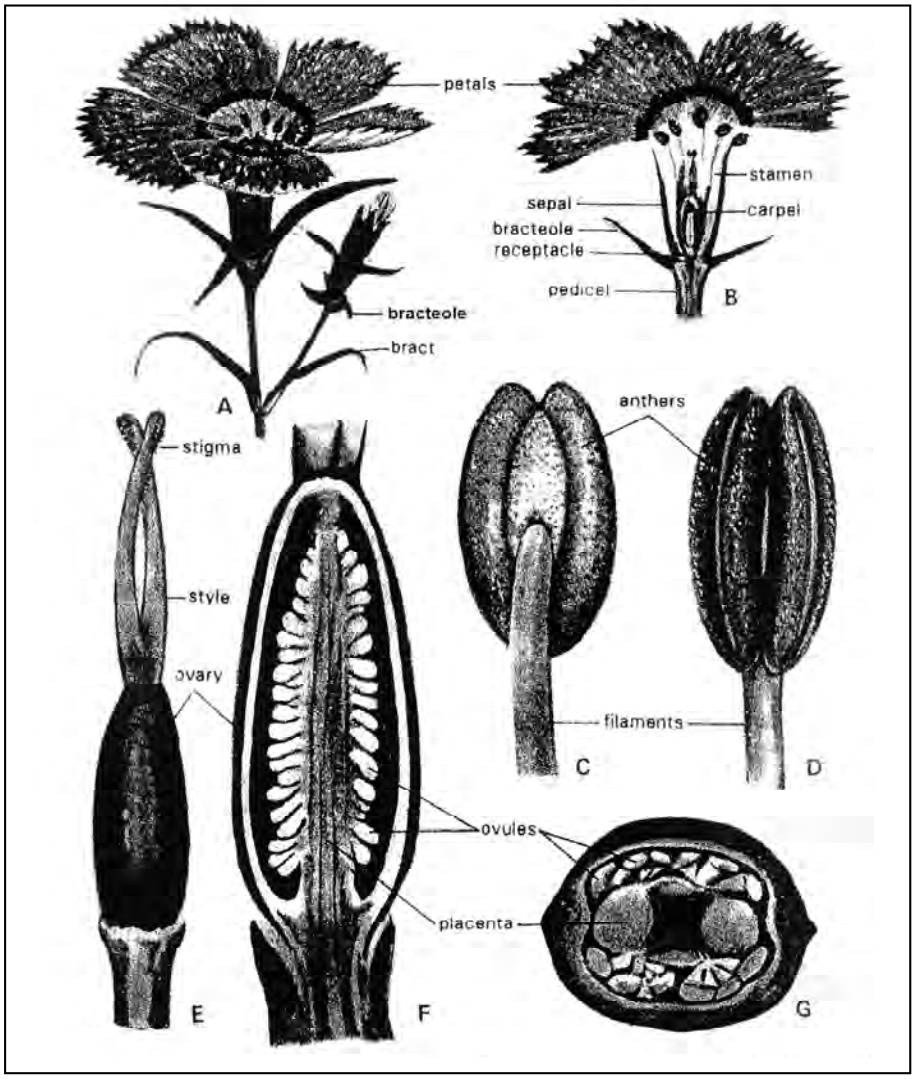

Fig. 2.2 : *Dianthus caryophyllus*. **A.** Flowering twig. **B.** Longitudinal-half of a flower to show its various parts. **C, D.** Anthers with a part of the filament; **C.** dorsal view, and **D.** ventral view. **E.** Carpels; the ovaries of both the carpels are fused completely whereas the styles and stigmas are free. **F, G.** Longitudinal **(F)** and transverse **(G)** sections of the ovary. The ovules are borne on a central axis, the placenta.

In unisexual flowers, if carpels are absent, it is called a male flower or **Staminate** flower and when stamens are absent the flower is female or **pistillate** flower. Sometimes, both the essential whorls are absent in a flower which is known as **neuter**.

A plant may have both male and female flowers borne separately (e.g. Maize, Cucurbita). It is called **monoecious**. When male and female flowers are present in different plants, such plants are called **dioecious** (e.g. Papaya, Mulberry, Date palm, Coccinia). When both perfect and imperfect flowers are borne on the same plant, they are called **polygamous** (e.g. Mango, Marigold).

In spite of limitless variations seen among the flowers, their basic organization (structure) is uniform.

2.3.1 The Structure of a Perfect Flower :

The stalk of the flower through which it is connected to the main plant is called a **pedicel**. The leaf-like structure(s) bearing a flower in it's axil is called a **bract**. Similar foliar (leaf-like) structure present at the summit or anywhere of the pedicel below the receptacle of the flower is called a **bracteole**. The swollen part of the flowering (floral) axis at which all floral parts such as; Calyx, Corolla, Androecium and Gynoecium are attached is called the receptacle. (Figs. 2.1, 2.2)

2.3.1.1 Calyx :

This is the outermost whorl of flower i.e. the lowermost whorl on the receptacle. The individual members (apendages) of calyx is called a **sepal**. The sepals may be united (gamosepalous) or free (polysepalous) and are generally green in colour or inconsipicuously coloured. Sepals are usually leaf-like (foliaceous) in texture and primarily serve to protect the flowering bud. They usually fall off soon after the flower opens or sometimes are persistent (e.g. Brinjal). When sepals look like a petal (petaloid) they attract the insects for pollination (e.g. *Clematis*). Green sepals perform photosynthetic function. Sepals sometimes form a spur which stores nectar. They may help in seed dispersal such as in fruits of some members of Asteraceae.

2.3.1.2 Corolla :

Inner to the calyx, lies the corolla whorl of the flower. The corolla whorl arises in the receptacle from a node just above that of calyx. The individual members (appendages) of corolla are called **petals**. The flowers owe their charm mostly to the bright and gorgeous colours and attractive forms of corolla. Corolla with free petals is called **polypetalous** and that with united or fused petals called **gamopetalous**. This character has been used in the system of classification of flowering plants by Bentham and Hooker (1862-1833). All dicotyledonous flowering plants in this system are further classfied into three groups, such as; (i) Gamopetalae (plants with gamopetalous Corolla), (ii) Polypetalae (with Polypetalous Corolla) and (iii) monochlamydeae (plants lacking a corolla)

When these sterile appendages of a flower are not clearly distinguishable into Calyx and Corolla as in Monochlamydeae (as above) they are collectively called a **perianth** and the individual members of it are knwon as **tepals**. The corolla or the perianth protect the young reproductive structures in bud condition and also help in pollination by attracting insects through their attractive colours and curious forms.

2.3.1.3 Androecium :

Androecium whorl is located inner to the corolla and its individual members (appendages) are called **stamens**. These are the male reproductive organs. A stamen typically consists by a long stalk-like sterile filament to which usually a fertile bilobed anther is attached at its distal end. Each of the anther lobes typically has two microsporangia or pollen sacs which contain microspores or pollen grains. The tissue that joins filament with the anther lobes is called **connective**. So each anther typically has two another lobes and four microsporangia. (Fig. 2.2 C, D & 2.3).

2.3.1.4 Gynoecium :

Inner to the Androecium whorl lies the gynoecium which is the female reproductive apparatus (organs) in a flower. The individual members constituting the gynoecium whorl are called **carpels**. As a rule, Carpels are borne laterally on the receptacle. A typical Carpel comprises of a basal swollen ovary with a terminal stigma held on stalk like structure called style.

A flower having a single carpal is called simple or monocarpellary (e.g. fabaceae). When more number of carpels constitute the gynoecium whorl it is termed as compound or multicepellary. The multicarpellary gynoecium may be **apocarpous** with totally free carpels (e.g. *Clematis*) or **syncarpous** with united (fused) carpels forming a compound gynoecium. Syncarpous condition is seen in most of the angiosperms, (such as; Mustard, Hibiscus, Datura etc.). Based on number carpels formed in the gynoecium a flower may be monocarpellary (single carpel), **bicarpellary** (with two carpels e.g. *Allium*), tetracarpellary (with four carpels, e.g. *Datura*, *Berberis*), Pentacarpellary with five carpels, (e.g. *Melia, Hibiscus*) and **multicarpellary** with more than five carpels (e.g. *Papaver*).

The ovary contains ovules which are enclosed within the ovary wall and hence the name angiosperms. The portion of the carpellary tissue to which ovules are attached is called the **placenta** (Fig. 2.2). The mode of distribution of ovlues inside the ovary is called placentation.

The elaborate description of the structure of the flower is in Volume-I of this book (Bureau's Higher Secondary (+2) Biology, Vol-I, 2016, Pages 163-181). The terminologies relating to a flower have great value in understanding the development of embryological processes in angiosperms.

Main reproductive structures associated with a noraml flower are given below :

Flower	-	A reproductive structure
Stamen	-	Male sex organ
Carpel	-	Female sex organ
Anther	-	Develops microsporangia (pollen sacs)
Ovule of ovary	-	Develops megasporangium
Pollen grain	-	Develops into male gametophyte
Embryosac within ovule	-	Female gametophyte
Sperm	-	Male gamete
Egg	-	Female gamete

2.4 DEVELOPMENT OF MALE GAMETOPHYTE :

To study the development of male gametophyte, one must start with the stamen and followed by structure of anther, microsporangia, microsporogenesis and microspores or pollen grains. The microspore develops into a male gametophyte.

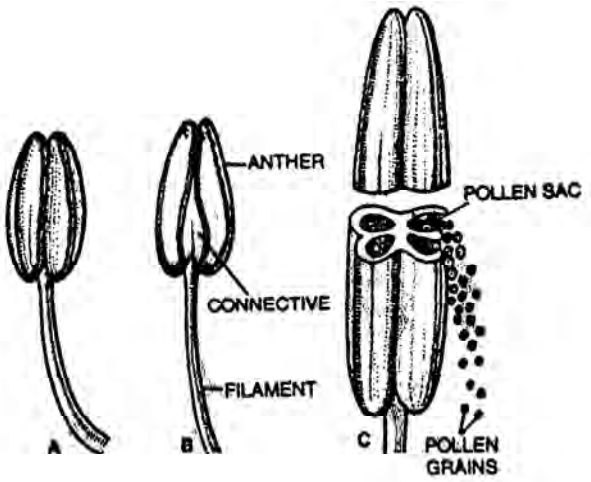

Fig. 2.3 : Stamen. **A.** Ventral view, **B.** Drosal view, **C.** T.S. of Anther (Enlarged)

2.4.1 The stamen :

As stated earlier , the stamen is the male reproductive organ and consists of the lower sterile, long, narrow stalk-like filament and upper fertile part, the broader knob-like **anther** (Fig. 2.3). The anther and filament are connected by a **connective**.

2.4.2 Structure of Anther :

The anther shows great variety in form but externally it is typically two lobed, called anther lobes. Each lobe contains two longitudinally running chambers or pollen sacs. Each pollen sac represents a **microsporangium** which contains several haploid microspores or pllen grains. Therefore an anther generally contains four microsporangia. (Figs. 2.3, 2.4)

2.4.3 Formation of Microsporangia (Pollen sacs) :

Young anther is a homogenous mass or meristematic cells surrounded by an epidermis (Fig. 2.5). Groups of hypodermal cells in each of the four corners of it, become distinguished from the surrounding cells by their larger size, dense cytoplasm and prominent nuclei. The

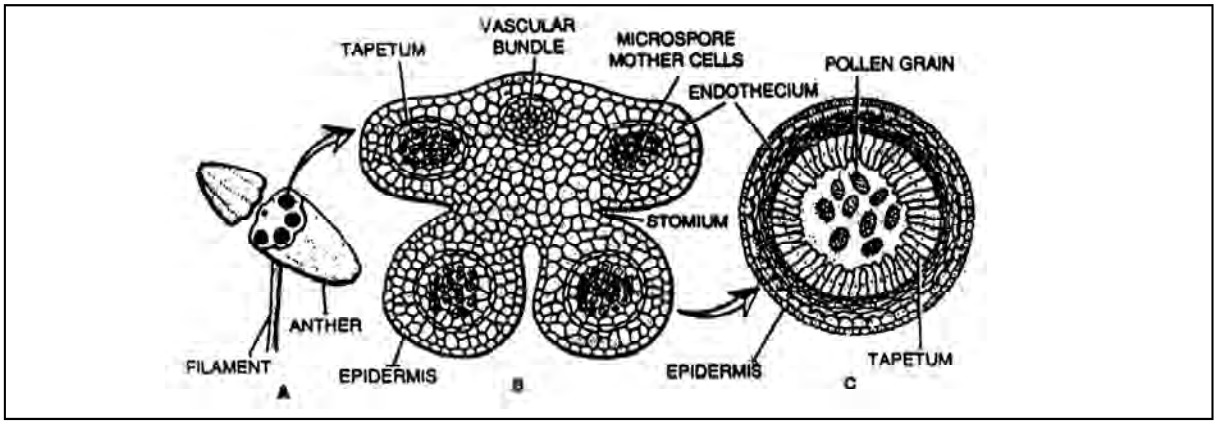

Fig. 2.4 : T.S. anther, showing stomium and pollen grain

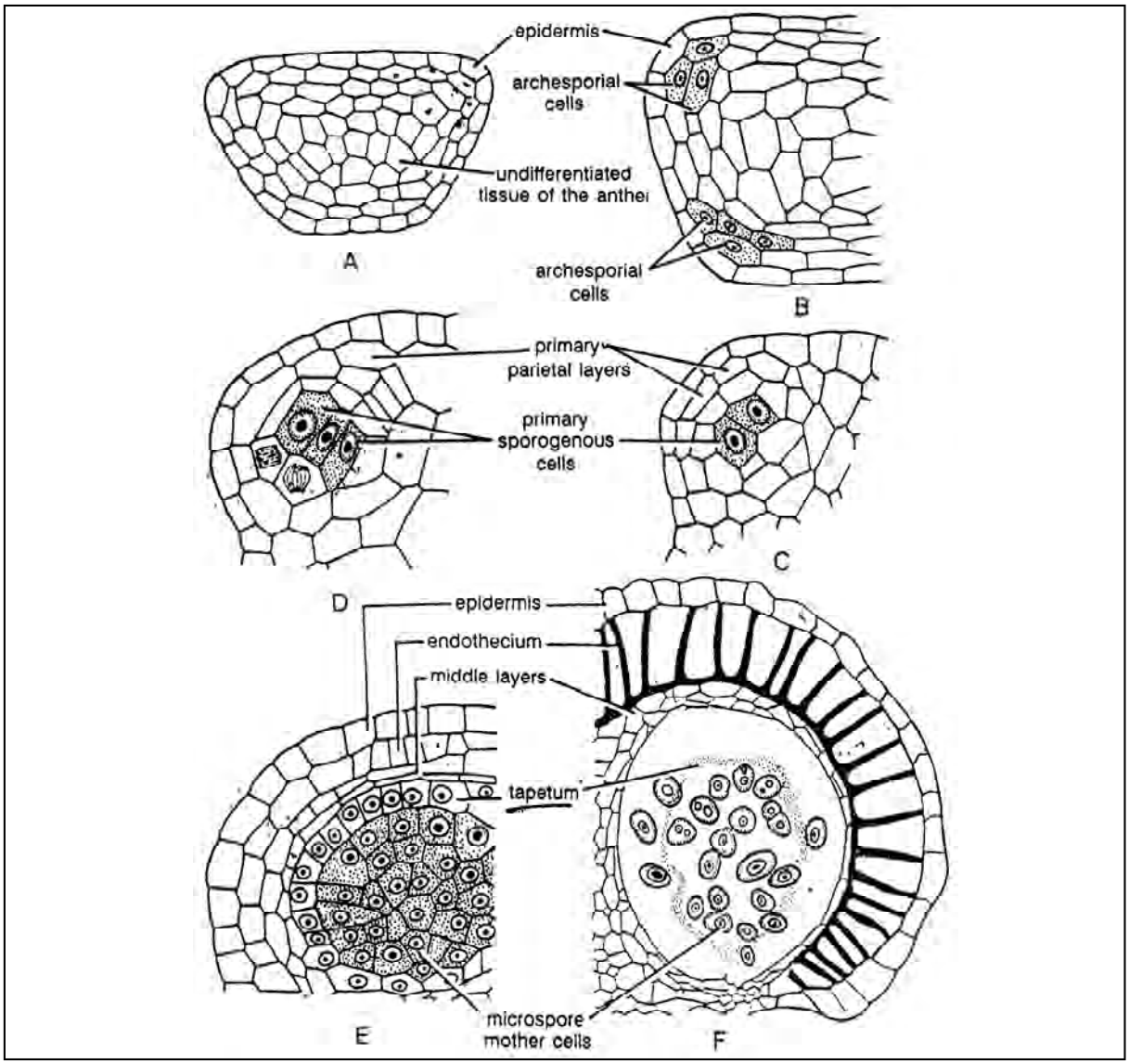

Fig. 2.5 : A-F. Development of microsporangium : **A-E** Successive stages of the development of microsporangium; **F. A** mature pollen sac in a transerve section.

conspicuous cells which are arranged in plate-like or crescent-shaped vertical rows form the **archesporium** of anther. The rows of archesporial cells may vary from one to few in different species. The archesporial cells enlarge radially and divide periclinally to form **outer primary parietal cells** and inner **primary sporogenous cells** (Fig. 2.5, 2.6). The primary parietal cells undergo repeated periclinal and anticlinal divisions giving rise to 3-5 concentric layers of cells which eventually form the wall layer of the anther, (anther wall). The primary sporogenous cells may directly function as **microspore mother cells** (MMC) or undergo several mitotic divisions and finally each of them function as MMC (Fig. 2.5 E).

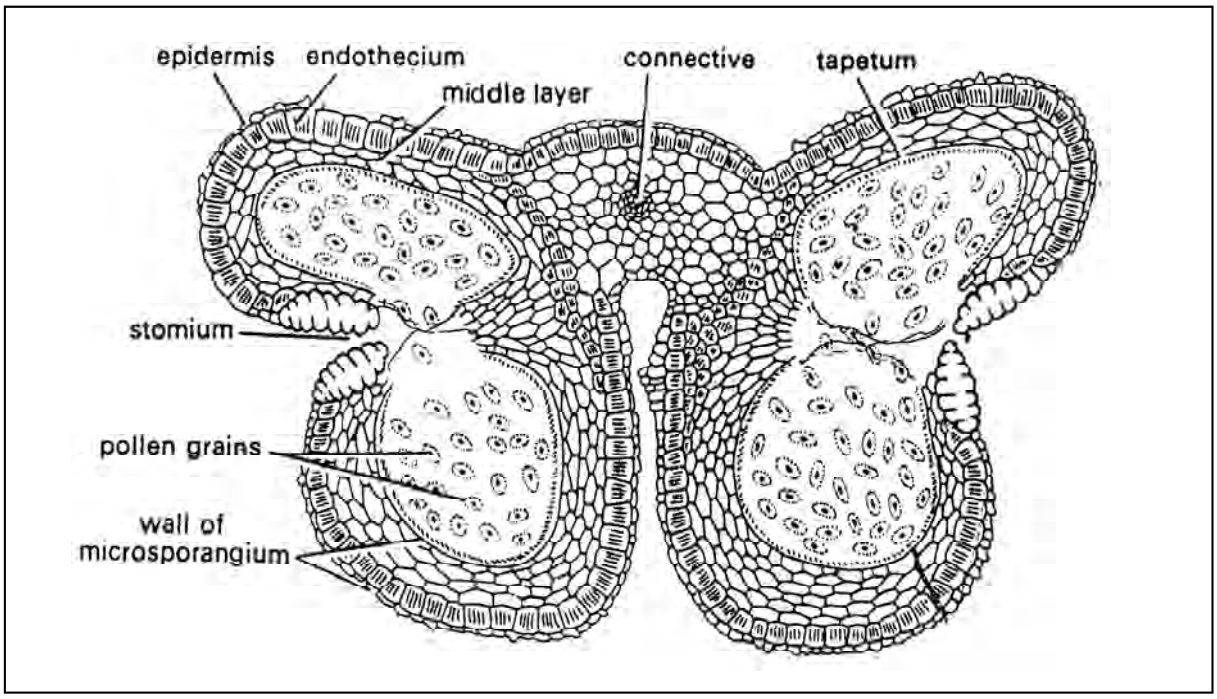

Fig. 2.6 : Transverse section of a mature anther.

Thus, at four corners of the anther, four microsporangia are formed. Each microsporangium consists of 3-5 layered anther wall which surrounds the core of microspore mother cells (Fig. 2.5). The anther wall is made up of (i) one layered outermost epidermis, (ii) single layer of endothecium (sub-epidermal layer), (iii) middle layers and (iv) tapetum. (Fig. 2.6). The cells of endothecium are radially elongated, may be U-shaped or ring shaped and they attain the maximum growth when pollen grains mature. They help in dehiscence of anthers.The middle layers lie inner to endothecium. Cells of middle layers are ephemeral and degenerate completely before microspore mother cells undergo meiosis. They store food materials in some taxa. The innermost of wall layers having larger and centripetally extended cells is the tapetum which surrounds the microspore mother cells (Fig. 2.7). Tapetal cells may be multinucleate and provide nutrition to the sporogenous cells and ultimately to the developing microspores. Thus the developing microspores consume products of middle layers and tapetum.

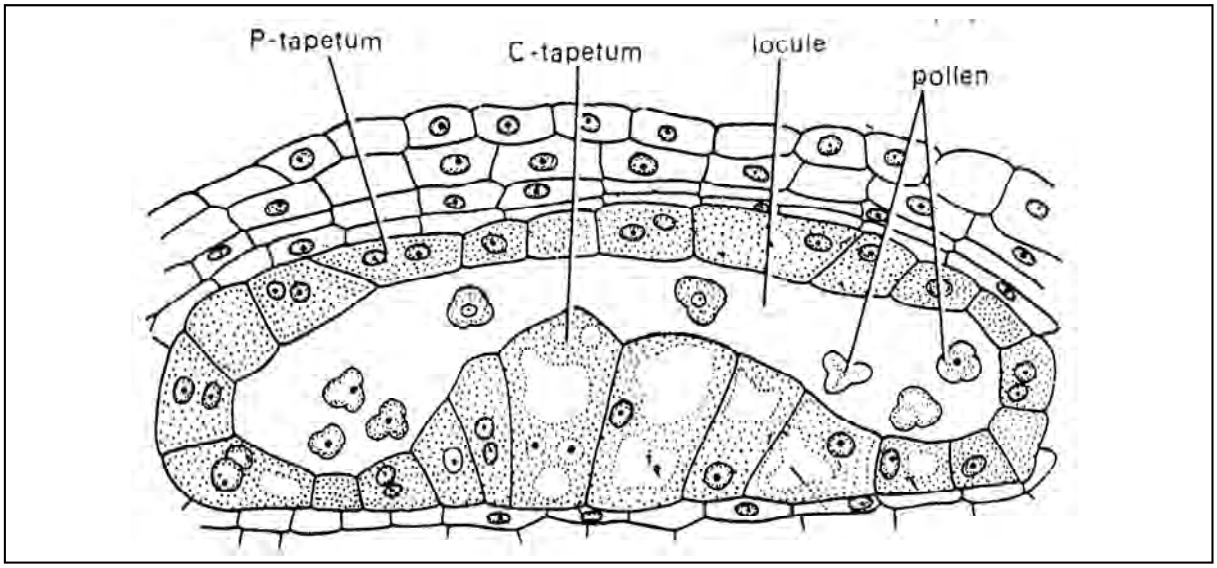

Fig. 2.7 : Dimorphic tapetum in *Alectra thomsonii*

2.4.4 Microsporogenesis :

The sporogenous cells as stated above function as microspore mother cells, which are polygonal in shape and closely packed. As the anther enlarges the pollen sacs (microsporangia) become spacious i.e. get loosely arranged. A few microspore mother cells become non-functional and are finally absorbed by the developing microspores.

Each viable or functional microspore mother cell undergoes meiotic cell division and forms four haploid microspores. This process of formation of microspores from the microspore mother cell through meiosis is known as **microsporogenesis**. (Fig. 2.8)

After microsporognesis, the mature anther dehisces by means of slits. Once the slit (opening) is made the microspores come out of the anther.

2.4.5 The Microspore or Pollen grain :

Typically, the microspore or pollen grain is a haploid and unicellular body with a single nucleus. The mature microspore or pollen grain may be oval, ellipsoidal, triangular, lobed or even crescent shaped. The microspore has a well defined two-layered wall, consisting of outer thick **exine** and inner thin **intine**. The intine surrounds the cytoplasm. The outer exine may have spines, ridges or furrows which may vary in different species. So the exine is either sculptured or smooth. The exine is chiefly composed of **sporopollenin**, a substance considered to be the oxidative polymer of carotenoids or carotenoid esters. Sporopollenin is a tough substance providing resistance to physical, chemical and biological decomposition and checks natural decay of pollen grains. In insect pollinated pollen grains, the exine is covered by a yellowish viscous and sticky substance called **pollenkitt** which emits smell. The definite function of pollenkitt is not known but it is believed that it helps in attracting insects and protects the pollen from ultraviolet radiation.

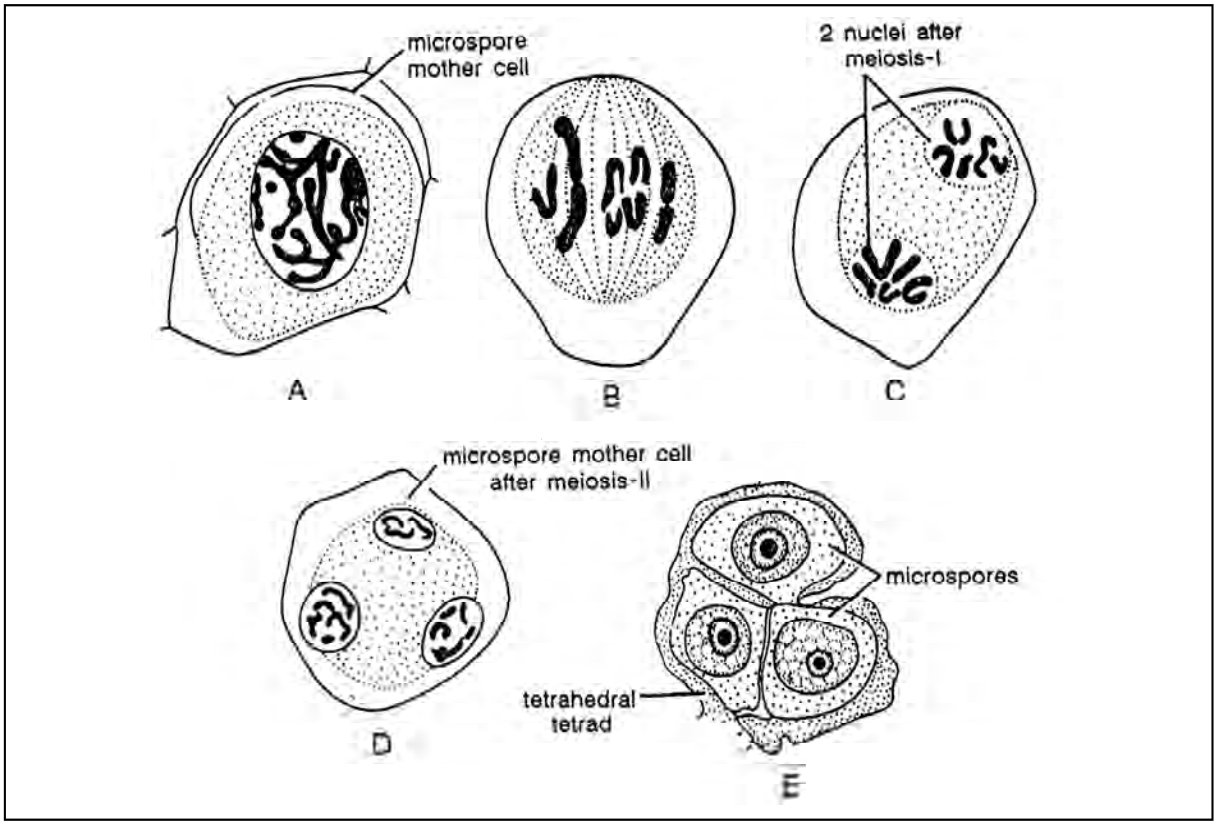

Fig. 2.8 : A-E. Simultaneous division of cytoplasm in microspore mother cell.

The intine is composed of pectin and cellulose. Intine is usually thicker near the germ pores and at these points also contain enzymatic proteins. Cytoplasm below the intine contains dictyosomes, mitochondria, endoplasmic reticulum, rich starch contents and unsaturated oils. Pollen grains are densely cytoplasmic as long as they are in tetrad condition. Later on when they become free from tetrad condition, their cytoplasm become considerably enlarged and highly vacuolated.

At certain places, the exine remains thin. These areas are called **germ pores** through (one germ pore) which the intine protrudes outside and forms the pollen tube (Fig. 2.10)

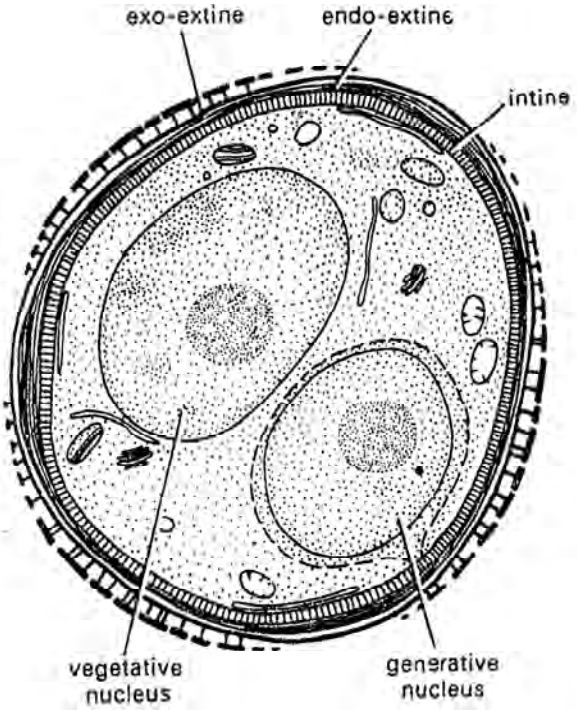

Fig. 2.9 : Structure of pollen grain.

Study of pollen grains is known as **palynology**. The pollen grain on further development forms a male gametophyte.

2.4.6 Formation of Male Gametophyte :

1. **Pre-pollination development :** The development of male gameophyte is more or less uniform in angiosperms (flowering plants). It may start in pollen grains while still within the microsporangium or pollen sac (precocious germination). Before the cell division, the nucleus of microspore migrates from the centre to periphery and many vacuoles appear in between the nucleus and the wall of the microspore. The microspore undergoes only two mitotic divisions. The first mitotic division leads to the formation of a bigger **vegetative cell** (also called **tube cell**) and a smaller **generative cell** (Fig. 2.9). There is no distinct cell wall between these two cells. Both the cells are bound by the cell membrane only. A temporary callose wall is laid down between vegetative and generative cells (2.10). The callose wall (plug) spreads between generative cell and intine to finally pinch the generative

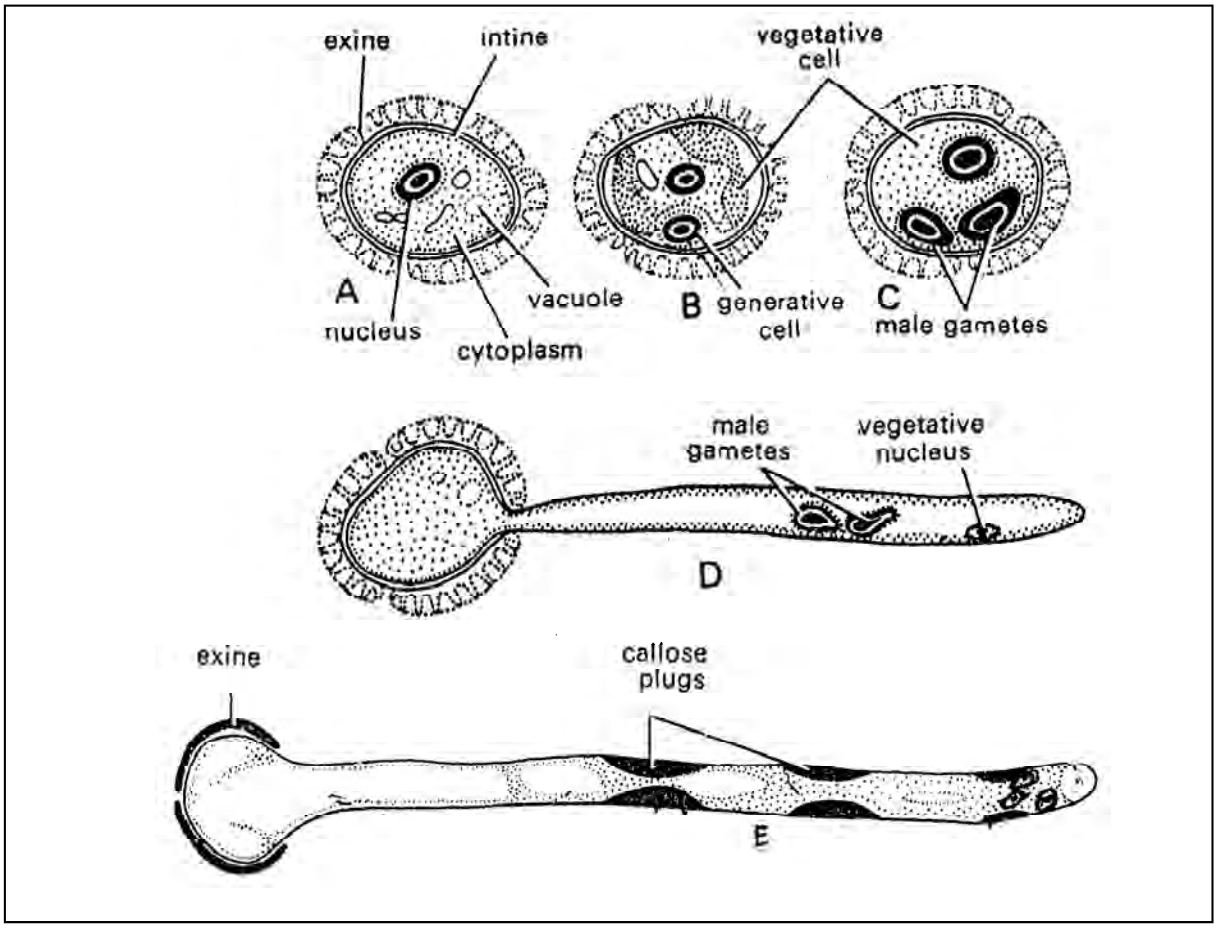

Fig. 2.10 : Successive stages of development of Male Gametophyte and callose plugs in developing pollen tube

cell off. The callose wall then dissolves and the generative cell (bound by membrane only) lies freely in the cytoplasm. The cytoplasm of the generative cell is almost hyaline and does not contain much of stored food. The texture of generative cell is relatively uniform. It may be elliptical, lenticular or even spindle shaped (Figs. 2.9, 2.10). The elongated form of generative cell however facilitates it's passage through pollen tube.

The larger vegetative cell contains various stored food such as; fat, starch and some protein granules. Now the microspore contains two cells such as vegetative cell and generative cell (Fig. 2.10B). It is usually at its two celled stage, the microspores are liberated from the microsporangia of the anther. Upto this stage, the development of male gametophyte is said to be under pre-pollination stage.

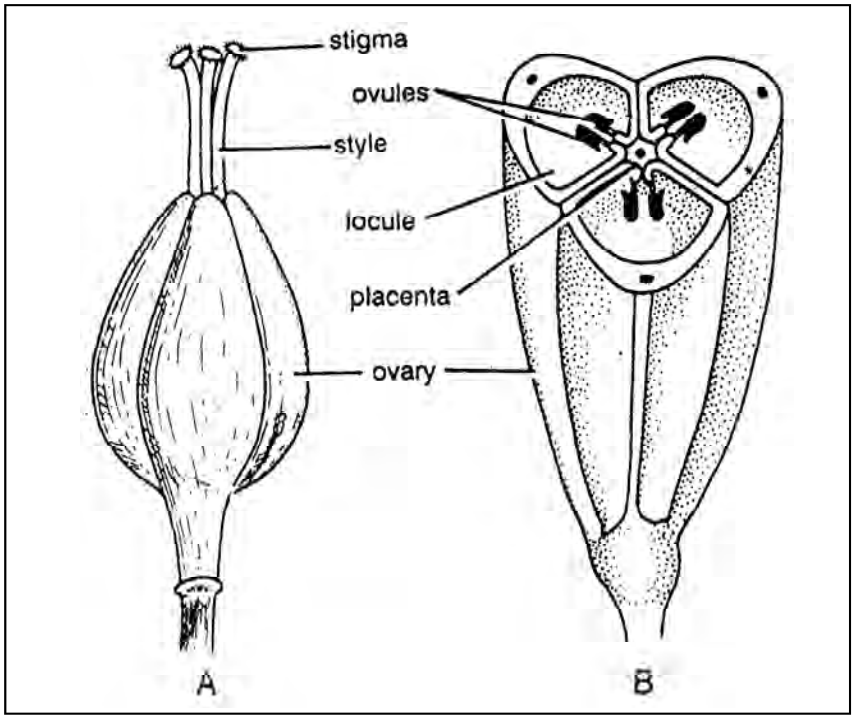

Fig. 2.11 : A-B. Gynoecium: **A.** External view, **B.** Transverse section of ovary.

2. Post-pollination development :

When the microspores or pollen grains fall on the stigma of the pistil, post-pollination changes occur. The pollen grains absorb water and nutrients available on the stigmatic surface. The intine of the pollen grain protrudes out through one of the germ pores and a pollen tube is formed. The pollen tube pierces the stigmatic surface and moves down through the style of the pistil (Figs. 2.11, 2.27). Now, in the generative cell, the nucleus divides mitotically to form two male nuclei which

become surrounded by a thin cytoplasmic sheath and appear as distinct non-motile male gametes. Since there is no cell wall in the male gametes, they may be called naked. The nucleus of the generative cell, migrates to pollen tube (Fig. 2.10D). Formation of male gametes may also occur prior to formation of pllen tube. This three-celled male gametophyte remains viable for a short time. As the pollen tube elongates, the distal part bcomes highly vacuolated and becomes separated from the anterior part containing the three nuclei, by formation of callose plug (Fig. 2.10E).

The male gametophyte in flowering plants is a highly reduced structure.

2.5 DEVELOPMENT OF THE FEMALE GAMETOPHYTE :

To study the development of the female gametophyte one must be thoroughly acquainted with structure of the ovary and ovule.

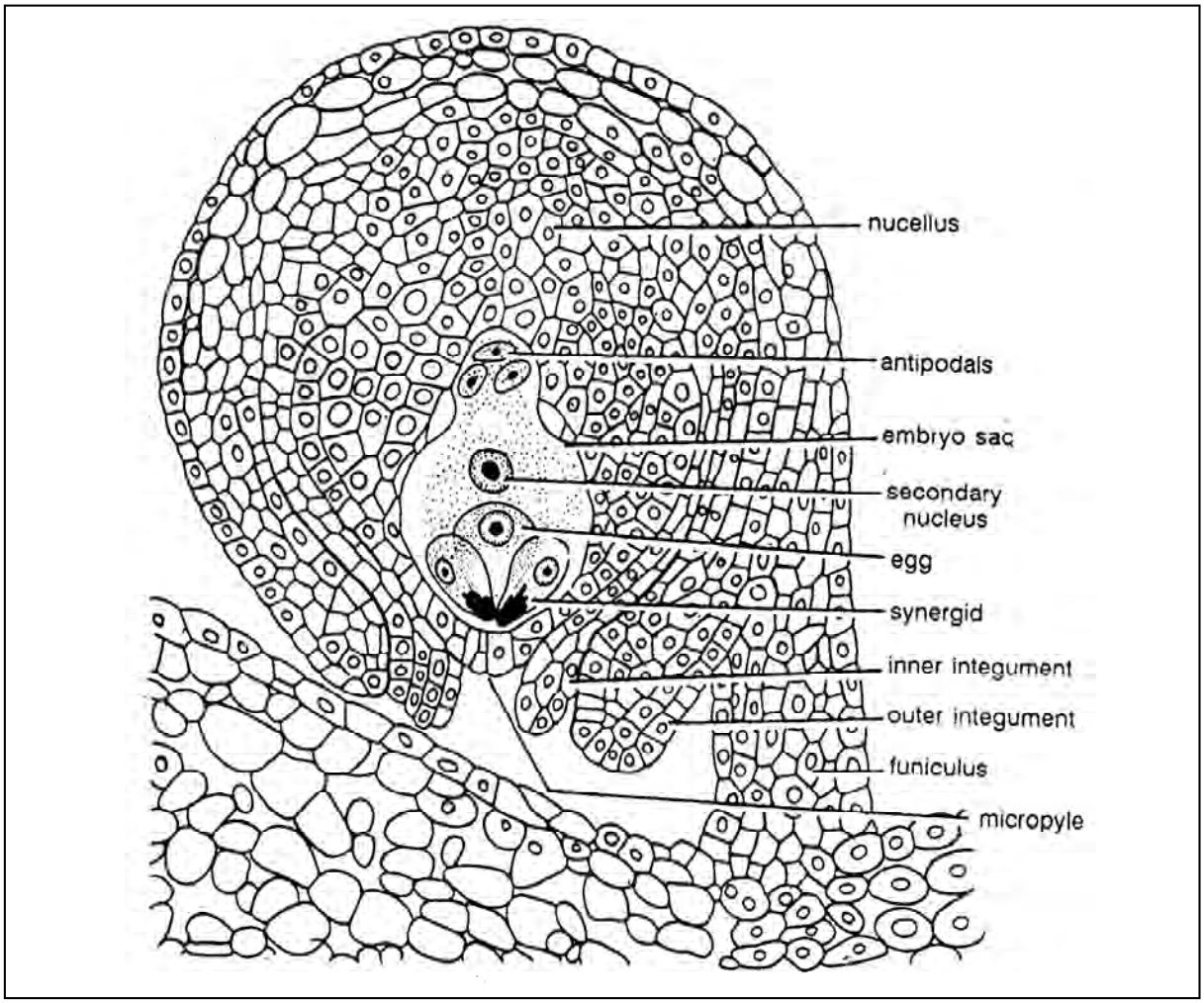

Fig. 2.12 : Ovule : Structure (Anatropous Ovule).

2.5.1 The Ovary and Ovule :

The gynoecium consists of one to many carpels (Fig. 2.11). A typical pistil (carpel) consists of a basal portion called **ovary**, a stalk (or **style**) and the terminal receptive disc (the **stigma**) (Fig. 2.11A). The ovary may contain a single or may ovules arranged in specific placentation. Similarly if more than one carpel is present in the syncarpous ovary, the ovary may contain a number of locules (chamber), usually corresponding to number of carpels. (Fig. 2.11B). The ovule after fertilization develops into a seed, ovary finally develops into a fruit.

The ovule is the **megasporangium**. The ovlue is attached to the **placenta** (Fig. 2.11). The placenta is a ridge of tissue (a parenchymatous mass) in the inner wall of the ovary to which the ovules are attached. The mode of arrangement of ovules along the placenta in the cavity of the ovary is known as **placentation** (axile, parietal, free central etc.)

2.5.2 Structure of Ovule :

Each ovule in a flowering (angiospormic) plant has the following structure. The ovule is attached to the placenta by a slender stalk called **funicle** or **funiculus** (Fig. 2.12). This point of attachment of the body of the ovule to its stalk (funiculus) is known as **hilum**. In an inverted (anatropous) ovule. The part of funiculus remains attached beyond the hilum alongside of the

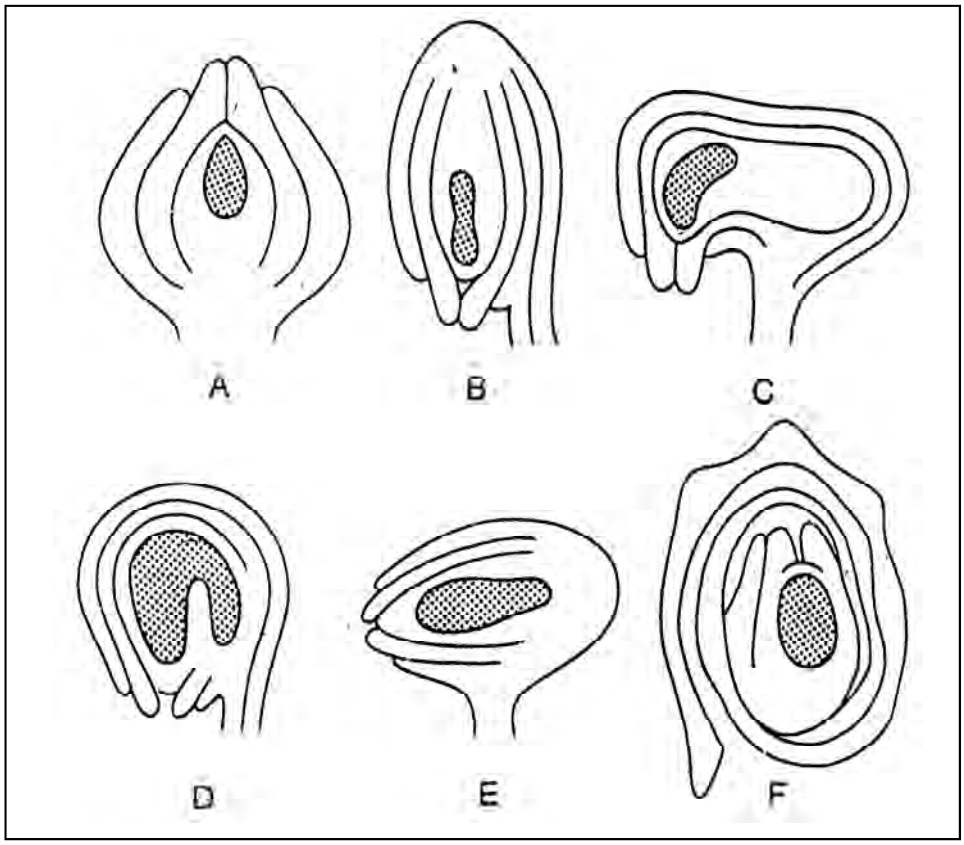

Fig. 2.13 : A-F. Various types of ovules : **A.** Orthotropous, **B.** Anatropous, **C.** Campylotropous, **D.** Hemi-anatropous, **E.** Amphitropous, **F.** Circinotropous.

body of the ovule forming a sort of ridge called **raphe**. The main body (swollen portion) of the ovule consists of a mass of thin walled parenchymatous cells forming a central body, called **nucellus**, The nucellus is surrounded and protected by one or two multicellular coats (or sheaths) called **integuments**. The ovules with one integument are called **unitegmic** (e.g compositae) and with two integuments are known as **bitegmic**. Majority of anigosperms have bitegmic ovules.

The small opening in the integumentary sheath at apex region of ovule where the tip of nucellus remains exposed is called a **micropyle**. The basal part of the ovule where the nucellus, integuments and funiculus merge is called the **chalaza** (Fig. 2.12). Depending upon the relative position of micropyle and chalaza at maturity of ovules, different types of ovules have been reported in angiosperms, such as (i) orthotropous (upright or erect ovule), (ii) anatropous (inverted ovule), (iii) campylotropous, (iv) hemianatropous, (v) amphitropous and (vi) circinotropous (Fig. 2.13).

2.5.3 Development of the ovule :

The ovule primordium arises on the placenta as a hemispherical projection or a parenchymatous mound (Fig. 2.14A). Periclinal division followed by anticlinal divisions in the very young protruberence (projection) results in enlargment of the same. There is an early differentiation of the **archesporial cell** which becomes conspicious due to their larger size and dense cell contents (Fig. 2.14B). Differentiation of archesporial cells (archesporium) is followed by initiation of the inner and outer integuments (Fig. 2.14 C, D). Integuments arise as a complete ring, right below the nucellus and grows upwards to cover the whole nucellus except at the micropylar opening at the tip region. The central part of the ovule inner to the integument is a parenchymatous mass of cells which becomes differentiated in due course of time to a mature nucellus containing a female gametophyte or embryo sac (Fig. 2.12).

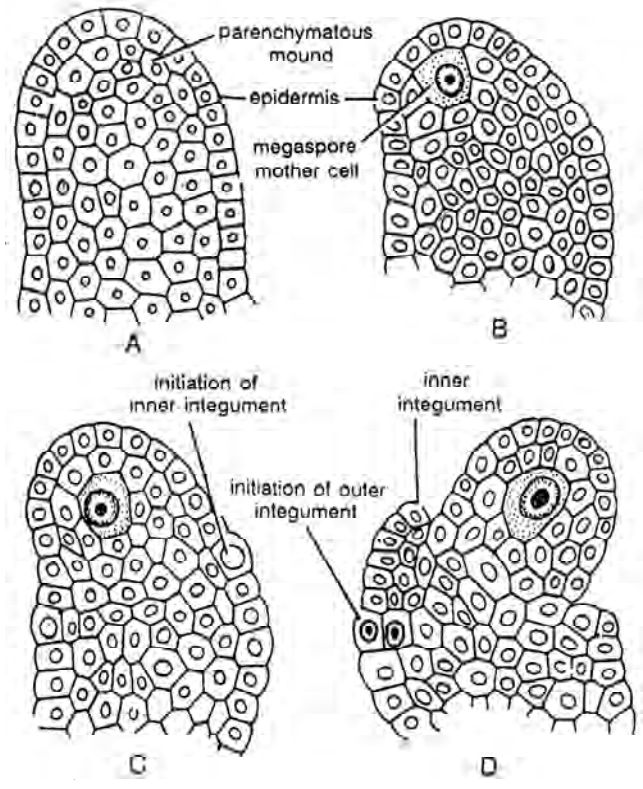

Fig. 2.14 : A-D. Successive stages in the development of ovule.

Thus the mature ovule consists of outermost shealths, the integuments and the nucellus which encloses an embryo sac (the female gametophyte) (Fig. 2.12).

2.5.4 Megasporogenesis :

The mucellus towards the micropylar end, differentiates a hypodermal cell into an **archesporial initial**. This archesporial initial divides periclinally to form outer primary parietal cell and inner primary sporogenous cell.

The primary sporogenous cell forms the **megaspore mother cell** (Fig. 2.14 B-D) which undergoes meiotic cell division to form four haploid **megaspores**. (Fig. 2.15 A). The formation of megaspores from the megaspore mother cells is known as **megasporogenesis**. Out of four megaspores in a linear tetrad, usually the upper three megaspore degenerate and the lower most megaspore (the chalzal one) enlarges to become functional megaspore (Figs. 2.15, 2.16 A). Thus the megaspore mother cell is the last cell of the female sporophytic (2n) or diploid generation. Similarly the haploid (n), functional megaspore represents the first cell of the female gametophytic generation.

This functional megaspore organises to form a female gametophyte or embryo sac. (Fig. 2.16)

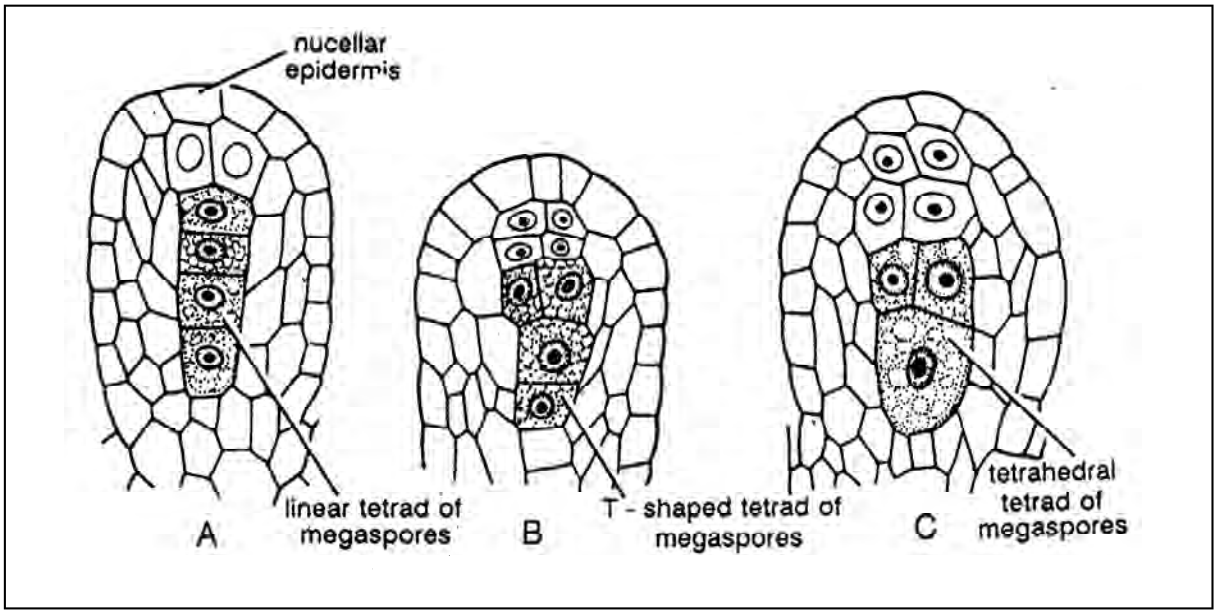

Fig. 2.15 : A-C. Megaspore tetrads : **A.** Linear tetrad, B. T-shaped tetrad, **C.** Tetrahedral tetrad

2.5.5 Organization of the female gametophyte (Embryo Sac) :

The functional megaspore grows in size and many small vacuoles appear in its cytoplasm. The vacuoles, later on, join together to form a large vacuole. The nucleus of the megaspore undergoes three mitotic divisions to form eight nuclei. Generally four nuclei are seen at micropylar end (pole) and the other four, seen at the chalzal region (pole) of the enlarged megaspore.

This enlarged megaspore organises itself to form an **embryo sac**. It has been observed that the separation of nuclei and presense of four nuclei at each pole is due to enlargement of the central vauole which pushes the nuclei towards the opposite poles of the embryo sac (Fig. 2.16 C-D).

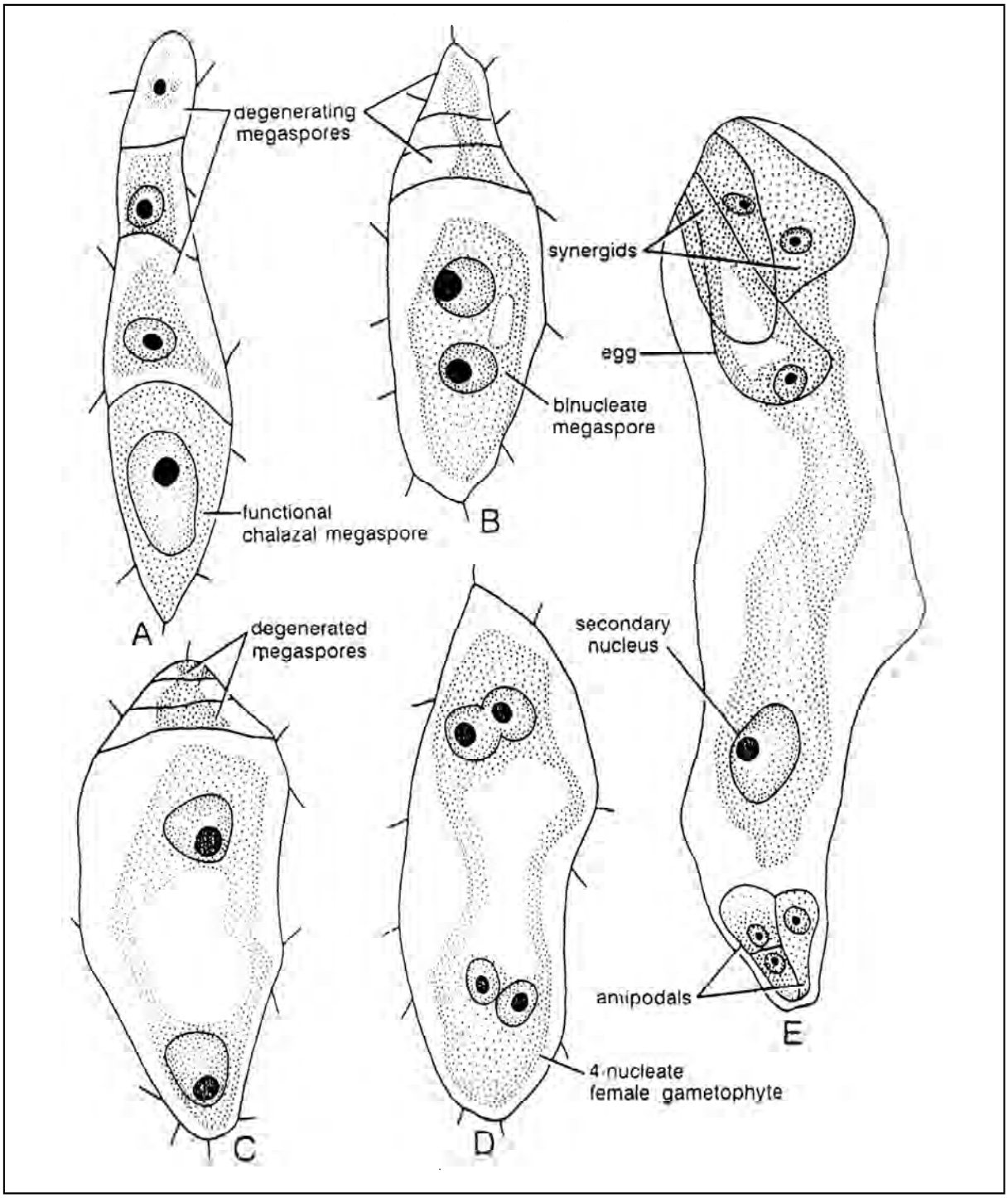

Fig. 2.16 : A-E. Successive stages of the embryo sac development.

The most common type of embryo sac is eight nucleate embryosac developing from a single megaspore. It is found in about 81% of flowering plants. Since this embryo sac develops from a single megaspore it is called **monosporic** type of embryo sac or (*Polygonum* type), which is described below.

Out of four nuclei, one nucleus from each group at a pole migrates to the centre of embryo sac. These two nuclei which have migrated to center are called **polar nuclei**. Two polar nuclei fuse to form a **definitive nucleus**. The three nuclei left at the chalazal pole are surrounded by walls and are called **antipodals** or antipodal cells. Again, out of three nuclei located at micropylar pole (or micropylar end) are organised to form the **egg apparatus** consisting of one egg (or oosphere) and two synergids (Fig. 2.17). The egg is the female gamete. The egg hangs between the synergids. The embryo sac is the female gamete bearing part or the female gametophyte (Figs. 2.16 E, 2.17). The egg (the female gamete) on fertilization with a male gamete forms a **zygote** which develop into an **embryo**. The synergids help the passage of pollen tube bearing two male gametes. Pollen tube passes through the synergids to effect fertilization. One male gamete fertilizes the female gamete (egg) called **syngamy** and the other male gamete fuses with the two polar nuclei (secondary nucleus) in the center of embryosac, forming the primary **endosperm cell**. Primary endosperm cell or the endosperm mother cell grows into an endosperm which provides nutrition to the growing embryo (Figs. 2.16, 2.17).

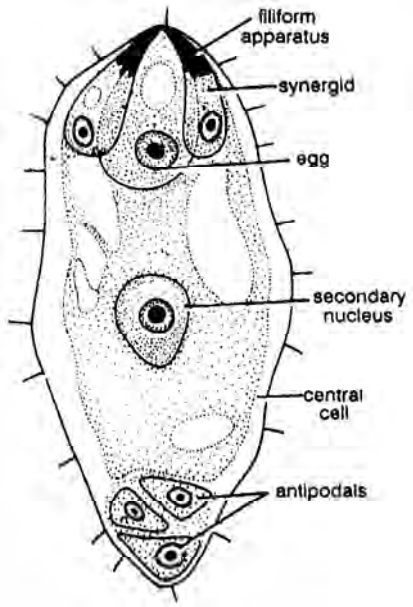

Fig. 2.17 : Mature Embryo sac.

The antipodal cells sooner or or later get disorganised.

2.6 POLLINATION :

When pollen grains are shed from the anther they are disseminated by means of various agencies. Some pollen grains may be by one means or the another, finally reach the stigma of a pistil, either of the same or another flower of same plant or another.

Therefore the process of transfer of pollen grains from anthers to the stigma is called pollination. The process of pollination ends when the pollen grain has reached the stigma.

2.6.1 Types of pollination :

The two broad categories of pollination are self pollination and cross pollination. The transfer of pollen grains from an anther to the stigma of the same flower or to a flower on the same plant is known as **self pollination** or autogamy. On the contrary, the transfer of pollen grains from anther of the flower to the stigma of a flower of another plant is called **cross pollination** or allogamy.

Pollination may also be divided into three types basing on the source of the pollens. They are (i) Autogamy (ii) Geitonogamy (iii) Xenogamy.

(i) **Autogamy -** Here the source of the pollen to fill on stigma is the same flower.

(ii) **Geitonogamy -** When transfer of pollen grains takes place between two flowers borne by the same plant it is called geitonogamy. It is functionally a cross pollination but genetically a self pollination.

(iii) **Xenogamy -** In this process, the transference of pollen grains between two different (separated) plants of the same species. Here pollination causes transference of genetically different types of pollen grains of a plant to the stigma of another plant.

2.6.2 Self Pollination :

Self pollination (as defined above) can take place in bisexual flowers in which both male and female sex organs mature at the same time. Self pollination can also occur in unisexual flowers (male and female) of the same plant (monoecious condition) when their sex organs mature at the same time.

The process of self pollination can be classified into the flowering two types.

1. Autogamy :

Autogamy means pollination of a flower by its own pollens. So it is the transfer of pollen grains from anther of a flower to the stigma of the same flower. Naturally autogamy is possible in bisexual plants only (e.g. Tea, Wheat, Rice etc.).

2. Geitonogamy :

This type of pollination is the transfer of pollen grains from the anther of a flower to the stigma of another flower borne on the same plant. In otherwords, this is a type of self pollination that occurs between two different flowers present on the same plant. Here, only one plant is involved. The flowers may be bisexual or unisexual borne by the same plant.

2.6.2.1 Contrivances (Adaptations) of self pollination :

There are certain adaptations or devices in the flowers to effect the self pollination.

1. **Homogamy :** It takes place in bisexual flowers in which both the anther and stigma mature at the same time (e.g. *Mirabilis,* Potato, Wheat, Rice etc).

2. **Dichogamy :** In many bisexual flowers, when the anthers and stigma mature at different times, it is known as dichogamy. Normally, it favours cross pollination. But, if cross pollination fails, the stigmas move back and touch the anthers to activate self pollination (e.g. Sun flower). In the flowers of *Ixora,* and *Vinca*, the sessile anther may lie at the mouth of narrow corolla tube, that may brush against stigma of the same flower. This results in self pollination.

3. **Cleistogamy :** The bisexual flowers which never open are called cleistogamous or closed flowers. In this case, pollen grains have to be pollinated on the stigma of the same flower, so that self pollination is obligatory. Such flowers are very small, not coloured and do not emit any smell. Cleistogamy is seen in the underground flowers of *Commelina benghalensis* (Fig. 2.18). It is also seen in the case of *Impatiens, Oxalis, Portulaca* etc. On the other hand, flowers which open and expose their reproductive organs to pollinating agents are called chasmogamous. *Commelina benghalensis* has both cleistogamous and chasmogamous flowers.

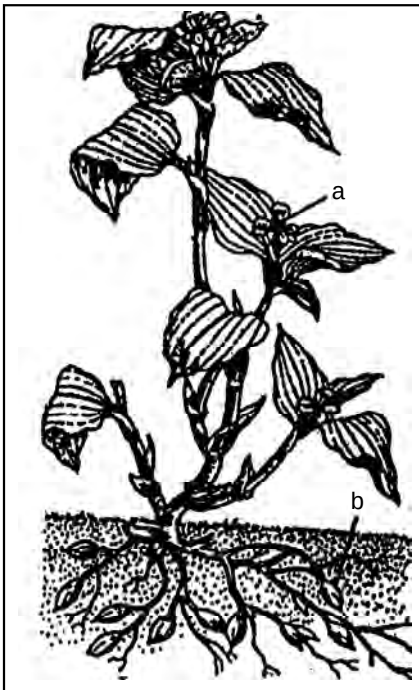

Fig. 2.18 : Chasmogamous and cleistogamous flowers in *Commenlina benghalensis*. **a-** normal chamogamous flower; **b-** underground cleistogamous flowers.

2.6.2.2 Advantages of self pollination :

1. Fertilization and production of the progeny are always certain by this method.
2. It maintains purity of race and superiority of the variety.
3. Here there is less wastage of pollens.

2.6.2.3 Disdvantages :

1. It leads to loss of viability and vigour of the plant in the long run.
2. If lethal genes become homozygous, the effect may be disastrous.

2.6.3 Cross pollination :

Cross pollination is also called **allogamy**. It is the transference of pollen grains from anther of one flower to stigma of flower on another plant of the same or allied species. Cross pollination within a species (different variety) is called **xenogamy**. Since it occurs between two different strains of the plant, it yields hybrids. It is effected by external agents which carry the pollens of one flower to the stigma of another flower. These flowers are borne by two seperate plants of the same or allied species. It occurs in both dioecious and monoecious species. But dioecious species are necessarily cross pollinated.

2.6.3.1 Contrivances (adaptations) of cross pollination :

There are various adaptations for the process of cross pollination. In unisexual and bisexual flowers, certain devices are present for effective cross pollination and avoiding self pollination. Some of these devices of cross pollination are as follows.

1. **Dicliny or unisexuality -** Here, the flowers are unisexual, borne either in a monoecious plant (bearing male and female flowers in the same plant) or in a dioecious plant (male and female flowers are borne in two separate plants). In monoecious plants although cross pollination takes place by several agents, geitonogamy may occur. Some of the such monoecious plants are *Cucurbita, Ricinus, Zea* etc. In dioecious plants, cross pollination is the rule. The examples are *Piper, Cannabis, Morus* etc.

2. **Self sterility -** It is the condition when a flower cannot be pollinated by the pollen grains of the same flower or from any flower of the same plant. It is found that stigma of some orchids wither away if the pollen grains from the same flower are deposited on it. Many species of Solanaceae (*Solanum, Nicotiana*) and tea plant are self sterile and are cross pollinated.

3. **Dichogamy -** It is found in bisexual flowers where stamens and carpels mature at different times, hence, the self pollination is prevented naturally. There are two conditions for dichogamy. When the gynoecium matures earlier than the anthers, the stigma receives the pollen grains from another flower. This condition is known as **protogyny**. Common examples of protogynous flowers are *Anona, Polyalthia, Magnolia, Michelia* etc. The other condition of dichogamy is **protandry** where the anther matures earlier than the stigma. Here self pollination is naturally impossible. Hence, the pollen grains are carried over to the respective stigma of another flower in which gynoecium is matured. It occurs in *Hibiscus, Gossypium, Helianthus, Tagetes, Coriandrum* etc. Under above conditions, geitonogamy or xenogamy can only occur.

4. **Herkogamy -** In some bisexual flowers, there are certain adaptations of floral parts like anthers and style which act as barriers to self pollination. Here autogamy is mechanically impossible (Fig. 2.19), and thereby, favouring cross pollination. In many cruciferous and caryophyllaceous plants, the style is much longer and the stigma is exerted far

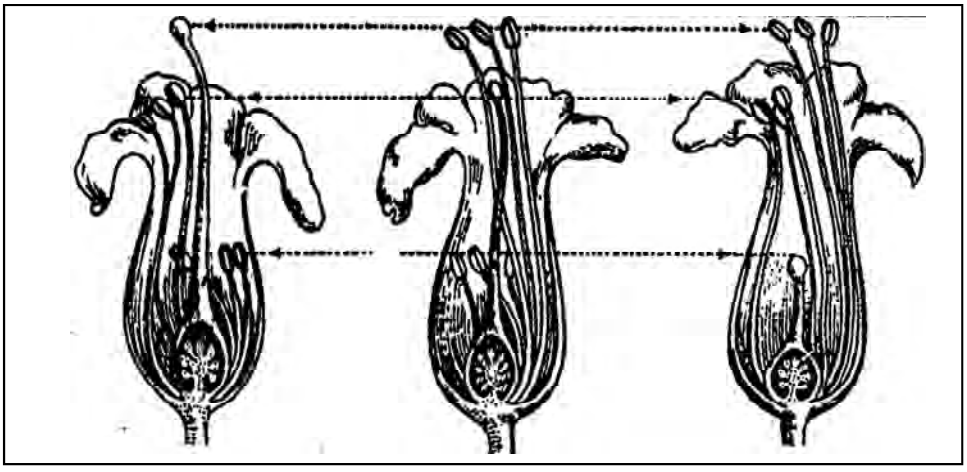

Fig. 2.19 : Trimorphism in flower of Lythrum salicaria. Three types of flowers show pistils and stamens of three different heights. Pollination usually takes place between organs of the same height.

beyond the stamens, preventing pollens to reach the stigma. In flowers of **Gloriosa**, the anthers are extrose-(facing outwards) and dehisce at a distance, Thus discouraging self pollination. The peculiar arrangement of stamens and pistils in *Salvia* achieve cross pollination only by insects. (Fig. 2.20)

5. Heteromorphism - Plants may have two (dimorphic), three (trimorphic) or different forms of flowers, based on the position of anthers and stigmas at differnt levels. Such heteromorphous flowers may have **heterostyly** (styles of different length) or **heteroanthy** (different types of anthers). One form has short stamens and long style while the other has long stamens and short style (e.g. *Primula, Lythrum*) (Fig 2.19).

In this case, one with short style will be cross pollinated by pollens from lower anthers and vice versa by insects having the capacity to enter in to particular depth of the flower. **Dimorphism** is observed in *Jasminum, Linum* etc. Some species of *Oxalis*, *Linum*, *Lathyrus* exhibit **trimorphism,** which show three types of flowers at three different positions of anthers and stigmas. It results in cross pollination only (Fig. 2.19).

2.6.3.2 Agents and types of Cross pollination :

Cross pollination is brought about by external agents, as it involves two separate plants of the same or closely allied species. These agents can be categorized (Table-2.1) as biotic agents (insects, birds, bats, snail etc.) and abiotic agents (wind, water).

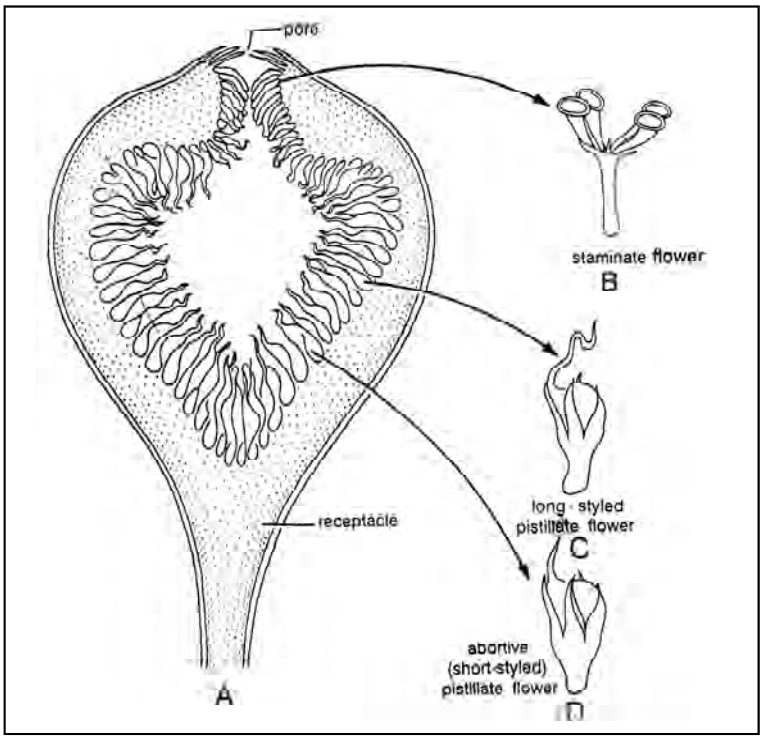

Fig. 2.20 : A-D. Structure of *Ficus carica* inflorescence : **A.** Longitudinal section of inflorescence. **B.** Staminate flowers, **C.** Long styled pistillate flower, D. Abortive (short-styled) pistillate flower.

Table - 2.1
Pollinating agents for cross pollination

Category	Agent	Type of cross pollination
Biotic Agents	Insect	Entomophily
	Animals	Zoophyly
	(Birds	(Ornithophilly
	Bats	Cheiropteriphily
	Snails)	Malacophily)
Abiotic Agents	Air	Anemophily
	Water	Hydrophily

1. Entomophily

Insect pollinated plants are entomophilous. In these cases, the flower attracts the insects in a variety of ways and the sticky pollens easily adhere to the body or body parts of the insects. Similarly, the stigma is also sticky to receive the pollen grains. The flowers develop the following adaptations to attract the insects.

(i) Conspicuous and coloured flowers

Here the petals of corolla are large sized, irregular and beautifully shaped to attract insects. Bracts, sepals or even stamens become coloured in some plants, (e.g. *Mussaenda, Bougainvillea, Musa*).

(ii) Nectar - Nectary glands secrete the nectar which attract the bees. Nectar provides nutrition to these insects, (e.g. Oranges).

(iii) Scent - Flowers that open during nights emit good scent which attract many nocturnal insects, (e.g. *Nyctanthes, Cestrum*). Flowers with offensive smell and nauseating to human beings attract swarm of carrion flies (e.g. mature inflorescence of *Amorphophallus, Rafflesia* and some aroids)

(iv) Edible sap - There are certain plants which do not have nectaries to attract insects. Edible sap secreted by such plants attracts insects. (e.g. Some Orchids).

(v) Edible pollens - Wax on the pollen is utilized to build the honeycomb and pollen may be required to nourish the young insects, (e.g. *Papaver, Rosa, Clematis*).

(vi) Special mechanisms

(a) In the case of *Bignonia*, if it has not been pollinated, the stigma gets exposed again by opening the flap closed by insect visit.

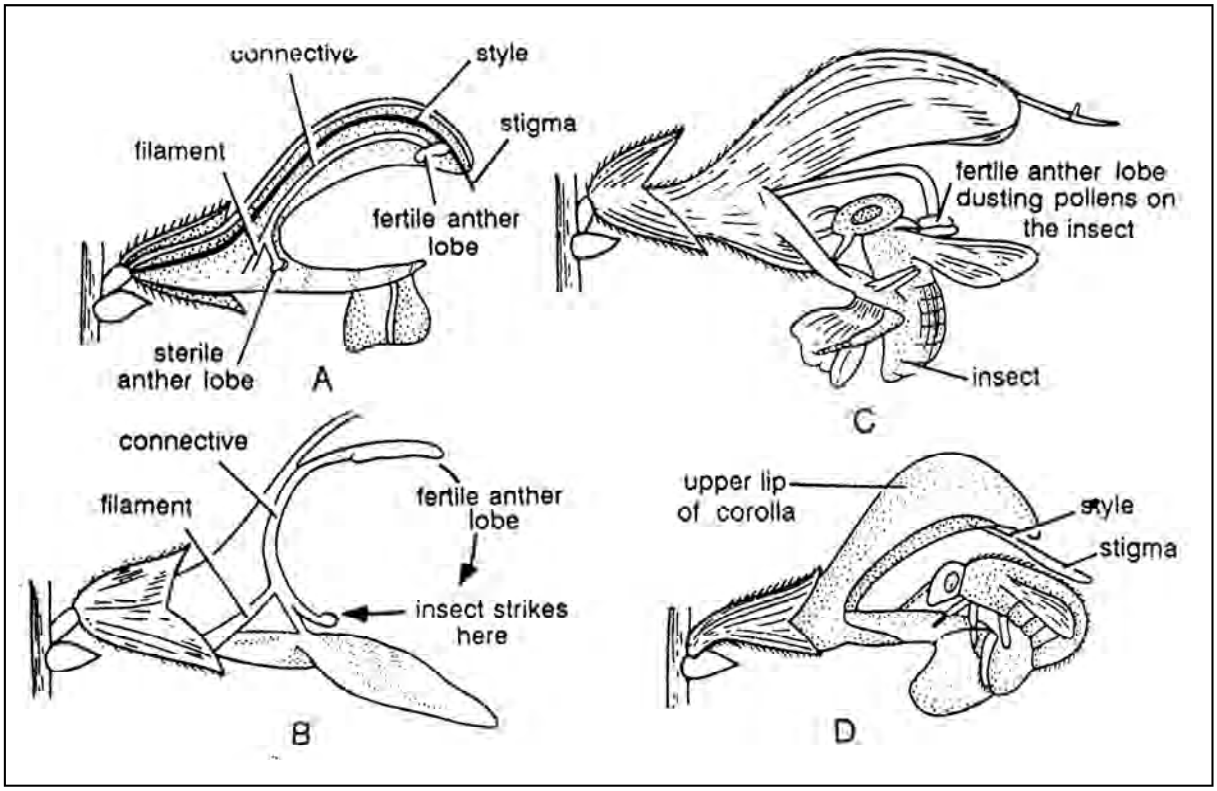

Fig. 2.21 : A-D. Insect pollination in **Salvia:** A Structure of flower, **B.** Diagrammatic presentation of changes taking place in the position of anther lobes during the entry of insect in the flower, **C.** Entry of insect in the flower (note the dusting of pollen grains on the back of the insect), **D.** Insect entering another flower and the pollens on its backdate beingjcollected by stigma.

(b) In dense, capitulum type of infloerscence, the inconspicuous, small individual flowers become attractive when grouped together so that these are visited by insects, (e.g. *Helianthus, Tridax, Tagetes).*

(c) In hypanthodium inflorescence, the insects enter through the ostiole and bring about pollination. (Fig. 2.20) Here the infloresence is a closed one and open to outside by a pore called ostiole. The receptacle is hollow with three types of flowers. Near the ostiole the flowers are male. The middle portion flowers are female with long styles. The lowest at base of cavity are sterile female flowers. The insect enters through the ostiole passes through anthers of male flowers and reaches upto the female flowers on the middle part of infbrescence. So the pollen grains are carried by the insect and passes onto the stigma of the female flowers (Fig. 2.20) .

(d) A peculiar type of adaptation is seen in the case of *Salvia* (Fig. 2.21) where there is occurrence of a bilabiate corolla with two epipetalous stamens. The bilobed anther of each stamen is widely separated by the elongated curved connective

which swings freely on the filament. Out of the bilobed anther, the upper lobe is fertile and the lower one is sterile. The lower sterile lobe of anthers receive the insect which enters the bilipped corolla tube. During this event, the connective swings down, so that the upper fertile lobe comes down, and strikes the back of the insect and dusts it with pollen grains. The flower is protandrous. So, when the stigma matures it bends down and touches the back of the insect covered with pollen grains and bring about pollination. This type of adaptation is called ballistics.

Fig. 2.22 : A humming bird collecting nector form *Bignonia capreolata* flowers, thereby pollinating them. (After Bates).

2. Zoophily :

Animals act as useful agents of pollination. Birds, squirrels, bats, snails and other animals take part in pollination. Based on the type of animals involved, the zoophily is classified into different types.

(a) **Ornithophily -** Several birds such as tiny **humming birds**, the **honey thrushes** feed on the nectar of the flowers and pollinate. Birds like crows also help in pollination. (e.g. *Bignonia*) (Fig. 2.22), *Bombax*, *Erythrina* and *Callistemon* also show this type of pollination.

(b) **Cheiropteriphily -** In this type of pollination, the flowers are pollinated by bats. They visit the flowers to collect nectar during which pollination occurs, (eg. *Anthocephalus, Bombax* etc.).

(c) **Malacophily -** Snails and slugs help in this type of pollination. *Chrysanthemum, Lemna* are some of the examples.

3. Anemophily :

In this case, plants are pollinated by wind. The flowers are inconspicuous and small. They are never coloured and showy. They neither emit any odour nor secrete any nectar to attract the insects. However, the pollens are produced in enormous numbers. For example, a *Cannabis* flower produces approxmately 5 lakh pollen grains. They are light and dry. Such pollens are easily carried away by wind and transferred to the stigmas. The occurrence of

branched bushy stigma and comparatively larger protruding stigma in grasses, bamboos, cereals, millets, sugarcane and other such plants help wind pollination. In *Zea mays* (Fig. 2.23), the male flowers are borne in a terminal panicle of spikelets. A few female spadices are borne, each in a axil of leaf surrounded by spathes. The style consists of long and silky threads. These are seen to hang in tufts from the spadix. When the anthers burst a cloud of pollen grains, these float in air, close round the plant. Some of these floating pollen grains are received by protruding stigmas which bring about pollination (Fig. 2.23).

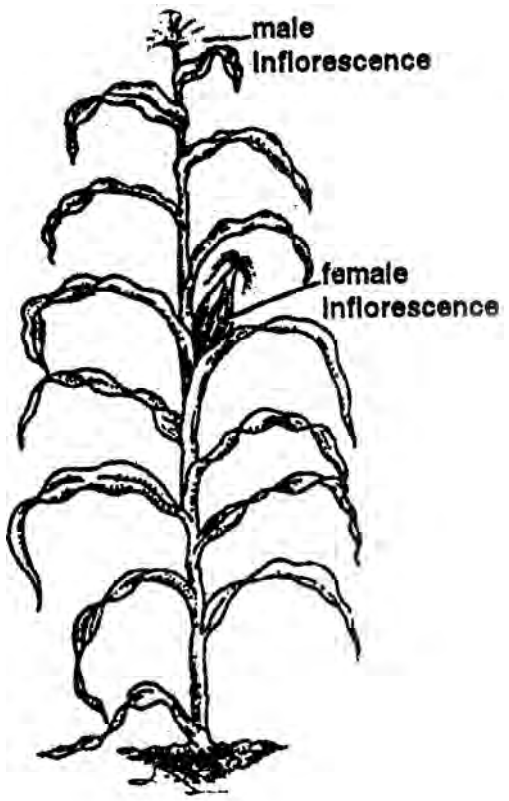

Fig. 2.23 : Maize-an anemophilous plant.

4. Hydrophily :

This type of pollination takes place in aquatic plants. Water is the medium for transfer of pollen grains. Particularly, the submerged plants are adapted for this type of pollination (e.g. *Naja, Vallisneria, Hydrilla* etc.) There are two possibilities, either pollination takes place completely under water or it takes place on the water surface. In the former case, it is known as **hypohydrogamous** (e.g. *Najas*) while in the latter, it is known as *epihydrogamous*. (e.g. *Vallisneria, Hydrilla*). In *Vallisneria*, the plant grows in mud (Fig. 2.24). It is dioecious and leaves are strap-shaped. The male plant bears flowers in small spadix surrounded by spathe. These are short stalked and borne low down amongst radical leaves. The female flowers are borne singly on long wiry stalks enabling the flowers to float on water when mature. The individual male flowers get detached and float freely in large numbers on the water surface. These flowers open on the water surface exposing two stamens vertically. The male flowers cluster around the female flower. As anthers burst and the sticky pollen grains get attached to the stigma.

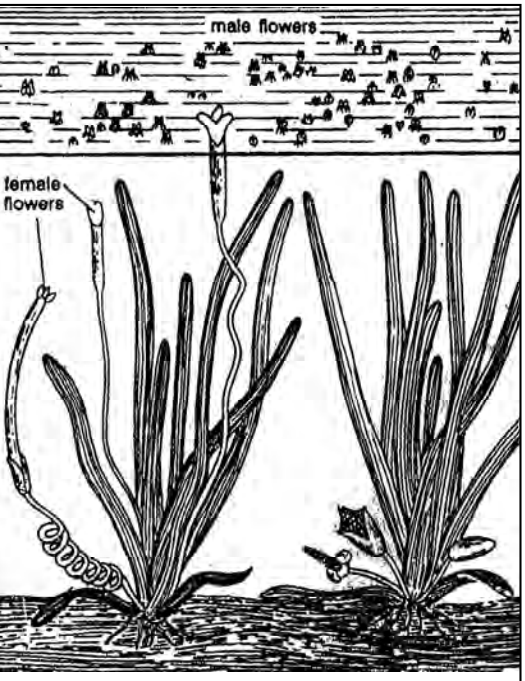

Fig. 2.24 : *Vallisneria spiralis* showing free-floating male flower.

2.6.3.3 Advantages of cross pollination :

(1) This always results in much healthier offsprings.

(2) The offsprings produced in this method are better adapted ones.

(3) As a result of the cross pollination, hybrids are produced.

(4) More abundant and viable seeds are produced which store greater quantities of food material.

(5) The process eliminates defective characters and is helpful in production of new varieties.

2.6.3.4 Disadvantages of Cross Pollination :

(1) This is wasteful process, as large number of pollen grains get damaged in the course of pollination.

(2) The chance of fertilization is limited here since it can be effected only if the pollen reaches the matured stigma.

2.7 OUTBREEDING DEVICES :

Pollination (as described in previous pages) is the process of transferring pollen grains from anther to stigma of the pistil. Pollination can be either cross pollination or self pollination. Majority of flowering plants are bisexual or hermaphrodite which commonly promote self pollination. But self pollination always is not desirable. Successive series of self pollination affects the progeny negatively and causes inbreeding depression. This results in formation of homozygous genes. Therefore nature has provided certain adaptations or devices to promote cross pollination in order to produce healthy progeny. This is known as outbreeding. Outbreading is a phenomenon where individuals within a species will tend to breed with others who are neither close relatives nor distant genetic relations but a middle ground of both. The devices or factors which encourage outbreeding are briefly presented below (More descriptions made in previous pages)

2.7.1 Unisexual flowers (Dicliny) :

Nature has created unisexual flowers which contain only one sex either male or female. So cross pollination is the only choice. Therefore formation of unisexual flowers or dicliny is one of the outbreeding devices.

2.7.2 Non-synchronisation (Dichogamy) :

Timing is important for successful pollination. Release of pollen grains from anther and receptibility of stigma should happen simultaneously. Sometimes pollen grains in a hermaphrodite flower mature and get released before stigma is open which leads to loss of pollen vitality or vice-versa. This phenomenon i.e. dicliny includes two processes such as protandry (anthers

mature earlier than carpels) and protogyny (carpels mature much earlier than it's anthers). So dichogamy is an outbreeding device in which cross pollination is the only method to develop seeds.

2.7.3 Heterostyly and Herkogamy :

In heterostyly due to great disparity in length of style and stigma, effective self pollination is not possible. In herkogamy, the homogenous flowers adapt certain devices in which only cross pollination is possible.

2.7.4 Self Incompatibility :

Incompatibility is the inability of certain gametes even from genetically similar plant species to fuse with each other. Here, even though pollination takes place, it cannot proceed to fertilization due to failure of the pollen tube growth. This is also known as intraspecific incompatibility, *self sterility* or self incompatibility, which has been reported in nearly 66 families of flowering plants.

Self incompability within a flower (or plant) may be due to prevention of some physiological and morphological mechanism. It involves very complex mechanisms associated with interactions of pollen and stigmatic tissues. Self incompatibility helps to prevent self pollination.

If the incompatibility is due to genotype of the sporophyte (e.g. stigamtic tissue), it is termed as **sporophytic incompatibility**. On the other hand if incompability is due to the genotype of the pollen, it is termed as **gametophytic incompability**. Self incompability usually develops with maturation of stigma. It may also be due to preventing of pollen germination, retardation of pollen tube growth, deorientation of pollen tube or even failure of nuclear fusion. Incompatibility is controlled by genes with multiple alleles.

In self incompatible fruit trees, it is necessary to plant two cross compatible varieties to ensure good results. Self incompatibility may be used in hybrid seed production.

2.8 POLLEN-PISTIL INTERACTIONS :

A special character of sexual reproduction in flowering plants is the interaction of pollen grain (the male gametophyte) with massive sporophytic tissue of pistil particularly stigma and style, before discharging the male gametes near the egg (female gamete) inside the embryo sac. All pollinations do not lead to a successful fertilization of male and female gametes. For successful fertilization, the stigma of the pistil has to recognise the pollen of the same species. Once a compatible pollen is recognised and accepted by the stigma, then the various subsequent events for fertilization will proceed. In this recognition process (pollen-stigma), the incompatible pollens are rejected by the stigma.

Pollen-pistil interaction is a long term (prolonged) interaction of pollen grains and pistil resulting in a successful fertilization. The chain of events of pollen-pistil interaction proceed as follows :

- Landing of pollen on the stigma which recognises its compatible pollen.
- Germination of pollen and formation of pollen tube where the pollen releases its contents (Fig. 2.25)
- Pollen tube growth through style of the pistil towards ovary and then into ovule (Fig. 2.27).
- Entry of pollen tube containing male gametes into the embryo sac of ovule and then to the synergid.

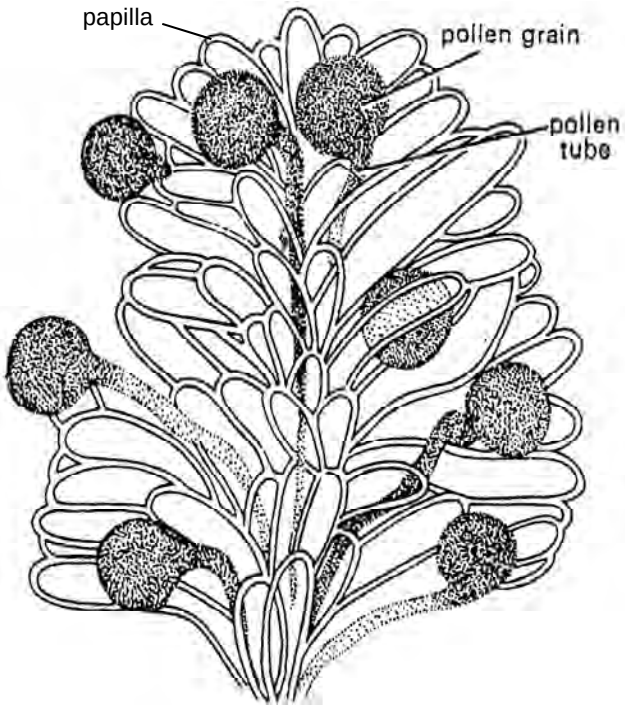

Fig. 2.25 : Germination of pollen grains on the stigma.

2.8.1 Salient features of pollen-pistil interactions :

1. Sexually reproducing organisms have the ability to recognise and select suitable gametes for fertilization. The function of recongnition and acceptance of making partners in flowering plants starts with pollen-pistil interactions. Female gametes produce chemicals to attract male gametes.

2. In flowering plants the pollen grains (microspores) do not have direct access to reach the female gamete (egg). The female gamete is deep seated in the embryo sac of ovule in the nucellus. The nucellus in turn is covered by the ovule inside the ovary of the pistil. The pollen tube has to push through the stigma, style, ovary, ovule, nucellus and egg apparatus (synergids) to reach the female gamete. (Figs. 2.26, 2.27)

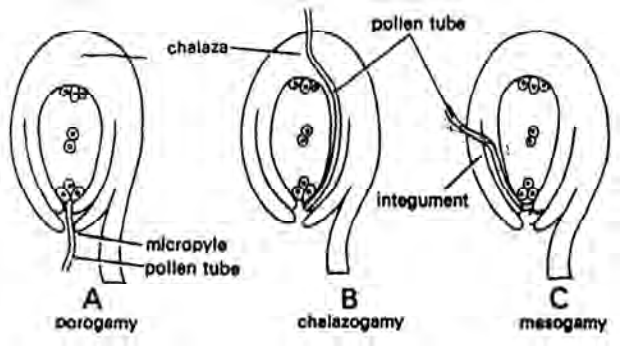

Fig. 2.26 : Entry of pollen tube into the ovule: **A.** Porogamy, **B.** Chalazogamy, **C.** Mesogary.

3. The pistil is adequately equipped with devices to allow the pollen of only right mating type to function normally, others are discarded. The stigma receives variety of air borne or insect-carried pollens, even also from other species, but only the compatible and right types are chosen to participate in the competition to effect fertilization.

4. The pollen-stigma interaction determines the germination of pollen grains on the stigma. The stigmatic surface recognises the specific compatible pollens and allows them to hydrate and finally the recognised pollens germinate. In self-incompatible plants, some factors on exine of their pollen grains may produce rejection response on stigmatic surface.

5. The stigma plays an important role in germination of pollen grain. The stigma has a number of adaptations to achieve this. The stigmatic surface secretes fluid containing liquids, gums, sugar and resins. The main function of the stigmatic secretion is to protect the pollen as well as stigma from dessication. In *Brassica* (Mustard), the pollen grains stick to the stigmatic papillae present on it's surface. Cytochemical studies have shown the presence of many hydrophilic proteins and hydrolytic enzymes (acid phosphatase, ribonclease, esterage etc.) in the papillae of stigma. The hydrophilic proteins keep the stigmatic surface moist. The stigmatic popillae collapse after pollination and form watery substances by degenration of their cytoplasm which also facilitates pollen germination. On landing the stigmatic surface, only the compatible pollens start germination. Pollens absorb liquid from the wet surface of stigma, expand in size, their intines protrude through the germ pores and the pollen tubes are developed.

6. Germination of pollen grain also depends upon their longevity i.e. the duration for which they remain viable. Pollen grains are viable for only 3 minutes in *Reseda*, 5 minutes in *Zea mays* 30 minutes in rice, 2 hours in *Beta vulgaris* 15 days in in *Prunas*, 56 days in *Primula* and so on. Only viable pollen grains, if find a suitable stigma can germinate.

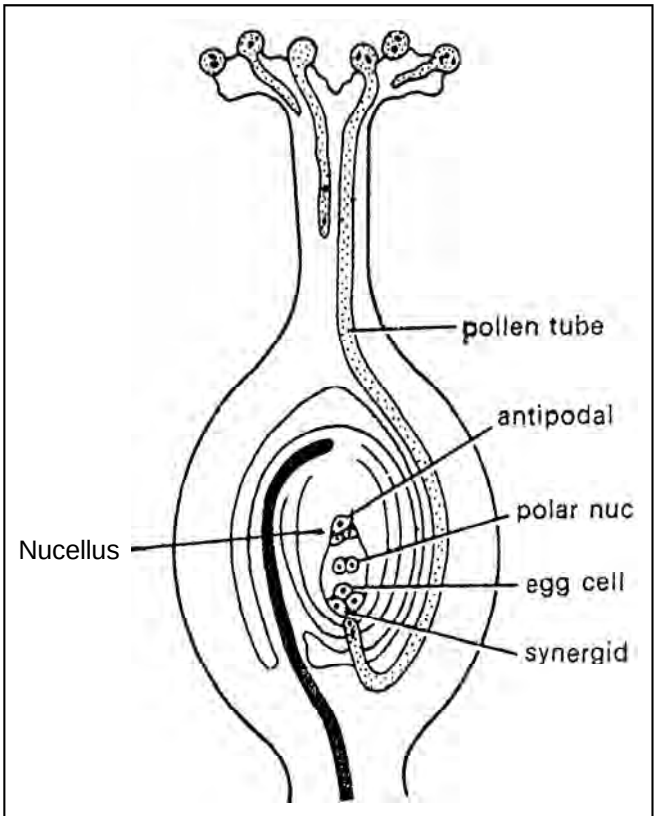

Fig. 2.27 : Longitudinal section of gynoecium showing growth of pollen tube from towards embryo sac.

7. The ungerminated pollens contain free ribosomes, but pollens start absorbing water from the stigmatic surface, get hydrated polyribosome assembly. The hydrated pollen shows high ratio of rRNA and tRNA. The enzymes required for pollen germination and pollen tube growth are produced at this time. Enzymes like alkaline phosphatase ribonuclease, esterase and amylase are present just below the germ pores of the pollen and help in pollen germination. Cutinase enzyme is present in pollen tube and dissolves the cutins present on the cuticle of the stigmatic surface at the point of contact. Cutinase also degrades the cuticle layer of stylar canals during pushing of the pollen tube through the style.

8. The pollen tubes after passing through the style always grow in the direction of the ovary and finally to the ovule of the pistil (Fig. 2.27). The unidirectional path of pollen tubes in the pistil is guided by hydrotropic and chemotropic secretion of the ovules.

9. Usually a single pollen tube is formed from a pollen grain (called monosiphonous). More than one pollen tubes (called polysiphonous) may be formed (e.g. cucurbitaceae, malvaceae) but only one pollen tube which carries male gametes grows upto the ovule and other tubes degenerate.

10. On reaching the ovary of the pistil the pollen tube grows towards one of the ovules it may enter into the ovule through the microphyle (called porogamy), chalaza (called chalazogamy) or through the integument (called mesogamy) (Fig. 2.26).

11. Irrespective of the place of entry into the ovule the pollen tube always enters the embryo sac through the micropylar region (Fig. 2.26). The pollen tube enters the embryo sac via one of the following routes; (i) between egg cell and one of the synergids, (ii) between wall of the embryo sac and one or both the synergids, (iii) between two synergids or (iv) directly penetrates one of the synergids.

12. When pollen tube finally reaches the vicinity of the egg, it discharges its two male gametes. Usually one male gamete fuses with the egg and other male gamete fuses with the definitive (2 polar nuclei) nucleus. This phenomenon is called double fertilization and triple fusion.

13. Due to pollen-pistil interaction, intense or tough competition develops in between the pollens as well as the male gametes for fertilization. This intense competition is responsible for success of reproduction of angiosperms over other plants.

2.9 FERTILIZATION :

The fusion of two sexual reproductive units such as male and female gametes is called fertilization. Here in Angiosperms, this process was discovered by Strasburger in 1884. It begins with pollination and completed with fusion of male and female gametes within the embryo sac present in the ovule.

2.9.1 Double fertilization and triple fusion :

In angiosperms, fertilization occurs in the embryo sac. So, the compatible pollen grains received by the stigma (Fig. 2.25) have to germinate, produce pollen tube and carry the male gamete to the egg cell. The growth of the pollen tube is stimulated by sugary substances secreted by the stigma. A mass of cytoplasm accumulates at the tip of the pollen tube, which also contains the male nuclei. The tube nucleus gets disorganized sooner or later while the pollen tube runs down through the style to finally reach the ovule (Fig. 2.26). There are different ways of entry of the pollen tubes into the embryo sac. Generally It reaches near the embryo sac by penetrating the nucellus through the micropyle or the chalaza.

One of the two male gametes (n) fuses with the egg of the egg apparatus of embryo sac and female a diploid zygote (2n). The process is called syngamy or true fertilization (Fig. 2.27). The other male gamete (n) through triple fusion (fusion of three nuclei) fuses with the definitive nucleus or secondary nucleus (2n) formed out of fusion of each two pollar nuclei and results usually in the formation of a triploid primary endosperm nucleus (3n). As there are two separate fusions taking place within the embryo sac by the two male gametes, the process is called double fertilization and triple fusion, one with the egg and other is with the definitive or secondary nucleus. As soon as fertilization is over, the cells other than the fusion products (zygote and primary endosperm nucleus) of embryo sac gets disintegrated. Antipodal cells disappear even before fertilization, since they probably have no function in the process. The process of double fertilization was first discovered by S.G. Nawaschin (1897) in *Lilium* and *Fritillaria*.

The time involved between pollination and fertilization varies among different plants. Generally, it is between few hours to a few days, but in some cases, it may take even many months. It depends on the rate of growth of the pollen tube.

Although many pollen grains are deposited on the stigma and some produce germ tubes, only one pollen tube becomes effective to reach the egg apparatus of the embryo sac prior to others. Other pollen tubes dry up and become nonfunctional (Figs. 2.25, 2.27).

2.10 POST FERTILIZATION EVENTS :

The withering and shedding of corolla usually indicate that fertilization has been completed. As a result of fusion between one of the two male gametes and egg cell a zygote is formed, which later develops into an embryo. The fusion product of the other male gamete with definitive nucleus (resulted from triple fusion) is the primary endosperm cell, which is the first cell of the endosperm. During post-fertilization period, the embryo and the endosperm are seen to develop simultaneously. The ovule containing the embryo is transformed into the seed while the ovary becomes a fruit.

2.10.1 Development of endosperm :

Endosperm is the nutritive tissue formed as a result of triple fusion i.e. fusion of three haploid nuclei in the embryo sac of the angiosperms. Endosperm is generally triploid in nature, meant for nourishment of the growing embryo. Endosperm formation starts prior to embryo formation with degeneration of the nucellar tissue. Based on the mode of development, there are three types of endosperms; (i) Nuclear (ii) Cellular and (iii) Helobial (Fig. 2.28 - 2.31).

1. **Nuclear endosperm :** Primary endosperm nucleus divides repeatedly to form a large number of free nuclei (Fig. 2.28 A-E). No cell plate formation takes place at this early stage. A central vacuole appears later. It is followed by cell plate formation which is centripetal. Hence, in a mature ovule a multicellular endosperm is formed (Fig. 2.28). The process of cell plate formation may not be complete as in the case of coconut. Its peripheral portion has outer oily multicellular solid endosperm called coconut meat and inner free nuclear, degenerated multinucleate liquid endosperm called coconut milk.

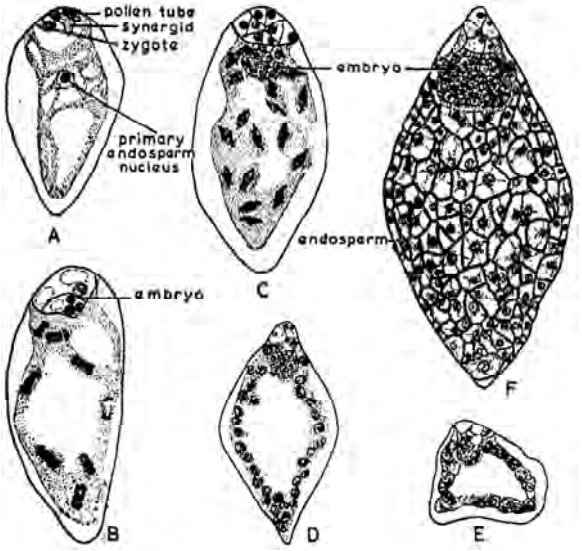

Fig. 2.28 : Nuclear endosperm : **A, B, C**- Free nuclear division; D, E- Vacuolation.

2. **Cellular endosperm :** Here wall formation occurs immediately after the first division of the primary endosperm nucleus. Subsequent divisions also are accompanied by cell plate formation. As a result, the endosperm becomes cellular from the beginning (e.g. Balsam, Petunia) (Fig. 2.29). Cellular endosperm is found in 25% of famillies of angiosperms.

3. **Helobial endosperm :** In the primary endosperm nucleus, wall formation takes place following the first division. However, inside each of these two newly formed cells, free nuclear divisions occur. But finally, the endosperm becomes cellular following the pattern of development of nuclear endosperms (Fig. 2.30). Hence, helobial endosperm is the combination of cellular and nuclear endosperms. The helobial endosperm is found in 19% of families of angiosperms.

Endosperms formed may remain in the seeds or it may be fully consumed by the developing embryo. In the later case, the food is generally stored in the cotyledons of mature seed. These are called exalbuminous or non-endospermic seeds. (e.g. Ground nut, Mustard, Sun flower).

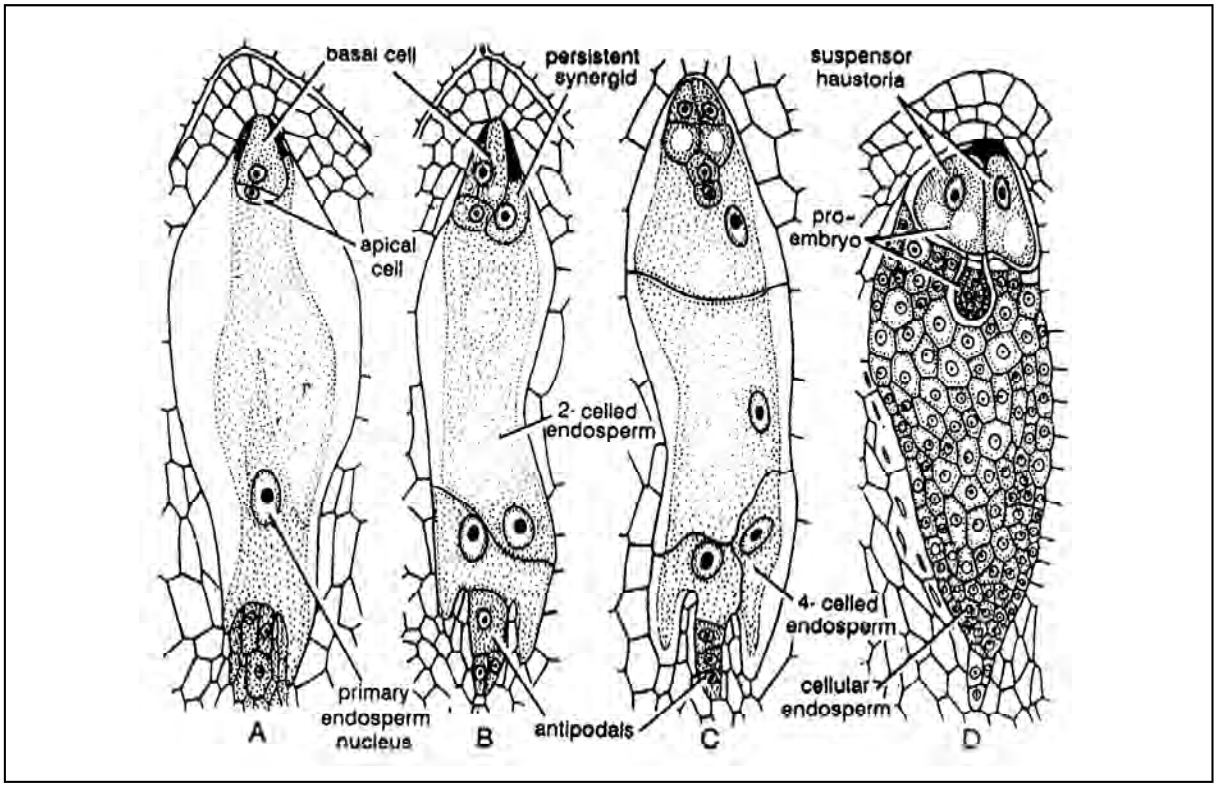

Fig. 2.29 : A-D. Successive stages of the development of cellular endosperm.

In albuminous or endospermic seeds, endosperm persists in the seed along with mature embryo. Here the cotyledons are thin, papery and have very less or no nutritive function, (e.g. Cotton, Castor, Papaya).

Endosperm formation is totally absent in the seeds of members of family Orchidaceae and Podostemonaceae. Endosperm is usually non-green, hard, containing cellulose in Ivory palm, Coffee, Black pepper; oily substances in coconut; castor, cotton; starch in cereals and proteins in aleurone layer in cereal grains (maize).

2.10.1 Development of the Embryo :

After fertilization, the fertilized egg within the embryo sac is called zygote or oospore. The zygote develops into the embryo. The process of development of embryos is called embryogeny.

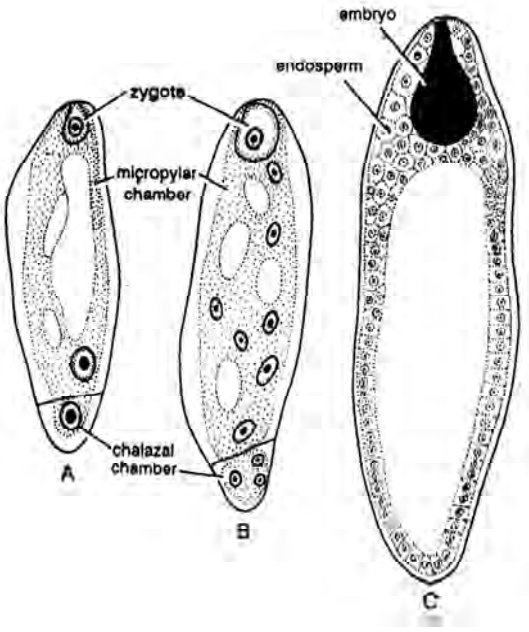

Fig. 2.30 : Successive stages of Development of Helobial Endosperm.

The embryo has the potentiality to form a seed and later on to a complete plant. After fertilization, the zygote takes rest for a period that varies greatly in different species from a few hours to several weeks. Generally, there are no fundamental differences in the early stages of development of dicotyledonous and monocotyledonous embryos. They differ only in ther later stages of development (Figs. 2.31 and 2.33).

2.10.2.1 Development of Dictyledonous Embryos :

The embryogeny of *Capsella bursa-pastoris*, (family Cruciferae) has been extensively studied and considered as a typical representative of dicotyledonous embryo development or embryogeny. Here the embryogenetic (developmental) pattern is classified as crucifer type or onagrad type, out of five categories described in embryological literature. It is very comprehensively described as follows :

1. The zygote divides transversely and produces two cells; an apical (or terminal) cell (ca) and a basal cell (cb). The basal cell (also called hypobasal cell) lies close to the micropyle while the apical cell lies on the inner side, being directed towards the chalaza (Fig. 2.31 AB).

2. The basal cell (cb) divides by a transverse wall to form two cells (cm, ci) (Fig. 2.31 D). The apical cell (ca) divides longitudinally, resulting in formation of four-celled proembryo which assumes the shape of a reverse 'T' (exactly looking like ' ⊥ ' in Fig. 2.31 E).

3. The first formation of two cells by the first longitudinal division of terminal cell is followed by another longitudinal division at right angles to the first and thus a quardrant (4 cells) is formed out of the terminal cell (ca). Again each cell of the quardant divides by transverse wall form a 8-celled octant. (Fig. 2.31).

4. Of the octant, the lower four cells directed towards chalaza finally form the epicotyl apex or stem tip and the cotyledons. The other four cells of octant directed towards micropyle form the hypocotyl the tip of which develop into root (radicle) of the embryo. All the eight cells of the octant undergo periclinal division, differentiating an outer dermatogen layer and inner core of cells. The dermatogen cell devide anticlinally along with growth of the embryo, eventually to form the epldermis of the embryo. The inner core of eight cells inner to the dermatogen layer, by further divisions differentiate into ground meristem and procambial system of the hypocotyl and cotyledons. Ground meristem gives rise to cortex and endodermal layers. The procambial system gives rise to the vasular bundles (vasculature) and pith of whole embryo.

5. Concomitant with division of apical cell (ca) and its development, the two basal cells (cm and ci) undergo a number of transverse division giving rise to an eloingated suspensor of 6 to10 cells (Fig. 2.31 D-N). The proximal cell (v) of this supensor which is close to the micropyle become swollen, enlarged and vesicular in shape to form a

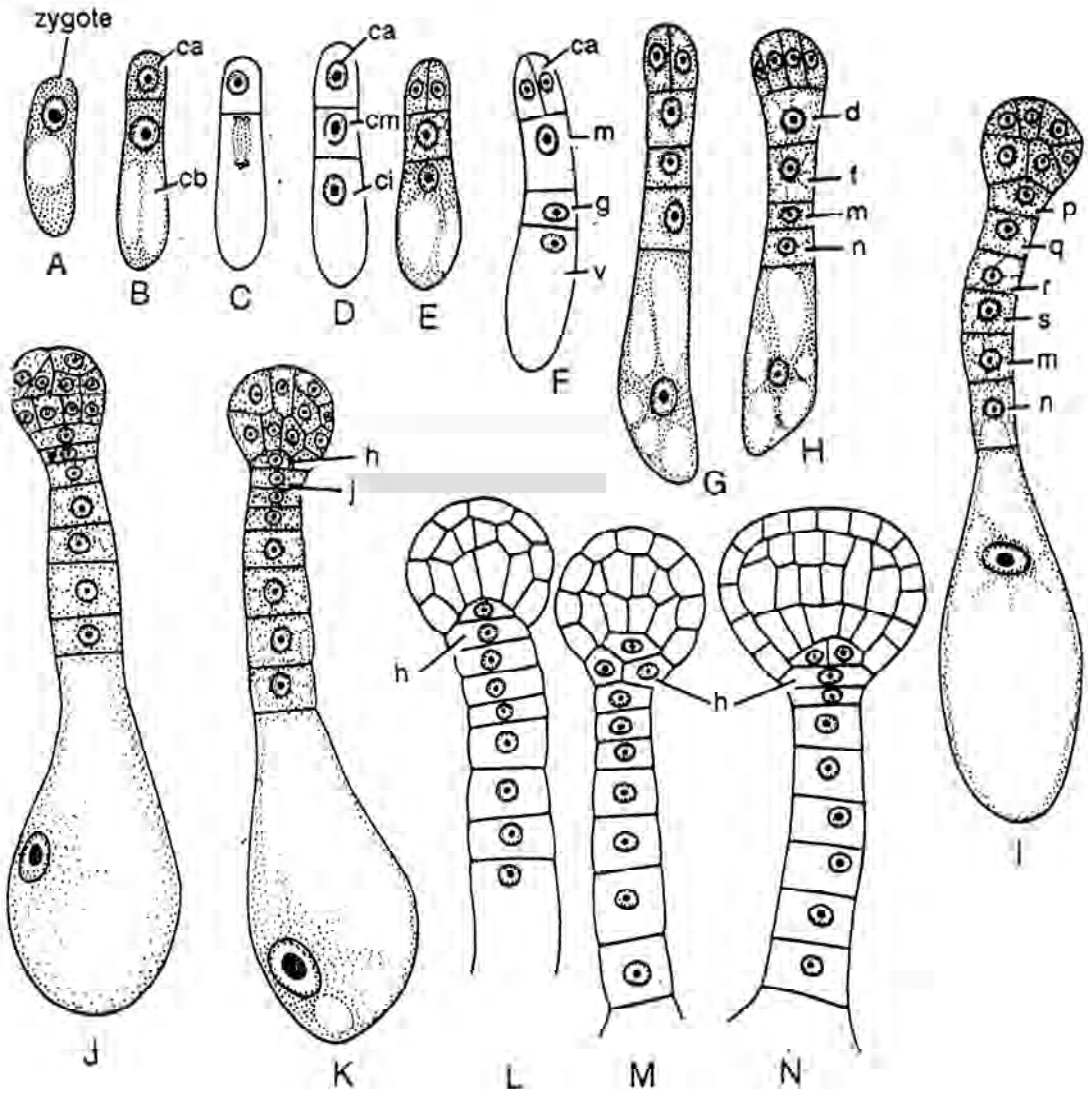

Fig. 2.31 : A-N. Successive stages of the development of proembryo in *Capsella bursa-pastoris*.

haustorium. Haustorium anchors the suspensor alongwith its terminal growing embryo with the embryo sac and also absorbs nutrition. The suspensor pushes the embryo proper (proembryo onwards) into the endosperm to enable the growing embryo to receive nutrition (Fig. 2.29D).

6. The lowermost (distal) cell of the suspensor contiguous with the distal part of embryo proper (octant and its products) functions as hypophysis (h) (Fig. 2.31 K, L, M, N). The hypophysis cell divides to form a group of eight cells arranged in two tires of cells. The lower tier (proximal group) of cells towards suspensor give rise to root cap and epidemis the other tier of four cells (distal group) form initials to give rise to root cortex (Fig. 2.31N, 2.32 A-D).

7. In the begining of embryo development starting from octant stage, the embryo has a globular shape hence called a globular embryo (Fig. 2.31 I-K). As growth proceeds the globular embryo become heart-shaped (cordate) with initiation of cotyledons (primordial cotyledons) (Fig. 2.31, 2.32A). The embryonal axis above the level of origin of two cotyledonary primoridia grows to develop the plumule with epicotyl apex (future shoot apex) at it's apex. The axis of the embryo below the level of cotyledons is known as hypocotyl. The tip of hypocotyl towards the suspensor forms the hypocotyl apex (the future radicle and apex) at its tip.

8. The embryo during development, passes through different morphological forms, such as; (i) Heart-shaped, (ii) Torpedo-shaped and (iii) Mature embryo (Figs. 2.32 A-D).

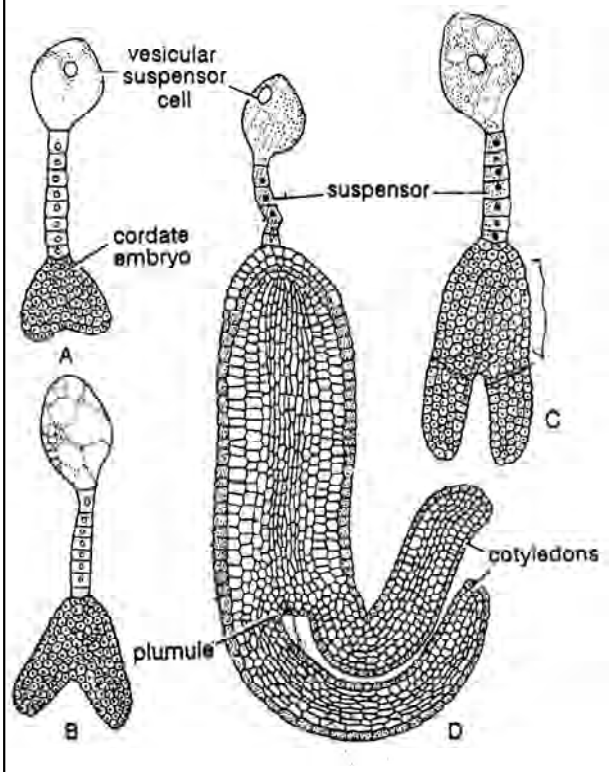

Fig. 2.32 : A-D. Successive stages of the development of embryo from proembryo in *Capsella bursa-pastoris*.

9. Finally, a mature dicotyletonous embryo while inside the seed, consists of (i) two colytedons (ii) an embryonal axis below the region of cotyledons upto to root apex called the hypocotyl and (iii) the hypocotyl apex (root-apex) (Fig. 2.32D). The plumule give rise to shoot apex of germinating seed and ultimately the shoot system. Similarly the radicle gives rise to future root system.

10. The ovulle develops into the seed and the ovary develops into the fruits.

2.10.2.2 Development of Monoctyledonous Embryo :

As describd earlier, the early development upto globular stage of embryo development in dicots and monocots is usually similar except some variations. Generally the *Sagittaria* type is considered as a typical embryogenetic pattern in monocotyledons. The mature monocot embryo has only one cotyledon, often called a scutellum. At the lower end the embryonal axis there is radicle covered by root cap. It is further covered by coleorrhiza. At the upper part of embryonal axis there is epicotyl apex surrounded by coleoptile (Fig. 2.33).

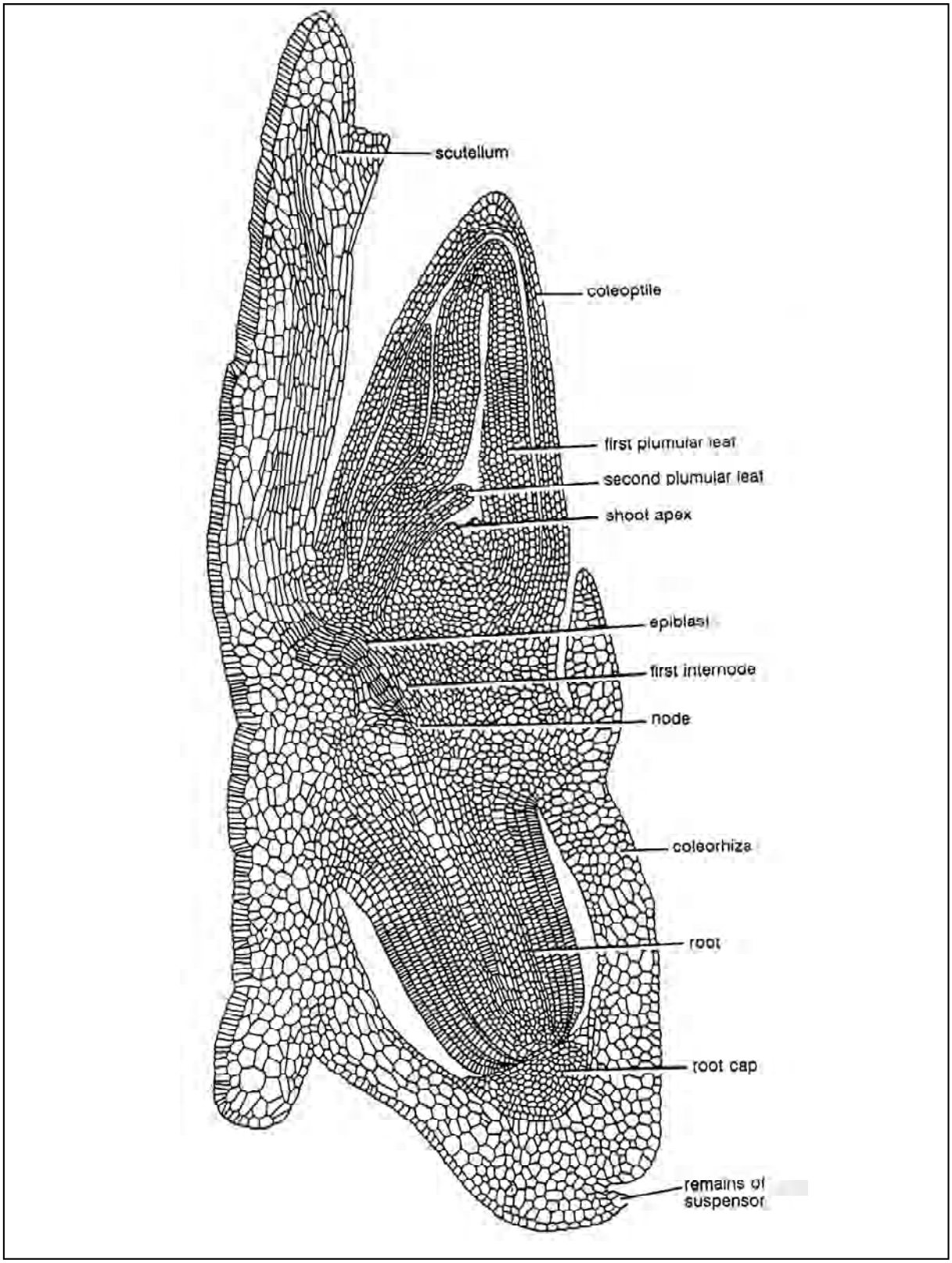

Fig. 2.33 : Longitudinal section of the embryo of *Triticum vulgare*.

2.11 SPECIAL MODES OF REPRODUCTION :

The vegetative and sexual methods of reproduction in flowering plants as described earlier are generally considered normal, they occur in nature. Nature also provides some special methods of propagation of plants, even, without act of fertilization, such as apomixis, parthenocarpy and polyembryony.

2.11.1 Apomixis :

In sexual reproduction (or amphimixis), meiosis and syngamy (fusion of male and female gametes) are two main characteristics. There is formation of haploid gametes (male and female) through meiosis and union of haploid gametes called syngamy to restore the diploid nature of sporophytic generation of the plant. The plants performing sexual reproduction with union of two gametes are called amphimictic plants. The haploid and diploid phases regularly alternate with each other to maintain the life cycle of the plant. This is known as alternation of generation.

In many plants there are other special asexual processes of reproduction without the acts of meiosis and syngamy which substitutes the process of sexual reproduction. The phenomenon of substitution of sexual process by asexual methods is known as apomixis and the plants which show apomitic methods of reproduction are called apomicts. The term apomixis (away from mixing) refers to substitution of sexual reproduction by any such method which does not involve meiosis and syngamy (Winkler, 1908). Even the plants which propagate only through vegetative reproduction are also regarded as apomictic plants. In simple words, apomixis is a modified form of reproduction in which seeds are formed without fusion of gametes.

The apomictic plants are not morphologically different from the amphimictic plants. The amphimictics species under certain cirumstances also show apomixis. Apomixis has been reported in more than 300 species of angiosperms belonging to 36 families.

2.11.1.1 Types of Apomixis :

According to a broader concept, there are catagories of apomixis, such as vegetative reproduction and agamospermy.

1. Vegetative Reproduction :

In vegetatives reproduction, new plants develop from parts other than seed. Those plants are regarded as apomictic where vegetative reproduction has replaced the sexual methods completely or essentially so. Vegetative propagation includes reproduction by means of bulbils, runners, suckers and so on which are formed only by the sporophytes. The structural units employed for this purpose are called propagules.

2. Agamospermy :

This is the phenomenon of formation of embryos through asexual reproductive process without the formation of gametes (by gametogenesis). The plants belonging to this category possess seeds. Their embryos inside the seed are formed by some process which lacks involvement of the processes of meiosis and syngamy.

Agamospermy is of the following three types.

(i) Recurrent Agamospermy (Recurrent Apomixis; Apospory and Diplospory) :

In this type of agamospermy, a diploid embryo sac is formed either from the diploid nucellar cells (a phenomenon called apospory, e.g., *Citrus*, mango, prickly pear etc.) or from the diploid megaspore mother cell (a phenomenon called diplospory, e.g. *Allium*, *Iberis*, *Taraxacum* etc.). In such embryo sacs, the egg and all other cells are diploid (unlike a normal egg that is haploid). The diploid egg develops parthenogenetically without the act of fertilization into an embryo. It has been observed that stimulus of pollination is often required for parthenogenesis.

(ii) Non-recurrent Agamospermy :

In non-recurrent Agamospermy, the megaspore mother cell divides meiotically and forms haploid embryo sac. The embryo is formed from such haploid egg (haploid parthenogenesis) or any other haploid cell of the embryo sac (haploid apogamy) without fertilization. The plants formed from such embryos are always haploid and are usually sterile. Haploid parthenogensis is seen in some species of *Solanum* and *Epipactus latifolia*. Haploid parthnogenesis is of considerable value in producing true breeding homozygous forms. Haploid apogamy (seen in *Lilium*) in which the synergid develops into an embryo in addition to normal zygotic embryo.

(iii) Adventive Embryony : (Adventive Polyembryony)

Here, the embryos which develop directly from the diploid cells of nucellus or integuments of the ovule and not from a fertilized egg are called adventive embryos. In such cases, the embryos formed from fertilized eggs either degenerate or compete with the adventive embryos. Usually, adventive embryony results in the formation of more than one embryo in seed (e.g. *Citrus*). Besides *Citrus* it is known to occur in members of Myrtacece, Cactaceac, Orchidaceae and Euphorbiacece. However, like parthenogenesis, stimulus of pollination is required for the formation of adventive embryos.

2.11.1.2 Importance of Apomixis :

1. As apomixis does not involve meiosis, there is no scope for segregation and recombination of genes in the chromosomes. It is only useful for preservation of desirable characters for indefinite periods. It has no role in evolution of species.

2. Due to above characteristics, apomixis was once regarded as evolutionary dead end because the meiotic segregation and recombination on the female side are eliminated. However now-a-days, apomixis is being regarded as a potential and powerful genetic factor for use in crop improvement. It is a fact that apomixis is not very common in major crop plants.

3. Apomictically produced plants are genetically identical to female plants. If apomixis can be introduced in crop plants it would provide an inexpensive way to perpetuate a given genotype and simplify commercial hybrid seed production. Plant breeders are making serious attempts to introduce apomictic reproduction through hybridization of sexually reproducing plants and apomictic relatives of such plants.

4. Adventive embryony, well known in *Citus* is used in production of uniform root-stock and virus free scion material.

2.11.2 Parthenocarpy :

Parthenocarpy is a phenomenon in which there is development of fruit from an unfertilized egg of the flower, resulting in usually a seedless fruit. Such fruits are called parthenocerpic fruits. The term parthenocarpy was coined by Noll (1902). Parthenocarpy is widespread in species which have usually large number of ovules per ovary, (e.g. Banana, Guava, Pineapple, Tomato, Melons and Figs). Such fruits may or may not be always seedless. When seeds are formed they are abortive i.e. do not germinate. Parthenocarpic fruits are produced normally in many cultivated plants such as, banana, citrus, grapes, pine apple and some varieties of apple and pear. So parthencorpy finds importance in horticulture because seedless fruits are ideal for consumption.

Parthenocarpic fruits in nature may be produced due to (i) absence of pollination, (ii) failure of fertilisation or (iii) zygotic sterility.

2.11.2.1 Types of Parthenocorpy :

Nitsch (1963) has recognised three types of parthenocarpy.

1. Genetic Parthenocarpy :

In this type, seedless fruits are formed parthenogenetically due to hybridization or mutation. The famous seedless novel variety of orange was developed from a normal seed bearing variety of *Citrus* through mutation in axillary bud that grew out into a branch bearing seedless fruits.

2. Environmental Parthencarpy :

Environmental factors such as frost, fog, high temperature, freezing etc. interfere with functioning of normal reproductive organs and bring about parthenocarpic seedless fruits. Examples of formation of parthenocarpic fruits are Capsicum by keeping the plants at low

temperature (6° – 10°C) at the time of anthesis and in pears by placing them at freezing temperature for 3 – 19 hours.

3. Chemically Induced Parthenocarpy :

Thimann (1934) reported that pollen grains have auxin and other growth regulatory substances that have stimulating effects on female sex organs. Parthenocarpy has been successfully induced by spraying flowers with 0.5-1.0% solution of the hormones like Indole Acetic Acid (IAA) and Napthalene Acetic acid (NAA), gibberellins etc.

Parthenocorpic seedless fruits of “Allahbad round” variety of guava were produced by simply applying aqueous extracts of pollen grains on the stigma of emasculated flowers.

2.11.2.2 Significance of parthenocarpy :

1. The parthenocarpic fruits are usually seedless and people love to eat this fruits without any trouble.
2. Parthenocarpic fruits have an increased proportion of edible part than normal fruits.
3. These fruits are of great significance in horticulture as they are very suitable for preparing jams and juices.

2.11.2.3 Parthenogenosis :

Parthenogenesis (Gr. Parthenos = Virgin and genesis = origin) may be defined as the development of the female gamete (egg) into a new individual plant without the act of fertilization. This is a type of apomixis in which the megaspore mother cell undergoes usual meiotic division to form megaspores which develop the female gametophyte containing female gamete (the egg). This haploid egg without fertilization develops into an embryo and ultimately forms the viable seed which germinates to form a plant. But in parthenocarpy, as described earlier, embryos abort but the seedless fruit is formed. If seeds develop in parthenocarpy, they are abortive and do not germinate to give rise to a plants. Here, in parthenogenesis, since embryos develop from haploid egg, usually the haploid homozygous plants are produced. Sometimes, a diploid egg may be found due to fusion of haploid cells of embryo sac and give rise to a parthenogenetic diploid plant, the process called as diploid parthenogenesis. Parthenogenesis can also be artificially induced and has practical application in producing homozygous haploids used extensively for breeding (crossing) experiments to produce pure haploid plants.

2.11.3 Polyembryony :

The phenomenon of developing more than one embryo inside an ovule or a seed is known as polyembryony. This phenomenon is very common in Gymnosperms than Angiosperms. In nature, there are many plants in which polyembryony is observed but in such plants only one embryo attains full maturity and the rest of the embryos degenerate during course of seed development.

2.11.3.1 Types of polyembryony :

Polyembryony can be broadly categorised into two groups.

1. **True polyembryony :** Many embryos are developed inside a single embryo sac.
2. **False polyembryony :** Here the ovule carries more than one embryo sac and emryos develop in each embryo sac.

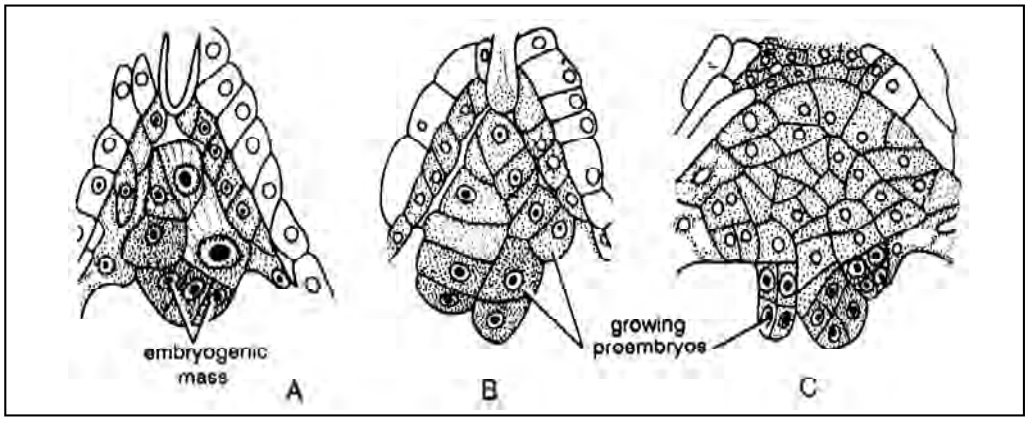

Fig. 2.34 : A-C. Cleavage polyembryony: A. Embryonic mass formed by the bassal cell of the zygote in *Erythronium americanum*, B-C. Differentition of embryos from the cells of the embryonic mass.

2.11.3.2 Origin of Polyembryony :

Based on origin, following types of polyembryony have been recognised.

1. **Cleavage polyembryony :** Polyembryony results from the cleavage of either zygote or earlier stages of development of the embryo (the proembryo), into two or more units which develop into embryos inside the embryo sac. This is widespread in Gymnosperms. But among Angiosperms, it is observed in *Nicotiana rustica* (solanceae), *Erythronium americanum* (Liliaceae), *Lobelia* etc. (Fig. 2.34).
2. **Origin of embryo from cells of embryo sac other than egg :** Additional embryos may develop from synergids (e.g. *Argemone mexicana*, *Phaseolus*). The haploid synergids may be fertilized by sperms from additional pollen tubes or may develop without fertilization. The antipodal cell may give rise to embryos (e.g. *Allium odorum* and *Ulmus americana*) (Fig. 2.35).
3. **Origin of embryo from additional embryo sacs :** In addition to the normal embryo sac, some additional embryo sacs may develop inside the same ovule. Fertilization of eggs in these additional embryo sacs may result in formation of extra embryos inside the same ovule (e.g. *Hydrilla*, Brinjal, *Casuarina*).
4. **Origin of embryos in embryo sacs from any sporophytic cell of ovule :** The cells of nucellus (e.g. *Citrus*, Mango) or integument (e.g. *Lymnanthes*) of ovule may give rise to embryos. These embryos developing from nucellus or integument are also known as adventive embryos (Fig. 2.35.C).

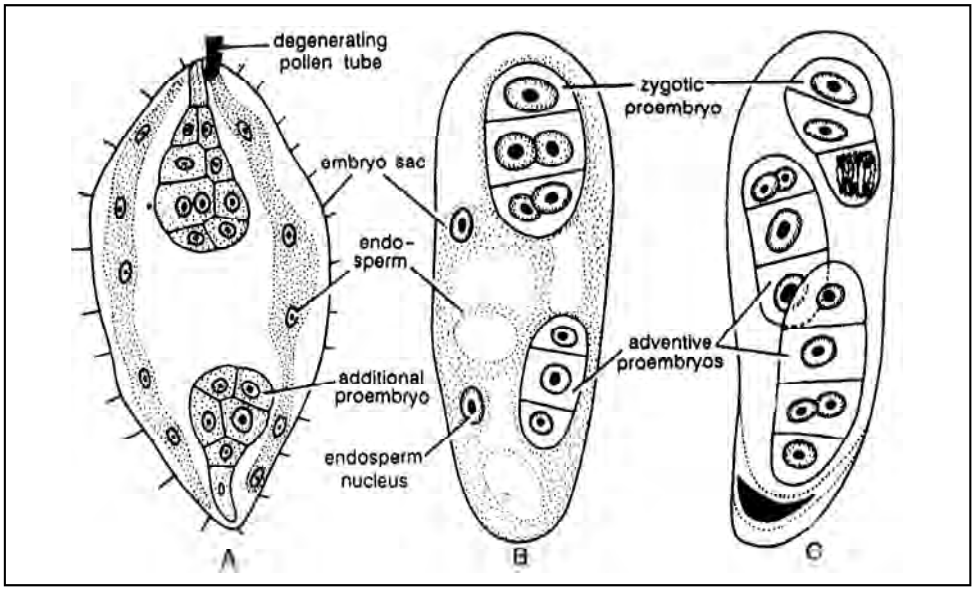

Fig. 2.35 : A-C. Polyembryony : **A.** Development of embryo from antipodal cells. **B.C.** Adventive pro-embryos developed from the cells of the nucellus (they grow along with the zygotic embryos).

2.11.3.3 Induced Polyembryony :

Polyembryony occurs in nature. But recent in vitro studies indicate that not only ovular tissue but all living cells of the plant which are totipotent can develop into embryos in tissue cultures. Such embryos developed through induction, applying tissue culture techniques are known as somatic embryos, supernumery embryos, adventitious embryos or simply embryoids. Embryoids are produced from pollen grains inside anthers (called androgenesis) under culture conditions. Besides pollen grains, somatic cells from roots, apical meristems, stems, leaves, fruits also develop embryoids under tissue culture conditions. Totipotency and formation of non-zygotic embryos have been proved in many plants like wheat, paddy, soya bean, apple, coffee, grapes, mustard, onion etc.

2.11.3.4 Role of Polyembryony in plant breeding and Horticulature :

Adventive polyembryony is of great significans in providing uniform seedings of parental types. By application of tissue culture techniques, large numbers of such uniform seedings with desired qualities (like high-yeilding, disease-free etc.) can be produced in definitely much shorter duration than the normally grown seedings from seeds. Nucellar polyembryony result in virus-free clones of Citrus varieties in nature. So polyembryony is of great use in plant breeding and horticulture. Adventive embryos are very useful in morphogenetic studies.

2.12 DEVELOPMENT OF SEED AND FORMATION OF FRUIT :

The seed and fruits have an important development in the success of flowering plants. Seed and fruit formation is stimulated by the act of fertilization in the ovules located in the ovary of the flower. The seeds contain the genes of both male and female parents and usually participation of both the male and female flowers are required to produce a seed. Sometimes

male and female flowers are from the same or separate plants. Sometimes flowers may also be fertilized by its own pollen. Without being fertilized (in most cases), the ovules will not develop into seeds. The seed contains the embryo and endosperm, surrounded by the maternally derived seed coat. The function of the seed is to protect the embryo to sense environmental conditions favourable to germination and to nourish the germinating seedlings.

The fruits develop from the ovary of the flower. The ovules grow into seeds after fertilization. Thus fruit development involves differentiation or redifferention of pre-existing organs (ovules). Evolutionarily floral organs are considered as representative of the modified leaves and the fruit is also modified leaf. Furits in a plant serve to protect the seeds during development and then to disperse the seeds after their maturation.

2.12.1 Development of seed : The Basic Structure of Seed :

A ture seed is defined as a fertilized mature ovule that possesses an embryonic plant (embryo), stored food materials (sometimes absent) and a protective seed coat or coats. A seed is a developed ovule and also a reproductive unit.

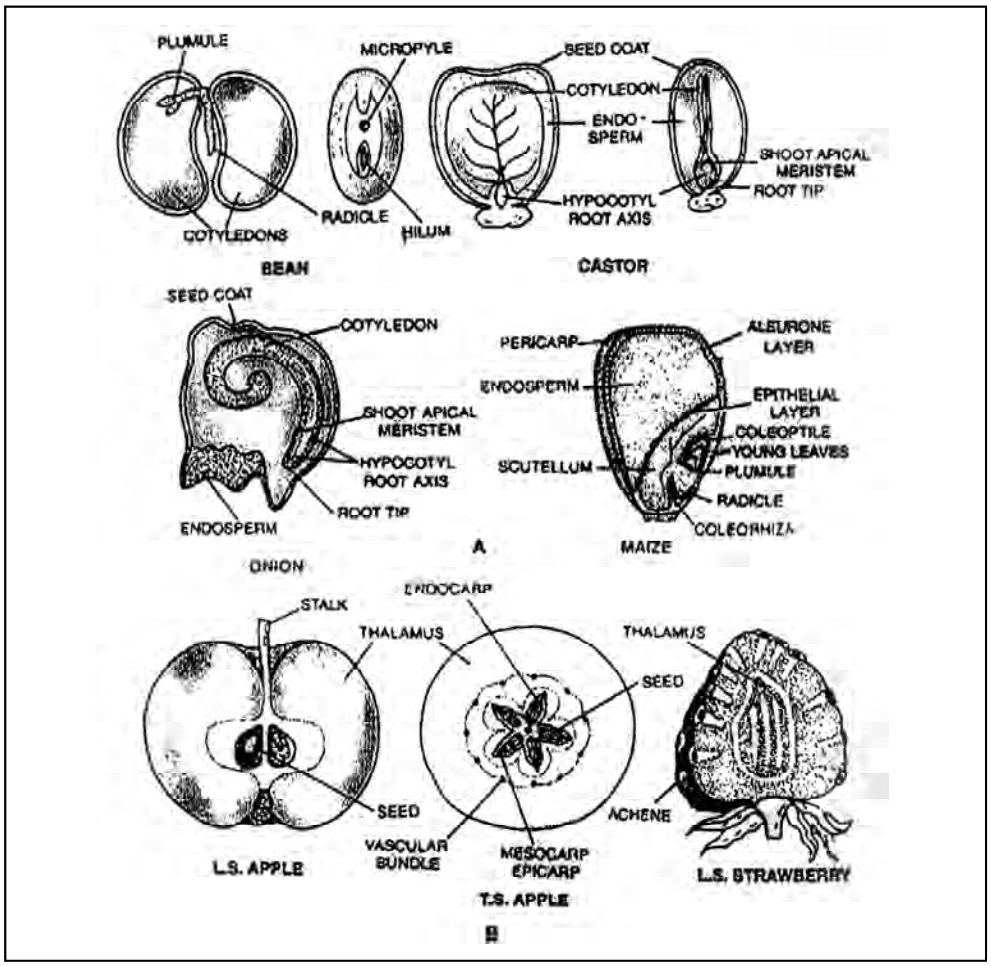

Fig. 2.36 : A. Structures of some seeds, **B.** False frutis of Apple and Strawberry.

2.12.1.1 The basic structure of a seed :

The main structural units of a seed are (i) the embryo, (ii) surrounding seed coat and, (iii) endosperm or stored food materials (if present) in between embryo and seed coat.

1. **Embryo :** The main components of the embryo are : (Fig. 2.36)
   - The Cotyledons (also called seed leaves) are attached to the tip of the embryonic axis. Monocot embryo has a single cotyledon. The dicot embryo has two cotyledons. Cotyledons are also the source of nutrition in the non-endospermic dicot seeds which are thick and leathery. In endospernous seeds the cotyledons are thin and papery.
   - The epicotyl region is the embryonic axis above the point of attachment of cotyledons.
   - The plumule is the tip of the epicotyl which bears the epicotyl apex that produces young leaf primorida. The pulmule develops into the shoot upon germination of the seed.
   - The hypocotyl is the embryonic axis present below the point of attachment of the cotyledons(s). Th hypocotyl connects the epicotyl and radicle. The hypocotyl is the stem-root transition zone.
   - The radicle is lower most tip of the hypocotyl and the tip of the radicle is called hypocotyl apex that grows into primary root on germination.

In monocotyledonous plants there are two additional structures in the form of sheaths. The plumule, here is covered with a coleoptile that forms the first leaf while the radicle is covered with a coleorhiza that connects to the primary root and adventitious roots are formed later from the sides (Fig. 2.33, 2.36). Here, the hypocotyl is a rudimentary axis between the radicle and the plumule. In maize or wheat plant, the seeds constructed with pericarp, scutellum (single large cotyledon that absorbs nutrients from the endosperm) plumule, radicle, coleoptile and coleorhiza (Fig. 2.33). The last two structures are sheath-like protective structures which enclose the flumule and radicle respectively (Fig. 2.33, 2.34).

2. **The seed coat :** The integuments of the ovule turn into seedcoat. The inner integument forms tegmen and the outer integument forms the testa. The testa of both monocots and dicots are often marked with patterns and textured markings. In some cases, it may also have wings (winged seeds) or tufts of hairs. When seed coat is single layered it is called a testa. The seed coats of some monocots such as grasses, are not distinct structures but are fused with fruit wall to form a pericarp. If seed coat has a single integument it is called unitegmic. If two integuments are present, such seed coat is bitegmic.

Anatomically the inner integument i.e. the tegmen in a mature seed consists of inner most fringe layer (Fig. 2.37F) follwed by (towards outer testa) multilayered (9-10) colourless zone, 4-5 layered inner pigmented zone and a palisade layer of elongated cells. Next to palisade layer starts the various layer of testa. The testa (the outer seed coat) from innermost to outerside consists 2-3 layered colourless zone (outer to palisade), outer 2-5 layers of pigmented zone and the outer most epidermis having hairs (Fig. 2.37 E, F).

3. **The food reserves :** Usually (not always) there is stored nutrients in between the embryo and seed coat required for seed germination and initial seeding growth. Endosperm is the chief source of nutrition. In some mature seeds, endosperm persists as a food storage tissue, such seeds are called endospermous or albuminous seeds (e.g. castor, maize, wheat, barly coconut). When endosperm is fully consumed by the growing embryo in a mature seed, it is called non-endospermic or exalbuminous seed (e.g. Pea, Gram, Bean etc.) In some mature seeds the residual nucellus (remains of nucellus) persists after consuming endosperm by embryo (e.g., Black pepper, Coffee, Castor, Cardamom). This residual nucellus is called perisperm. Endosperm is rich in oil, carbodydrates and starch. Proteins are also present among other stored food materials.

The funiculus of the ovule on maturity of seed, detaches itself at a fixed point called abscission zone and a scar is left forming an oval depression called the hilum. The anatropous ovules have a region where the funiculus is fused to the seedcoat it leaves a longitudinal ridge called a raphe, located just above the hilum.

2.12.1.2 The Shape Appearance and Size of Seeds :

Seeds have many shapes and appearances, specific to the different species. The shapes may be reniform (resembling a kidney), lobed, square, oblong, spherical, ovoid, globose, discoid etc. Similarly, seeds may be coloured (brown, black, red, cream coloured). Common colours are brown and black. The surface may be rough, polished hairy or winged.

Size of seeds may be dust-like (Orchid seeds) or medium to very large. The largest seed weighs 23 kg produced by coco de mor (double coconut palm-*Lodoicea maldivica*) the fruit of which contains a single seed.

2.12.1.3 Sequence of seed development (Salient features) :

The development of the fertilized ovule into the seed involves several steps. From the outside to inwards, the development steps are (i) seed coat formation, (ii) storage of food materials in cotyledons for future use during germination and (iii) maturation of the embryo inside the seed.

4.12.1.4 Overall Development Features of Seed Development :

1. **Preparation for developmental arrests :** Most of cell divisions are compete at the early stage of maturation phase of embryo development. But the embryo still increases in size upto 100 fold by cells expansion. This accompanies a massive accumulation of storage compounds. Gradually embryo growth is arrested due to action of growth inhibitors.

2. **Accumulation of storage products :** Storage proteins accumulated in the seeds are important source of amino acids, nitrogen and carbon for the germinating seedling. Massive accumulation of storage compounds occurs during maturation phase. These products are very valuable to humans and other animals for food such as carbohydrates, oils and starch.

   But finally the funiculus of seed dries out and gets detached. As a result, the food and water supply to the seed ceases.

3. **Acquisition of property of desiccation tolerance :** Gradually, the moisture contents of the seed decreases due to desiccation. Seeds dehydrate to almost 5% level of moisture content which forces lethal effects on plant tissue. To overcome the desiccation process the seed acquires the power of dessication tolerance. The seed coats become very tough and hard to release any moisture to outside. The outer surface of seed coat turns from green to brown. The embryos also express an internal developmental programme that allows them to survive. This acquisition of desiccation tolerance is a part of seed maturation programme.

4. **Dormacy and viability :** Dormacy is a characteristic feature of flowering plants. Embryos in a dry seed remain in state of inactivity called dormancy. Most of the seeds undergo dormancy prior to germination. Once dormant phase (period) is over, the seed germinates. Only the viable seeds germinate. The ability of seeds to retain the power of germination over a period of time is called viability of the seeds. Period of viability of seeds differs from generation to generation. When viability period is over, seeds do not germinate.

2.12.1.5 Significance of seeds (or importance of seeds) :

1. **Dependable method :** Unlike cryptogams, the pollination and fertilization of flowering plants (seed plants) are free from dependency on external water. In non-seed plants, the embryo once formed as to develop immediately into a sporophyte. There is no mechanism to store the embryo. But in seed plants, the embryo gets protection inside the seed and can germinate to form a seeding even after many months and years. Therefore, formation of seeds as reproductive units is a more dependable method.

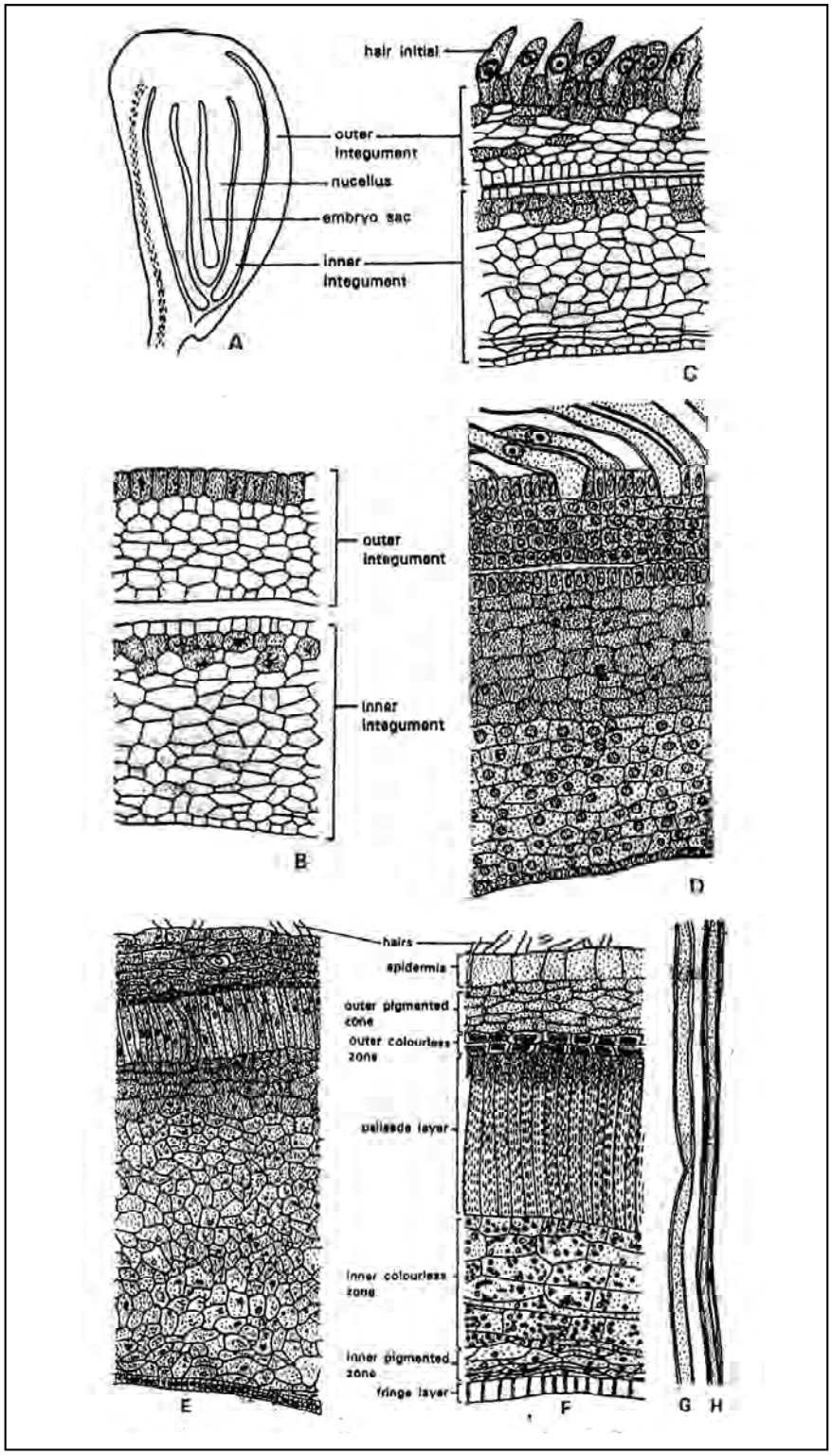

Fig. 2.37 : Seed-coat development in Gossypium herbaceum. **A.** Longisection of ovule at the mature embryo sac state. **B.** Portions of the outer and inner integuments enlarged from A to show their cellular details. **C-E.** Portions of integuments from ovules 2-3, 5-6 and 15 days after pollination, respectively. **F.** Portion of the mature seed-coat. **G.** A lint hair, **H.** A fuzz hari. (after Pamchandani *et al* 1965).

2. **Perennation :** Seed is a dry (water content 10-15%) structure with a dormant embryo and thick protective seed coat. It is most suitable for perennation through unfavourable periods.
3. **Dispersal :** Seeds have adaptive strategies to get dispersed to new habitats and colonise the same.
4. **Reserve Food :** Seeds have reserve food for nourishing the young seedlings till these become nutritionally independent.
5. **Variations :** As seeds are formed through sexual reproduction, they carry a number of variations. Variations are essential for adaptability to diverse environmental conditions.
6. **Storage :** Seeds can be stored for later (future) use. This is helpful for supply of food throughout the year and to overcome drought and famine conditions.
7. **Agriculture :** Seed is the basis of agriculture. Agriculture originated when humans learnt to eat, store and sow seeds. Agriculture becomes an easier method of food procurement due to use of seeds. It becames a turning point for evolution of human civilization, industrialization and development of modern science and technology.
8. **Importance for humans :** Large number of edible seeds and majority of human calories come from seeds, especially cereals, legumes and nuts. Seeds also provide many cooking oils, beverages, spices and many food additives. Seeds provide may medicines.

2.13 FRUIT DEVELOPMENT IN ANGIOSPERMS :

A fruit (L. fructus-fruit) in common botanical terms is matured ovary or a clusture of matured ovaries.

2.13.1 Contribution of different flower parts to the fruit :

1. **True and false fruits :** Most fruits develop from the ovary. Some authors classify fruits derived from a single ovary as “true fruits” while “false fruits” are composed of tissues derived from flower parts other than the ovary or from more than one ovary.
2. **Modes of fruit development :** There are three general modes of fruit development.
   (i) Apocarpous fruits develops from a single flower having one or more seprate carpels and they are called simple fruits.
   (ii) Syncarpous fruits develop from a single gynoecium having two or more carpels fused together.
   (iii) Multiple fruits develop from many different flowers of the same plant.

3. Types of fruits (For more details Vol-I of this book may be seen)

(i) Simple fruits : Simple fruits can be either dry or fleshy and result from simple or compound ovary (with one or more carpels) in a flower with single pistil, Dry fruits may be dehiscent or indehiscent (e.g. wheat, coconut, pea, beet, radish)

(ii) Aggregate fruits : They develop from a single flower that has multiple carpels (pistils) which are not jointed together, i.e. each pistil contains one carpel. Each pistil forms a fruitlet and collectively the fruitlets are called eterio (e.g. Calotropis Annona)

(iii) Multiple fruits : They develop from a cluster (many) of flowers (an inflorescence). Each flower produces a fruit but these mature into a single mass (e.g. Pineapple).

2.13.2 The salient features of Fruit Development :

1. As the ovules develop into seeds, the ovary begins to ripen and the ovary wall develops into pericarp.
2. The pericarp can be dry and papery (e.g. Maple, Dandelion), woody (e.g. nuts), fleshy (e.g. berries-grapes and tomatoes) or stony (called stone fruits as in Cherries and Peaches)
3. The variation in pericarp composition reflects adaptations to different dispersal mechanisms (e.g. wind for papery pericarp) and animal consumption (for fleshy fruits).
4. The fruit may develop a single seed (e.g. corn) or many seeds (e.g. Pea pod or Pumpkin).
5. Pericarp of some fruits may differentiate to form different specialized layers called outermost epicarp, middle mesocarp and innermost endocarp. In Citrus, the rind is the epicarp, the white covering is the mesocarp and juice sacs are the endocarp.
6. Many fruits we call berries, (such as Rasp berries and Straw berries), are botanically not classified as berries. Rasp berries are examples of aggregate fruits. Each juicy little sphere is actually an individual fruit of the same class as Cherries, and what we consider as the fruit is really an aggregation of fruits.
7. Straw berries and apples are examples of accessory fruits, where some of the fleshy tissue is derived from flower parts other than the ovary. Straw berry fruits are actually the seeds. They are called achenes. The fleshy part that we eat develops from the receptacle. Most of the fleshy tissue in apples develops from the thalamus or hypanthium which is a region of the flower where sepals, petals and stamens are all fused to the ovary. Thus all floral organs contribute to the fleshy portion of apples.

8. **Phases of Fruit Development :** Fruit development can generally be considered to occur in four phases: (i) fruit set (whether to abort or to proceed for fruit development), (ii) a period of rapid cell division, (iii) a cell expansion phase, and (iv) ripening (maturation).

9. **Fruit ripening :** Ripening represents the functions of shift from the protective function to dispersal of the fruit. Ripening occurs synchronously with seed and embryo maturation. In dry fruits (cereals, nuts) ripening consists of desiccation and is considered maturation. Ripening in fleshy fruits is designed to make the fruit appealing to animals that eat the fruit as a means for seed dispersal. Ripening involves the softening, increased juice and sweetness, and color changes of the fruit. Fleshy fruits are either climacteric or non-climacteric. Climacteric fruits produce a burst in ethylene synthesis, as the fruits ripen. These include fruits with high degrees of flesh softening, like Tomato, Banana, Avacado, peach etc.

   Fruit ripening has been extensively studied in tomato. External application of ethylene accelerates ripening. The fruit responds to ethylene only during end of cell expansion phase (mature green phase).

   Fruit softening involves a partial breakdown of cell walls and several enzymes are known to be involved in this process. Polygalacturonase hydrolyses bonds in pectins of cell walls.

2.13.3 Significane of Fruit Formation :

1. **Protection :** Developing fruits protect the developing seeds from mechanical injury, insects and unfavourable climatic conditions.
2. **Dispersal :** Fruits help the seeds in dispersal to distant places.
3. **Food to Animals :** Fleshy fruits provide food to animal who also act as dispersal agents of their seeds. Fleshy fruits generally have hard seeds (e.g., Guava) while hard shelled fruits have soft seeds (e.g., Almond).
4. **Nutrition to Germinating Seeds :** Some fruits provide nutrition to germinating seeds and developing seedings.
5. **Importance to Humans :** Fruits are a source of food, protein, oil, organic acids, vitamins, minerals and sugars, for human consumption.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the blanks with correct answers from choices given in the bracket:

(i) When gynoecium matures first it is called _____ to effect cross pollination.
(protogyny, protandry, herkogomy, unisexuality)

(ii) In Ornithophily, the agents for cross pollination are _____ .
(ants, birds, snails rats)

(iiii) Zygote develops from _____ cell of the embryo sac.
(egg, synergid, antipodal, nucellus)

(iv) Fertilization was discovered by _____.
(Strasburger, Mendel, Nitsch, Bower)

(v) Due to triple fusion, _____ is formed.
(Zygote, Embryo, Endosperm, Zoospore)

(vi) The innermost layer of wall layers is _____.
(Tapetum, Epidermis, Endodermis, Endothecium)

(vii) Straight ovules are called _____.
(Anatropous, Campylotropous, Orthotropous, Hemitropous)

(viii) Contrivance of self pollination is _____.
(Dicliny, Herkogamy, Self sterility, Cleistogamy)

2. Answer the following question in one word :

(i) Androecium and gynocium whorls are present in the same flower.

(ii) Both the essential whorls are absent in a flower.

(iii) Petals are united in a flower.

(iv) Free carpels in a flower.

(v) Transfer of pollen grains from anther to stigma of the same flower.

(vi) The process in which the male gamete fertilizes with egg.

(vii) Pollination in aquatic plants.

(viii) Fusion of one male gamete with definitive nucleus.

3. Correct the sentences in each bit without changing the underlined word/words:

(i) Anemophilous fowers are pollinated by ants.

(ii) Dichogamy is found in bisexual flowers where stamens and carpels mature at same time.

(iii) The ovule is attached to the placenta of ovary by means of nucellus.

(iv) Animals acting as agents of pollination is called anemophily.

(v) Polyembryony involves development of one embryo.

4. Fill in the blanks :

(i) The cells present on two sides of egg in the egg apparatus are called _____.

(ii) The outer wall of the pollen grain is called _____ .

(iii) The male gametes are formed from _____ cell.

(iv) Parthenogenesis mens development of fruits without _____.

(v) The endosperm in which first division is cellular and subsequintal cellular is called _____ endosperm.

(vi) In grafting, the part of the plant detached is called _____.

(vii) In self pollination, pollen is transfered to stigma of the _____ flower.

(viii) The fertile cells from which microspores or megaspores developed are called _____ cells.

(ix) In maize plant male inflorence is borne at _____ portion of the plant.

(x) The fusion product of male gamete and egg cell in angiosperms form _____.

GROUP - B
(Short Answer-type Questions)

1. Write note on the following in 2 to 5 valid and relevant points :

(i) Parthenogenesis

(ii) Allogamy

(iii) Herkogamy

(iv) Geitonogamy

(v) Xenogamy

(vi) Self sterility

(vii) Entomophily

(viii) Embryo sac

(ix) Embryo

(x) Micropropagation

(xi) Polymbryony

(xii) Incompatibility

2. Differentiate the following with at least three valid and meaningful points:

(i) Pollination and fertilization

(ii) Dechogamy and herkogamy

(iii) Protogyny and protandry

(iv) Self pollination and cross pollination

(v) Embryo and endosperm

(vi) Gamete and zygote

(vii) Micropyle and chalaza

(viii) Zoophily and anemophily

(ix) Double fertilization and triple fusion

(x) Porogamy and chalazogamy

(xi) Apospory and apogamy

(xii) Moncot and Dicot embryo

(xiii) Nuclear and cellular endosperm

GROUP - C
(Long Answer-type Questions)

1. Distinguish between self and cross pollination. Describe three conditions that favour cross pollination.
2. What is cross pollination ? Give an account of the contrivances of cross pollination.
3. Describe how different agents help in cross pollination.
4. Discuss the important outbreading devices for cross pollination.
5. Describe how double fertilization and triple fusion occur in the angiosperms.
6. With diagrams, describe the development of male and female gametophytes in angiosperms.

❑ ❑ ❑

CHAPTER 3

HUMAN REPRODUCTION

All organisms have two fundamental objectives i.e. to live and produce offsprings for the continuation of generation. The later part is achieved by the process of reproduction. Hence, reproduction is an essential feature of all organisms and is the process by which an individual multiplies in number by producing more individuals of its own type. In animals, it is of two types: (1) **asexual** and (2) **sexual**.

3.1 ASEXUAL REPRODUCTION :

In asexual reproduction, a single parent splits, buds or fragments to give rise to two or more young ones that have hereditary traits similar to that of the parent. It is mostly found in lower groups of organisms like some plants protozoa, sponges, coelenterates etc.

3.2 SEXUAL REPRODUCTION :

Sexual reproduction involves two parents, each contributing a gamete, an egg in the female and sperm in the male which fuse to form a fertilized egg or zygote in the act of fertilization. It occurs in almost all types of animals. In some lower grade animals both gametes are produced by the same individual. These animals are called **bisexual** or **hermaphrodite** or **monoecious**. But, never the less, self fertilization is prevented by one mechanism or other. Higher grade animals including human are **unisexual** or **dioecious** i.e. the testes and ovary are borne by separate individuals.

Human is **sexually dimorphic**. Each individual has either male reproductive system or female reproductive system. Human reproductive system consists of **primary reproductive organs** or **gonads** (ovaries and testes) which produce gametes and **accessory organs** (prostate gland and seminal vesicles in male and fallopian tubes in female), which do not form gametes but are essential for reproduction. **Secondary sexual characters** are those which help distinguish the two sexes morphologically.

Human is **viviparous**, i.e. the fertilization is internal and the development is internal. It gives birth to young ones. The reproduction process in human include formation of gametes (**gametogenesis**), i.e., sperms in male and ovum in females; transfer of sperms into female genital tract (**insemination**) and fusion of sperm and ovum (**fertilization**) leading to the formation of zygote of fertilized egg. It is followed by cleavage and formation of blastocyst. The attachment of blastocyst to the wall of uterus (**implantation**), further embryonic development (**gestation**)

and finally the delivery of the baby (**parturition**). All these events occur after puberty and it is different in male and female as sperm production continues till old age but formation ovum stops after 45- 50 years of age.

3.3 MALE REPRODUCTIVE SYSTEM (FIG. 3.1) :

The human male reproductive system consists of a **pair of testes**, enclosed in an **extra-abdominal scrotum**; **numerous excurrent ducts**; and **several accessory glands.**

3.3.1 Scrotum :

There is a pair of testes, situated outside the abdominal cavity in a scrotum. The scrotum communicates with the abdominal cavity through **ingunal canals**. Embryonic testes are abdominal i.e. lie in the abdominal cavity. They descend into the scrotum during the gestation period and 90% male babies are born with completely descended testes. Testicular descent to the inguinal region is effected by the **anti mullerian hormone (AMH)** and descent from the inguinal region to the scrotum depends upon other factors. However in 10% of the new-born males, the testes are retained in the abdominal cavity. In such cases, gonadotrpin

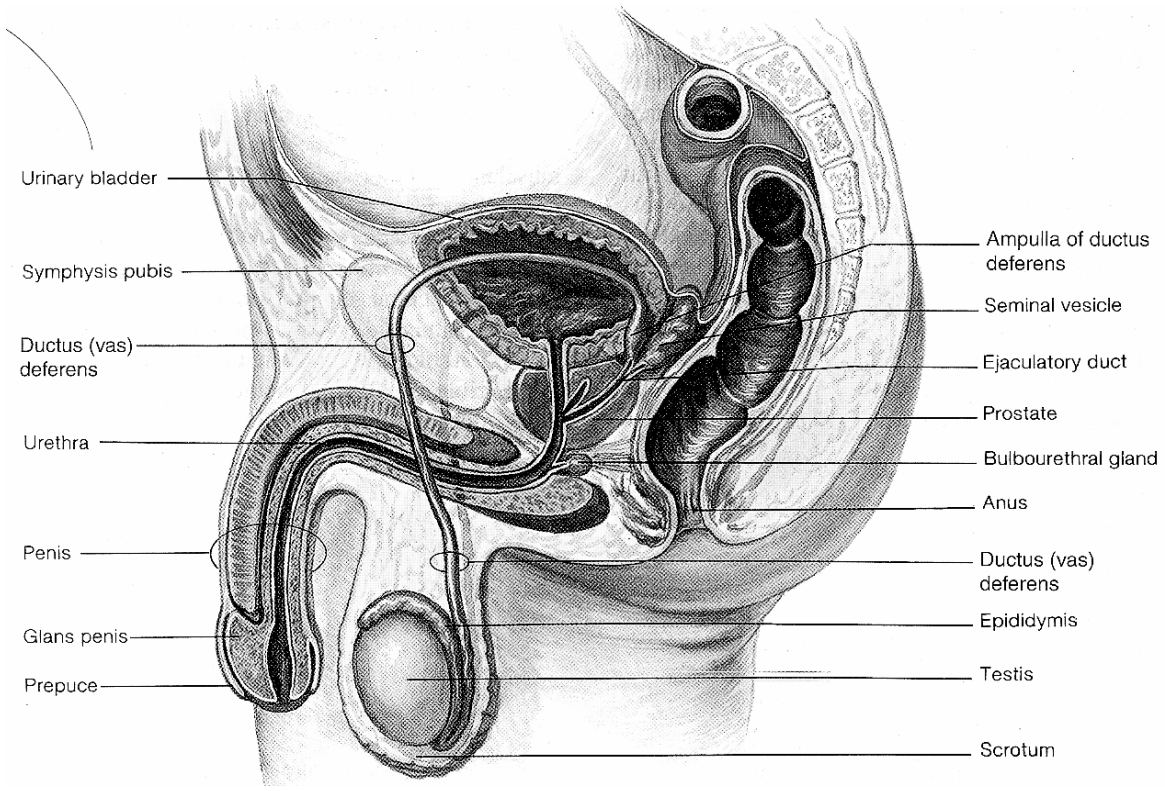

Fig. 3.1 : Human male reproductive system (Lateral view).

treatment or surgery rectifies the defect. This phenomenon of retention of the testes in the abdominal cavity is known as **cryptorchidism (also cryptorchism)**. In cold weather, the testes are elevated by the contraction of a band of muscle, known as **cremasteric muscle** to get the warmth of the trunk. This effect is known as **cremasteric reflex**. The same effect occurs, when the thigh of a man is stroked. In the baby this stimulus causes the testes to ascend up into the abdominal cavity through the inguinal canal.

The temperature of the testis is 2°–3° C lower than the normal body temperature. This temperature is vital for spermatogenesis to continue. Perspiration from the scrotal surface and evaporation maintains the testis at a temperature lower than the body temperature. This mechanism is supplemented by a special arrangement of the blood vessels that supply the testis. Testicular arteries that descend into the scrotum are surrounded by a plexus of veins, which ascend from the testis and form a **pampiniform plexus**. Blood that returns from the testis through the pampiniform plexus is cooler than the blood in the testicular arteries. The arterial blood is cooled by the venous blood by a **countercurrent heat-exchange mechanism**.

3.3.2 Testis : Microscopic Anatomy

Each testis is surrounded by a thick connective tissue capsule, called **tunica albuginea**. It thickens and extends inwardly into each testis as a **mediastinum testis**. A thin connective tissue septum extends from the mediastinum testis and subdivides it into about 250 compartments, called **testicular lobules**, each containing 1-4 coiled **seminiferous tubules** (Fig.3.3). Each seminiferous tubule is lined by stratified cuboidal epithelium containing dividing spermatogenic cells and large non-dividing somatic cells, called **Sertoli** or **sustentacular cells** [(Fig.3.2(a), (b) & (c)].

The Sertoli cells serve as the supporting and nourishing cells for the spermatogenic cells in different stages of their differentiation. **The basal lamina of the germinal epithelium, muscle-like myoid cells at the base of the basal lamina and tight junctions between adjacent Sertoli cells constitute a blood testis barrier**. This barrier prevents many macromolecules from moving it into the tubular lumen. It also prevents the blood-borne noxious chemical agents from entering into the tubule. It prevents the passage of antigenic agents from the tubule into the blood, which are likely to generate an autoimmune response.

The seminiferous tubules lie in a mass of loose connective tissue, containing fibroblasts, muscle-like cells, nerves, blood vessels, lymphatic vessels and epithelial cells. This tissue is known as the interstitial tissue and the epithelial cells as **interstitial cells of Leydig**. These cells secrete male steroid hormones, collectively called **androgens**. The more important among the androgens is **testosterone**.

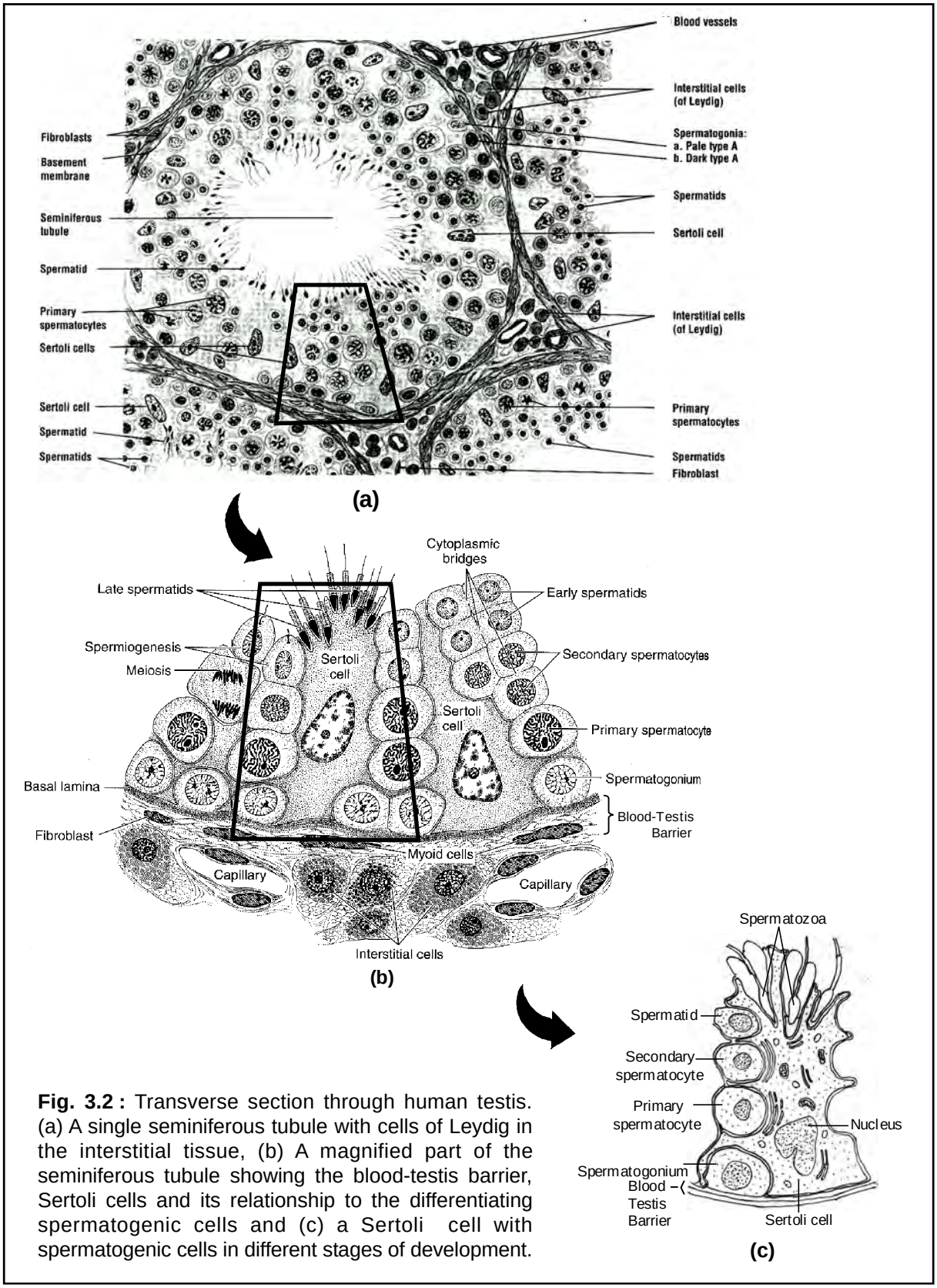

Fig. 3.2 : Transverse section through human testis. (a) A single seminiferous tubule with cells of Leydig in the interstitial tissue, (b) A magnified part of the seminiferous tubule showing the blood-testis barrier, Sertoli cells and its relationship to the differentiating spermatogenic cells and (c) a Sertoli cell with spermatogenic cells in different stages of development.

3.3.3 Excurrent Ducts (Fig.3.3) :

There is a system of ducts, which help convey the mature sperms with the secretions of the glands to the exterior. The mature sperms pass from the seminiferous tubules to the **rete testis** through **straight tubules**. The rete testis is formed by irregular anastomosing network of tubules, whose inner lining is formed by squamous to low cuboidal epithelium. Twelve short tubules, called **ductuli efferentes** or **efferent ductules** arise from the rete testis and form a coiled mass outside the testis, constituting the **head of epididymis**. All the efferent ductules join into a singular duct that passes through the middle part of the epididymis, known as the **body of epididymis**. This duct enlarges to form the **tail of epididymis**. This duct continues further as **ductus** (**vas**) **deferens**, which then opens into the **ejaculatory duct**. Shortly before opening into the ejaculatory duct, the ductus deferens dilates as an ampulla. Each ejaculatory duct receives a ductule from the **seminal vesicle**. The ejaculatory ducts enter into the **prostate** and join to form a single **prostatic urethra**. This duct receives small ductules from the prostate. The prostatic urethra then enters into the penis forming a **penile urethra**. The prostatic urethra, before entering into the penis, receives two small ductules, one each from the **bulbo-urethral or cowper's glands**.

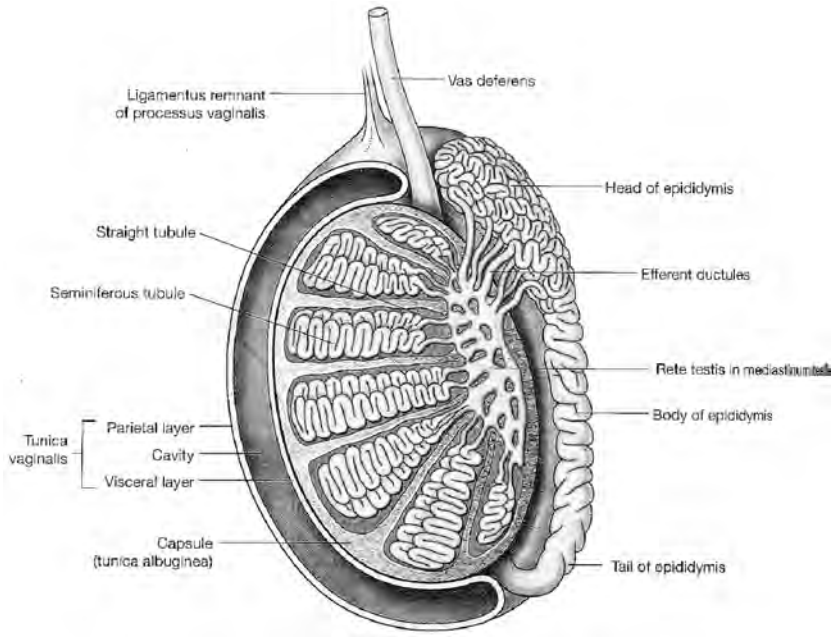

Fig. 3.3 : Duct system in the human testis

3.3.4 Glands :

The male reproductive system is associated with many accessory glands, whose secretions mix with the spermatozoa forming a fluid, known as the **semen**. The glands consist of a pair of seminal vesicles, a pair of bulbo-urethral (cowper's) glands and a single prostate gland.

3.3.4.1 Seminal vesicle :

There is a pair of seminal vesicles, situated just posterior to the bladder above the prostate. The duct of each seminal vesicle empties into the ductus deferens at the base of a dilated terminal part, called ampulla, consequently forming an ejaculatory duct. **The seminal vesicles secrete a yellowish viscous fluid containing fructose, which serves as the main energy source for the spermatozoa.** This fluid accounts for 60% of the volume of the semen.

3.3.4.2 Prostate :

There is a single prostate gland, just inferior to the bladder. The two ejaculatory ducts enter into the prostate and join to form a prostatic urethra. The prostatic urethra receives small ductules from the prostate. **The prostate secretes a thin watery fluid, which contains citric acid, calcium, coagulation proteins, prostate-specific antigens and an enzyme, fibrinolysin.** The coagulation proteins cause the semen to coagulate after ejaculation. However, fibrinolysin later causes the coagulate to assume a liquid form. The seminal vesicles and prostrate are androgen dependent glands i.e. they atrophy following the removal of the testes.

3.3.4.3 Bulbo-urethral (Cowper's) gland :

There is a pair of small spherical bulbo-urethral glands, whose ductules discharge into the prostatic urethra. These glands secrete a clear mucous-like fluid that acts as a lubricant. Its secretion precedes the secretion of the semen.

3.3.5 External Genitalia (Penis) :

The penis is an erectile copulatory organ in human male. The slightly swollen free end is known as **glans penis**, which is covered by a loose fold of skin, called **prepuce**. The

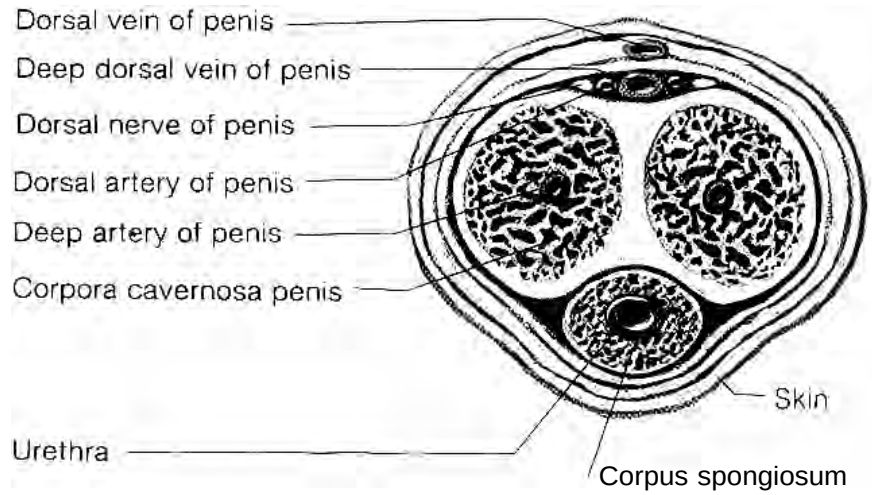

Fig. 3.4 : Transverse section through the penis showing the erectile tissues and blood vessels.

prepuce can be made inside out. The penis consists of erectile tissues, comprising of a paired dorsal **corpora cavernosa** (singular; cavernosum) and a single ventral **corpus spongiosum** (Fig.3.4). The corpus spongiosum extends into the glans penis. The passage through which the spermatozoa pass through the penis is known as **penile urethra**, surrounded by the corpus spongiosum. The corpora cavernosa are surrounded by a thick connective tissue capsule, called the tunica albuginea. It forms a median septum between the two corpora cavernosa. The corpus spongiosum is surrounded by a thin tunica albuginea, which contains smooth and elastic fibers. The blood is drained from the testis by two types of veins: a superficial dorsal vein and a deep dorsal vein. Similarly, there are two types of paired arteries supplying blood to the penis: dorsal arteries and deep arteries.

3.3.6 Semen :

The secretions of the male accessory glands, such as seminal vesicles; prostate; bulbo-urethral glands; and possibly the urethral glands mix with the spermatozoa forming a fluid, known as the **semen**. The main constituent of the semen is live spermatozoa present in an alkaline viscous medium, maintained at a pH range of 7.2-7.8. Another main constituent is fructose contributed by the seminal vesicles. It is the chief energy source for the spermatozoa. The semen is forcibly expelled through the urethra, a phenomenon known as **ejaculation**. The volume of ejaculate is 1.5-5.0 mL. The normal count of sperm is 40-250 million / mL. Fifty per cent of men with a count between 20 to 40 million / mL and all those with a count below 20 million / mL are said to be sterile. This low count is known as **oligospermia**.

3.4 FEMALE REPRODUCTIVE SYSTEM (Fig. 3.5) :

Human female reproductive system consists of **a pair of internal ovaries**; **a pair of uterine** or **fallopian tubes; a single uterus**; **a cervix** and **a vagina**.

3.4.1 Ovary : Microscopic Anatomy

There is a pair of almond-shaped ovaries, situated in the pelvic cavity. Unlike the testes, the ovaries are not extra-abdominal. A part of the ovary is attached to the **broad ligament** by a peritoneal fold, the **mesovarium**. Another part is attached to the wall of the uterus by an **ovarian ligament**.

Histologically, the ovary consists of a single surface layer of squamous to cuboidal epithelial cells, constituting the germinal epithelium (Fig.3.6 & 3.8). Inner to this layer is a mass of dense irregular connective tissue, called **tunica albuginea**. Internal to the tunica albuginea is a **cortex**. Below the cortex is a highly vascularized connective tissue, called **medulla** or **stroma**. There is no distinct boundary between the cortex and medulla.

The female germ cells differentiate as oogonia during the embryonic life. The oogonia divide by mitosis (equational division) in the multiplication phase and then enter into the

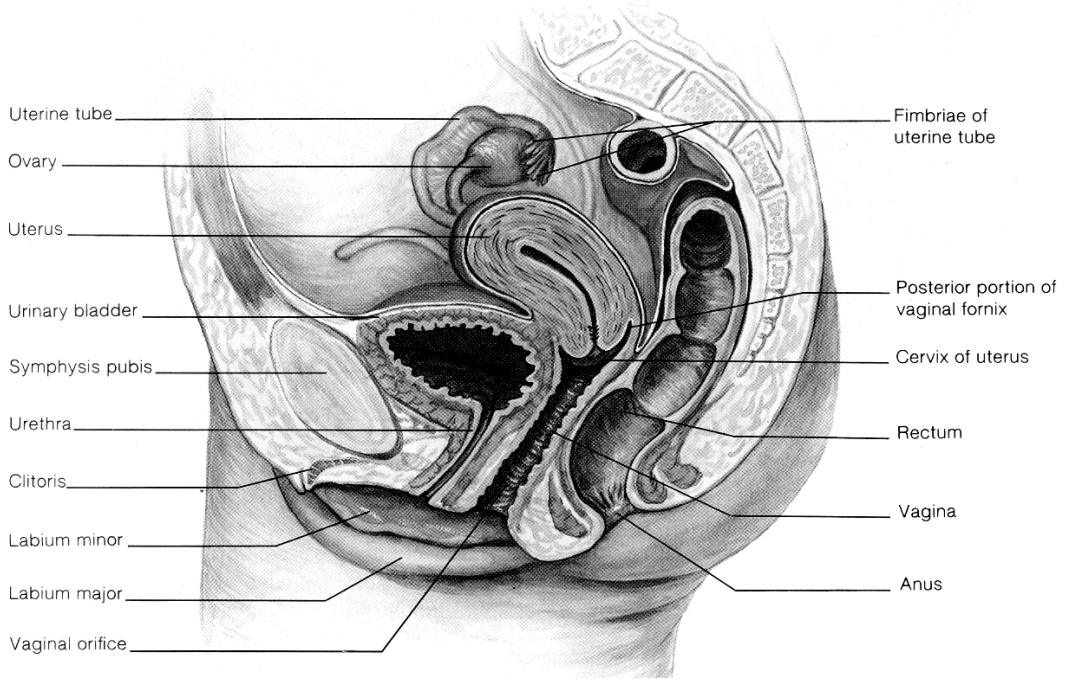

Fig. 3.5 : Human female reproductive system (Lateral view)

phase of maturation through a phase of growth as **primary oocytes**. The phase of maturation consists of two meiotic divisions. The first meiosis is reductional. **The primary oocytes are arrested at the diplotene stage and remain as such until the onset of puberty.** A primary oocyte is surrounded by a single layer of squamous follicle cells. This structure constitutes a **primordial follicle** (Fig.3.6).

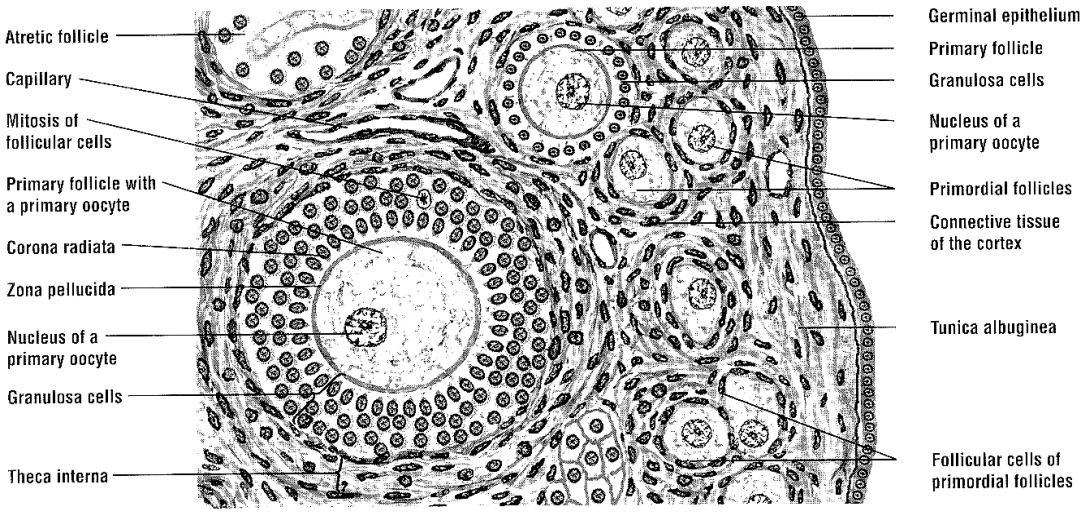

Fig. 3.6 : Primordial and primary follicles in the cortex of the ovary.

The primordial follicles are situated in the cortex. At puberty, the primordial follicles are stimulated by the pituitary gonadotropins (FSH and LH) to differentiate into several later stage follicles, such as **primary, secondary** and **mature follicles**. As the primordial follicle grows, the squamous follicular cells change to cuboidal or low columnar. This follicle is known as **primary follicle** (Fig. 3.6). The developing oocyte has an eccentric nucleus. The follicular cells of the primary follicle grow by mitosis and form layers of cuboidal cells called the **granulosa cells**. The innermost layer of granulosa cells that surrounds the oocyte is known as the **corona radiata**. A glycoprotein protective layer, known as the **zona pellucida** appears between the oocyte and corona radiata. Cells from the stroma surround the granulosa cells and differentiate as the **thecal cells**. The theca differentiate as an outer **theca externa** and an inner **theca interna**. A thin **basement membrane** separates the granulosa cells from the theca interna.

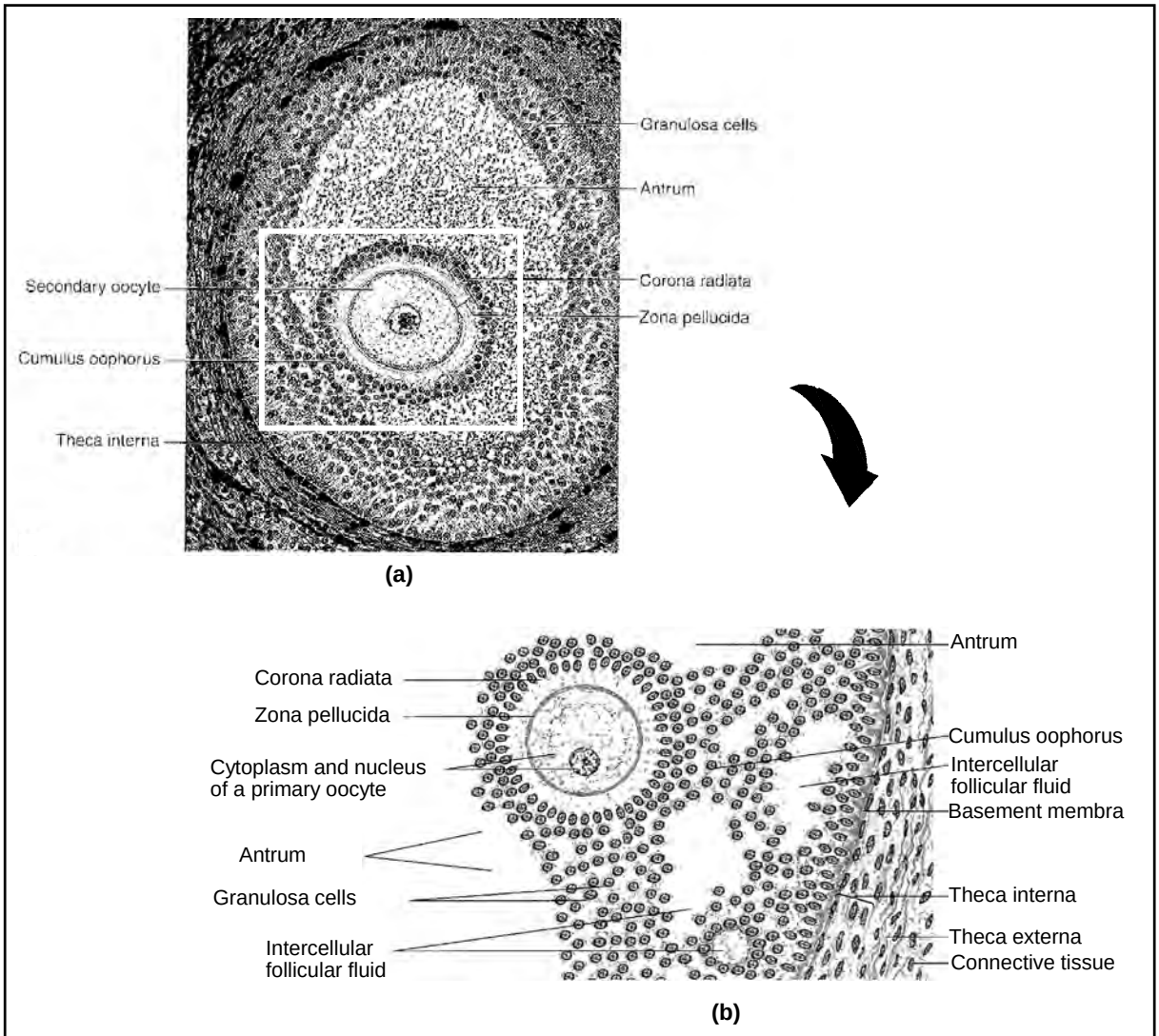

Fig. 3.7 : Structure of a graafian (mature) follicle. (a) A complete graafian follicle and (b) a part (inset) is magnified to show the elaborate structure of the egg with the associated follicle cells.

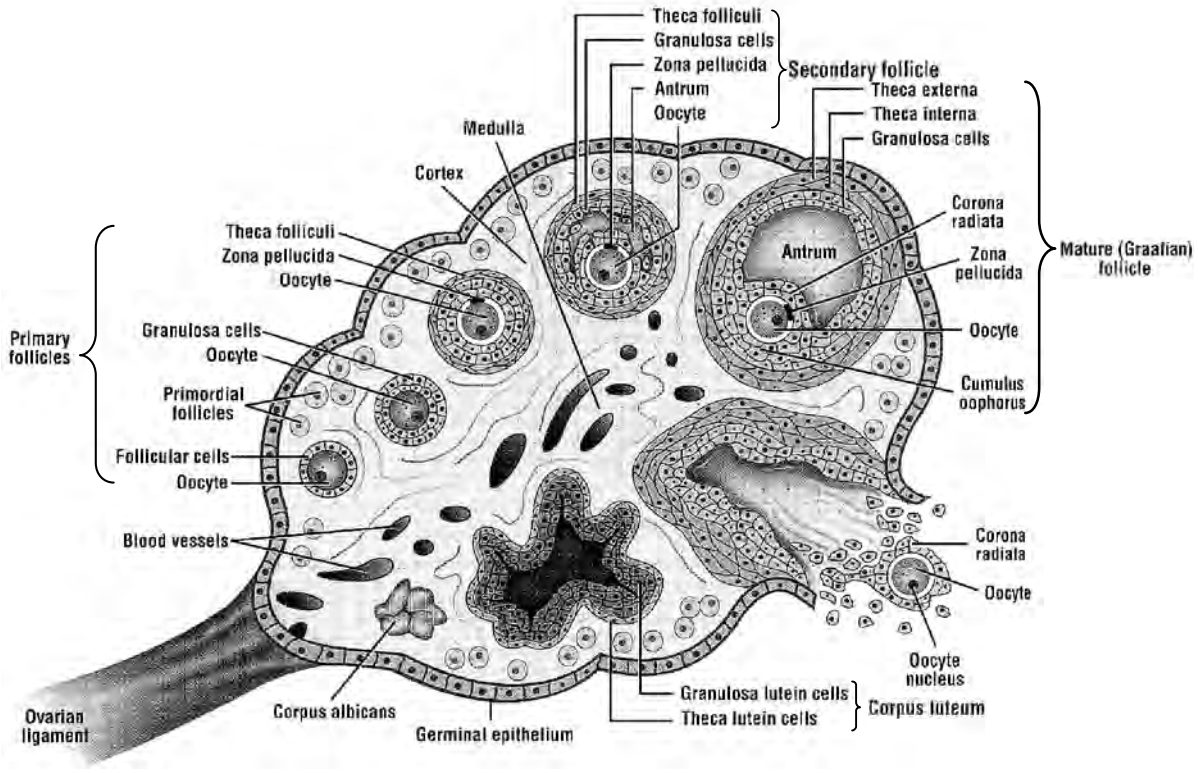

Fig. 3.8 : A diagrammatic cross section of the human ovary to show different stages of ovarian follicle development.

The primary follicle grows in size and a fluid, known as **liquor folliculi** (**follicular liquid**) accumulates between the granulosa cells. The fluid accumulates in a cavity, called **antrum**. Follicles with antrum are known as **antral** or **secondary follicles**.

As more liquor folliculi is synthesized, the antrum grows in volume and the granulosa cells segregate and some cells surround the oocyte and some others are displaced to the periphery. The oocyte with its surrounding granulosa cells remains attached to the peripheral cells by a hillock of granulosa cells. This structure is known as the **cumulus oophorus**. The theca interna cells are now surrounded by several layers of **theca externa** of stromal origin. This follicle is known as the **mature** or **graafian follicle** [Fig. 3.7(a) & (b)]. The arrested first meiosis is completed shortly before ovulation. Thus **the graafian follicle contains a secondary oocyte**. It ruptures and the secondary oocyte is released, which is caught by the fimbrae of the fallopian tube and then is transported to the uterus.

Following the rupture, the follicle fills with blood forming a **corpus haemorrhagicum**. The thecal and granulosa cells proliferate, become glandular and fill the antrum as **theca lutein** and **granulosa lutein cells**, respectively. These cells are collectively called luteal cells. The luteal cells are the source of **estrogens** and **progesterone**. This post-ovulatory

follicle is known as **corpus luteum**. Corpus luteum is an endocrine structure that secretes two steroid hormones, such as **estradiol** and **progesterone** and a peptide hormone, called **relaxin**. Relaxin helps maintain pregnancy by inhibiting the contraction of smooth muscles in the myometrium of the uterus. If fertilization occurs and pregnancy follows, the corpus luteum persists and there is no more menstruation. If there is no pregnancy, the corpus luteum degenerates into a structure called **corpus albicans**. In case of fertilization and pregnancy, the zygote undergoes further embryonic development and implants in the uterine epithelium (**endometrium**) through a structure called placenta. The placenta produces sufficient estrogen and progesterone and takes over the functions of the corpus luteum after the sixth week of pregnancy.

Most ovarian follicles do not reach to maturity. Only one follicle, termed as the **dominant follicle** progresses through the usual process of development and the egg is ovulated. Others undergo degeneration or **atresia** at one stage of development or other. Such degenerating follicles are known as **atretic follicles**, which are replaced by connective tissue.

3.4.2 Fallopian (Uterine) Tubes (Fig. 3.9):

A fallopian (uterine) tube extends from the ovary to the uterus. One end of the fallopian tube opens into the peritoneal cavity near the ovary. The other end opens into the uterus. It is divided into four segments: **infundibulum**; **ampulla**; **isthmus**; and **interstitial**

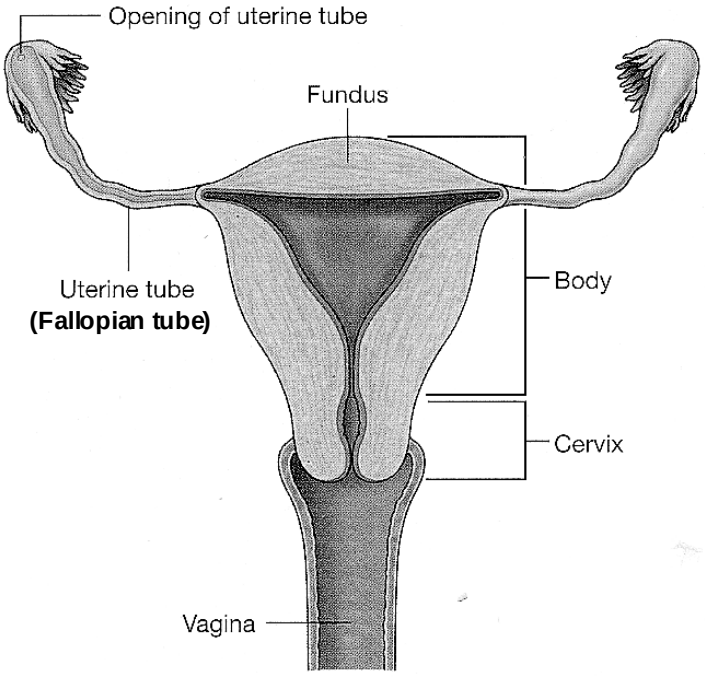

Fig. 3.9 : Structure of fallopian (uterine) tubes and uterus of human female.

region. The end, close to the ovary is a funnel shaped infundibulum. The margin of the infundibulum bears finger-shaped processes, called **fimbriae** (singular; fimbria). The infundibulum opens into a wider and longer ampulla. The isthmus is a short and narrow tube, joining the uterine tube to the uterus. The last part is known as interstitial region. It passes through the thick uterine wall to open into the uterine cavity.

3.4.3 Uterus (Fig.3.9) :

The uterus is a pear-shaped organ with a thick muscular wall. It is divided into three regions: the **body** or **corpus**; **fundus**; and **cervix**. The upper rounded part is the fundus. The body or corpus constitutes the major part of the uterus and the cervix is the terminal part. It opens into the vagina. The wall of the uterus is composed of three layers: **an outer perimetrium**; **a middle myometrium**; and **an inner endometrium**. The perimetrium is a single layer of squamous epithelial cells. The myometrium consists of thick layers of smooth muscle. The endometrium is the innermost layer and consists of simple cuboidal to columnar epithelium. The endometrium descends into the lamina propria forming numerous uterine glands. Functionally, the layer is divided into two layers: the luminal **stratum functionalis** and the basal **stratum basalis**. The stratum functionalis with the uterine glands and blood vessels is sloughed or cast off during menstruation. The basalis layer regenerates a new functionalis layer.

The blood supply plays an important role during menstruation. The uterine arteries supplying blood to the uterus, break up into **arcuate arteries**. These arteries spread circumferentially in the myometrium. **Straight** and **spiral arteries** from arcuate arteries supply the endometrium. The straight arteries are short and supply the basalis layer, while the spiral arteries are long and coiled supplying the functionalis layer. These arteries are very sensitive to changes in the estrogen and progesterone concentrations during the menstrual cycle. Blood supply to these vessels decreases and consequently, the functionalis layer degenerates and cast off.

3.4.4 Vagina (Fig. 3.5, 3.9 & 3.10) :

The uterus narrows to form the **cervix**, which opens into the tubular vagina. There is a plug of **cervical mucous** between the vagina and uterus. **The structures, namely the vagina, uterus, and fallopian tubes constitute the accessory sex structures of human female.** The vaginal opening is situated just posterior to the opening of the urethra. Both openings are covered by longitudinal folds: **inner labia minora** (singular; labium minorus) and **outer labia majora** (singular; labium majorus). An erectile organ, the **clitoris** is situated at the anterior margin of the labia minora.

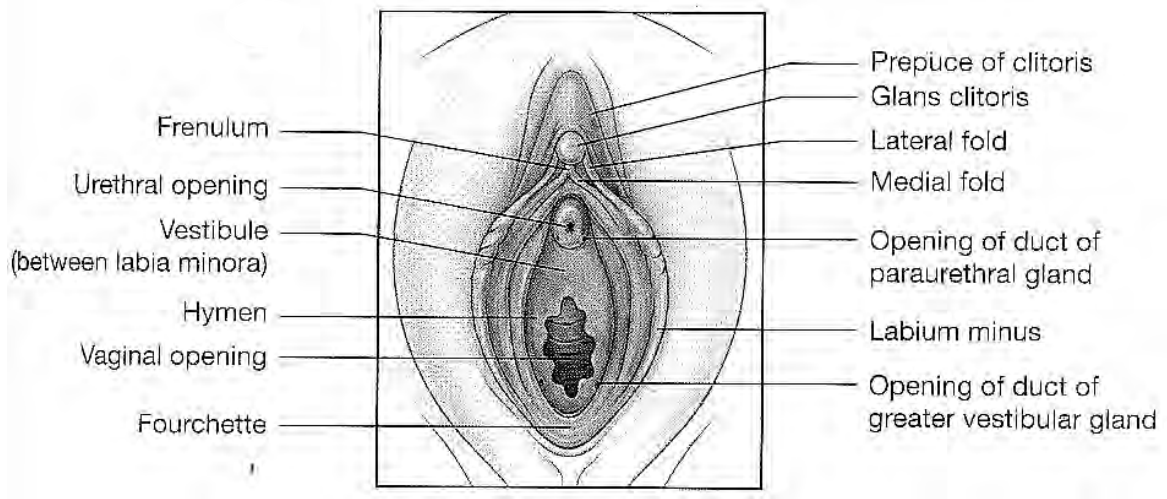

Fig. 3.10 : Frontal view of vagina with its associated structures.

3.4.5 Accessory Glands :

The glands, associated with female reproductive system are known as vestibular glands, which are of two types.

(i) **Lesser vestibular or paraurethral glands or glands of Skene :** These are numerous minute glands present on either side of the urethral orifice; homologous to male prostate and secrete mucous.

(ii) **Greater vestibular or Bartholin's glands :** These are paired glands, situated on each side of vaginal orifice; homologous to bulbo-urethral or cowper's gland of male and secrete viscous fluid that supplements lubrication during sexual intercourse.

3.5 GAMETOGENESIS :

The process by which the gametes are formed in the gonads of the sexually reproducing organisms is called **gametogenesis**. The sexually reproducing organisms contain two types of cells in their body : **somatic cells** and **germinal cells**. The germinal cells in the gonads multiply by mitosis and meiosis to produce gametes. The male gametes are known as spermatozoa or sperms and female gametes as ova or eggs. The formation of sperms in the testis is known as **spermatogenesis** and that of eggs in the ovary as **oogenesis**.

3.5.1 Spermatogenesis (Fig. 3.11) :

At puberty, the immature male germ cells, known as **spermatogonia** start producing sperms by spermatogenesis. A seminiferous tubule is lined by an epithelium known as germinal

epithelium. The epithelium consists largely of cuboidal cells, known as primodial germ cells and large columnar somatic cells called Sertoli cells or sustentacular cells. Spermatogenesis completes in two steps : (i) formation of spermatids (spermatocytogenesis) and (ii) transformatin of immotile spermatids into motile sperms (spermiogenesis).

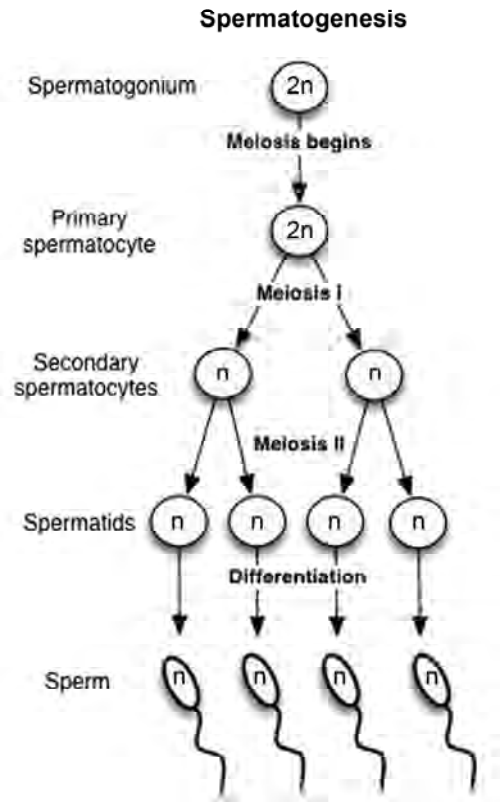

Fig. 3.11 : Three phases in spermatogenesis

3.5.1.1 Formation of Spermatids (spermatocytogenesis) :

It is completed in three phases :

(i) **Multiplication phase :** The undifferentiated primodial germ cells undergo repeated mitotic cell divisions that produce large number of sperm mother cells or spermatogonia. Each spermatogonium is diploid and contains 46 chromosomes.

(ii) **Growth phase :** Due to repeated mitosis, the cell size of spermatogonia are reduced. Some of them actively grow in size by obtaining nourishment from Sertoli cells. These cells are called **primary spermatocytes**.

(iii) **Maturation phase :** The primary spermatocytes periodically undergo meiosis. A primary spermatocyte completes the **first meiotic division** (**reduction division**) leading to the formation of two equal, haploid cells called **secondary spermatocytes** having 23 chromosomes each. Both secondary spermatocytes undergo second maturation division (equational division) to form four haploid **spermatids**.

3.5.1.2 Formation of Spermatozoa (Spermiogenesis) :

Each haploid spermatid is a typical immotile cell containing a haploid nucleus, cytoplasm and cytoplasmic organelles. The metamorphosis of spermatids into motile sperms occurs because a sperm possesses many structures which are not typical to a spermatid. All the structures confer motility to the sperm. After spermiogenesis, sperm heads are embedded in the Sertoli cell, and finally released from the seminiferous tubule by the process of **spermiation**.

Spermatogenesis starts at the onset of puberty due to significant increase in the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus. The increased level of GnRH then stimulates the anterior pituitary gland to secrete two gonadotropins : LH and FSH. LH acts on the Leydig dells and stimulates the synthesis and secretion of androgens, which in turn drive the process of spermatogenesis. FSH acts on the Sertoli cells and stimulates the secretion of some factors which help in the process of spermiogenesis.

3.5.1.3 Structure of a mature Sperm (Fig. 3.12) :

A mature sperm consists of four parts: **head**, **neck**, **middle piece** and a **tail**. A plasma membrane envelops the sperm. The sperm head contains an oval haploid nucleus, the anterior part of which is capped by structure called **acrosome**. The acrosome secretes enzymes that help dissolve the egg barriers during fertilization. The neck harbours a pair of centrioles, a proximal and a distal, both placed perpendicular to each other. The distal centriole continues through the middle piece to the tail, where it forms the core (axoneme). The middle piece contains mitochondria, which provide energy for motility. The tail is made by a bundle of microtubles in an arrangement, typical to a flagellum (9 peripheral doublets and two central singlets). This structure confers motility to the sperm. The human male ejaculates about 200 to 300 million sperms during a coitus. For normal fertility at least 60% of the sperms must have normal shape and size and at least 40% of them must show vigorous motility. Human sperm servive for 3-4 days in the female reproductive tract.

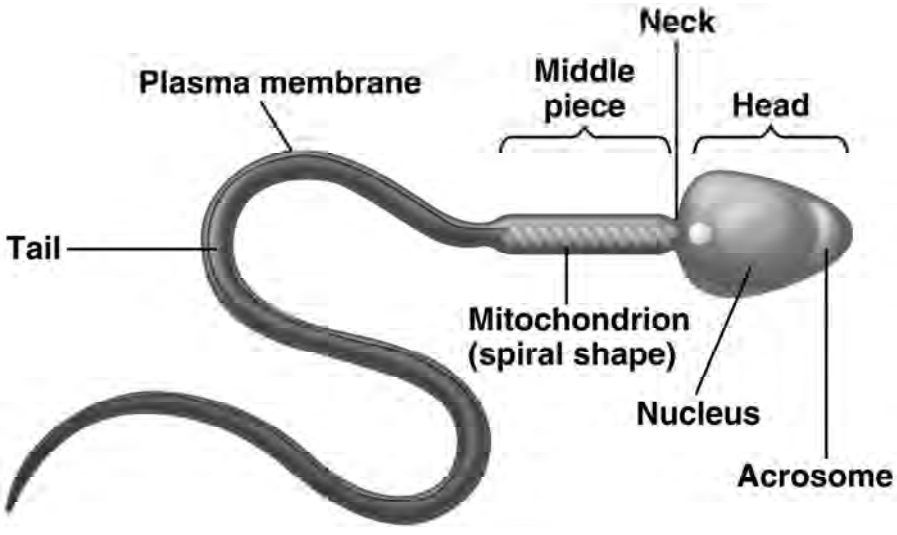

Fig. 3.12 : Structure of a mature human sperm

3.5.2 Oogenesis (Fig. 3.13) :

It occurs in the ovary. It is completed in the three usual phases like that of spermatogenesis : multiplication, growth and maturation phases.

3.5.2.1 Multiplication phase :

Oogenesis is commences during embryonic developmental stage, when some cells in the germinal epithelium of the ovary of the fetus divide by mitosis, producing undifferentiated germ cells called **egg mother cells** or **oogonia**. No more oogonia are formed after birth. The oogonia multiply by mitotic divisions, which project in to the stroma of the ovary as a chord, the **egg tube** of **pfluger**. The egg tube becomes a round mass called the **egg nest**. One cell in the egg nest grows and enters into the prophase-I of the meiosis and gets temporarily arrested at the diplotene stage, becoming the **primary oocyte**. Other oogonia in the nest form the follicular epithelium, round the primary oocyte to protect and nourish it. The structure thus formed is known as a **primary follicle**. A large number of these follicles degenerate during the phase from birth to puberty. At birth around 2.5 millions of primary follicles are found in each ovary but at puberty only 60,000-80,000 primary follicles are left. The rest degenerate, in a process called **follicular atresia**. The follicle that is destined to develop further is known as a **dominant follicle**. The primary oocyte in the follicle becomes the future ovum after passing through growth and maturation phases.

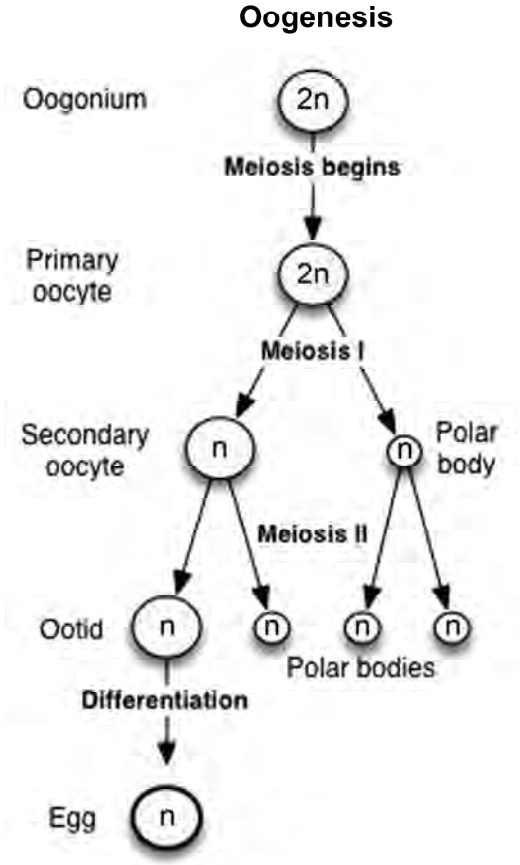

Fig. 3.13 : Three phases in oogenesis

3.5.2.2 Growth phase :

This phase is prolonged. It may extend over many years. The primary follicles get surrounded by more layers of **granulosa cells** and **theca cells** to become secondary follicles. A secondary follicle soon transforms into a tertiary follicle, which is characterized by a fluid filled cavity called **antrum**. The theca layer is organized into an inner **theca interna** and outer **theca externa**.

3.5.2.3 Maturation phase :

The primary oocyte undergoes an unequal first meiotic division and results in the formation of a large haploid **secondary oocyte** and a tiny **first polar body** or **polocyte**. The secondary oocyte retains bulk of the nutrient-rich cytoplasm of the primary oocyte. In the second

maturation division the first polar body may further divide or degenerate. The tertiary follicle further changes into the mature follicle or **graafian follicle** (Fig. 3.7). The secondary oocyte forms a new membrane called **zona pellucida**, surrounding it. The graafian follicle now ruptures to release the secondary oocyte from the ovary by the process called **ovulation**. The maturation of secondary oocyte is completed in the fallopian tube by an unequal second maturation division, which produces an **ootid** and a **second polar body**. The ootid undergoes a few changes and transforms into a mature ovum or egg, ready to be fertilized. The onset of second maturation division is triggered by the penetration of a sperm, a process known as **egg activation**.

3.5.2.4 Structure of Ovum (Fig. 3.14) :

The mature ovum or female gamete is spherical in shape. The human ovum is almost without yolk and hence is classed as **alecithal**. Its cytoplasm is called **ooplasm** containing a large nucleus termed as **germinal vesicle**. The nucleus contains a prominent nucleolus. The cytoplasm is enclosed by a plasma membrane. The membrane forming the surface layer of ovum is called **vitelline membrane**. The plasma membrane of the ovum is surrounded by a thick and noncellular **zona pellucida**. It is surrounded by a layer or two of follicle or nurse cells

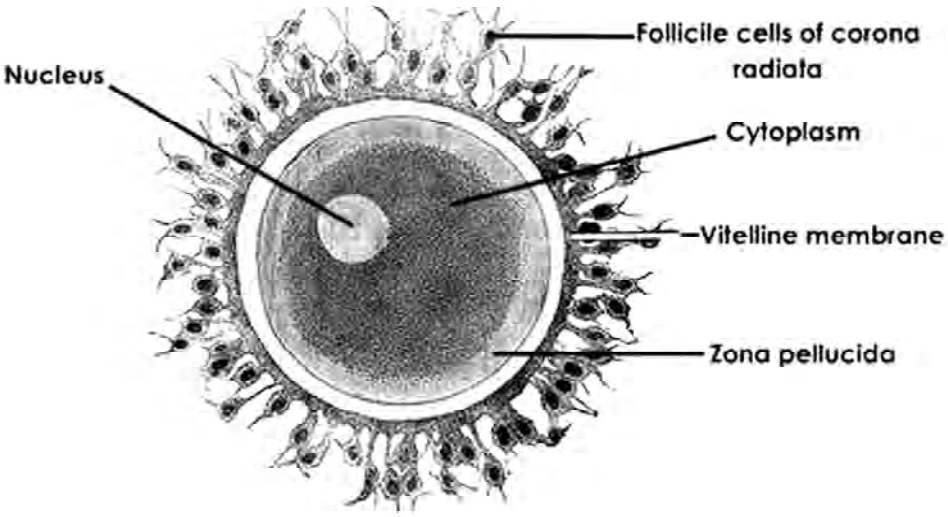

Fig. 3.14 : Structure of a mature human ovum

constituting **corona radiata**. Zona pellucida is secreted by the ovum itself and is thus, a primary egg membrane. Corona radiata, on the other hand, is a secondary egg membrane. A narrow **perivitelline space** is present between zona pellucida and plasma membrane. The side of the ovum which forms and extrudes polar bodies is termed as **animal pole** and the opposite is the called **vegetal pole**.

3.6 MENSTRUAL CYCLE (Fig. 3.15) :

Menstrual cycle is the cyclic changes in the female reproductive tract of primates (monkeys,apes and human). Menstruation is the bleeding from the uterus of an adult human female at an interval of one lunar month (28 days). The first menstrual cycle begins at puberty called **menarche** in girls at around 10-13 years. It is regulated by hormones of the hypothalamus, pituitary and ovary. The menstrual cycle consists of three phases :

1. Menstrual phase (3-4 days),
2. Proliferative phase (10-11 days),
3. Secretory phase or luteal phase (13-14 days).

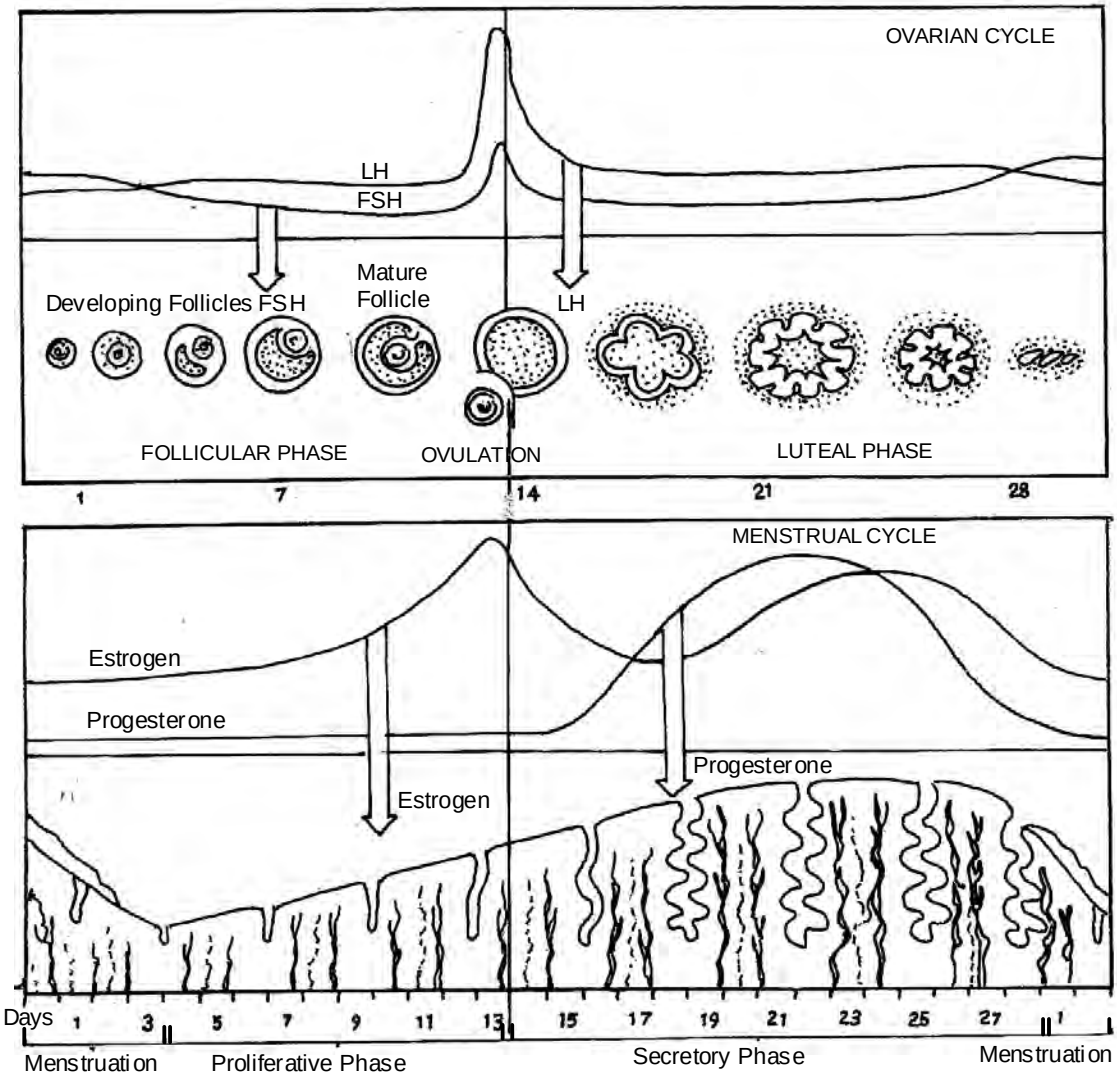

Fig. 3.15 : Correlation among pituitary gonadotropins, estrogen and progesterone levels; ovarian; and uterine endometrium changes during the menstrual cycle.

3.6.1 Menstrual phase :

The cycle starts, with a menstrual flow (bleeding), which lasts for 3-4 days. The menstrual flow results due to breakdown of endometrial lining of the uterus and its blood vessels. The blood with damaged tissue comes out through the vagina. Menstruation only occurs if the released secondary oocyte is not fertilized. Absence of menstruation may indicate pregnancy. The cycle is regulated by LH and progesterone.

3.6.2 Proliferative phase :

The menstrual phase is followed by the **proliferative phase** or **follicular phase**. During this phase, a primary follicle in the ovary grow to become a mature Graafian follicle and simultaneously the endometrium of the uterus regenerates through proliferation. These changes in the ovary and uterus are induced by changes in the level of pituitary gonadotrophins (LH and FSH) and estrogen. During this phase a rising level of FSH stimulates the growth of the ovarian follicle and secretion of estrogen. When the blood level of estrogen rises to a peak near the middle of the cycle, it gives positive feedback and stimulates more LH secretion. The LH stimulates ovulation. It occurs usually on the 14^{th} day or midway during the menstrual cycle which is called as the **ovulatory phase**.

3.6.3 Secretory phase :

During this phase the remaining part of the Graafian follicle transforms as a corpus luteum. The corpus luteum secretes large amount of progesterone which is essential for the maintenance of endometrium, necessary for implantation of the fertilized egg and other events of pregnancy. During pregnancy all other events of the menstrual cycle stop and there is no menstruation. Corpus luteum keeps growing for the first nine days after its formation, if fertilization does not take place corpus luteum starts regressing and ultimately transforms into a white body, called **corpus albicans**. This causes disintegration of the endometrium leading to menstruation, the beginning of a new cycle.

In human, menstrual cycle ceases around 45-50 years of age, termed as **menopause**. Cyclic menstruation is an indicator of normal fertility period in women and extends from menarche to menopause.

3.7 FERTILIZATION (Fig. 3.16) :

Fertilization is the act of union of a male gamete (spermatozoon or sperm) and a female gamete (egg or ovum) resulting in the formation of a zygote or fertilized egg. The union leads to the fusion of two gametic nuclei in a process called **amphimixis** or **syngamy**. In human, the semen is released by the male into the vagina (insemination) through sexual intercourse. The motile sperms swim rapidly, pass through the cervix, enter into the uterus and finally reach the ampullary-isthmic junction of the fallopian tube. The secondary oocyte released by the ovary is also transported to the ampullary-isthmic junction where fertilization takes place. Fertilization can only occur if the secondary oocyte and sperms are transported simultaneously towards the

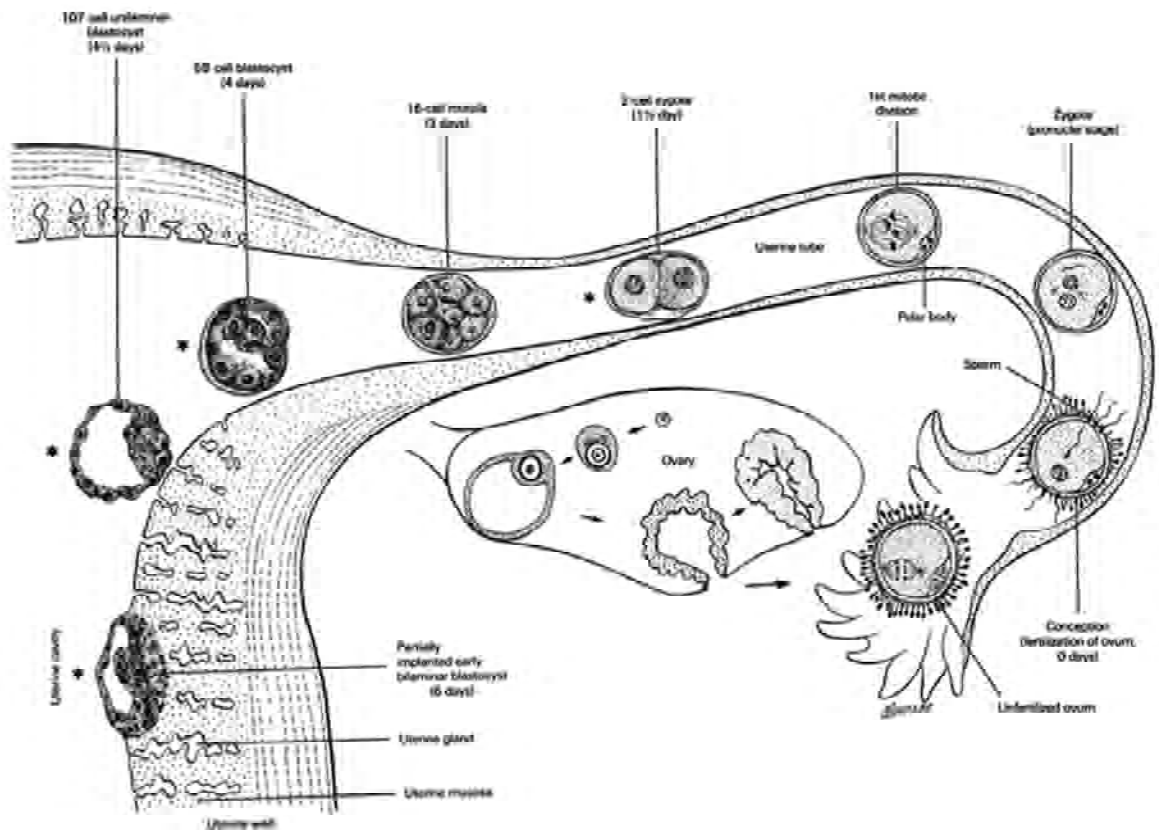

Fig. 3.16 : Embryonic development

junction. The secondary oocyte secretes a chemical substance called **fertilizin**. The sperm, too, secretes another chemical substance, anti-fertilizin. The fertilizin and anti-fertilizin of the same species are complementary and react in a reaction known as **agglutination reaction**. This is a mechanism to prevent the progress of large number of sperms towards the egg.

Before fertilization, sperm undergoes **capacitation** and **acrosomal reaction**. Acrosomal reaction involves the release of sperm lysins like (i) **hyaluronidase**, (ii) **corona penetrating enzyme (CPE)** and (iii) **zonalysin** or **acrosin** from the acrosome. All these enzymes digest the corona radiata and zora pellucida and facilitate the march of the sperm to the egg cytoplasm.

A sperm comes in contact with the zona pellucida layer of the secondary oocyte and induces a depolarization in the membrane that blocks the entry of additional sperms. This is known as fast block. Within minutes of the fast block, a slow block takes place by cortical reaction and the formation of a **fertilization membrane**.

The ovum is released from the ovary at the secondary oocyte stage. Meiosis II starts soon after the release, but it is temporarily arrested in metaphase II because of the release of MPF (M-phase Promoting Factor). The entry of sperm into the secondary oocyte restarts the cell cycle by breaking down MPF and turning on the APC (Anaphase Promoting Complex) and

completes the second meiotic division. The second polar body, thus formed is extruded and the ovum is ready for fertilization. The nucleus of the ovum now condenses and turns into a **female pronucleus**. At the same time the head of the spermatozoon separates off from the middle piece and tail, and transforms into a **male pronucleus**. The male and female pronuclei fuse to from the **zygote nucleus** (2n = 46 chromosomes).

3.8 CLEAVAGE BLASTOCYST FORMATION AND IMPLANTATION (Fig. 3.16 & 3.17) :

Immediately, the zygote undergoes **cleavage** to form 2, 4, 8, 16 daughter cells called **blastomeres** and develops into a **morula**. Morula moves from the fallopian tube into the uterus. The morula continues to divide and transforms into a **blastocyst**. The cavity of the blastocyst is called **blastocoel**. The blastomeres of the blastocyst are arranged into an outer layer called **trophoblast** and inner group of cells attached to the trophoblast called the **inner cell mass**. The trophblast layer becomes attached to the endometrium of the uterus and inner cell mass differentiates as the embryo. After attachment, epithelial cells lining the uterine cavity divide rapidly and cover the blastocyst. Now, the blastocyst is completely embedded in the endometrium. This is called **implantation**, which occurs 7 days after fertilization and leads to pregnancy.

3.9 PREGNANCY AND PLACENTA FORMATION (Fig. 3.17) :

After implantation, finger like projections appear on the trophoblast called chorionic villi which are surrounded by the uterine tissue and maternal blood. The chorionic villi and uterine tissues interdigitate with each other and jointly form a structural and functional unit between developing embryo (fetus) and uterine wall, called **placenta**.The placenta is of **haemochorial**, **metadiscoidal** and **deciduous type**.

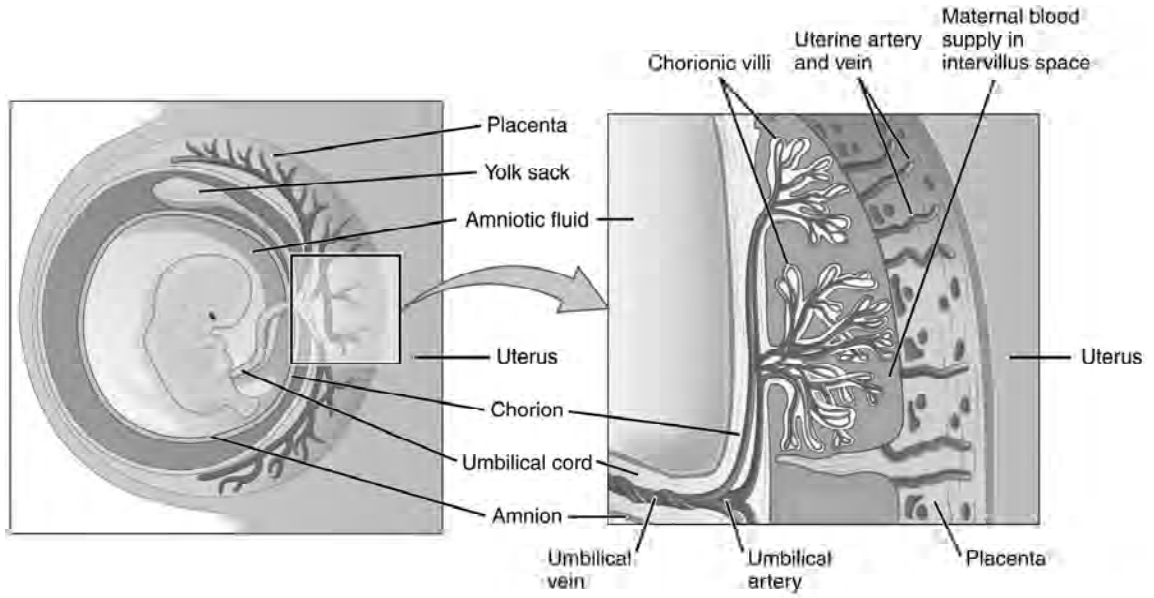

Fig. 3.17 : Implantation and placenta formation

The placenta facilitates the transprat of oxygen and nutrients to the fetus and removal of carbon dioxide and excretory waste materials from the fetus. The umbilical cord of the embryo connected to placenta helps in the transport of materials between the mother and the fetus.

The placenta also acts as an endocrine tissue and produces several hormones like **human chorionic gonadotropin** (hCG), **human placental lactogen** (hPL), estrogen, progesterone etc. In the later phase of pregnancy, a hormone called **relaxin** is also secreted by the placenta. Hormones such as hCG, hPL and relaxin are produced in women only during pregnancy. In addition, during pregnancy the levels of other hormones like estrogens, progesterone, cortisol, prolactin, thyroxine etc. are increased several fold in the maternal blood. Increased production of these hormones is essential for supporting fetal growth, metabolic changes in the mother and maintenance of pregnancy.

3.10 EMBRYONIC DEVELOPMENT (Fig. 3.16) :

Following implantation, the inner cell mass differentiates into an **outer layer called epiblast (ectoderm and mesoderm)** and the **inner layer called hypoblast (endoderm)**. This marks the begining of gastrulation. During gastrulation, which occures around 17 days following fertilization, mesodermal cells, present in the epiblast migrate and position as a layer called mesoderm between esctoderm and endoderm. This occures by the formation of a structure is known **primitive streak**.

These three layers give rise to different organs in the fetus (Table-3.1). Organ formation in the embryo takes place through two composite process known as **cell differentiation** and

Table - 3.1

Differentiation of germ layers into respective tissues and organs in human

Zygote → Blastula → Gastrula

Ectoderm	Mesoderm	Endoderm
Epidermis	Dermis	Gut
Cutaneous glands	Muscular tissue	Visceral organs
Nervous system	Connective tissue	Glands of stomach and intestine
(Brain and spinal cord)	Endoskeleton	Tongue
Eye (Retina, lens and cornea)	Vascular system	Lungs, trachea and bronchi
Nasal epithelium	Blood (heart and blood vessels)	Urinary bladder
Internal ear and external ear	Kidneys	Gills
Lateral line sense organ	Gonads	Liver and pancreas
Stomodaeum	Urinary and genital ducts	Thyroid gland
Proctodaeum	Coelom and coelomic epithelium	Parathyroids
Pituitary	Choroid and sclerotic coats of eye	Thymus
Pineal gland	Adrenal cortex	Eustachian tube
Adrenal medulla	Spleen	

organogenesis. The inner cell mass contains certain cells called stem cells which have the potentiality to differentiate into any type of cell. In human, after one month of pregnancy, the embryo's heart is formed. By the end of the second month of pregnancy, the fetus develops limbs and digits. By the end of twelve weeks (first trimester), most of the major organ systems have developed, for example, the limbs and external genital organs are well developed. The first movement of the fetus and appearance of hair on the head are usually observed during the fifth month. By the end of 24 weeks (second trimester) the fetus is covered with fine hair, eyelids separate and eye lashes are formed. By the end of nine months of pregnancy, the fetus is fully developed and is ready for delivery.

3.11 PARTURITION :

The average duration of human pregnancy is about nine months, which is called the **gestation period**. Vigorous contraction of the uterus at the end of pregnancy causes expulsion of the fetus. This process of delivery of fetus is called **parturition**. Parturition is induced by a complex neuro-endocrine mechanism. The signals of parturition originate from the fully developed fetus and the placenta, which induce mild uterine contraction called **fetal ejection reflex**. This triggers the release of oxytocin from the mother's pituitary gland. Oxytocin acts on the uterine muscle and causes stronger contraction, which in turn stimulates further secretion of oxytocin. The stimulatory reflexes between the uterine contraction and oxytocin secretion continues resulting in stronger contractions. This leads to the expulsion of the fetus out of the uterus through the birth canal. Soon after the baby is delivered, the placenta is expelled out of the uterus.

3.12 MAMMARY GLANDS AND LACTATION (Fig. 3.18) :

Human female has a pair of mammary glands, which develop after puberty. These glands proliferate during pregnancy and start producing milk towards the end of pregnancy by the process called **lactation**.

It is induced by hormones like **prolactin (PRL)** and **oxytocin** secreted from the mother's pitutary gland. Lactation helps the mother in feeding the newborn. The milk produced during the initial few days of lactation is called **colostrum**, which contains **immunogloblin (Ig A)**. Ig A passively immunizes the baby. Breast feeding during the initial period of infant growth is recommended by doctors. Thus, **breast feeding is best feeding**.

These are modified sweat glands that lie over the pectoral muscles. In the female, breasts are undeveloped until puberty. At puberty they begin to develop under the influence of estrogen and progesterone. Externally, the breast is covered with skin and has a nipple surrounded by a pigmented area, the areola. Each gland consists of glandular, fibrous and adipose tissues. The glandular part is constituted by 15-20 **lobes**. Each lobe is made up of a number of **lobules**, which end up in grape like clusters of milk secreting glands called **alveoli**

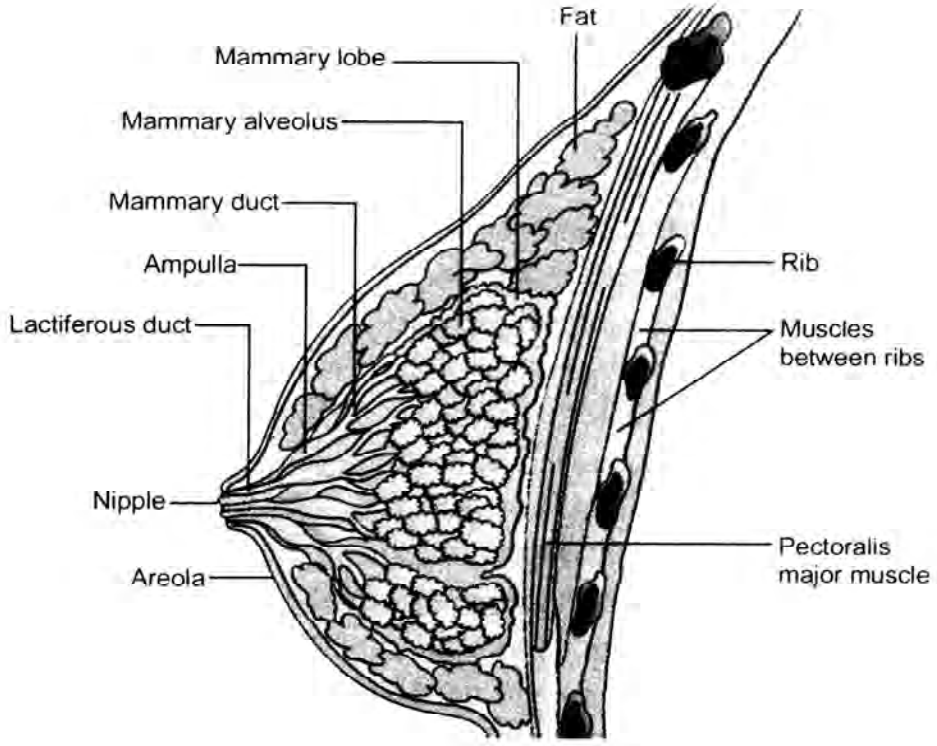

Fig. 3.18 : Structure of human mammary gland (breast)

(Fig. 3.18). When milk is produced, it passes from alveoli into the mammary lobules and then into mammary ducts. Near the nipple, each mammary duct expands to form a mammary ampulla, where some milk may be stored, before going to lactiferous ducts from which, it is secreted out. Fibrous tissue supports the alveoli and ducts. Fatty or adipose tissue is found between the lobes and covers the surface of the gland. The amount of adipose tissue determines the size of the breasts. Main function of mammary gland is secretion and ejection of milk. Milk production is stimulated by hormone prolactin and ejection of milk by the hormone oxytocin. Suckling of the baby acts as a reflex for the secretion of oxytocin from the posterior pituitary. Oxytocin brings about the contraction of the smooth muscle of the breast, which results in milk ejection.

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer :

(i) Which of the following is not a gonadotropin.
(a) FSH (b) hCG
(c) LH (d) Testosterone

(ii) Which of the following hormone is not a steroid.
(a) Ralaxin (c) Estradiol
(b) Progesterone (d) Testosterone

(iii) Which of the following is not secreted by the acrosome
(a) Hyaluronidase (c) Corona penetrating enzyme
(b) Zonalysin (d) Fertilizin

(iv) Blastocyst formation follows
(a) Fertilization (c) Spermiogenesis
(b) Gametogenesis (d) Cleavage

(v) Placenta secretes the hormone
(a) Testotorone (c) Oxytocin
(b) Human chorionic gonadotropin (d) Growth hormone

(vi) Fallopian tube is part of
(a) Ureter (c) Uterus
(b) Oviduct (d) Vas deferens

(vii) In human, fertilization usually occurs in the
(a) Vagina (c) Uterine cavity
(b) Cervix (d) Uterine tube

(viii) Which of the following is not a male secondary sexual character?
(a) Beard (c) Coarse voice
(b) Enlarged penis (d) Increased fat in the buttocks

(ix) The chief source of circulating estrogen is
(a) Theca interna (c) Theca externa
(b) Granulosa (d) Stroma

(x) Which of the following is not an accessory sex organ
(a) Testis (c) Bulbo-urethral gland
(b) Epididymis (d) Seminal vesicles

(xi) Delivery of a human baby following pregnancy is know as
(a) Ovulation (c) Abortion
(b) Parturition (d) Conception

(xii) Sertoli cells are regulated by
(a) GH (c) FSH
(b) LH (d) TSH

(xiii) Which of the following is a source of progesterone
(a) Corpus luteum (c) Corpus albicans
(b) Corpus spongiousm (d) Corpus haemorrgagicum

(xiv) Milk ejection form the breasts of a woman following the birth of a baby is stimulated by
(a) LH (c) GH
(b) FSH (d) Oxytocin

(xv) Find the mismatch
(a) Acrosome - Dissolution (c) Mitocondria - Energy production
(b) Tail - Nutrition (d) Centriole - Cleavage

2. Fill in the blanks with appropriate words :

(i) All but one X chromosomes in human female cells are condensed and inactive. Such X chromosmes are known as _____ .

(ii) The testis determining factor (TDF) is a polypetide, expressed by _____ gene present on the Y chromosome.

(iii) The factor responsible for the regression of the mullerian duct in the human male fetus is known as _____ secreted by _____ cell of the testis.

(iv) Gonadotropins (FSH and LH) are secreted from _____ .

(v) FSH stimulates the Sertoili cells to synthesize three polypeptides, namely inhibin, _____ and _____ .

(vi) The early development of the ovarian follicles is stimulated by _____ and estrogen.

(vii) Lutenzing hormone stimulates _____ cells of the testis.

(viii) The final maturation of the ovarian follicles and ovulation are stimulated by _____.

(ix) The prostatic fluid contains an acid called _____ .

(x) Prostate specific antigens (PSA) help in the diagnosis of _____.

(xi) The swollen tip of the penis is known as _____ .

(xii) The erectile tissue of the penis is constituted by _____ and _____ .

(xiii) The seminal vesicles discharge into vas deferens through _____ .

(xiv) The peritoneal fold by which the ovary is attached to the broad tigament is called _____ .

(xv) The primary oocytes are arrested at _____ stage of first meiosis until the onset of puberty.

(xvi) The layers of cuboidal follicular cells surrounding the primary oocyte constitutes _____ .

(xvii) Stromal cells, surrounding the granulose cells are known as _____ cells.

(xviii) The egg is ovulated at _____ stage.

(xix) the noncellular layer surrounding the primary oocyte is known as _____.

(xx) The inner epithelial lining of the uterus is known as _____ .

(xxi) The menstrual cycle spans _____ days and the ovulation occurs on the day _____.

(xxii) The secondary oocyte is arrested at _____ before fertilization.

(xxiii) Penetration of spermatozoon into the egg at fertilization triggers metaphase II in the secondary oocyte. This phenomenon is known as _____.

(xxiv) Following the failure of fertilization, the corpus luteum regresses into a structure called _____ .

(xxv) Corpus luteum is the main source of estrogen and _____ .

3. Answer the following in one word :

(i) Retention of testis in the abdominal cavity.

(ii) The canal through which the testis descends into the scrotum.

(iii) The plexus of blood capillaries that helps maintain the temperature of the testis for normal functioning.

(iv) The connective tissue capsule of the testis.

(v) The seminal fluid contains a manosaccharie as the energy source.

(vi) The passage through which both the urine and semen are discharged.

(vii) The glans penis is covered by a fold of loose skin.

(viii) The forcible explusion of semen through the urethra.

(ix) The low count of sperms in human semen.

(x) The muccopolysaccharide layer surrounding a primary ovarian follicle.

(xi) The hillock of granulosa cells connecting the granulosa cells surrounding the oocyte with the peripheral granulosa cells layer in a graafian follicle.

(xii) The loose mass of connective tissue, in which are present different stages of ovarian follicles.

(xiii) The regressing follicles and the act of regression.

(xiv) The uterine layer that is sloughed off during menstrual cycle.

(xv) The arteries of the uterine wall that undergo disintigration during the menstrual cycle.

(xvi) The height of LH secretion, 16-26 hours before ovulation.

(xvii) The tissue formed by the apposition of both the maternal and fetal tissues during pregancy.

(xviii) The modified sweat glands in the female that serve as the source of food for neonatal babies.

(xix) The fertilzin-antifertilizin reaction that stops the march of a large number of sperms towards the egg.

(xx) The penetration of the spermatozoon into the egg sets in a reaction in the cortical cytoplasm, which results in the formation of a fertilization membrane.

GROUP - B
(Short Answer-type Questions)

1. Answer the following within 50 words each :

(i) What are the disadvantages of asexual reproduction.

(ii) Explain sexual dimorphism.

(iii) How do gametes acquire haploid number of chromosomes?

(iv) Is a Y chromosome essential for the development of testis in human? Explain.

(v) What is the role of antimullerian hormone? Where is it secreted from?

(vi) Explain, what is puberty.

(vii) Name two gonadotropins. Where are these secreted from?

(viii) Describe the major role of LH in both male and female.

(ix) What is a cremasteric reflex?

(x) Explain the countercurrent heat exchange mechanism in human testis.

(xi) Describe the functions of the sertoli cells.

(xii) What is a blood testis barrier? How does it help the testis?

(xiii) What are the function of epididymis?

(xiv) Name five secondary sexual chracters in human male.

(xv) What do you mean by accessory sex organs? Give five examples in human male.

(xvi) What is the function of the prostate gland?

(xvii) What is the role of corpus luteum following fertilization and implantation?

(xviii) Explain LH surge.

(xix) What is spermiogenesis?

(xx) What is the role of acrosome in fertilization.

(xxi) Placenta is an endocrine tissue- explain.

(xxii) Enlist the hormones regulating menstrual cycle and mention the role of each.

(xxiii) What do you understand by follicular atresia ? Where does it occur ?

(xxiv) Where do the granulose and thecal cells originate from and what are their functions.

(xxv) How is the mammary glad hormonally regulated?

2. Write brief notes on the following :

(i) Secondary sexual characters

(ii) Accessory sex organs

(iii) Seminiferous tubule

(iv) Graafian follicle

(v) Corpus luteum

(vi) Prostate gland

(vii) Seminal vesicles

(viii) Bulbo-urethral gland

(ix) Blood testis barrier

(x) Luteal phase

(xi) Menopause

(xii) Gonadotropins

(xiii) Placenta

(xiv) Parturition

(xv) Spermiogenesis

(xvi) Lactation

3. Differentiate between :

(i) Sertoli cell and Leydig cell

(ii) Corpus luteum and Corpus hemorrhagicum

(iii) Follicular phase and Luteal phase

(iv) Antral follicle and Graafian follicle

(v) Granulose and Thecal cells

(vi) First maturation division and Second maturation division

(vii) Spermatogenesis and Oogenesis

GROUP - C
(Long Answer-type Questions)

1. Discuss about different methods of asexual reproduction in animal, as studied by you.
2. Describe the male reproductive system in human.
3. Describe the female reproductive system in human.
4. What is menstrual cycle? Describe the cyle in human with a reference to cyclic changes in the ovary and uterine endomtrium.
5. Draw a neat labeled diagram of the male reproductive system in human (Description is no required).
6. Draw a neat labeled diagram of the female reproductive system in human (Description is not required).
7. Draw a neat labeled diagram of the corss section through the human ovary (Description is not required).
8. Draw neat labeled diagram of seminiferous tubule (Description is not required).
9. Draw a neat labeled diagram of a graafian folicle (Description is not required).

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CHAPTER 4

REPRODUCTIVE HEALTH

Each year some eight million of the estimated 210 million women, who become pregnant, suffer from life-threatening complications related to pregnancy. In 2000, an estimated 529,000 women died during pregnancy and childbirth, 2.7 million infants are stillborn every year and 3 million die within the first seven days of life. Globally, the maternal mortality ratio has not changed over the past decade. Nintynine per cent of all maternal deaths occur in developing countries. The International Conference on Population and Development (ICPD) held in Cairo in 1994 declared that a reproductive health is a state of complete physical, mental and social well being and not merely the absence of disease or infirmity in all matters related to the reproductive system and to its functions and processes. Sexual and reproductive health is closely associated with socio-cultural factors, gender roles and the respect and protection of human rights.

Human rights issues pertaining to sexual and reproductive health that have been considered as fundamental in drafting this document includes :

- the right of all persons to the highest attainable standard of health,
- the fundamental right of all couples and individuals to decide freely and responsibly the number, spacing and timing of their children and to have the information and means to do so,
- the right of women to have control over and decide freely and responsibly on matters related to their sexuality,
- the right of men and women to choose a spouse and to enter into marriage only with their free and full consent,
- the right of acess to relevant health information and
- the right of everyone to enjoy the benefits of scientific progress and its applications.

The Government of India guarantees better healthcare services to all its citizens. The Government have implemented several welfare programmes to improve the healthcare services rendered to its citizens, especially to children and women with a view to minimizing mortality rate. Some of these welfare programmes are mentioned in a chronological order.

1952 - National Family Planning Programme

1977 - National Family Welfare Programme

1983 - National Health Policy

1985 - Universal Immunization Programme

1997 - Reproductive and Child Health Programme (RCH - I)

2002 - National Health Policy (Reviewed)

2005 - Reproductive and Child Health Programme (RCH - II)

2013 - Reproductive, Maternal, Newborn, Child and Adolescent Health Strategy (RMMCH+A)

2017 - National Health Policy (Reviewed)

The Ministry of Health and Family Welfare of Government of India with generous help of United States Agency for International Development (USAID) launched a five year (2012 - 2017) flagship programme of National Rural Health Mission (NRHM) in 2013 to address the major causes of mortality among women and children as well as the delays in accessing and utilizing the healthcare services. The programme is entitled as "A Strategic Approach to Reproductive, Maternal, Newborn, Child and Adolescent Health in India (RMNCH+A).

4.1 SEXUALLY TRANSMITTED DISEASES (STD) :

Sexually Transmitted Diseases (STDs) or Sexually Transmitted Infections (STIs) are infectious diseases that spread from person to person through intimate contact. STDs can affect human male and female of all ages and backgrounds, who are having intimate contact through sexual intercourse. It does not matter if they are rich or poor.

Unfortunately, STDs have become prevalent among teens, since teens are at more risk for getting STDs. It is important to be conscious about ones own protection. STDs are sometimes referred to as sexually transmitted infections, since these conditions involve the transmission of infectious organisms between two sex patners. More than 20 different STDs have been identified and about 19 million men and women are infected every year in the United States.

The infection may spread through any type of sexual contact involving external genitalia, the anus or the mouth. An infection may also spread through contact with blood during sexual activity. STDs are infrequently transmitted by any other type of superficial contact. However, people who share hypodermic needles markedly increase the chance to contract such diesases, especially Hepatitis B. Some diseases which are not officially documented as STDs (e.g., Hepatitis A, C and E) but still are infrequently noted to be transferred during sexual activity.

- ✶ STDs affect men and women of all ages and backgrounds, including children. Many states require that child protective services be notified if children are diagnosed with STDs.
- ✶ STDs have become more common in recent years, partly because people are becoming sexually active at a younger age, are having multiple partners and do not use preventive methods to lessen their chance of acquiring STDs.
- ✶ People can pass STDs to sexual partners, although themselves do not have any symptoms.
- ✶ Frequently, STDs may be present but express no symptons, especially in women (e.g., chlamydia, genital herpes, gonorrhea). This situation may also occur in men.
- ✶ Health problems and long-term consequences from STDs tend to be more severe in women than men. Some STDs can cause pelvic infections such as pelvic inflammatory disease (PID), which may cause a tubo-ovarian abscess. The abscess, in turn, may lead to scarring of the reproductive organs, which may result in an **ectopic pregnancy** (a pregnancy outside the uterus), infertility or even death of a woman.
- ✶ **Human papilloma virus infection** (HPV infection), an STD, is a known cause of **cancer of the cervix**.
- ✶ Many STDs can be transmitted from the mother to the baby before, during or immediately after birth.

4.1.1 Common STDs :

- ✶ Chlamydia
- ✶ Genital Herpes [caused by Herpes Simplex Virus (HSV)]
- ✶ Genital Warts
- ✶ Gonorrhea
- ✶ Hepatitis B [(caused by Hepatitis B Virus (HBV)]
- ✶ HIV and AIDS
- ✶ Pelvic Inflammatory Disease (PID)
- ✶ Pubic lice Infection (caused by Crabs)
- ✶ Syphilis
- ✶ Trichomoniasis

4.1.2 Prevention and Treatment :

It is much easier to prevent STDs than treating. The only way to completely prevent STDs is to abstain from all types of sexual contact. If some one is going to have intercourse, the best way to reduce the chance of getting an STD is by using a condom.

People who are in the habit of having frequent sexual contract should get regular gynaecological or male genital examinations. There are two benefits from this. First, these examinations give the doctor an opportunity to teach people about the consequences of contracting STDs and the methods of protecting themselves. Secondly, regular examinations give a better opportunity for an effective treatment, while the infections are in their earliest.

The visiting person needs to disclose all facts about his / her sexual contact to the physician without fear, so that the physician decides on a course of action. Consequently, the physician will prescribe an investigation and then an effective treatment schedule. If the person feels embarassed to visit a physician, with whom he / she is familiar, he / she may seek the assistance of experts by calling STD hotline operated by some national organisations. The experts will advice in respect of the STD clinics undertaking the investigation and treatment. In doing so a confidentiality about the identity of the person in question is maintained.

Not all infections in the genitalia are caused by STDs. Sometimes, people may express symptoms that are similar to those of STDs, although they have never had sex. For example, in girls, a yeast infection is sometimes confused with an STD. Males may worry about bumps on the penis that turn out to be pimples or irritated hair follides. It is therefore, important to visit a physician to solve many of the above mentioned problems.

4.2 BIRTH CONTROL :

Birth control is the regulation of the number of children of a couple through deliberate control of conception. It is carried out by family planning or family welfare measures. Family planning is a programme aimed at limiting the size of families through prevention of conceptions and spacing the birth of children. **Contraception** is a temporary or permanent measure that prevents pregnancy or conception. For using contraception, motivation is important. It is provided through mass media (e.g. television, radio, newspapers, magazines, hoardings and posters), books, lectures, school or college curriculam and personal contacts by family welfare and community health workers, students and educated persons.

Couple Protection is the process of bringing eligible couples under family planning programmes. The success is above 60% at present and is voluntary in nature. Family planning by contraceptive measures are of two types : (1) spacing and (2) teminal methods.

4.2.1 Spacing method :

These are temporary methods, which are used to postpone or space appropriately the birth of children. The spacing measures are (i) Barrier method (ii) Use of intrauterine devices (ii) Chemical methods (iv) Hormonal method, (v) Natural method and (iv) Medical Termination of Pregnancy (MTP).

4.2.1.1 Barrier Method :

There are mechanical devices which prevent the release of sperms into the vagina and hence their passage into the uterus. The common barrier methods are condoms, diaphragm, fem shield and cervical cap.

(i) Condom - It is a tubular latex sheath which is rolled over the penis during intercourse. The common brand provided by family welfare services is NIRODH. Other improved brands are also available in the medicine stores. The device also provides protection against sexually transmitted diseases including AIDS.

(ii) Diaphragm - It is a tubular rubber sheath with a flexible metal or spring ring at the margin which is fitted inside the vagina.

(iii) Fem shield (Female condom) - The device is a polyurethane pouch with a ring at either end. The inner ring is smaller and present at the inner closed end. The device covers the external genitalia as well as the lining of the vagina. Fem slield provides protection from sexually transmitted diseases.

(iv) Cervical cap - It is a rubber nipple, which is fitted over the cervix and is designed to remain there by suction. The device prevents the entry of sperms into the uterus.

(v) Vault cap - It is a hemispherica domelike rubber or plastic cap with a thick rim, which is meant for fitting over the vaginal vault over the cervix.

4.2.1.2 Intrauterine devices (IUD) or Intrauterine contraceptive devices (IUCD) :

These are devices made from plastic, metal or a combination of both, which are inserted into the uterus to prevent conception. IUCDs are called loops, spirals, rings, bows, shields, based on their shapes, IUCDs are of three types - inert, copper releasing and hormone releasing. The inert IUCDs are made up of polyethylene, impregnated with barium sulphate or stainless steel. The exact mechanism of inert IUCD contraception is not clear. However, It prevents conception in the following manner :

(i) There is impairment of sperm ascent.

(ii) There is a quick tubal motility resulting in the premature migration of the fertilised eggs into the uterus before it is ready for receiving it.

(iii) Histological and biochemical changes in the endometrium, which have gametotoxic and spermicidal effects.

Copper IUCDs are commonly called copper Ts having ionised copper. It slowly diffuses at the rate of some 50 mg / day. It has a local antifertility effect by bringing about the release of toxic cytokines. The device is to be replaced every 3-5 years when copper release slows down due to calcium deposition.

Hormone releasing IUCDs include progesterone IUCD and levonorgestrel IUCD. The devices release small quantities of hormones which suppress endometrial changes and cervical mucus, cause anovulation and insufficient luteal activity.

4.2.3.1 Chemical methods :

They are contraceptives which contain spermicidal chemicals. The chemical contraceptives are available in the form of **creams** (e.g., delfem), **jelly** (e.g., perception, volpar paste), **foam tablets** (e.g., aerosl foam, chlorimin T or contab). They commonly contain lactic acid, boric acid, citric acid, zinc sulphate and potassium permanganate. The contraceptives are introduced into the vagina prior to sexual intercourse. Sponge (Today) is a foam suppository or tablet containing nonoxynot-9 as a spermicide. It is moistened before use to activate the spermicidal effect. The device also absorbs the ejaculate.

4.2.1.4 Hormonal method :

These are hormones possessing contraceptive properties, usually employed by women for suppressing ovulation. Hormonal methods are three types : oral contraceptives (oral pills), non-oral hormonal contraceptives and emergency contraceptives.

(i) Oral contraceptives - The pills are taken orally for 21 days in a menstraul cycle starting from 5th day and ending on 25th day. However, it is advisable to repeat the course after a gap of 7 days, irrespective of the onset or nonset of menstruation. If a pill is missed, it should be taken, when one remembers, sometimes two at a time. This helps in maintaining the hormonal levels required for contraception. Hormonal pills act in four ways (a) inhibition of ovulation (b) alternation in the uterine endometrium to make it unsuitable for implantation (c) changes in cervical mucus secretion, impairing its ability to allow passage and transport of sperms and (d) inhibition of motility and secretory activity of fallopian tubes. Oral pills are of two types : **combined pills** and **mini pills**. Combined pills contain both estrogen and progestin (progesterone). They are synthetic products. Estrogen inhibits FSH secretion. Progestin inhibitis LH secretion. Progestin protects the endometrial lining from the adverse effects of estrogen. This hormone also changes cervical mucus secretion. The most commonly

used progestin is **levonorgestrel** or **desogestrel**. The most common estrogen is **ethinyl estradiol** or **menstranol**. In **multiphasic combined pill**, both oestrogen and progestin are present in nearly the same amount. (e.g. **Mala D**, **Mala L**). **Minipills** are progestin pills only, with no estrogen. These are taken daily without break.

(ii) **Non-oral Contraceptives -** These are of two kinds : **injectable** and **implant**.

(a) **Injectable contraceptives** - Two types of progestin preparations are used singly. They are **depot-medroxy progesterone acetate** (DMPA) with a dose of 150 mg every 3 months or 300 mg every 6 months and **norethisternone enanthate** (NET EN) with a dose of 200 mg every 2 months. **Cyclofem** and **mesigna** are combined injectable contraceptive which are given once every month. These contain progestin preparation (DMPA 25 mg or NET EN 50 mg) as well as oestradiol (5 mg).

(b) **Implants** - These are hormones containing devices which are implanted subdermally (bellow the dermis) for providing long term contraception. **Norplant** is a progestin only device having six small permeable capsules (34 mm × 2.4 mm) each having about 36 mg **levonorgestrel**. They are inserted under the skin in a fan shaped manner in side upper arm or fore arm through a small incision. Norplant remains effective for about 5 years. **Implanon** is a single rod-like device (40 mm × 2 mm) which is implanted through a wide bored needle. It contains about 60 mg of **3-keto desogestrel**. It remains functional for three years.

(iii) **Emergency contraception** - It is a treatment for unprotected sex, sexual assult, missed pills and other reasons which have a risk of pregnency. The drugs used in emengency contraception are called **morning-after pills**. These are also available in India under the family welfare programme since 2002-2003. Two **Ovral** tablets at the begining and two tablets after 12 hours do the needful. Other morning after pills are **noral**, **norgynon** and **ovidon**. An **antiprogesterone pill** (**mifepristone**) is a single pill treatment. Insertion of an IUCD within five days of unprotected sex prevents implantation.

4.2.1.5 Natural Methods :

These are methods, which donot require any device, medicine or religious sanction. Natural methods are of three kinds- safe period, withdrawal and breast feeding.

(i) Safe period (Rhythm Method) - Ovulation occurs roughly about in the middle of the menstrual cycle. The fertility period is upto 48 hours after ovulation. Avoiding sexual intecourse during the fertility period prevents conception.

Ovulation period can be known from the body temperature, as the temperature dips below average and then rises by around 1ºF, which is maintained for the rest part of the cycle.

Cervical mucous is slippery and can be drawn into a thread (spinnbarkeit test) when stretched between two fingers. Period prior to ovulation is safe. Period after fourth day of rise in temperature (or last positive spinnbarkeit test) is also considered safe.

(ii) Withdrawal Method (Coitus interruptus) - The method is based on withdrawal of penis before ejaculation.

(iii) Breast feeding - In the amenorrhoeic period after delivery, breast feeding prevents pregnancy.

4.2.1.6 Medical Termination of Pregnancy (MTP) :

Unwanted pregnancy can be terminated medically, provided it is carried out early during the first trimester. **Misoprostol** (**a prostaglandin**) alongwith **mifepristone** (**antiprogesterone**) is an effective combination. Vaccum aspiration and surgical procedures follow the treatment.

4.2.2 Terminal Methods of Family Planning :

These are permanent methods of family planning where there is no need of replacement or augmentation. The methods are surgical or operative procedures which block the passage of semen in males and ova in females. The techniques are also called **sterilisation procedures**. They are called **vasectomy** in males and **tubectomy** in females.

4.2.2.1 Vasectomy :

It is a surgical method of sterilisation of males. Vasa deferentia are blocked by cutting and occluding them so that sperms are unable to pass down the male reproductive duct.

(i) Conventional Vasectomy (Scalpel surgery) - Under local anasthesia, a transverse (1 cm) incision is made on the skin of the scrotum with the help of a scalpel over the area of vas deferens. Each vas deferens is exposed and cut. The two ends are separated and tied. A gap of 1-4 cm must be maintained between the two ends otherwise reunion may occur.

(ii) Non-Scalpel Vasectomy - In this case, instead of a scalpel, a dissecting foreceps and a ringed forceps are used. The skin is punctured and the vas deferens is taken out. It is occluded by removal of 1-2 cm followed by ligation of ends. Occlusion can also be achieved by heat and clips. Vasectomy is a reversible procedure as the two ends may rejoin to open the sperm passage.

4.2.2.2 Tubectomy :

It is a surgical procedure of female sterilization, where a part of both the fallopian tubes is excised or ligated to block the passage of ova through them. Tubectomy is performed by conventional transabdominal surgery, conventional laparoscopy and milaparotomy. In surgical procedure, the fallopian tubes are cut and the cut ends tied to prevent reunion. The procedure is reversible as the cut ends may rejoin. In laparoscopic procedure, sterilization is achieved by loop development and constricting the basal region of loop with the help of silistic ring.

4.3 AMNIOCENTESIS :

Amniocentesis (also referred to as amniotic fluid test or AFT) is a medical procedure used in prenatal diagnosis of chromosomal abnormalities and fetal infections, in which a small amount of amniotic fluid from the aminiotic sac is removed and sampled. The fluid contains fetal tissue. This tissue is separated and the chromosomes from the cells are karyotyped. The DNA is isolated from the cells and purified. It is then analyzed for genetic abnormalities. The fluid is also analyzed for the presence of abnormal metbolites, if any. Amniocentesis was first introduced by an American obstetrician, Fritz Friedrich Fuchs and Danish gastroenterologist, Polv Riis in 1956 for fetal sex determination. Another process known as **chorionic villus sampling (CVS)** can also diagnose these problems of the fetus. CVS was first performed by the Italian biologist Giuseppe Simoni in 1983. Now real-time ultrasound has been used in place of invasive procedures like that of amniocentesis, because it provides the safety to the fetus and yeilds accurate result.

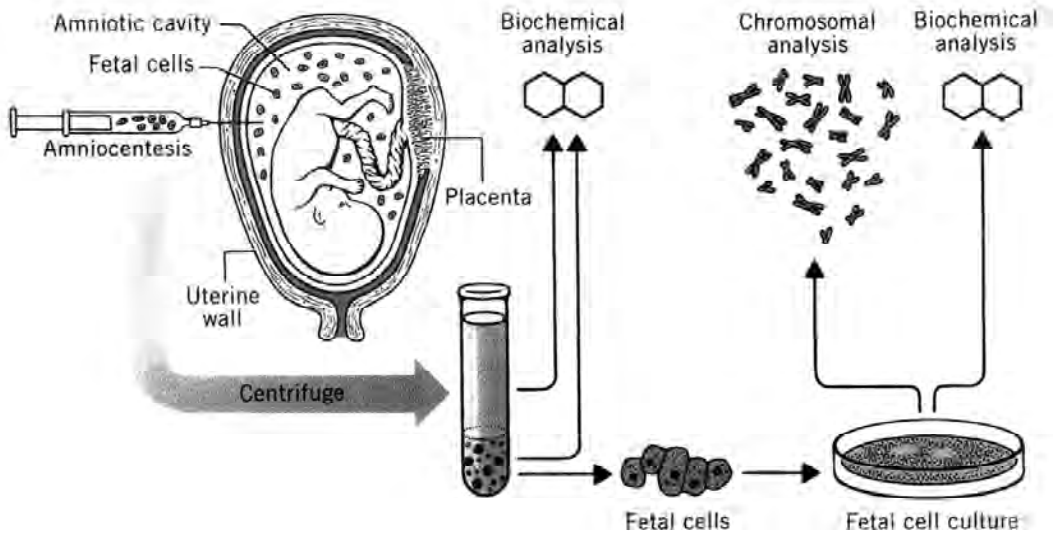

Fig. 4.1 : Practice of amniocentesis

Amniocentesis is performed for ascertaining the following :

- ✶ Abnormal genetic conditions, such as Down Syndrome and spina bifida.
- ✶ Baby's lungs are mature enough for birth.
- ✶ Evaluation of a baby for infections or illness.
- ✶ Rarely, amniocentesis is used to decrease the volume of the amniotic fluid.

Although amniocentesis can provide valuable information about the baby's health, the decision to pursue this invasive diagnostic testing is serious. It's important to understand the riks of amniocentesis and be prepared for the consequence, if any.

4.3.1 Procedure :

Before the start of the procedure, a local anesthetic is given to the mother in order to relieve the pain felt during the insertion of the needle used to withdraw amniotic fluid. After the local anesthetic is in effect, a needle is inserted through the mother's abdominal wall, then through the wall of the uterus, and finally into the amniotic sac. With the aid of ultrasonographic guidance, a physician punctures the sac with precision in an area away from the fetus and extracts approximately 20ml of amniotic fluid. Fetal cells are separated from the extracted sample. The cells are grown in a culture medium, then fixed and stained. Under a microscope the chromosomes are examined for abnormalities. The most common abnormalities detected are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Turner syndrome (monosomy XO). The puncture later heals and the amniotic sac replenishes the liquid over the next 24-48 hours.

Two types of amniocentesis are classed to diagnose two types of fatal abnormalities.

4.3.2 Genetic Amniocentesis :

Genetic amniocentesis is performed if :

- ✶ **A prenatal screening test yeilds a positive result :** If the results of a screening test, such as the first trimester screen or noninvasive prenatal testing are positive amniocentesis may be formed to confirm or rule out a diagnosis.
- ✶ **There was an abnormal chromosomal complement or a neural tube was defective in a previous pregnancy :** If a previous pregnancy was affected by Down syndrome or a neural tube defect, a serious condition affecting the brain or spinal cord in the present pregnancy might be at higher risk, too.
- ✶ **The age is 35 or more :** Babies born to women of 35 years and older have a higher risk of chromosomal disorder, such as Down syndrome.

- **There is a family history of a specific genetic condition, or the partner is a known carrier of a genetic condition.** In addition to identifying Down syndrome and spina bifida, amniocentesis may be used to diagnose many other conditions, such as cystic fibrosis.

4.3.3 Maturity amniocentesis :

Maturity amniocentesis can determine whether a baby's lungs are mature for birth. This type of amniocentesis is done only when premature delivery, either through induction or C-section is being considered to prevent pregnancy complications for the mother. It's usually done between 32 and 39 weeks of pregnancy. Earlier than 32 weeks, a baby's lungs are unlikely to be fully developed.

4.3.4 Safety concerns of amniocentesis :

On safety grounds, second-trimester amniocentesis has been found to be comparatively safer than early amniocentesis. According to global statistics, about one woman in every 100 having an amniocentesis experiences a miscarriage. The World Health Organisation suggests the risk of having a miscarriage or getting an infection following an amniocentesis will be lower if the procedure is done by an experienced practitioner at a hospital which undertakes these procedures as a routine.

4.3.5 Amniocentesis and stem cells :

Recent studies have discovered that amniotic fluid can be a rich source of multipotent mesenchymal, hematopoietic, neural, epithelial, and endothelial stem cells.

A potential benefit of using amniotic stem cells over those obtained from embryos is that the process skips ethical concerns among pro-life activists by obtaining pluripotent lines of undifferentiated cells without harm to the fetus or destruction of an embryo. These stem cells would also, skip the donor / recipient issue, if used to treat the same individual they came from.

Artificial heart valves, working trachea, as well as muscle, bone, heart, neural and liver cells have all been engineered through the use of amniotic stem cells. Tissues obtained from amniotic cell lines show promise for patients suffering from congenital diseases / malformations of the heart, liver, lungs, kidneys, and cerebral tissue.

The first amniotic stem cell bank is active in Boston, Massachusetts, USA.

4.4 INFERTILITY :

Infertility refers to an inability to conceive after having regular unprotected sexual intercourse. Infertility also refers to the biological inability of an individual to contribute to conception or to a female who cannot carry a pregnancy to full term. In many countries, infertility refers to a condition in which couples have failed to conceive after 12 months of regular sexual intercourse without the use of contraception.

4.4.1 Risk factors of infertility :

1. **Age -** A woman's fertility starts to drop after she is about 32 years old and continues doing so. A 50 year old man is usually less fertile than a man in his 20s (male fertility progressively drops after the age of 40)
2. **Smoking -** Smoking significantly increases the risk of infertility in both men and women. Smoking may also undermine the effects of fertility treatment. When a woman gets pregnant, she has a greater risk of miscarriage, if she is a smoker.
3. **Alcohol Consumption -** A woman's pregnancy can be seriously affected by alcohol consumption. Alcohol abuse may lower male fertility. Moderate alcohol consumption has not been shown to lower fertility in most men, but is thought to lower fertility in men who already have a low sperm count.
4. **Obese or Overweight -** In industrialized countries overweight (obesity) and a sedentary lifestyle are often found to be principal causes of female infertility. An overweight man has a higher risk of having abnormal sperms.
5. **Eating disorders -** Women who become seriously underweight as a result of dieting may have fertility problems.
6. **Being vegetarian -** If a person is a strict vegetarian he / she must make sure your intake of iron, folic acid, zinc and vitamin B-12 are adequate, otherwise, fertility may be affected.
7. **Over exercise -** A woman who exercises for more than seven hours every week may have ovulation problems.
8. **Sedentary life-style -** Leading a sedentary lifestyle is sometimes linked to lower fertility in both men and women.
9. **Sexually Transmitted Infections (STIs) -** Chlamydia can damage the fallopian tubes as well as influence man's scrotum. Some other STIs may also cause infertility.
10. **Exposure to some chemicals -** Some pesticides, herbicides, metals (lead) and solvents have been linked to fertility problems in both men and women.
11. **Mental stress -** Studies indicate that ovulation and sperm production may be affected by mental stress. If at least one partner is stressed, it is possible that the frequency of sexual intercourse is less, resulting in a lower chance of conception.

4.4.2 Causes of infertility in women :

4.4.2.1 Ovulation disorders :

Problems with ovulation are the common causes of infertility in women. Ovulation is the monthly release of an agg. Some women never release eggs, while some do not release eggs during cycles. Ovulation disorders can be due to :

- Premature ovarian failure - The ovaries stop functioning properly before 40 years of age.
- PCOS (Polycystic ovary syndrome) - The ovaries function abnormally. Such women have abnormally high levels of androgens. About 5% to 10% of women in the reproductive age range are affected by this syndrome.
- Hyperprolactinemia - If the prolactin level is high and the woman is not pregnant or breast feeding, it may affect ovulation and fertility.
- Poor egg quality - Eggs that are damaged or develop genetic abnormality cannot sustain a pregnancy. Older women are at a higher risk.

4.4.2.2 Problems in the uterus or fallopian tubes :

The egg travels from the ovary to the uterus (womb) through the fallopian tube, where the fertilization of the egg takes place. If there is something wrong in the uterus or the fallopian tubes the woman may not be able to conceive naturally. This may be due to :

- **Surgery -** Pelvic surgery may sometimes cause damage to the fallopian tubes. Cervical surgery may sometimes cause shortening of the cervix. The cervix is the neck of the uterus.
- **Submusosal fibroids -** Benign or non-cancerous tumours found in the muscular wall of the uterus, occurr in 30% to 40% of women in the child bearing age. They may interfere with implantation. They may also block the fallopian tube preventing sperm from fertilizing the egg. Large submusosal uterine fibroids make the uterus cavity bigger, increasing the distance the sperm has to travel.
- **Endometriosis -** Cells that are normally found within the lining of the uterus start growing elsewhere in the body.
- **Previous sterilization treatment -** If a woman has her fallopian tubes blocked, it is possible to reverse the process. But the chances of becoming fertile again are low.

4.4.2.3 Medications :

Some drugs can affect the fertility of a woman. These include :

- **NSAIDs (Non-steroidal anti-inflammatory drugs) -** Women who take aspirin or ibuprofen in a long-term may find it harder to conceive.

- **Chemotherapy -** Some medications used in chemotherapy can result in ovarian failure. In some cases, this side effect of chemotherapy may be permanent.
- **Radiotherapy -** If radiation therapy is aimed near the woman's reproductive organs, there is a higher risk of fertility problems.
- **Illegal drugs -** Some women who take marijuana or cocaine may have fertility problems.

4.4.3 Causes of infertility in men :

Abnormal semen is responsible for about 75% of all cases of male infertility. The following semen problems are possible.

- **Low Sperm count (oligospermia) -** Sperm count should be 20 million sperms / ml^3 of semen. If the count is under 10 million, there is a low spem concentration.
- **No sperm -** when the man ejaculates, there is no sperm in the semen.
- **Low sperm motility -** The sperms cannot swim as effectively as it should.
- **Abnormal sperm -** Sometimes the sperms have unusual structures, making these more difficult to swim towards the egg and fertilise it.

The following reasons may cause the semen to be abnormal :

- Testicular infection
- Testicular cancer
- Testicular surgery
- Overheating of the testicles
- Ejaculation disorders
- Undescended testicles
- Genetic abnormality
- Mumps
- Radio therapy
- Diseases like anaemia, diabetes, thyroid malfunctioning

4.4.4 Diagnosis of Infertility :

4.4.4.1 Tests for males :

1. **General Physical Examination -** The andrologist (physician specialised in male reproduction) may ask the man about his medical history, medications and sexual habits. The physician will also carry out an examination of his genitals. The testicles will be checked for lumps or deformities, while the shape and structure of penis will be examined for any abnormality.

2. **Semen analysis -** The semen sample will be analysed in a laboratory for sperm count, motility, colour, quality and infections.
3. **Blood analysis -** The blood is analyzed for testosterone and other male hormone concentrations.
4. **Ultrasound Test -** Any ejaculatory duct obstruction, retrograde ejaculation or other abnormal functioning will be assertained by untrasonography.
5. **Chlamydia test -** If the man is found to have chlamydia, which can affect fertility, he will be prescried antibiotics to treat it.

4.4.4.2 Tests for females :

1. **General Physical Examination -** The gynaecologist will interogate the woman about her medical history, menstrual cycle and sexual habits. She will also undergo a general gynaecological examination.
2. **Blood Test -** The blood sample is analyzed to check if the female hormone levels are correct and of the woman is ovulating.
3. **Hysterosalpingography -** A radio-opaque fluid is injected into the woman's uterus which shows up in the x-ray film. X-rays are taken to know about blockages in the uterus and fallopian tubes. This is followed by a surgery to reactify the problem.
4. **Ovarian Reserve Testing -** This is done to find out how effective the eggs are after ovulation.
5. **Pelvic Ultrasound -** Ultrasonography of the pelvic region of the female is undertaken to know about the normal structures of the ovary, uterus and fallopian tube.
6. **Thyroid function test -** According to National Health Service (UK), between 1.3% and 5.1% of infertile women have a thyroid malfunctions. This test is undertaken and the problem, if any, is corrected by medication.

4.4.5 Treatment options for infertility :

The treatment schedule depends on many factors, including the age of the patient, duration of infertility, personal preferences and the general state of health. Male sperms can survive inside the female reproductive tract for upto 72 hours, while an egg can be fertilized for upto 24 hours after ovulation.

4.4.5.1 Fertility treatment for men :

- **Erectile dysfunction or premature ejaculation -** Medication and / or behaviour approaches can help men with general sexual problems, resulting in improved fertility.
- **Blockage of the ejaculatory duct -** In case of blockage sperm can be extracted directly from the testicles and injected into an egg *in vitro* in the laboratory condition.
- **Retrograde ejaculation -** Sperm can be taken directly from the bladder and injected into an egg *in vitro*.
- **Surgery for epididymal blockage -** If the epididymis is blocked, it can be surgically repaired and sperms can be ejaculated properly.

4.4.5.2 Fertility treatment for women :

- **Ovulation disorders -** If a woman has an ovulation disorder, she will be prescribed with fertility drugs, which regulate or induce ovulation. These include Clomifene, Metformin, HMG, FSH, Human chorionic gonadotropin , Gn-RH, Bromocriptine etc.
- Surgical procedure for women

  1. **Fallopian tube surgery -** If the fallopian tubes are blocked or scarred, surgery may repair them, making it easier for eggs to pass through them.
  2. **Laparascopic surgery -** A small incision is made in the woman's abdomen. Laparoscope is inserted through the incision. In endometriosis, laparoscopy removes implants and scar tissue restoring fertility.

4.5 ASSISTED REPRODUCTIVE TECHNOLOGY (ART) :

Due to miscellaneous infertility problems (in both men and women), some women fail to conceive. However, research and investigations in this area have opened up new vistas for conception and giving birth to healthy babies. Several methods have successfully been practiced, which are collectively classed under Assisted Reproductive Technology (ART).

1. **Intrauterine insemination (IUI) -** A fine catheter is inserted through the cervix into the uterus to place a sperm sample directly into the utreus. This procedure may be done when ovulation occurs. The woman may be administered a low dose of lutenizing hormone (LH) before the practice to initiate ovulation.

   IUI is more commonly practiced, when the man has a low sperm count and decreased sperm motility. This procedure is also helpful for males, suffering from severe erectile dysfunction.

2. ***in vitro* fertilization (IVF) -** Among all ARTs, this technology has become more popular among couples for begetting children. Sperms are placed with unfertilized eggs in a petridish. Fertilization takes place *in vitro*. The embryo is then implanted into the uterus to begin a pregnancy. Sometimes the embryo is frozen for future use (cryopreservation). Before IVF is practiced, the female is administered with fertility drugs to induce ovulation and prepare the uterus for implantation.
3. **Donation of sperms or eggs -** If there is either no sperm or egg in one of the partners, it is possible to receive sperms or eggs from a donor. The egg is fertilized *in vitro* and transplanted into the uterus of a pseudopregnant woman.
4. **Assisted hatching -** This improves the chances of the embryo's implantation, to the wall of the uterus. An expert practitioner opens a small hole in the outer membrane of the embryo, known as **zona pellucida**. The opening improves the ability of the embryo to leave its shell and implant into the uterine lining. Patients who benefit from assisted hatching include women with previous IVF failure, poor embryo growth rate and older women.
5. **Electrical or vibratory stimulation to induce ejaculation -** Ejaculation is achieved with electrical or vibratory stimulation. This procedure is useful for men who cannot ejucaulate normally, such as those with a spinal cord injury.
6. **Surgical sperm aspiration -** The semen is removed from the male reproductive tract such as the vas deferens or epididymis.

4.5.1 *in vitro* fertilisation (IVF) :

in vitro fertilisation (IVF) is a process, by which an egg is fertilized by a sperm outside the body, under controlled laboratory conditions. The process involves monitoring a woman's ovulatory process, removing egg from the ovaries and allowing sperms fertilize the egg in a medium in the laboratory. The fertilized egg (zygote) is cultured for 2–6 days in a growth medium and is then transferred into the uterus of a pseudopregnant woman with the intention of establishing a successful pregnancy.

IVF techniques can be used in different types of situations. It is a technique of assisted reproductive technology for the treatment of infertility. IVF techniques are also employed in gestational surrogacy, in which case the fertilised egg is implanted into a surrogate mother's uterus. In some situations, donated eggs or sperms may be used. Some countries have surrogacy legislation in force, which prohibits women to act as surrogate mothers. Secondly, surrogacy is very expensive. These two barriers, have opened up a new area of tourism, i.e. **fertility tourism**. Less expensive and legislative regulation free surrogate mothers are hired for this purpose.

The first successful birth of a test tube baby, named Louise Brown, took place in 1978. Robert G. Edwards and Patrick Steptoe are credited with the birth of Louise Brown in London. For this invaluable work Robert G. Edwards was awarded Nobel prize in Psyology or Medicine in 2010. Patrick Steptoe could not be considered, since Noble Prize is not awarded posthumously. With egg donation and IVF, women in post-menopause period can still be pregnanat. Adriana Iliescu holds the record as the oldest woman to give birth to a child by using IVF and donated egg. She gave birth to a child in 2004 at the age of 66. After the IVF treatment many couples are able to get pregnant without any fertility treatments.

4.5.1.1 Practice of IVF :

Normally, an egg is fertilized in the woman's reproductive tract. If the fertilized egg transplants to the lining of the womb and continues to grow, a baby is born about nine months later. This process is called natural or unassisted conception.

IVF is a form of assisted reproductive technology (ART). Special medical techniques are used to help a woman become pregnant. It is most often tried when other, less expensive fertility treatments fail.

There are five fundamental steps in the IVF practice :

Step 1: Stimulation, also called super ovulation

- ✶ Medicines, called fertility drugs, are given to the woman to boost ovulation.
- ✶ Normally, a woman produces one egg per month. Fertility drugs stimulate the ovaries to produce several eggs.
- ✶ During this step, the woman will have regular transvaginal ultrasounds to examine the ovaries and blood tests be conducted to check hormone levels.

Step 2: Egg retrieval

- ✶ A minor surgery, called follicular aspiration, is done to remove the eggs from the woman's body.
- ✶ The surgery is done as an outpatient procedure in the practitioner's office most of the time. The woman is then administered with medicines to relieve the pain during the procedure. Using ultrasound images as a guide, the practitioner inserts a thin needle through the vagina and into the ovary containing mature eggs. The needle is connected to a suction device, which pulls the eggs with the fluid, one at a time.
- ✶ The procedure is repeated for the other ovary. There may be some cramping after the procedure, but it will go away within a day.
- ✶ In rare cases, a pelvic laparoscopy may be needed to remove the eggs. If a woman does not produce any egg, donated eggs may be used.

Step 3: Insemination and Fertilization

- The man's semen is placed together with the best quality eggs. The mixing of the sperm and egg is called insemination.
- Eggs and sperms are then stored in an environmentally controlled chamber. A sperm fertilizes an egg a few hours after insemination.
- The chance of fertilization is extremey low. In this case the sperm may be directly injected into the egg. This is called intracytoplasmic sperm injection (ICSI).
- Many fertility programs routinely practice ICSI on some of the eggs, although the situation appears normal.

Step 4: Embryo culture

- Following fertilization, the zygote undergoes cleavage and turns into an embryo. A laboratory technician is supposed to check the embryo regularly to make sure that it is growing properly. In 5 days time, a normal embryo having many actively dividing cells is formed.
- Couples who have a high risk of passing a genetic disorder to a child may consider pre-implantation genetic diagnosis (PGD). The procedure is done about 3 to 4 days after fertilization. Laboratory scientists remove a single cell from each embryo and screen its genetic material for specific genetic disorder.
- According to the American Society for Reproductive Medicine, PGD can help parents decide which embryos to implant. This decreases the chance of passing a disorder onto a child. The technique is not offered at all centers since it may raise ethical issues.

Step 5: Embryo transfer

- Embryos are placed into the woman's womb 3 to 5 days after egg retrieval and fertilization.
- The physician inserts a thin tube (catheter) containing the embryos into the woman's vagina and the through the cervix, up into the womb. The embryo will be transferred into the womb and if an embryo implants in the lining of the womb and grows, pregnancy is established.
- More than one embryo may be placed into the womb at the same time, which will lead to twins, triplets, or more. The exact number of embryos transferred is a complex issue that depends on many factors, especially, on the woman's age.
- Unused embryos may be frozen and implanted or donated at a later date.

4.5.1.2 Advantages of IVF :

IVF is done to help a woman become pregnant. It is used to treat many causes of infertility, including:

- ✶ Advanced age of the woman
- ✶ Damaged or blocked fallopian tubes
- ✶ Endometriosis
- ✶ Male infertility, including decreased sperm count and blockage
- ✶ Unexplained infertility

4.5.1.3 Risks and Disadvantages of IVF :

IVF involves a large quantium of physical and emotional energy, time, and money. Many couples, inflicted with infertility problems suffer from stress and depression.

A woman taking fertility medicines may have bloating, abdominal pain, mood swings, headache and other side effects. Many IVF medicines must be given by injection, often several times a day. Repeated injections may cause bruising.

In rare cases, fertility drugs may cause ovarian hyperstimulation syndrome (OHSS). This condition causes a buildup of fluid in the abdomen and chest. Symptoms include abdominal pain, bloating, rapid weight gain (10 pounds within 3 to 5 days), decreased urination despite drinking plenty of fluids, nausea, vomiting, and shortness of breath. Mild cases can be treated with bed rest. More severe cases require draining of the fluid with a needle.

Risks of egg retrieval include reactions to anesthesia, bleeding, infection, and damage to structures surrounding the ovaries, including the bowel and bladder.

There is a risk of multiple pregnancies when more than one embryo is placed into the womb. Carrying more than one baby at a time increases the risk of premature birth and low birth weight.

IVF is very expensive. Some, but not all, states have laws that spell that health insurance companies must cover a defined part of the IVF expenditure. But, many insurance plans do not cover infertility treatments. Fees for a single IVF cycle include costs for medicines, surgery, anesthesia, ultrasounds, blood tests, processing the eggs and sperm, embryo storage, and embryo transfer. The exact expenditure of a single IVF cycle varies, but may cost in the range of 6-9 lakh rupees.

4.5.1.4 Post-IVF and Embryonic Transfer Recomendations :

After the embryo transfer, the woman may be adviced to take rest for the remainder of the day. Complete bed rest is not necessary, unless there is an increased risk of OHSS. Most women return to normal activities the next day.

Women, who undergo IVF are prescribed to take daily doses of progesterone for 8 to 10 weeks after the embryo transfer. Progesterone is a hormone produced by the ovaries that

helps prepare the lining of the uterus for implantation. Less progesterone than the prescribed dose during the early weeks of pregnancy may lead to a miscarriage.

About 12 to 14 days after the embryo transfer, the woman needs to return to the clinic so that a pregnancy test can be undertaken.

The health care provider must be informed instantly, if the IVF and embryo transfer beneficiary has :

- A fever over 100.5°F (38°C)
- Pelvic pain
- Heavy bleeding from the vagina
- Blood in the urine

4.5.1.5 Outlook (Prognosis) :

Statistics of success varies from one clinic to another.

- Pregnancy rates reflect the number of women who became pregnant after IVF. But not all pregnancies result in live births.
- Live birth rate reflects the number of women who give birth to live babies.

According to the Society of Assisted Reproductive Technology (SART), the approximate chance of giving birth to a live baby after IVF is as follows:

- 41% to 43% for women under the age of 35 years.
- 33% to 36% for women in the age group of 35 to 37 years.
- 23% to 27% for women in the age group of 38 to 40 years.
- 13% to 18% for women at the age of 41 years and more.

4.5.2 Zygote Intra-fallopian Transfer (ZIFT) :

Zygote Intrafallopian Transfer (ZIFT) is an infertility treatment used ,when a blockage in the fallopian tubes prevents the migration of sperms to the egg. Eggs are removed from ovulating woman's ovaries, and *in vitro* fertilised. The resulting zygote is placed into the fallopian tube by the use of laparoscopy. The procedure is a spin-off of the gamete intrafallopian transfer (GIFT) procedure. ZIFT has a success rate of 64.8%.

4.5.3 Gamete Intrafallopian Transfer (GIFT) :

Gamete Intrafallopian Transfer (GIFT) is an assisted reproductive technology to counter infertility. Eggs are removed from a woman's ovaries, and placed in one of the fallopian tubes, along with the man's semen. The technique, first attempted by Steptoe and Edwards and later pioneered by endocrinologist Ricardo Asch, allows fertilization to take place the woman's uterus. The zygote then implants and the woman becomes pregnant.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer :

(i) The method of directly injecting a sperm into an ovum is assisted by reproductive technology called :

(a) GIFT (b) ZIFT
(c) ICSI (d) ET

(ii) Intensity lactating mothers do not generally conceive due to the :

(a) Suppression of gonadotropins (b) Hypersecretion of gonadotropins
(c) Supression of gametic transpat (d) Suppression of fertilization

(iii) Which is not a spacing method of family planning ?

(a) Natural method (b) Terminal method
(c) Chemical method (d) Hormonal Method

(iv) Intrauterine devices (IUD) are not made up of :

(a) Plastic (b) Metal
(c) Rubber (d) Plastic and metal

(v) Creams, Jelly and foam tablets are chemical contraceptions of which methods of birth control ?

(a) IUD (b) Chemical method
(c) Hormonal method (d) Natural method

(vi) Which is not a constituent of chemical method ?

(a) Lactic acid (b) Boric acid
(c) Malic acid (d) Citric acid

(vii) Which is not a method of tubectomy ?

(a) Conventional transabdominal surgery (b) Conventional laparotomy
(c) Implants (d) Milaparatomy

(viii) In which type of pill, both oestrogen and progestin are present in nearly the same amount ?

(a) Monophasic combined (b) Multiphasic combined
(c) Mini (d) Antiprogesteron

(ix) Which is not a type of Intrauterine device (IUD) ?

(a) Vaginal vault (b) Loops
(c) Spirals (d) Ts

(x) Which is not a common type of Sexually Transmitted Disease (STD) ?
(a) Genital warts (b) Syphilis
(c) Cancer (d) Gonorrhea

(xi) Which is a fertility treatment for men ?
(a) Intra Uterine Insemination (b) Erectile dysfunction
(c) Assisted hatching (d) In-Vitro fertilisation

(xii) Emergency contraceptive are effective if used within :
(a) 72 hrs. of coitus (b) 72 hours of ovulation
(c) 72 hrs. of menstruration (d) 72 hours of implantation

(xiii) The correct surgical procedure as contraceptive method is :
(a) Ovariectomy (b) Hysterectomy
(c) Vasectomy (c) Castration

2. Fill in the blanks :

(i) The scientific study of human population is called _____
(ii) In India, the sex-ratio of 1:1 is found in _____
(iii) The common brand provided by family welfare services is _____
(iv) Fem shield is otherwisely known as female _____
(v) Loops and bows are the type of _____
(vi) Copper Ts has a local _____ effect.
(vii) Sponge (Today) is a foam suppository or tablet containing _____ as spermicids.
(viii) The exampl of chemical contraceptive in the form of cream is _____
(ix) An antiprogesterone pill _____ is a single pill treatment for oral contraceptive.
(x) The sterilisation produre in males is called _____ and in females is called _____
(xi) _____ and _____ are combined injectable contraceptives.
(xii) Human _____ infection is a known cause of cancer of the cervix.
(xiii) The method of preserving sperm in frozen condition is called _____
(xiv) The monthly release of eggs is called _____
(xv) The ejaculatory duct obstruction is males is confirmed by _____
(xvi) Fertility treatment with donor eggs is usually done using _____

3. Answer each of the following in one word or a few words :

(i) In which state of India, the sex-ratio is favourable for females ?
(ii) Which device provides protection against sexually transmitted diseases including AIDS ?

(iii) Which device prevents the entry of sperms into the uterus ?

(iv) Which toxic substance is released by local antifertility effect of copper Ts ?

(v) What activities are caused due to hormone releasing IUDs ?

(vi) The chemical method of birth control absorbs what ?

(vii) STDs can be considered as self-invited diseases– comment.

(viii) Mention the primary aim of the "Assisted Reproductive Technology" (ART prgramme.

(ix) What is the significance of progeteron-estrogen combination as a contraceptive method?

(x) Males whose testes fail to descend to the scrotum are generally infertile, why?

(xi) Name the process of bringing eligible couples under family planning measures.

GROUP - B
(Short Answer-type Questions)

1. Differentiate between the two words in the following paris :

(i) Vasectomy and Tubectomy

(ii) Spacing mehod and Terminal method

(iii) Chemical method and Natural method

(iv) Safe period and unsafe period

(v) Conventional vasectomy and Non-Scalpel vasectomy

2. Answer the following in one or a few sentences :

(i) Mention the different barrier methods of family planning.

(ii) What are the different types of IUDs ?

(iii) Why copper Ts are to be replaced every 3-5 years ?

(iv) What changes occur in the endometridium due to invert IUD contraception ?

(v) What are the hormone releasing IUDs ?

(vi) What are the different types of hormonal methods of family planning ?

(vii) What are the morning - after pills of oral contraceptive ?

(viii) Mention the different natural methods of birth control.

(ix) What are the different ways of tubectomy ?

(x) What are the different diseases, which show no symptoms ?

(xi) What are the STDs in women, which show no symptoms ?

(xii) What is ''Ectopic pregnancy'' ?

(xiii) Name the different types of semen problems.

GROUP - C
(Long Answer-type Questions)

1. Discuss the mode of action and advantages / disadvantages of harmonal contraceptives.
2. What are advantages of natural methods of contraceptive over artificial methods?
3. Why are the Assisted Reproductive Techniques practised to help infertile couples ? Describe any three techniques.
4. Give an account of medical termination of pregnancy (MTP).
5. Discuss the spacing method and intra-uterine devices of family planning.
6. Give an account of risk factors of infertility.
7. Describe the various causes of infertility in women.
8. What is sexually transmitted diseases (STDs) ? Give an account of the prevention and treatment of STDs.
9. How infertility in men and women can be diagnosed by tests ?
10. Describe the treatment options for infertility in men and women.

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UNIT - II : GENETIC AND EVOLUTION

CHAPTER 5

HEREDITY AND VARIATION

Like begets like is an important and universal phenomenon of life. The living beings produce offsprings of their own kind. The young ones resemble their parents and also with each other; yet there are considerable variations amongst themselves. The people from different parts of the world differ in appearance and people from one family have many characters in common. These similarities and variations in characters pass from one generation to another. The process of transmission of characters through generations is known as **heredity**. The degree of differences among the offsprings, and between the offsprings and parents is known as **variation.** The science dealing with the study of heredity and variation is known as **genetics** (**Bateson,** 1905).

5.1 EARLY CONCEPT OF HEREDITY :

For many years, philosophers and scientists attempted to understand and explain the science of heredity. Many ideas and views were put forward from time to time. Greek philosophers suggested that “elements” from all the body parts of both the parents were passed directly to the offsprings. **Hippocratus** called these reproductive materials as “**Gonos**”-meaning seeds. **Charles Darwin**, (1868) proposed that all body parts of parents excrete microscopic granules or “**gemmules**” which pass directly to the offsprings. All these views support that the characters of the parents got mixed or amalgamated in the offsprings.

In 1760, **Koelreuter**, a German botanist carried out breeding experiment in two varieties of tobacco plants and concluded that characters never got amalgamated in the offsprings. In 1790, **T.A.Knight** crossed two varieties of common garden pea *(Pisum sativum)* plants and concluded that some characters appeared in more numbers than others in the offsprings**.** **John Goss**, 1822, crossed two varieties of garden pea plants and found that the parental characters again appeared when the hybrids were self pollinated. These experimental results proved that, the earlier concept of mixing of parental characters in the offsprings was wrong. The findings were further corroborated by **Naudin** in 1862. He commented from his breeding experiments in garden pea that on repeated crossings of hybrids, the parental types appeared in the offsprings showing that the hybrids contained the parental characters without mixing or amalgamation, though they were not externally visible.

The first scientific study to understand the principle of heredity was carried out by an Austrian monk **Gregor Johann Mendel** (Fig. 5.1) in garden pea plants. Though many workers

had earlier conducted breeding experiments in pea plants they could not arrive at any conclusion. Where others failed, Mendel succeeded as he planned and conducted his experiments in scientific manner keeping all the mathematical records of his experiment and analyzing those statistically. For his pioneering work in this field, he is known as the **father of genetics**. Born to a peasant family in 1822, Mendel was educated in a monastery and went for higher studies in science and mathematics to the University of Vienna. After his return from the University, he joined the monastery of Brunn as a monk and spent the rest of his life there eventually becoming an abbot. In the garden of the monastery, Mendel started his breeding experiments on pea plants in the year 1856 and continued the experiments for eight years. He submitted his findings to the Natural Society of Brunn in (1865) in a paper titled “Experiments in Plant Hybridization” which was published in the Proceedings of the Society in 1866. His findings were not accepted by the then scientific communities and Mendel died in 1884 perhaps considering himself a failure. During his life time, Mendel did not receive the recognition for his work which he deserved. His works were rediscovered in 1900 when three workers independently established Mendelism. They were a Dutch, **Hugo de Vries**, a German, **Carl Correns**, an Austrian, **Erich Von Tschermark**. After that, Mendel’s observations were divided into a fundamental generalization and two laws of inheritance.

5.2 MENDELIAN INHERITANCE :

Mendel’s Breeding Experiment :

For his experiment, Mendel selected the same garden pea plant which Knight and many others had studied earlier. This choice was good for several reasons:

Fig.5.1 : G.J.Mendel (1822 - 1884)

1. Pea plants are small and easy to grow. They have relatively short generation time.
2. Flowers are bisexual and self pollinated. The sex organs are very well enclosed within the flower.He could allow the flowers either for self pollination or cross-pollination as per the requirements of the experiment.
3. Many varieties of pea plants showing alternative forms of characters, (Tall plant and Dwarf plant, Yellow or Green seeds) were naturally available. Mendel carefully selected only seven pairs of contrasting or alternative characters (Fig. 5.2).
4. Many earlier breeders had produced hybrids by crossing different varieties of pea plants with alternative characters. Hence, from the very beginning, Mendel expected separation of characters in the offsprings.

5.2.1 Working Methods :

(i) Mendel carefully carried out preliminary investigations to familiarize himself with experimental specimens.

(ii) He considered only one or a few specific differences between the plants used at a time and ignored countless other differences. He only considered the differences that could be compared easily like the height of the plant or colour of the cotyledons etc.

(iii) He took special care to avoid undesirable cross-pollination.

(iv) He kept statistical records of all his findings.

(v) He collected sufficient data for analysis.

Mendel usually carried out his breeding experiments in three stages :

1. **Obtaining Pure-lines:** He allowed pea plants of a given variety (say tall plants) to produce progeny by self pollination for several generations. By doing so, he ensured that the progeny produced by a tall plant are all tall plants. Such plants which produce similar progeny for a particular character are called pure-lines or true breeding or pure breeding for that particular character. For example, a plant can be pure breeding or true breeding or pure-line for tall or for round seed.

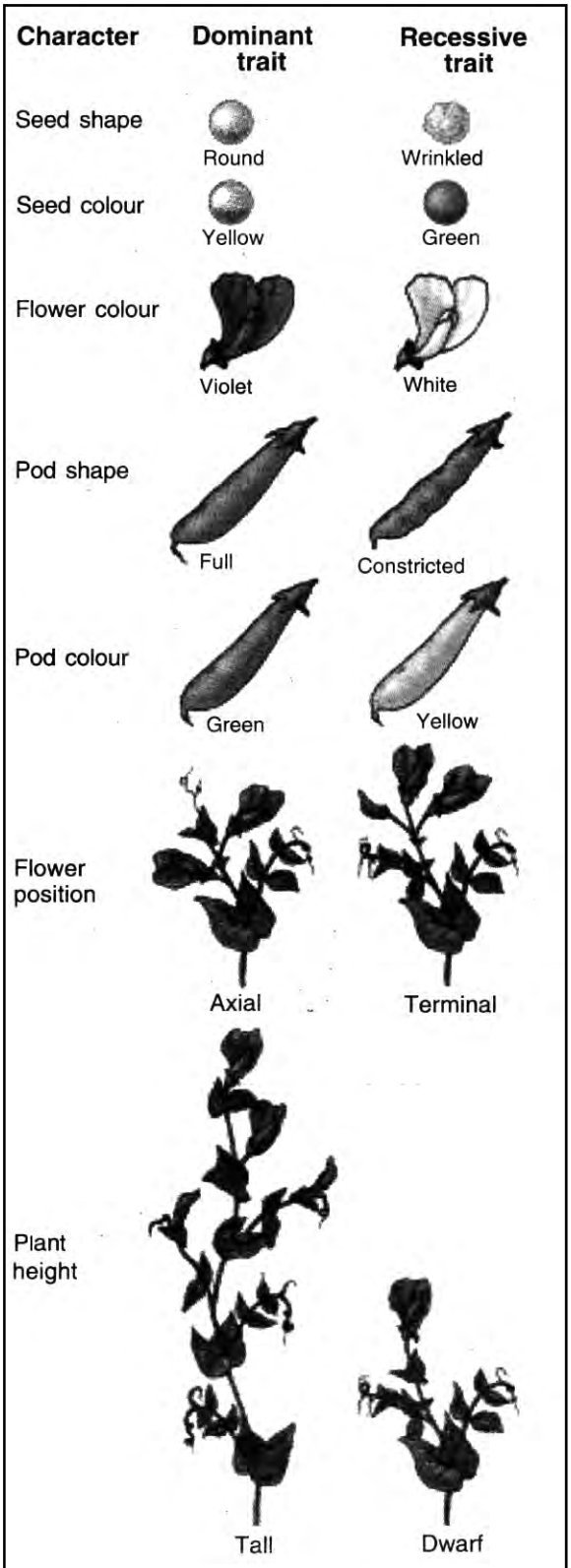


Fig.5.2 : Contrasting charaters selected by Mendel

2. **Hybridization :** Mendel performed crosses between two varieties of plants showing contrasting or alternative forms of characters such as tall plant and dwarf plant. For this he removed male parts from the flowers of one plant which then was used as female plant. The pollens from the other plant were then dusted on the stigmas of the female plants. After pollination, all the flowers of the female plants were covered to prevent unwanted cross-pollination . The pollen contributing plant was considered as male plant. Then he repeated his experiment by **reciprocal cross** where he used the pollen contributing plant as female and the other plant as male. For example, in the first case he used tall plant as male and dwarf plant as female and then in the reciprocal cross he used tall plant as female and dwarf as male. In both the crosses, he obtained the same results. The offspring and seeds of such crosses (**hybridizations**) constituted the **first filial generation** or **F1-generation**.

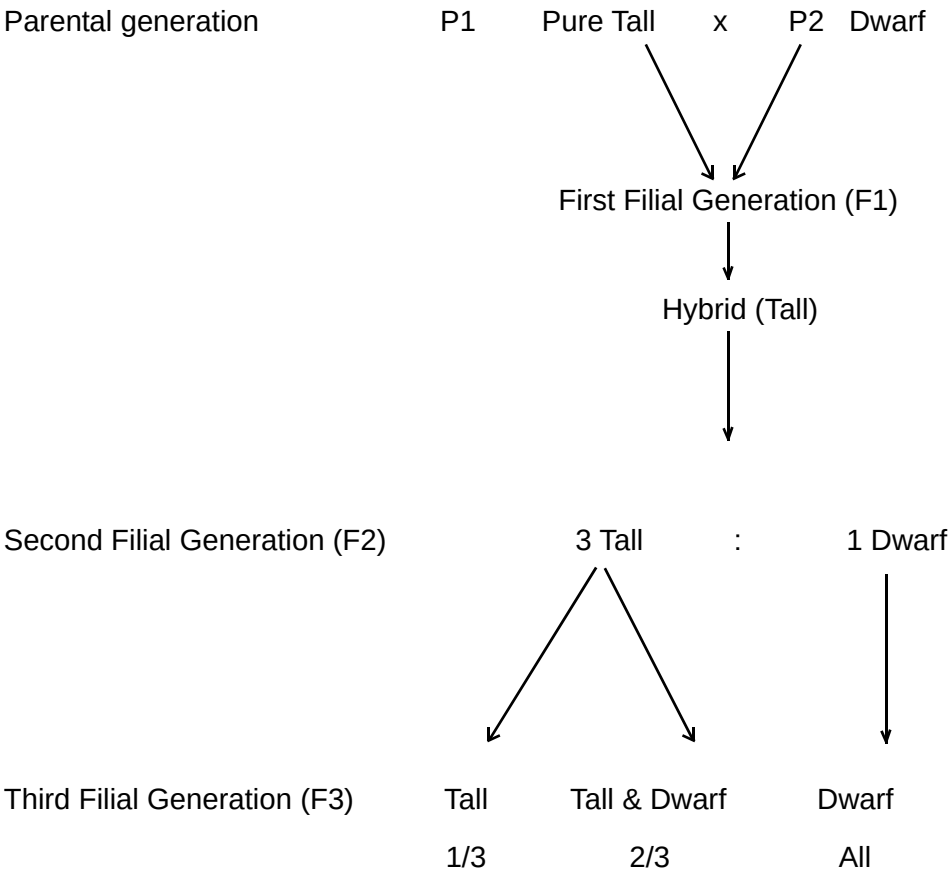
3. **Selfing:** In the third and final stage, Mendel allowed the hybrids of F1-generation to self pollinate. The offsprings and seeds produced from the F1-generations constituted the **second filial generation** or **F2-generation**. The original plants used in the hybridization were denoted as **P1** and **P2**.

5.2.2 Mendel's Findings :

The alternative forms of characters studied by Mendel had only two variants those were easy to identify and score. In one set of experiments, he considered only one pair of contrasting characters and ignored all other differences. The cross in which only one pair of alternative characters is taken into consideration is known as **monohybrid cross**. In another set of experiment, he considered the inheritance pattern of two pairs of contrasting characters. This type of cross where two pairs of alternative characters taken into consideration is known as **dihybrid cross**.

5.2.3 Monohybrid cross :

When Mendel crossed a true breeding tall plant with the dwarf plant the F1-plants were all tall. Then, he subjected the F1-tall plants to self pollination and in the F2-generation, the tall and dwarf plants appeared in the ratio of almost 3:1. Then individual plants of F2-generation were self pollinated and F3-generation was raised. In F3, all the dwarf plants produced only dwarf plants; the dwarf plants were thus true breeding. Out of the tall plants two third tall plants produced tall and dwarf plants in the ratio of 3:1. Hence, two third tall plants were not true breeding. The rest one third tall plants of F2 produced only tall plants; which means that they were true breeding. The same results were obtained in reciprocal crosses and also with all the seven pairs of alternative forms of characters.



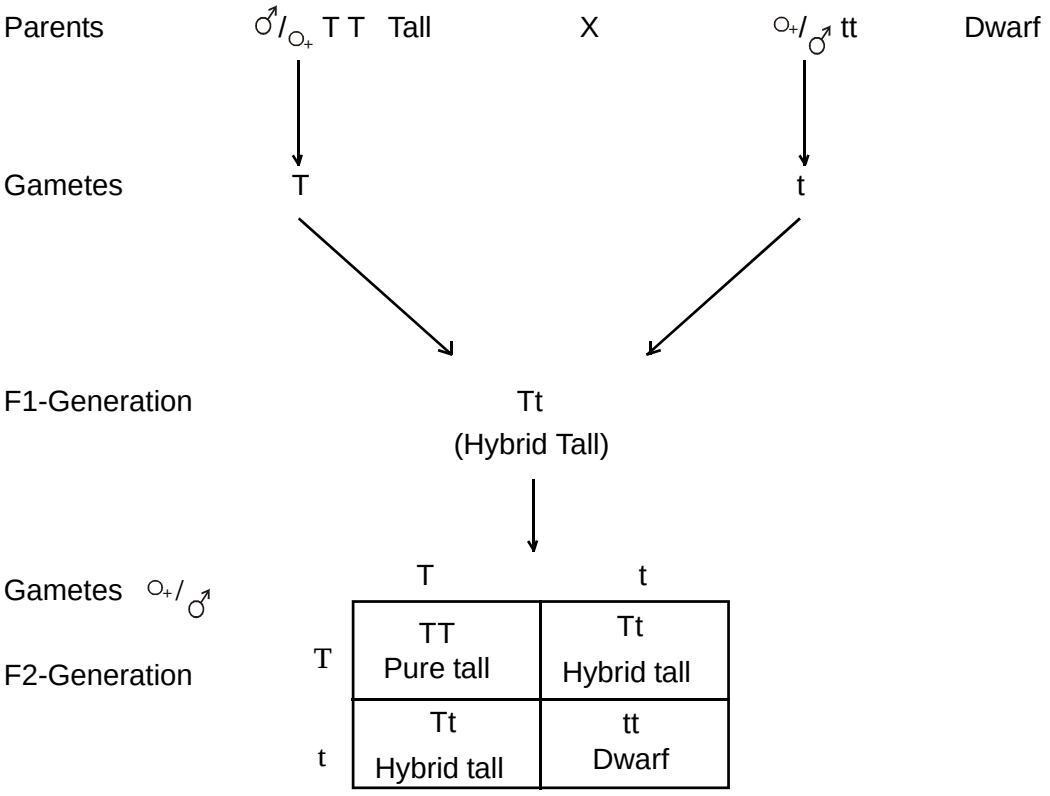
(Mendel's Monohybrid Cross)

Explanation: Mendel's monohybrid cross suggested that, in F1-generation out of the two alternative characters (Tall and Dwarf) only one (Tall) is expressed and the other(Dwarf) is masked. The character which appeared or expressed in the F1-generation (Tall) is called **dominant** and the character which was masked or suppressed (dwarf) is called **recessive**. In F2-generation the dominant and recessive (Tall and Dwarf) appeared in the ratio of 3:1. But, in the F3-generation, it became clear that the F2 ratio of 3:1 is actually 1:2:1 as one third of the total population of F2 are pure tall, two third are hybrid tall like that of F1 and rest one third are dwarf.

In order to keep the records of the crosses Mendel used certain symbols for each pair of alternative characters. He used english capital letters for dominant characters and small letters for recessive characters as given below:

Characters	Dominant	Recessive
Seed shape	Round (R)	Wrinkled (r)
Seed colour	Yellow (Y)	Green (y)
Pod shape	Full (F)	Constricted (f)
Pod colour	Green (G)	Yellow (g)
Flower/Pod position	Axial (A)	Terminal (a)
Seed coat colour/ Flower colour	Red/Violet(R/V)	white (r/v)
Plant height	Tall (T)	Dwarf (t)

According to Mendel a character is inherited by “elemente” or factors. Thus a diploid organism contains a pair of factors for a character. A pure breeding tall plant, therefore, contains two similar factors for tallness as “TT”and a pure breeding dwarf contains “tt”. Such offsprings containing two similar factors for a character are called **homozygous**. The hybrid tall is “Tt” and is called **heterozygous** tall. The morphological expression of a character is called **phenotype** and the internal factors (now known as genes) responsible for such expression is called **genotype**. A tall phenotype can have TT or Tt genotypes. The dwarf phenotypes have (tt) genotype only. One can now represent Mendel’s monohybrid cross as follows:



Phenotypically-tall: dwarf:: 3:1 ; Genotypically-pure tall: hybrid tall: dwarf :: 1:2:1

The two Mendelian factors which determine the character of diploid organism can be called as **alleles**. Thus a homozygous tall plant carries two similar alleles (TT) and heterozygous tall plant carries two different alleles (Tt).

Basing on the observations of Mendel, the German scientist Carl Correns formulated certain principles of heredity. These, **now known as Mendel's Laws of Inheritance,** are as follows:

1. Principle of dominance.
2. Principle of segregation or purity of gametes.
3. Principle of independent assortment.

Out of these three principles the first two are based on monohybrid crosses and the last one is based on dihybrid crosses.

5.2.3.1 Principle of dominance :

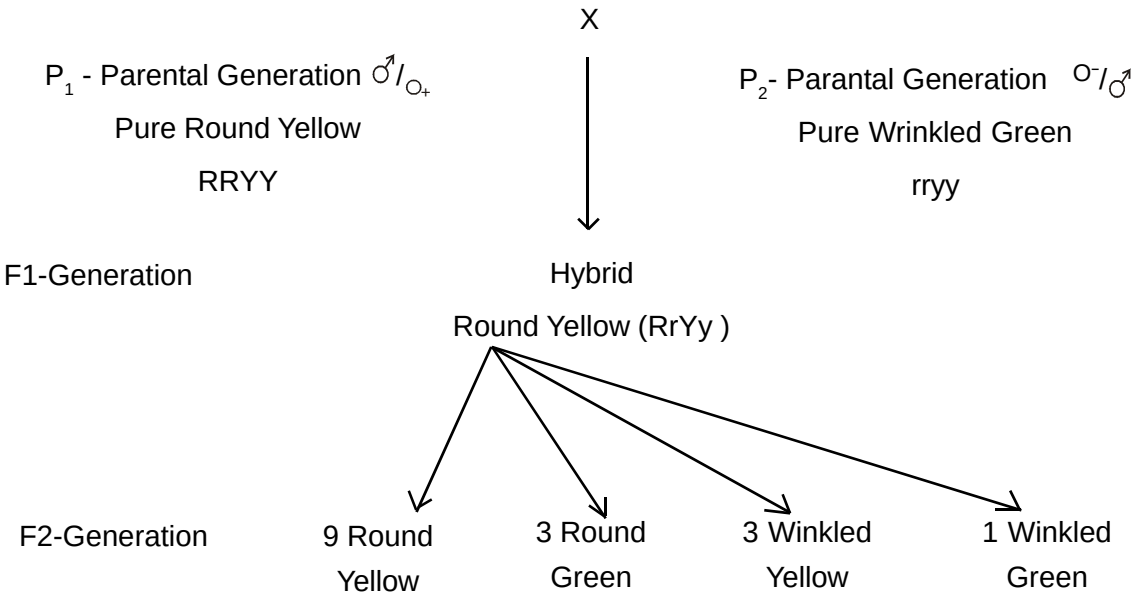
When two homozygous parents for two contrasting characters are crossed a hybrid results in F1-generation.This hybrid shows only one of the two alternative characters in its phenotype. Hence in a heterozygous, having a copy each of both types of alleles, only one is able to express its phenotypic effect.This is known as the dominant factor or dominant allele. The other factor or allele whose effect is completely masked or suppressed is known as recessive factor or allele. The phenotypic effect of recessive allele is seen in the F2-generation when the recessive alleles occur in homozygous form. Thus characters pass from parents to offspring in particulate forms as factors without mixing or amalgamation.

5.2.3.2 Principle of segregation :

In any diploid organism the two factors of a given character remain together without mixing and keeping their identity distinct. At the time of gamete formation, the two factors or alleles segregate and a gamete receives only one of the two factors of any character randomly as per the principle of probability. As the gamete receives only one allele of a character, the gamete is said to be pure for a character and the principle is also known as purity of gamete.

5.2.3.3 Dihybrid cross :

A cross where two pairs of alternative characters are considered for study is known as dihybrid cross. Two traits or characters such as shape of the seed and colur of the seed can be considered together to understand the dihybrid cross.In these two pairs of alternative characters round seed(R) is dominant over wrinkled seed(r) and yellow seed(Y) is dominant over green(y).Like the monohybrid cross, Mendel selected pure breeding plants for both the traits for the cross. Thus true breeding plants having round and yellow seed(both dominant) was crossed with plant having yellow and wrinkled seed (both recessive).

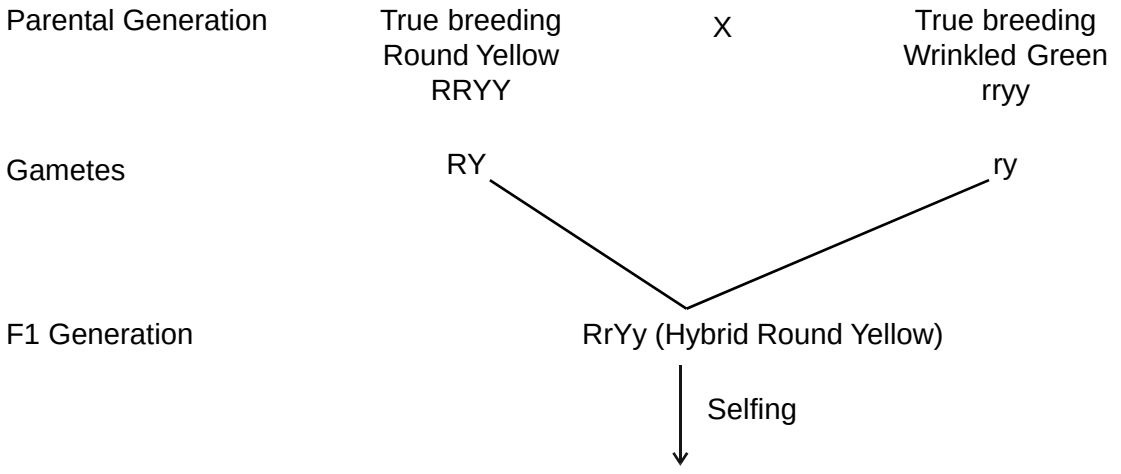


(Mendel's Dihybrid Cross)

In this dihybrid cross the F1 plants were all having round and yellow seeds. Then after selfing in F2 generation parental as well as different combinations appeared in the following ratios:

Plants with round yellow seeds – 9
Plants with round green seeds – 3
Plants with wrinkled yellow seeds – 3
Plants with wrinkled green seeds – 1
The phenotypic ratio of the dihybrid cross is 9:3:3:1

Explanation: Taking the genes or factors as the bases of characters or traits and assigning each factor a symbol the dihybrid cross can be explained by **chequer board** or **Punnet square** as below :



F2 Generation

♀/♂	RY	rY	Ry	ry
RY	RRYY Round Yellow 1	RrYY Round Yellow 2	RRYy Round Yellow 3	RrYy Round Yellow 4
rY	RrYY Round Yellow 6	rrYY Wrinkled Yellow 1	RrYy Round Yellow 6	rrYy Wrinkled Yellow 2
Ry	RRYy Round Yellow 7	RrYy Round Yellow 8	RRyy Round Green 1	Rryy Round Green 2
ry	RrYy Round Yellow 9	rrYy Wrinkled Yellow 3	Rryy Round Green 3	rryy Wrinkled Green 1

In this dihybrid cross four types of plants appeared in the F2 generation which is two more than the parental types. The genotypic ratio can be grouped into four categories considering the principle of dominance as follows:

R-Y - group - 9 Round Yellow phenotypes

rr -Y – group - 3 Wrinkled Yellow phenotypes

R –yy – group - 3 Round Green phenotypes

rr-yy – group - 1 Wrinkled Green phenotypes

The factor or allele for round (R) seed is dominant over wrinkled (r) seed. Similarly, Yellow colour (Y) is dominant over green colour (y).In the F2 generation of the dihybrid cross, each pair of dominant and recessive factors are inherited independently as if the other pair does not exist at all. This can be verified from the following example :

Number of plants with seeds having yellow colour = 9+3=12

Number of plants wth seeds having green colour = 3+1 =4

Hence the F2 ratio of Yellow : green is 12:4 or 3:1

Similarly, number of plants with round seeds = 12

Number of plants with wrinkled seeds = 4

Hence the F2 ratio of Round : wrinkled seeds = !2:4 or 3:1

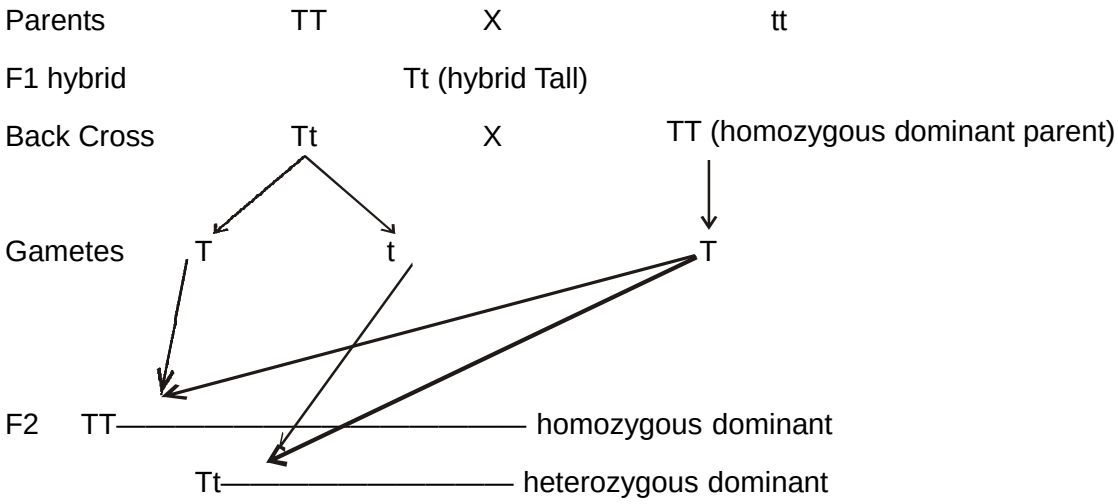
Though the factors for both the characters are present together, their inheritance ratio is the same as the monohybrid cross.This proves that characters are inherited independent of each other.

5.2.3.4 Principle of independent assortment :

As shown in the above results, law of independent assortment states that in a cross between parents with two or more contrasting characters, the inheritance or one pair of contrasting character is independent of the other pair of such characters. The two characters are transmitted independently. Thus, the two factors for seed colour (Y and y) and the two factors for shape of the seed (R and r) present together in the F1 hybrid assort independently and randomly during gamete formation so that a gamete contains only one of the two factors of a character. As a result, four types of gametes are formed,viz. RY, Ry,rY and ry. These four types of gametes unite randomly as per probability to give rise to four types of F2 offspring. Hence, inheritance of two or more factors or genes is independent of each other. However, it is now well established that genes or factors located very close to one another on the same chromosome are **linked** and are not assorted independently. Only those factors or genes located on different chromosomes or on same chromosome but distantly apart from one another assort independently.

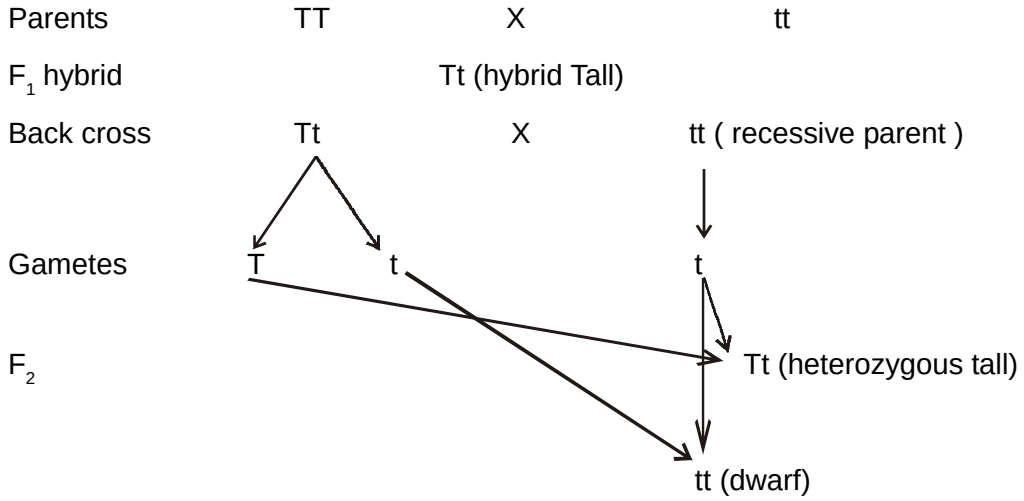
5.2.3.5 Back Cross :

Back cross is a cross between the F1 hybrid with any one of the homozygous parents. When F1 hybrid is crossed with the homozygous dominant parent then all the offspring will be with dominant phenotype.



Phenotypically all plants in F2 are tall.

When F1 hybrid is crossed with recessive parent, both dominant and recessive phenotypes appear in equal proportion.



tall : dwarf :: 1 : 1 (phenotypic ratio)

5.2.3.6 Test Cross :

This cross is actually a back cross employed to know the genotype of the dominant phenotype. A dominant phenotype can be a homozygous dominant (TT) or heterozygous dominant (Tt). If a dominant phenotype with unknown genotype is crossed with a recessive one, then the cross is known as test cross. After crossing if the F2 gives all dominant phenotypes then the test plant is a homozygous dominant. If the F2 gives equal proportion of dominant and recessive phenotypes then the test plant is a heterozygous dominant. This was a powerful tool employed by Mendel to know the genotypes of dominant phenotypes.

The test cross can also be employed for a dihybrid cross to know an unknown genotype. When an unknown genotype for round seed with yellow cotyledon is crossed to its double recessive parent (wrinkle seed and green cotyledon), the offspring may be either all with round seed and yellow cotyledon, or with phenotypes round yellow, round gree, wrinkled yellow and wrinkled green in the ratio of 1:1:1:1. The former result will indicate the unknown genotype to be homozygous for round and yellow (RRYY), while the later to be heterozygous for round and yellow (RrYy) characters. This is explained as follows:

Round Yellow — Wrinkled Green (Double recessive)

RRYY x rryy

Gametes	RY	RY
ry	RrYy	RrYy
Ry	RrYy	RrYy

All are round and yellow

Round Yellow Wrinkled Green (Double recessive)

RrYy x rryy

Gametes	RY	Ry	rY	ry
ry	RrYy Round yellow	Rryy Round green	rrYy Wrinkled yellow	rryy Wrinkled gree
Ratio	1	1	1	1

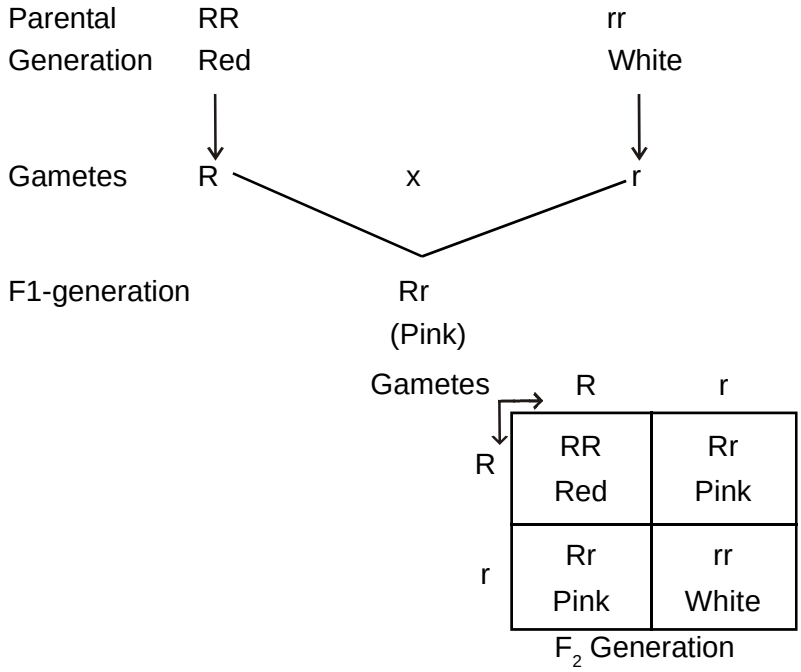
5.3 DEVIATIONS FROM MENDALISM :

In all his experiments, Mendel observed that the homozygous dominant as well as heterozygous genotypes were showing the same phenotypes. This meant, in a paired allele controlling alternative forms of characters, one allele is completely dominant over the other in its expression. Subsequently, workers discovered that such a simple dominant-recessive relationship was not always true. Some case studies revealed intermediate phenotypes in the heterozygotes and some heterozygotes even had the equal expression of both the alleles. These findings laid to establish the concepts of incomplete dominance and codominance respectively. Further it was observed that one character might be controlled by more than one gene (polygenic inheritance) and one gene might control more than one characters(Pleiotropic effect).

5.3.1 Incomplete Dominance :

Incomplete or partial or mosaic dominance is the phenomenon where there is absence of complete dominance so that in the heterozygote condition, an intermediate phenotype is observed. The F1 hybrid shows intermediate character of the two alternative forms. This is not a blending or mixing of characters as the F2 again shows the parental types. **Carl Correns**, for the first time reported incomplete dominance in the petal colour inheritance of *Mirabilis jalapa* (Four O' Clock plant).In *Mirabilis jalapa* and *Antirrhinum majus* (Snapdragon or Dog flower), the cross between red and white flower varieties yielded all pink flowered F1 hybrids. In F1 hybrids, neither the red nor the white trait was dominant rather the hybrid showed intermediate colour.

This can be explained by using genetic symbols. The pure red (RR) is crossed with pure white (rr). Both the forms produce only one type of gametes, i.e., either R or r. The F1 receives one R allele and one r allele and is heterozygote (Rr).



Here each parent is diploid and thus receives two alleles for petal colour. In red flower variety, the parent has two functional alleles (RR), both producing mRNA for the translation of necessary enzymes involved in the synthesis of red pigments. In pink hybrids, only half of the mRNA are transcribed by the only functional allele(R), so insufficient red pigments are synthesized and the flower becomes pink (intermediate). In white flower plants, both the alleles are defective. So no red pigments are synthesized. The defective allele may give rise to defective proteins or even may not be transcribed at all due to defect in the promoter.

5.3.2 Co-dominance :

Co-dominance is a condition in which both alleles of a gene pair in a heterozygote are fully expressed, with neither one being dominant or recessive to the other. In incomplete dominance, on the other hand, the quantitative interaction of allele products produces an intermediate phenotype, as has already been described. For example, in co-dominance, a cross between a homozygous red flowered plant and a homozygous white flowered plant will produce heterozygote offspring (F1 generation) which produce flowers with distinct red and white spots. When plants of F1 generation are self-pollinated, the phenotypic and genotypic ration of F2 generation will be 1:2:1 (Red:Spotted:White). Another typical example showing co-dominance is the ***ABO*** blood group system in human beings. An individual having ***A*** allele and ***B*** allele has a blood type ***AB*** because both ***A*** and ***B*** alleles are co-dominant with each other. Here allelic products of both ***A*** and ***B*** co-exist in the phenotype. More information about blood type is discussed under 'Multiple alleles'.

The distinction between incomplete dominance and co-dominance is often not easily appreciated. For example, Andalusian fowls show incomplete dominance. There are two pure forms of fowls as black feathered and white feathered. A cross between these two forms produce Fl hybrid which is blue feathered.F2 generation offspring are in the ratio of pure black: hybrid blue: pure white (1:2:1). Careful observation reveals that the blue feathered hybrid fowl is actually fine mosaic of distinct black and white areas that appear to be blue, which means that both the alleles are expressed in the heterozygote and it is a case of co-dominance.

5.3.3 Multiple Allelism and Inheritance of Blood Groups :

Alleles are alternative forms of a gene. In any diploid organism two copies of a particular gene occur one each on the homologous chromosome occupying the same loci. The alternative forms of a gene arise due to mutation in the original or wild gene. In some cases there may not be any alternative forms at all. Mutation that completely eliminates a gene is called a **null mutation**. But sometimes the mutation may not have any effect at all. This type of mutation is known as **silent mutation**. Null mutation or any other kind of mutation that impedes gene function but does not eliminate it completely result in loss of function gives rise to alternative allele. Thus it is possible that mutation can occur in different directions in a wild gene to give rise to many alternative alleles. More than two alternative forms of a gene present on the same locus are known as multiple allelism.

But one should remember that a single organism can have only two alleles. Multiple alleles occur in a population. Further in case of multiple alleles, different pairs of alleles may show different dominant-recessive relationships. Some may be completely dominant over others, some incompletely dominant and some may be co-dominant.

There are many examples of multiple allelism. The gene for coat colour in rabbit has four alleles and human blood group gene has three alleles. An extreme example is the wild gene controlling eye colour in *Drosophila*. So far over 100 different alleles of this gene have been identified.

Characteristic feature :

1. There are more than two alleles of a gene in a population.
2. Any diploid individual contains only two alleles as a chromosome contains only one allele of the group.
3. Multiple alleles occupy the same locus on a chromosome or its homologous chromosome.
4. There is no crossing over between the members of a multiple alleles group.
5. The wild allele whose mutations give rise to the multiple allele series may be always the dominant alleles and the mutant alleles may show co-dominance, partial dominance or even complete dominance among themselves.

Human gene that determines the blood group is an example of multiple allelism showing both complete dominance and co- dominance among the alleles. It is important to note that co-dominance is a case where both the alleles of a heterozygote have equal phenotypic expressions.There are four different blood groups found in human population. These are A, B, AB and O. The different blood groups are defined by the presence of different antigens on the surface of the erythrocytes. The genes responsible for producing these cell surface antigens is called **I**. This gene has three alleles: I^A, I^B and I^o/i. I^A and I^B are **codominant**, and both are dominant over I^o/i. I^A encodes an enzyme that adds **galactosamine** to the surface of RBC. I^B encodes the enzyme that adds **galactose** to the surface of RBC.I^o or *i* code a protein that does not add any sugar to the cell surface of RBC. The different combinations of three alleles produce four different phenotypes:

1. **Blood group A** individuals are either I^AI^A homozygotes or I^Ai heterozygotes. They have **only galactosamine** added to the cell surface of RBC.
2. **Blood group B** individuals are either I^BI^B homozygotes or I^Bi heterozygotes. They have **only galactose** added to the cell surface of RBC.
3. **Blood group AB** individuals have **both sugars** added to the surface of RBC and they are always I^AI^B heterozygotes.
4. **Blood group O** individuals have **neither sugar** added to the surface of RBC and are always homozygotes ii/I^oI^o

The four different cell surface phenotypes are called **ABO** blood groups. If type A receives blood transfusion from type B then recipient's immune system identifies the "foreign" antigen galactose on RBC of received blood and attacks donated blood cells causing **agglutination** or **clumping**. The same thing happens if type B individual receives blood from type A individual. This also happens if donated blood is type AB to either type A or B. If the donated blood is type O then no agglutination occur as the RBC in this case has no cell surface antigen. Therefore, O group is considered as **universal donor**. However, AB individuals can receive blood from type A, type B as well as type O. But for this another factor called Rh should match between recepient and donor. Rh negative (–) cannot receive from Rh possitive (+). Hence O^- (negative) is universal donor and AB^+ (positive) is universal acceptor.

Another example of multiallelism is the coat colour gene in rabbit. The gene for coat colour has four alleles: wild type C^+, Chinchila C^{ch}, Himalayan C^h and Albino C. The wild type is dominant to all other alleles. The Himalayan allele is dominant to albino but recessive to all other. Chinchila is partially dominant to Himalayan and completely dominant to albino but recessive to wild.

5.3.4 Pleiotropy :

Gregor Mendel, during his hybridization experiments in pea plant, made several interesting observations regarding the colour of various plant components. He noticed that plants with coloured seed coats always had coloured flowers and coloured leaf axils. He also observed that pea plants with colourless seed coats always had white flowers and no pigmentation in their axils. Mendel gave no explanation for these observations. But his results indicate that a single gene controls more than one trait. The phenomenon of a single gene contributing to multiple phenotypic traits is called as **pleiotropy.** In Mendel's pea plant, the seed coat colour gene was not only responsible for seed coat colour, but also for flower and axil pigmentation.

The term pleiotropy is derived from the Greek words ***pleio,*** which means "many", and ***tropic,*** which means "affecting". Genes that affect multiple, apparently unrelated, phenotypic traits are called pleiotropic genes.

One of the most widely cited examples of pleiotropy in humans is phenylketonuria. This genetic disorder is caused by the deficiency of the enzyme phenylalanine hydroxylase, which is necessary to convert the essential amino acid phenylalanine to tyrosine. As a result, phenylalanine accumulates in all body fluids because it cannot be converted into tyrosine, and tyrosine is less available to meet body's requirements. Phenylalanine is then converted into phenylpyruvate which is a major problem in phenylketonurics. Almost all untreated phenylketonurics are severely mentally retarded. Tyrosine is needed not only for general protein biosynthesis, it is also a precursor for several neurotransmitters (e.g., dopamine, norepinephrine), the hormone thyroxine, and the pigment melanin. Thus, mutations in any one of the genes that affect tyrosine biosynthesis or metabolism also affect multiple body systems. Pleiotropy reflects the fact that any genetic change that alters gene expression or function can potentially have wide-ranging phenotypic and physiological effects in a variety of tissues.

5.3.5 Polygenic Inheritance :

Mendel's laws of inheritance give us basic ideas of inheritance of characters from parents to offsprings. But this inheritance refers to **qualitative characters** only i.e. traits which are easily classified into distinct phenotypic categories. For example, we find in Mendelian experiments, how many of the plants became tall or dwarf and how many had yellow cotyledons or green cotyledons etc. The number (of plants) refers to a qualitative trait of the plant but does not let us know "how tall" or "how much yellow" the characters are. Such phenotypic categories are under the control of one or very few genes with little environmental modifications to obscure the gene effect. In contrast to this, the variability observed in many crop plants which fail to fit into separate phenotypic classes but forms a spectrum of phenotypes. Technically speaking, the phenotypic classes exemplify **"discontinuous variability"** and the spectrum of phenotype illustrate continuous variability. Characters such as grain weight, yield per acre, milk production, egg production are quantitative or **metric traits** with continuous variability. The basic difference between qualitative and quantitative traits involves the number of genes contributing to the phenotypic variability and the degree to which the phenotypes are modified by environmental

factors. Quantitative traits are generally governed by a large number of genes each contributing to the trait. The contribution of each such gene is so small to the phenotype that the individual effects cannot be detected by Mendelian methods. The number of genes affecting a single traits are together called 'Polygenes'. Generally speaking ,the quantitative characters are influenced more by the environmental factors than by the polygenes.This makes the study complicated much beyond the simple Mendelian genetics. To make the task easy, geneticists use statistics to arrive at definite conclusions on inheritance pattern in a given environment and to determine the magnitude of the genetic and environmental components to the phenotypic variability.

MAJOR DIFFERENCES BETWEEN

Qualitative Inheritance	**Quantitative Inheritance**
01. Characters of Kind	01. Characters of Degree
02. Discontinuous variation	02. Continuous variation
03. Single gene effects visible	03. Single gene effects not seen
04. Analysis is rather simple	04. Analysis needs appropriate satistical method and mostly complicated.

Multiple gene model, developed by the Swedish geneticis Nilsson-Ehle in 1910 to explain inheritance of kernel colour in wheat is treated as a classical example of a bridge between the two types of inheritance pattern.

When he crossed a red strain to a wheat strain, he observed that F_1 plants had light red wheat and in F_2 approximately 1/16 were red and 1/16 were white and the others showed a gradation from one extreme to the other. He interpreted these results in terms of two genes but each with a pair of alleles exhibiting cumulative effects.

P	:	$R_1R_1R_2R_2$	x	$r_1r_1r_2r_2$		
		(Red)		(White)		
F_1	:		$R_1r_1R_2r_2$			
			(Light Red)			
F_2	:	$R_1R_1R_2R_2$	$R_1R_1R_2r_2$	$R_1R_1r_2r_2$	$R_1r_1r_2r_2$	$r_1r_1r_2\ r_2$
			$R_1r_1R_2R_2$	$R_1r_1R_2r_2$	$r_1r_1R_2r_2$	
				$r_1r_1R_2R_2$		
		(Red)	(Medium) red	(Light Red)	(Very Light) red	(White)

The presumption is that each of the R1 or R2 (dominant) adds to the redness of the kernel in the phenotypes so that the phenotype with neither of these alleles turned out to be white. The F 2 distribution is an expression of (a+b)4 where a =b=$^1/_2$

These multiple gene models help us understand the origin of continuous variation characterizing truly quantitative traits. But, it should be remembered that environment does modify the phenotypes to different degrees in different systems. So, it is important to discount the environmental effects from the observed inheritance pattern to assess if heritability of quantitative trait is high. These findings help plant breeders in their selection methods.

5.4 CHROMOSOMAL BASIS OF INHERITANCE :

The knowledge on the behaviour of chromosomes during mitosis and meiosis helped to advocate chromosomal basis of inheritance. In 1900, Mendelism was reestablished by three workers, namely Hugo de Vries, Correns and Tschermark. Correns coined the term factors for hereditary unit, Which Mendel referred as “elemente”. An American graduate student, **Walter.S.Sutton** and a German biologist, **Theoder Bovery** observed close parallelism between the Mendelian factors and the behaviour of chromosomes during gamete formation and fertilization. Basing on their observations, Sutton and Bovery in 1902 independently put forward the chromosomal basis of inheritance. The parallelism between chromosomes and Mendelian factors are summarized below:

Chromosomes	**Mendelian Factors**
1. Chromosomes occur in homologous pairs in the diploid organisms.	Mendelian factors also occur in pairs in diploid organisms.
2. During gamete formation(meiosis) homologous chromosomes separate.	Mendelian factors also segregate during gamete formation.
3. Each gamete receives only one of the two chromosomes of a homologous pair.	Each gamete receives only one of the two alternative factors.
4. After fertilization, in a diploid cell, chromosomes again occur in two sets; one contributed by father and the other by mother.	After fertilization, Mendelian factors also occur in pairs, one each contributed by father and mother.

The chromosomal basis of inheritance advocates that Chromosomes are the bearer of hereditary units or genes. As chromosomes occur in pairs, each pair of Mendelian factors is carried by a pair of chromosome separately. The Mendelian factors or genes have specific loci on chromosomes. Factors segregate due to the segregation of chromosomes during meiosis Later on, in 1909**, Johannsen** coined the term gene for Mendelian factor. The two alternative

forms of genes occur in two homologous chromosomes and corresponds to the same loci on the two homologous chromosomes.

5.5 LINKAGE AND CROSSING-OVER :

Several experiments involving dihybrid crosses, are found to deviate from the Mendel's ratio of 9:3:3:1. Mendel was lucky to deduce the principle of independent assortment because the chosen traits (contrasting pairs of characters) were present on separate homologous sets. Deviations from dihybrid cross ratio are due to lack of independent assortment of chosen characters (traits). The pairs of characters (traits) are somehow coupled and do not assort independtly. It is reasonable to predict that such traits are present on the same chromosome. Traits (genes) present on the same chromosomes are said to be linked. All genes on a chromosome form **linkage group**. The chromosome having set of genes generally pass to a gamete. So genes belonging to a linkage group do not show independent assortment.

The phenomenon of linkage was demonstrated by **T.H. Morgan** in 1910. Morgan conducted a test cross between heterozygous grey bodied and long winged *Drosophila* (fruit fly, n = 4) and homozygous recessive black bodied and vestigial winged fly. The following result was obtained :

Phenotype		Per cent of occurrence
(i)	Grey body long wing	41.5
(ii)	Black body vestigial wing	41.5
(iii)	Grey body vestigial wing	8.5
(iv)	Black body long wing	8.5

The above results do not follow the possible outcomes of the test cross as shown by Mendel.

If the trait, grey and black body were on one chromosome of a homologous pair and the trait, long and vestigial wing on a different chromosomes of the same homologous pair, they would show independent assortment producing the following result.

Phenotype		Per cent of occurence	Proportion
(i)	Grey body long wing	25	1
(ii)	Grey body vestiged wing	25	1
(iii)	Black body long wing	25	1
(iv)	Black body vestigial wing	25	1

If both the traits [body colour (black and grey)] and [wing length (long and vestigial) are present on the same pair of homologous chromosomes (i.e. they are linked) the result would be theoretically as follows.

Phenotype	**Per cent of occurence**	**Proportion**
Grey body long wing	50	1
Black body vestigial wing	50	1

These theoretical assumptions can be illustrated by assigning the alphabets G & g for grey and black body colours and L & l for long and vestigial wings, respective and analysing the result of the test-cross through chequer board as follows :

Case I : G and L are on different homologous chromosomes.

Test cross

Phenotype	Grey body Long wing	X	Black body Vestigial wing
	G gL l		g gl l
Genotype	GgLl (heterozygous)		ggll (homozygous)

♀ GgLl / ♂ ggll	GL	Gl	gL	Gl
gl	GgLl Grey body Long wing	Ggll Grey body Vestigial wing	ggLl Black body Long wing	ggll black body Vestigial wingg
Proportion	1	1	1	1

In other words, the ratio of 1:1:1:1 would indicate that the two genes, G and L, are on different homologous chromosomes.

Case II : G and L are on same chromomsomes.

Test cross

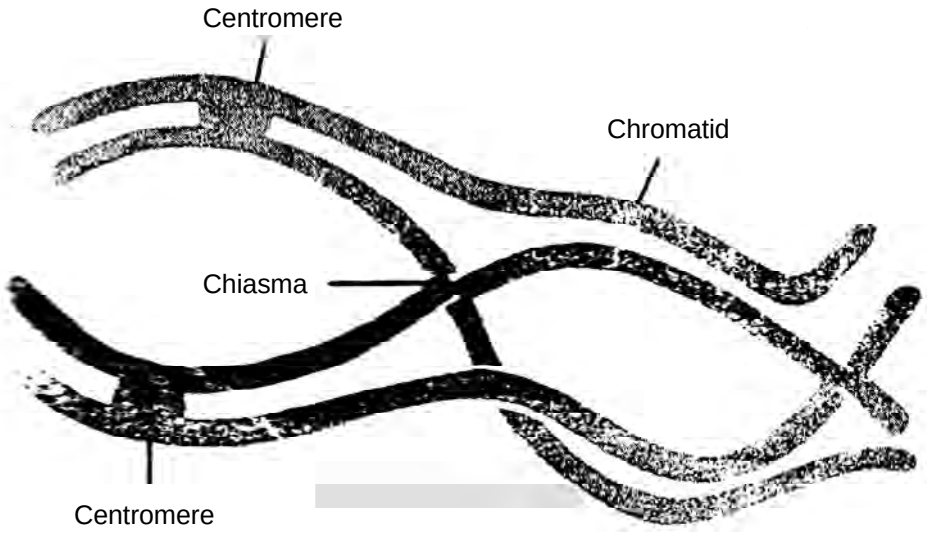
Phenotype	Grey body Long wing	X	Black body Vestigial wing
	G g L l		g g l l
Genotype	GgLl (heterozygous)		ggll (homozygous)

Gametes of / Gametes of ggll	G L	g l
g l	G g L l Grey body Long wing	G g L l Black body Vestigial wing
Proportion	1	1

In other words the 1:1 ratio would indicate that G and L are present in same homologous pair of chromosome :–

The deviation of the result from the theoretical predictions allows to postulate that both the traits are present on the same chromosome i.e. they are linked. The alleles of the traits (genes) are exchanged during meiosis in which homologous chromosomes undergo a process of synapsis (pairing) and exchange of fragments (Fig. 5.3) explaining the phenomenon of crossing over. Due to the exchange of the chromosomal fragments, two types of gametes result, one having normal parental combination (parental) and other having recombination (recombinants) (Fig**.** 5.4) Such gametes fuse and produce offspring of normal type and recombinant type. The experimental result of Morgan can be explained through crossing over and appearance of

(a)



(a)

Fig. 5.3 : Crossing over and chiasma formation in paired homologous chromosomes (a) as seen under microscope, (b) its schematic presentation.

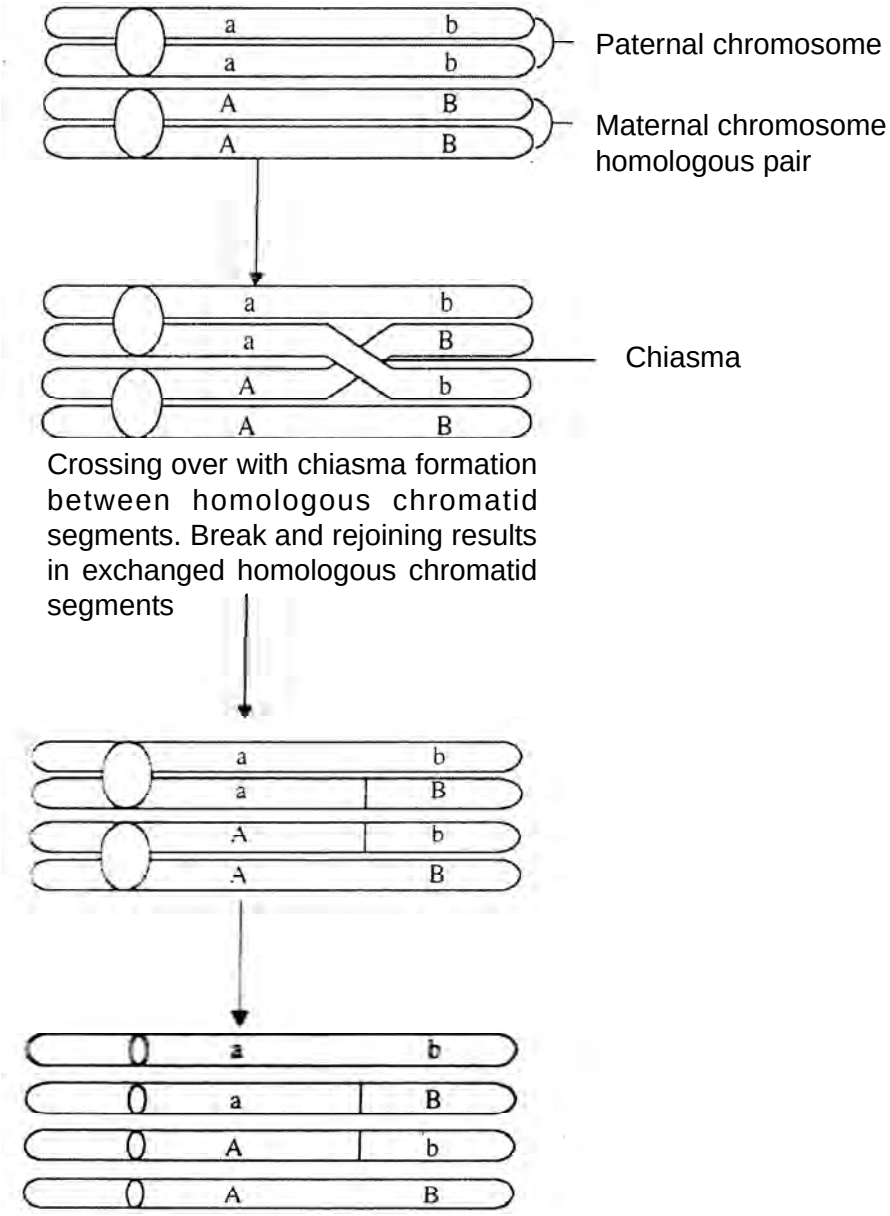


Fig. 5.4. : Recombination of the alleles of two genes due to crossing-over. Four allelic combinations are formed (AB, Ab, aB and ab). In the absence of crossing over only two parental combinations of alleles (AB, ab) would be produced.

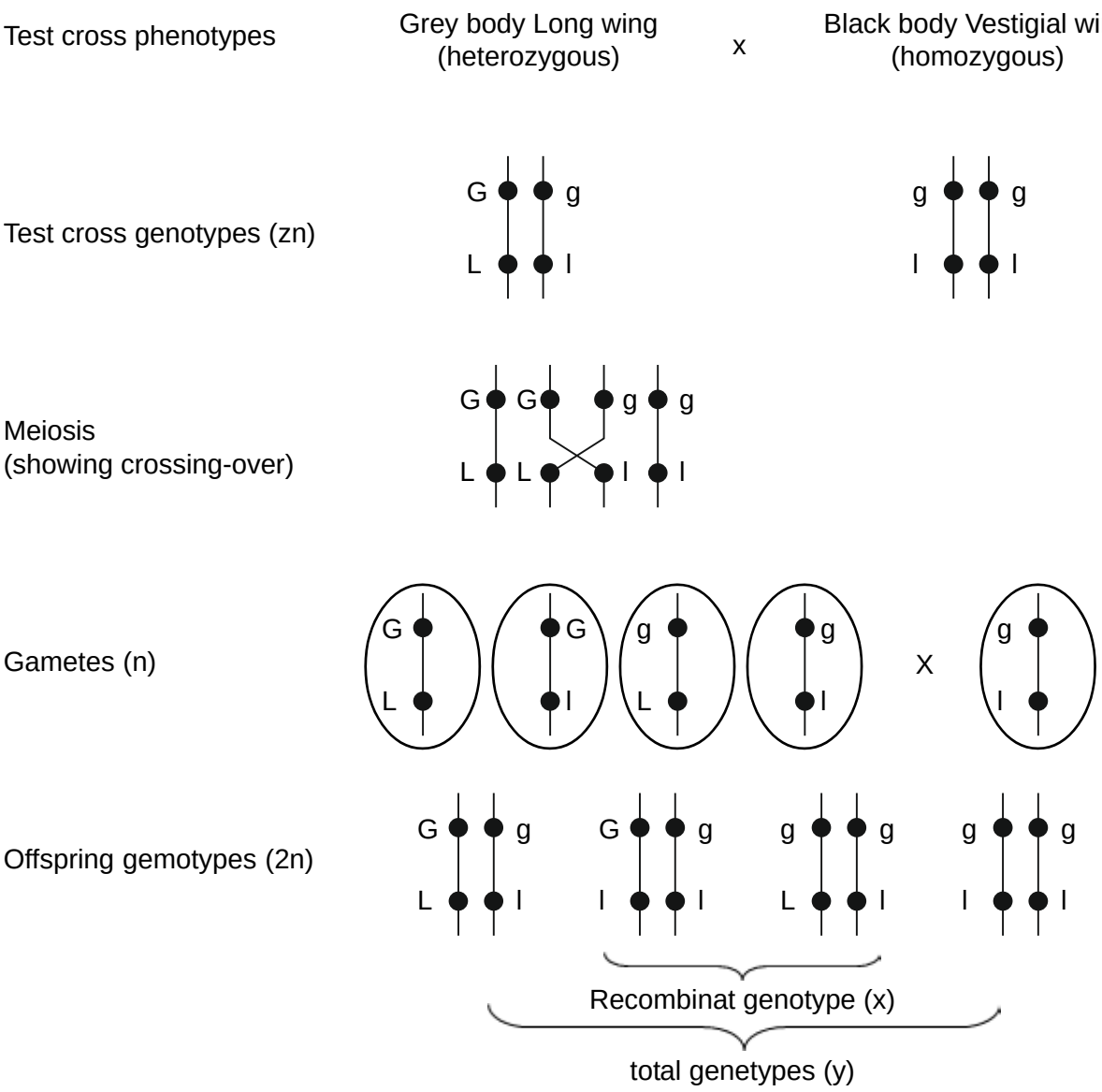


Fig. 5.5 : Genetic expression of crossing over and appearance of recombinants for the cited Morgan's experiment.

recombinant genotypes (Fig. 5.5). Most of the breeding experiments involving linkage, produce two types of offsprings. Two parental combinations, having an approximately equal proportion, are much more than the two recombinants, also in equal proportion.

Two or more genes are said to be linked when recombinant phenotypes with new gene combinations occur less frequently than the parental phenotypes. Traits present on the same chromosome, but do not show the production of recombinants in breeding experiment are said to the completely linked (complete linkage). This is a rare phenomenon. Generally recombinant

types are produced with different proportions. If the proportion is high, then the linkage distance between genes (traits) is also high or *vice versa.*

The production of recombinant types of offspring in breeding experiments is an outcome of crossing over which is observed as chiasma in Prophase I of meiosis. The major source of genetic variation within populations is crossing over.

Genes on one chromosome do not show complete linkage due to the occurrence of crossing over. It yields recombinants. The frequency of occurrence of recombinants is known as **recombination frequency** or **crossover values** or **crossover frequency**. The recombination frequencies observed for different genes or a chromosome suggest that such genes are linearly or arranged on that chromosome. Moreover cross over values reflect the relative position of genes on a chromosome. More is the recombination frequency between two genes, more apart they are or vice versa. This is becuase if the relative position of two genes on a chromosome is more then there is a more chance of crossing over between these two genes.

If A, B and C are three genes present on a chromosome linearly (Fig. 5.6) the crossover or separation of genes is more likely to occur between A and C than between B and C or A and B.

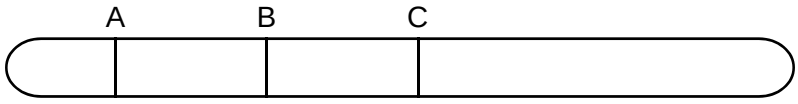


Fig. 5.6 : A chromosome showing gene loci A, B and C

The formula used to calculate recombination frequency is

$$\text{Recombination frequency} = \frac{\text{Number of Individuals showing recombination}}{\text{Number of offspring}} \times 100$$

Calculation of recombination frequencies for linked genes enable to produce genetic linkage map on a chromosome. This shows the relative position of genes on a chromosome. If recombination frequencies between A and B, B and C, and A and C are 5, 6 and 1 1 respectively, then the gene loci for A, B and C will be as follows (Fig. 5.7)

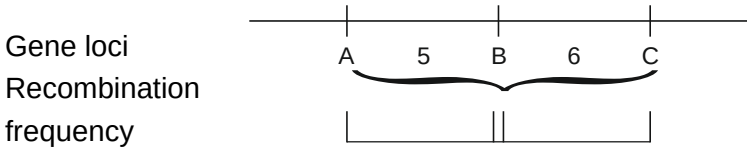


Fig. 5.7 : Position of gene loci on chromosome

The distance between genes in constructed gene map is expressed as map unit. It is also expressed as **Morgan unit**.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Answer the questions from the choices given under each bit :

(i) The experimental plant material used by Mendel was :

(a) Cow pea (b) Garden pea

(c) Wild pea (d) Sweet pea

(ii) Which of the following characters is not among the seven characters considered by Mendel for his hybridization experiments?

(a) Seed colour (b) Pod shape

(c) Flower position (d) Flower shape

(iii) Which law Mendel would not have proposed, if the phenomenon of linkage was known to him?

(a) Law of unit character (b) Law of dominance

(c) Law of segregation (d) Law of independent assortment

(iv) The number of genotypes produced in F2 generation in Mendel's monohybrid cross was:

(a) 1 (b) 2

(c) 3 (d) 4

(v) In which of the crosses, half of the offspring show dominant phenotype?

(a) Tt X Tt (b) TT X tt

(c) Tt X tt (d) TT x TT

(vi) Two alletic genes are located on the :

(a) Same chromosome (b) Two homologous chromosomes

(c) Two non-homologous chromosomes (d) Any two different chromosomes

(vii) Red (RR) *Antirrhinum* is crossed with white (rr) one. The Fl hybrid is pink. This is an example of:

(a) Complete dominance (b) Co-dominance

(c) Incomplete dominance (d) Complete recessive

(viii) In a dihybrid cross, in F2 generation, the parental types are far greater in number than the recombinants. This is due to:

(a) Linkage (b) Incomplete dominance

(c) Multiple allelism (d) Complete dominance

2. Express in one or two words :

(i) A pair of Mendelian factors (genes) that appear at a particular location on a particular chromosome and control the same characteristic.

(ii) Phenomenon where in the heterozygous condition an intermediate phenotype is observed.

(iii) The phenomenon of a single gene contributing to multiple phenotypic traits.

(iv) Genes which move together and do not show independent assortment.

(v) A cross between the F_1 hybrids with any one of the homozygous parents.

3. Correct the sentences, if requred, by changing the underlined word(s) only:

(i) The process of transmission of characters through generations is know as <u>variation</u>.

(ii) In Mendel's monohybrid cross, the dwarf phenotype is <u>always homozygous</u>.

(iii) In Mendel's dihybrid cross in F_2 generation <u>nine</u> phenotypes are produced.

(iv) The phenomenon of linkage disproved the principle of <u>independent assortment</u>.

(v) In a test cross, always <u>dominant</u> parent is used.

(vi) The distance between genes in a constructed gene map is expressed as <u>Mendel</u> unit.

4. Fill in the blanks :

(i) Monohybrid cross in F2 generation yields _____ number of phenotypes.

(ii) Monohybrid cross in F2 generation yields _____ number of genotypes.

(iii) The name of scientist often coined with linkage is _____ .

(iv) Genotype of a plant showing the dominant phenotype can be determined by _____ cross.

(v) In a cross between AaBB and aaBB, the genotypic ratio in Fl generation will be _____ .

GROUP - B
(Short Answer-type Questions)

1. Answer within 3 sentences:

(i) Law of independent assortment

(ii) Multiple alleles

(iii) Chromosomal basis of inheritance

(iv) Co-dominance

(v) Incomplete dominance

(vi) Law of segregation

(vii) Linkage

(viii) Recombination
(ix) Test cross
(x) Back cross

2. Differentiate between:

(i) Homozygous and Heterozygous
(ii) Genotype and Phenotype
(iii) Dominant and Recessive genes
(iv) Test cross and Back cross
(v) Qualitative and Quantitative inheritance

GROUP - C
(Long Answer-type Questions)

1. Give an account of Mendel's monohybrid cross. What inference did Mendel draw from this experiment?
2. State and explain Mendel's laws of inheritance.
3. What do you mean by back cross and test cross? Explain test cross through an example.
4. Describe Mendel's dihybrid cross.
5. Give an account of linkage and recombination.

❑ ❑ ❑

SEX DETERMINATION

CHAPTER 6

Sexually reproducing organisms are either bisexual or unisexual. Most plants and lower grade animals are bisexual, where both male and female gonads (testis and ovary) are present in the some individual. In these cases there is no sexual differentiation of individuals to establish them either as male or female. However, in unisexual organisms (e.g., higher grade animals) male and female gonads are borne by different individuals. During the embryonic development, gonads differentiate at a specific point of time. At the begining, they develop as indifferent gonads. Later in the development, the indifferent gonad either differentiates as testis or ovary. Against this backdrop, a fundamental question strikes as to when the sex is determined. Is it determined when the indifferent gonad differentiates either as testis or ovary or at some point of time before this ? Studies in genetics have revealed that the actual sex is determined at fertilization by the sex chromosomes. Thereafter sex chromosomes induce the differentiation of male or female gonads and then the secondary sexual characters to establish sexual dimorphism. Therefore sex determination occurs in three steps : **chromosomal sex determination** by sex chromosomes, **gonadal sex determination** by the differentiation of gonads and **phenotypic sex determination** by sex hormones secreted from the gonads.

The objective of this chapter is to acquaint the students with an elementary mechanism of sex determination by sex chromosomes or any other mechanism, as the case may be.

A **sex-determination system** is a biological system that determines the development of sexual characters in an organism. Biologically, sex is an aggregate of those morphological, physiological and behavioural characters, which differentiate the male from the female. Most organisms that produce their offsprings using sexual reproduction have two sexes. Occasionally, there are hermaphrodites in place of one or both sexes. There are also some species that have only one sex and reproduce by parthenogenesis, in which the female reproduces without fertilization.

In many species, sex determination is genetic: males and females have different sex chromosomes, bearing genes which express their sexual morphology. In animals this is often accompanied by chromosomal differences, generally through combinations of sex chromosomes at fertilization.

In some other cases, the sex is determined by environmental factors such as temperature and hormones, irrespective of the sex chromosome combination, which they possess.

6.1 SEX CHROMOSOME :

In the process of evolution, the genes responsible for sex determination segregated to specific chromosomes, the **sex chromosomes or allosomes**. These chromosomes are designated as **'X'** and **'Y'** or **'Z'** and **'W'**. Such chromosomes are morphologically distinguished from each other. The remaining chromosome of the cell are known as **autosomes** and designated as **'A'.**

The X and Y chromosomes differ from each other in many respects. They are heteromorphic and this is due to the location of sex determining genes on the respective sex chromosomes. There is practically no crossing over between X and Y chromosomes. This helps to conserve gene combinations favouring distinct sexual differences. The consequence is that the Y chromosomes bears mostly the genes essential for maleness while all other genes become inert. It is reversed in Z and W chromosomes. The individual, where the sex is determined by two similar sex chromosomes is known as **homogametic** or **homomorphic** producing similar type of gametes. Conversely, when the sex is determine by two dissimilar chromosomes, the individual is **heterogametic** or **heteromorphic** producing dissimilar types of gametes.

Despite the differences in shape and size, the homologus part of Y chromosome pairs with X chromosome during meiosis. The non homologous part of Y chromosome carries only **Y-linked genes** or **holoandric genes**. The Y chromosome carries a gene ***'sry'*** (sex determining region Y) that codes for a protein called **testis determining factor (TDF)**. TDF is required for the development and differentiation of the testis and its duct system and its absence leads to the development of ovaries (Fig. 6.1).

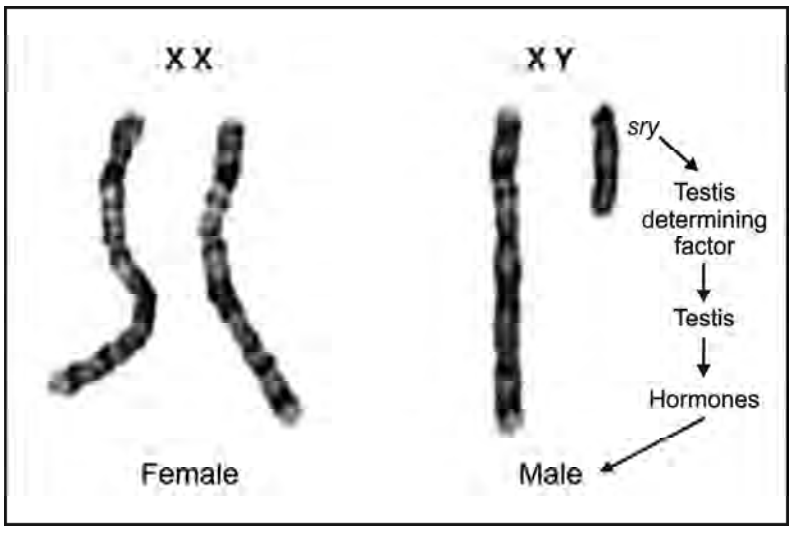


Fig. 6.1: The role of Y chromosomes in male sex determination

Females homozygous for genes on X chromosomes do not express phenotypes more elaborately than do hemizygous males. For long, geneticists believed that a compensation mechanism might be operating for making the dose of genes on X chromosomes equal in both

male and female sexes. Mary F Lyon, indeed established that the compensation is achieved by the inactivation of one of the two X chomosomes in the female. He termed it as **dosage compensation**. The inactivated X chromosme is termed as the **Barr body**.

The sexual character is inherited in a mendelian fashion. Its inheritance follows the law of segregation. In many species, the two sexes are phenotypically indistinguishable, while the two sexual phenotypes are quite easily distinguished in human.

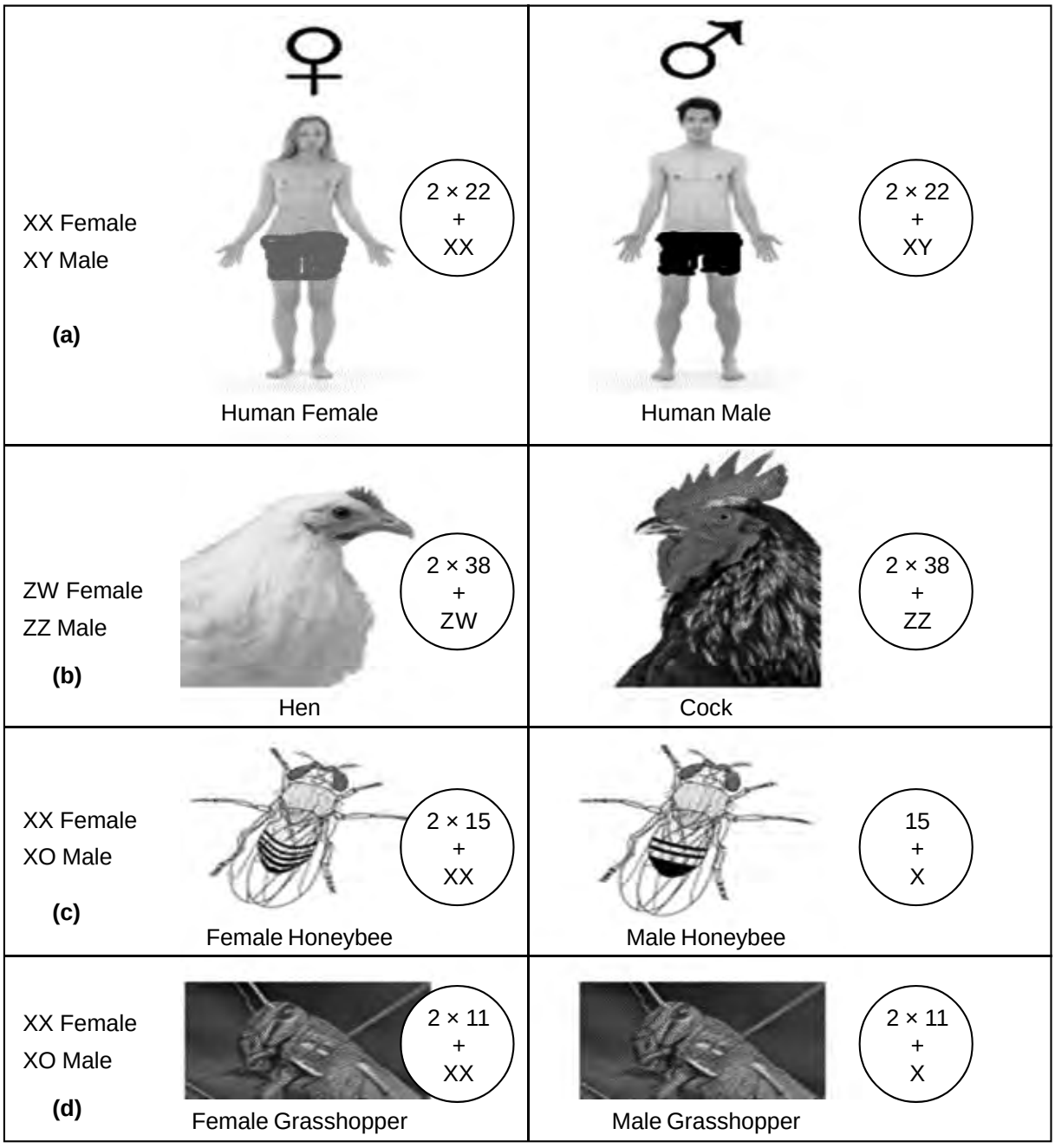


Fig. 6.2: Different types of chromosomal sex determination mechanisms.

Several genetically controlled sex determination mechanisms have been discovered. These are :

1. Chromosomal mechanism
2. Haplo-diploid mechanism (a variant of chromosomal mechanism)
3. Genic balance mechanism
4. Single gene effect

6.2 CHROMOSOMAL MECHANISM OF SEX DETERMINATION :

In a majority of diploid animals a pair of sex chromosomes, designated as X and Y is found, which determine the sex of an individual. Henking (1891) discovered X-chromosome in male bug and described it as X-body. Wilson and Stevens (1902-1905), proposed chromosomal theory of sex-determination and named X and Y chromosomes as sex chromosomes or **allosomes** and other chromosomes as **autosomes**.

In the animal kingdom many variants of chromosomal sex determination mechanisms have been described (Fig. 6.2). These are :

1. XX - XY mechanism
2. XX - XO mechanism
3. ZZ - ZW mechanism
4. ZZ - ZO mechanism
5. Haplo - Diploidy mechanism

6.2.1 XX-XY type or lygaeus mechanism :

Wilson and Stevens first studied it in the milk weed bug, *Lygaeus turcicus* and hence this mechanism is identified with his name. The female is homogametic (XX) and the male is heterogametic (XY). (e.g., Human and Drosophila.)

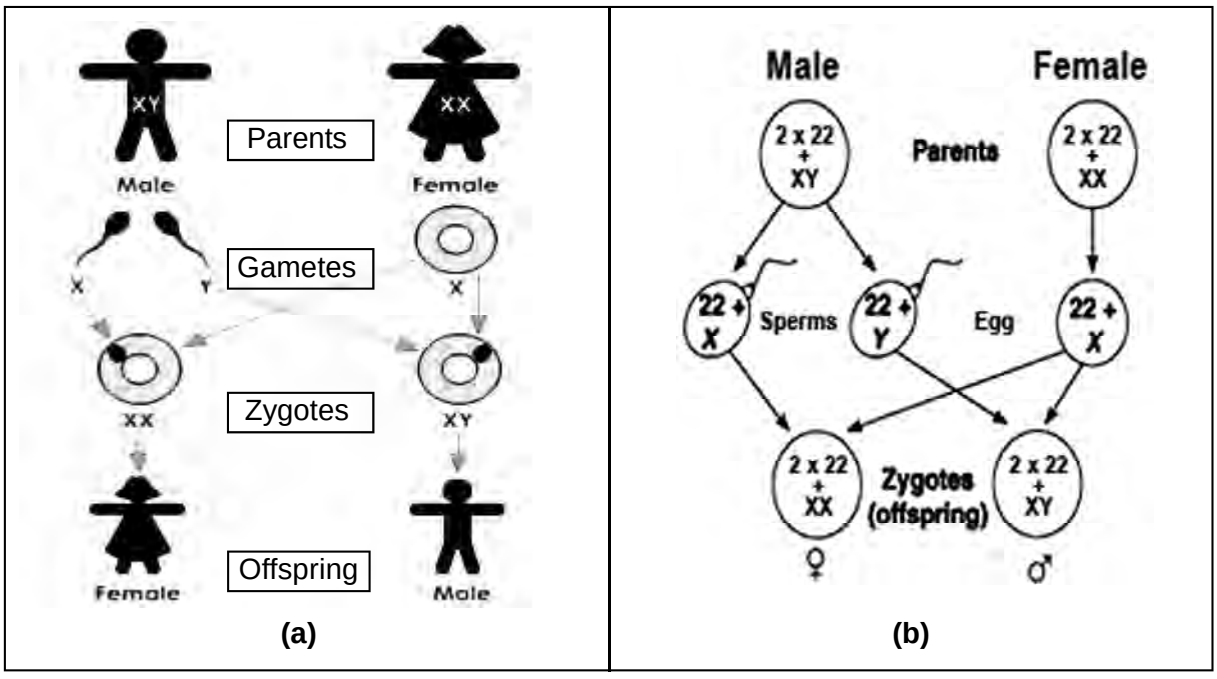


Fig. 6.3 : (a) Pattern of sex chromosomal inheritance in human, and **(b)** sex determination in human.

The female produces one type of egg i.e. with only 'X' and male produces two types of sperms i.e. with 'X' or 'Y'. Fertilization of female gamete with any one of the male gametes will determine the sex of the offspring. Fig. [6.3(a) & (b)] explains the mechanism.

6.2.2 XX - XO mechanism :

In this mechanism, only the X chromosome is present, the Y being absent.

The female is homogametic (XX) and the male is heterogametic (XO) (Fig. 6.4). Maleness is determined by a single X chromosome. O denotes the absence of a Y chromosome. (e.g., Grasshopper and Bug)

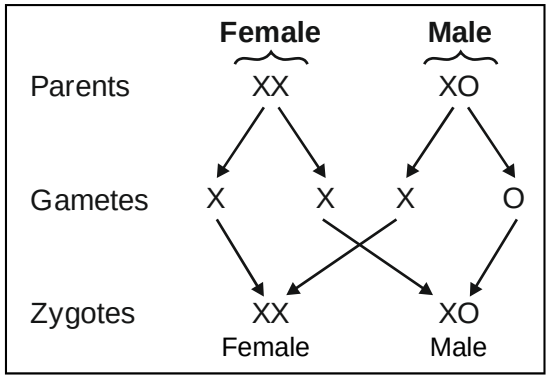


Fig. 6.4 : XX - XO mechanism of sex determination in grasshopper.

6.2.3 ZW - ZZ mechanism :

In this mechanism, the female is heterogametic (ZW) while the male is homogametic (ZZ). The inheritance of Z and W chromosomes occur in a simple mendelian fashion (Fig. 6.5). (e.g., Birds).

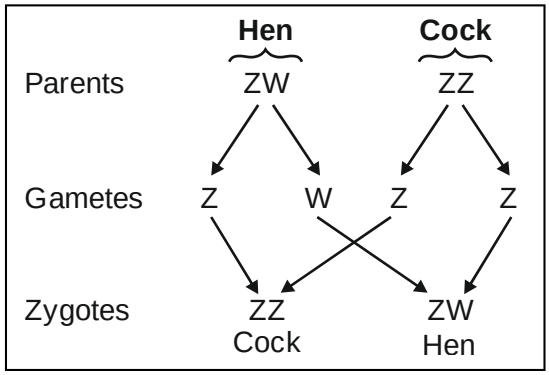


Fig. 6.5 : Sex chromosomal inheritance in fowl.

6.2.4 ZZ - ZO mechanism :

In Lepidoptera (e.g., moths and butterflies), the male is homogametic with two Z chromosomes (ZZ), while the female is heterogamatic with one each of Z and W (ZW). However, some females have ZO complement indicating that W is not essential for femaleness.

6.2.5 Haplo-diploidy mechanism :

The female is diploid and male is haploid. Haploid male is produced when the egg is not fertilized (Fig. 6.6). This type of development is termed as **parthenogenesis**. However, fertilized eggs develop as females. (e.g., Honeybee, Wasp and Ant).

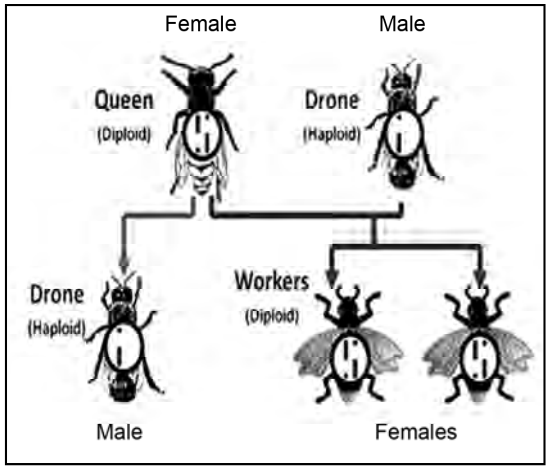


Fig. 6.6 : Haplo-diploidy mechanism of the sex determination in honey bee.

6.3 GENIC BALANCE MECHANISM :

Unlike in human and other animals, autosomes play an important role in addition to the sex chromosomes in the sex determination process in *Drosophila melanogaster*. C.B. Bridges proposed the genic balance theory of sex determination based on the ratio of the number of X chromosomes and sets of autosomes. According to him, the female determining genes are located on the X chromosome, while the male determining genes on autosomes. Table 6.1 describes about the phenotypic sex of *Drosophila melanogaster*, based on X / A values.

Table - 6.1

X / A ratios and corresponding sexual phenotypes in Drosophila melanogaster

Sl.No.	Chromosome complement	Ratio = X/A	Sex
1	2A + XX	2 / 2 = 1.0	Female
2	3A + XXX 4A + XXXX	3 / 3 = 1.0 4 / 4 = 1.0	Triploid Female Tetraploid Female
3	2A + XXX	3 / 2 = 1.50	Super Female
4	3A + XX	2 / 3 = 0.67	Intersex
5	2A + XY	1 / 2 = 0.50	Male
6	3A + XY	1 / 3 = 0.33	Super male

6.3.1 Gynandromorph in *Drosophila* as a proof of chromosomal mechanism of sex determination.

In *Drosophila*, occasionally flies are obtained in which a part of the body exhibits female characters, while the other part male characters. Such flies are known as **gynandromorphs.** These develop due to **failure of segregation (nondisjunction)** of X chromosomes at cleavage. The zygote starts with 2A+2X chromosome complement. During first cleavage one of the X-chromosomes is lost in one of the blastomeres. The consequnce is that, one of the blastomeres acquires **2A+2X** complement, while the other **2A+X.** At the end of development, the descendant blasomeres with 2A + XX complement differentiate as female phenotype, while those with 2A + X differentiate as male phenotype. Thus, half of the body is female while the other half is male (Fig. 6.7).

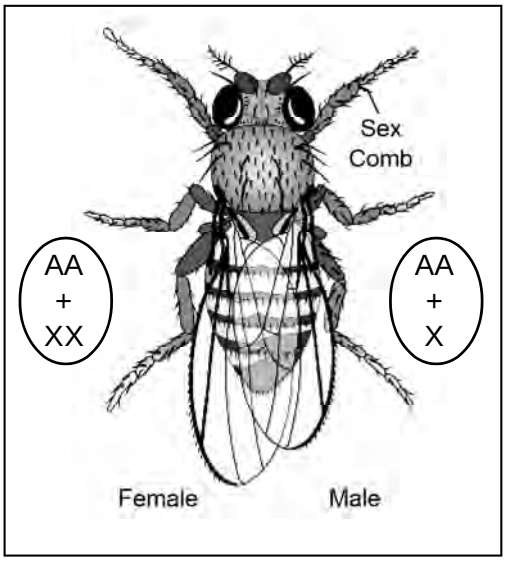


Fig. 6.7 : Gynandomorplism in *Drosophila*

6.4 SINGLE GENE EFFECT :

- In some organisms like *Drosophila*, human and several fishes, a single gene is responsible for the expression of sex.
- In *Drosophila tra* (tansformer), a recessive gene, present on the third autosome expresses the sex.
- Most males and females with dominant allele (tra^+) are fertile.
- But a normal female (AA + XX) when homozygous recessive with both *tra* alleles develop as sterile male.
- In human a *sry* (sex determining region Y), present on the Y chromosome influences the development of testes in the male and its absence develops ovaries in the female.
- Therefore , XX female with *sry* gene is a sterile female and a XY male without *sry* gene too is a sterile female.

6.5 SEX DIFFERENTIATION :

- The chromosome theory and genic balance theory of sex determination successfully apply to lower grade animals but in vertebrates and under certain conditions, in invertebrates, an embryo develops some characters of the opposite sex together with its own characters.
- It means that the sex changes under specific conditions.
- This may be due to hormones secreted by the gonads of the animal. Some examples of sex differentiation are as discussed bellow :

6.5.1 Sex Reversal :

- It is observed in fishes, amphibians, birds and even in some mammals.
- Artificial removal of gonads of either sex before puberty in mammals and even in human (castration or ovariectomy) results in the development of secondary sexual characters of the opposite sex reversal in chick is explained in Fig. 6.8.

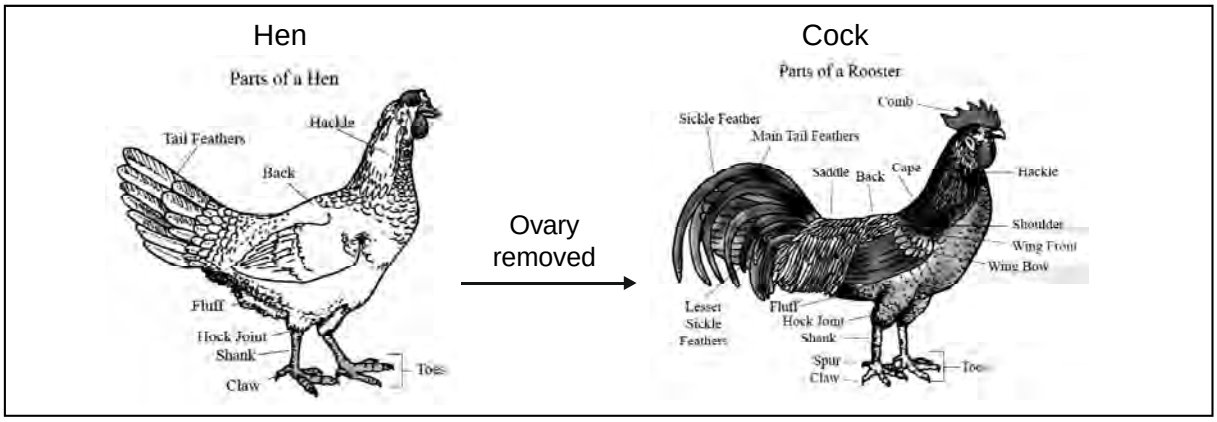


Fig. 6.8 : Sex reversal in chick

6.5.2 Free Martin :

In cattle when the twins of the opposite sex are born, the male is normal but the female is sterile with many male characteristic features. Such sterile females are called **free martin** (Fig. 6.9).

During the development, fetal membranes of the twins have a common blood circulation.

The female hormones are produced a little later than the male hormones. The male hormones influence the female fetus to become sterile.

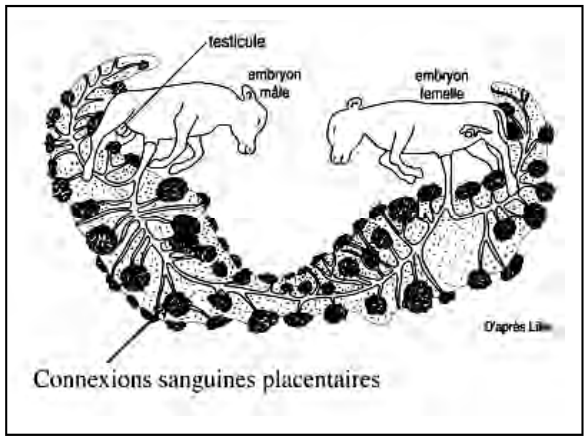


Fig. 6.9 : Freemartin in cattle

6.6 ENVIRONMENTAL FACTORS IN SEX DETERMINATION :

6.6.1 Chemotactic sex determination :

- In some animals, the environment plays an important role in the differentiation of sex or in the expression of genes encoding male and female characters.
- In a worm (*Bonnellia*), the larvae are potentially hermaphrodite.
- If a newly hatched worm is reared from a single egg in isolation, it develops as a female. If newly hatched larvae are reared in water containing mature females, some larvae adhear to the proboscis. These are transformed into males, which eventually migrate into the reproductive tract, where they become parasitic. It has been established that the proboscis of the mature female *Bonellia* secretes a chemotactic substance, which induces the larve to differentiate as males (Fig. 6.10).

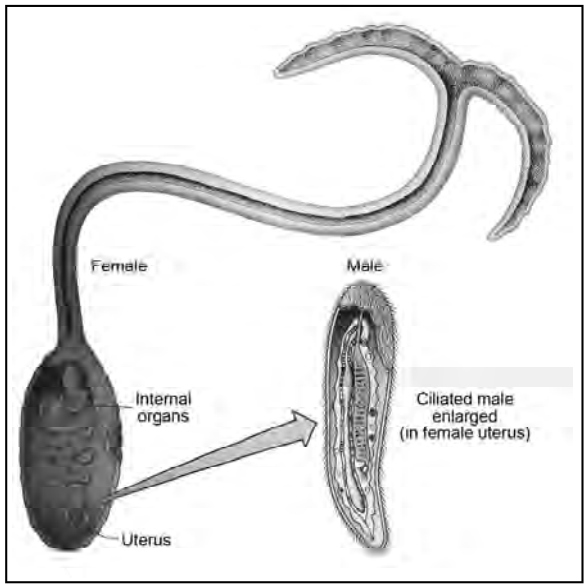


Fig. 6.10 : Sex determination in *Bonellia*

6.6.2 Temperature-dependent sex determination :

- This is a type of environmental sex determination, in which the temperature experienced during embryonic development determines the sex of the offspring.

- It is most prevalent and common among reptiles, especially turtles.
- In turtles, males are generally produced at lower incubation temperatures than females, with this change occurring over a range of temperatures as little as 1–2°C.
- At a lower temperature ranging between 22.5 and 27°C, mostly male turtles hatch, while at higher temperatures, around 30°C, only female turtles hatch.
- In lizards and crocodiles, this pattern is reversed.

6.7 SEX LINKED INHERITANCE :

Sex linkage is the phenotypic expression of an allele located on sex chromosomes. This type of inheritance is in contrast to the inheritance of alleles on autosomes, where both sexes have the same probability of inheritance. Since human has many more genes on the X than on the Y, there are many more X-linked characters than Y- linked. In mammals, the female is homogametic, with two X chromosomes (XX), while the male is heterogametic, with one X and one Y (XY). The male is hemizygous relative to the female, because it has half the number of X chromosomes a female possesses. Genes on the X or Y chromosome are called sex-linked genes and their mode of inheritance is called **sex linked inheritance**.

6.7.1 Sex linked Genes :

The sex linked genes are of the following types :

1. **X-linked genes :** Genes located on X chromosomes are called X-linked . Such genes do not have alleles on Y chromosome. In man about 300 genes are X-linked.
2. **Y-linked genes or Holandric genes :** only a few genes are located on the Y chromosome.
3. **Pseudoautosomal genes :** Genes located on homologous parts of both X and Y chromosomes.

6.7.2 Inheritance of sex-linked characters :

The alleles of the sex-linked characters are recessive to their normal alleles. Therefore, these are expressed in the heterogamatic sex or male. In the homogametic sex, i.e. the female, it is expressed in a homozygous condition. The chance of expression of a recessive X-linked character in the female is the square of that of the male. For example, if 1 in 20 males in a population expresses a sex-linked character, then the chance of expression of the same character is 1 in 400 in females.

When the female is heterozygous for the sex-linked gene, it is not expressed, but then the female is a carrier for the said allele. The female parent transmits one each of her X chormosomes to her sons and daughters in an equal propertion, while the male transmits it to his daughters in the F1 generation. The daughter, while forming the F2, transmits her

X chromosomes to her sons and daughters in equal propertion. Thus, a male parent bearing a sex-linked character transmits it to 25% of his grandsons (F2 generation) through his daughter (F1 generation). The character criss-crosses the F1 generation while passing on to the F2 generation i.e. transmitted to grandson through daughter. This type of inheritance is known as **criss-cross inheritance**.

In the following sections the inheritance of haemophilia and red-green colour blindness in human is considered to have a better understanding of the afore mentioned discussion.

6.7.3 Inheritance of Haemophilia :

Haemophilia is a sex-linked character in human. It is also known as bleeders disease. In the event of an injury, the blood fails to coagulate. This trait is inherited in the British royal family and has been passed on to other royal houses across Europe.

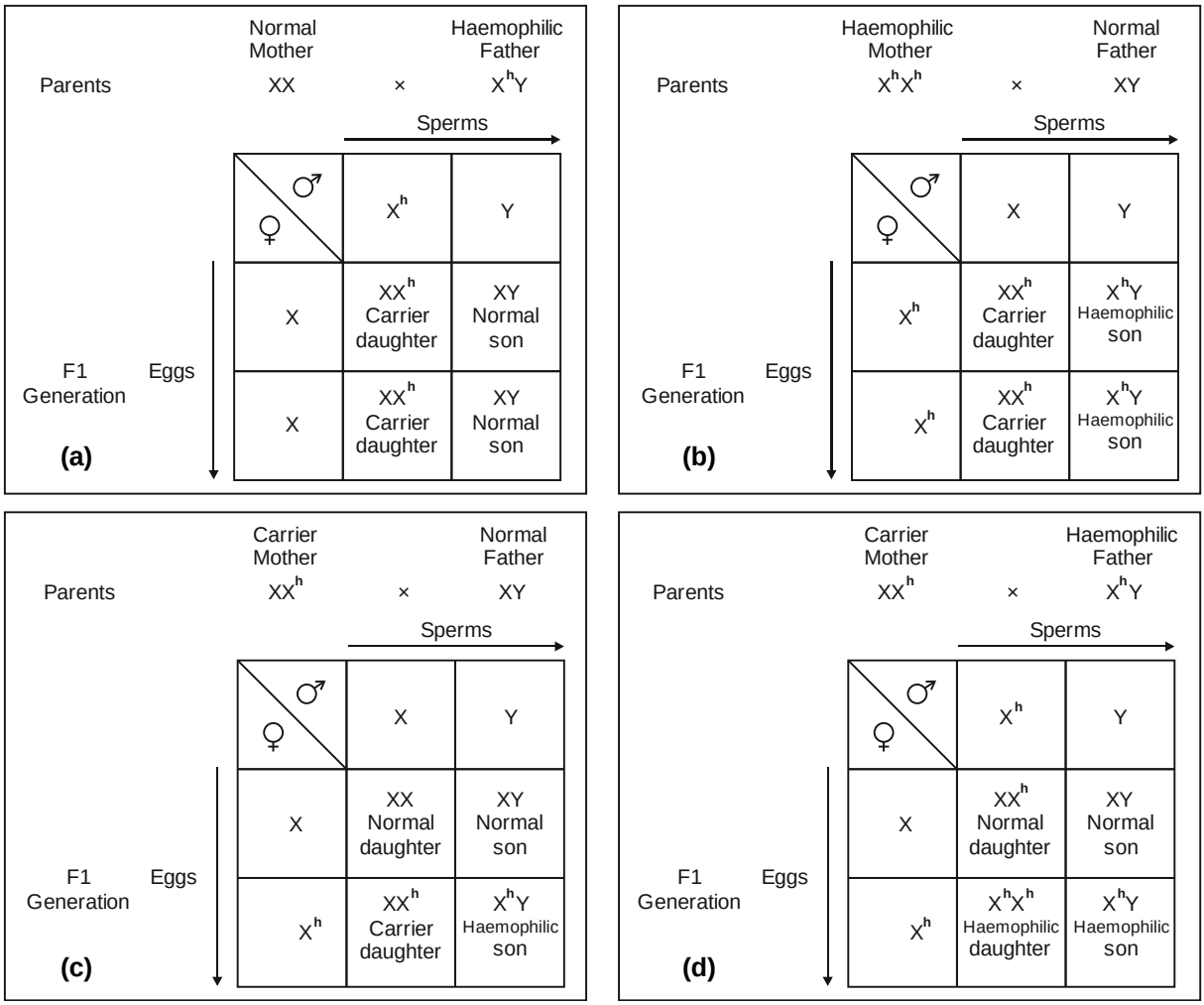


Fig. 6.11 : Four crosses describing the inheritance of haemophilia allele in human.
(a) normal mother and haemophilic father, **(b)** haemophilic mother and normal father,
(c) carrier mother and normal father & **(d)** carrier mother and haemophilic father.

The haemophilia allele is recessive to its normal allele. Homozygous recessive females and males with haemophilia allele on its X chromosome express the trait and become haemophilic. Females with one normal and one haemophilia allele do not express the train. They are known as carriers of the haemophilia allele. The allele follows a cris-cross inheritance in a simple mendelian fashion. The inheritance is explained in Fig. 6.11 (a -d).

6.7.4 Inheritance of red-green colour blindness :

It is an inherited disorder which results in the failure to distinguish red and green colours. The gene enabling a person to identify red-green colours lie on the X chromosome. When it is mutated, there is a loss of the ability to distinguish these colours. Thus, the gene has two alleles : a normal and a mutant that is recessive the normal allele. Homozygous females for this mutant allele and hemizygous males for the same allele are colourblind, while heterozygous females have normal vision and are considered as carriers for the mutant allele. The mutant allele is inherited in the same way as that of the haemophilia allele and follows a criss-cross inheritance. The situation can be understood by considering the Punnet squares in Fig. 6.11 by substituting X^h with X^c, which denotes the colourblind allele.

6.8 MENDELIAN DISORDERS IN HUMAN :

Normal genes encode normal proteins, which regulate normal physiological functions of the body. When a gene undergoes mutation, it encodes an abnormal protein, which fails to regulate the body functions, it is meant for. In this situation, the abnormal body functions express some abnormal phenotypic characters. The expression of these characters has been referred to as a **genetic disorder** or **syndrome**. Most of the human disorders are inherited in simple mendelian fasion.

In the following section, a few such disorders and / or syndromes are discussed.

6.8.1 Thalassemia :

Thalassemia is an inherited blood disorder, in which the body makes an abnormal form of haemoglobin. The disease is prevalent in Asia, Middle-East, Africa and Mediterranian countries like Greece and Turkey.

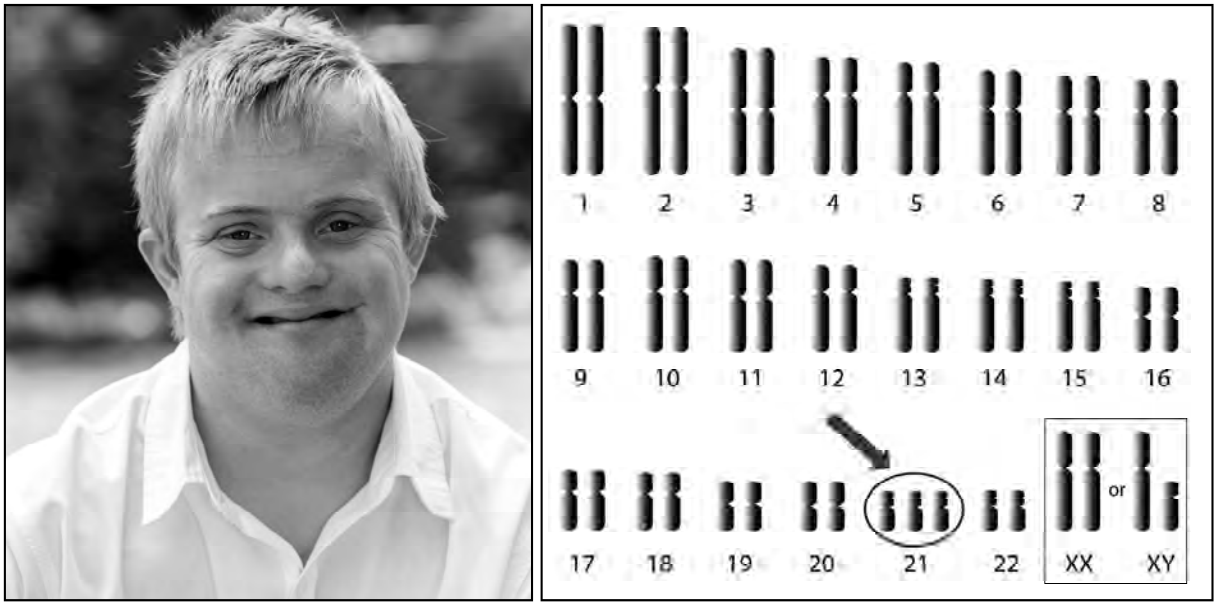
There are three forms of thalassemia : **α-thalassemia**, **β-thalassemia** and **thalassemia minor**. α-thalassemia is again of two types : haemoglobin H disease and Hydrops Fetalis. Hydrops Fetalis is the more severe from. It is expressed, when all the four globin genes are mutated. Most babies afflicated with this form are either stillborn or die shortly after birth. Haemoglobin H disease is expressed, when three out of four α-globin genes are mutated. β-thalassemia is expressed when the body cannot produce β-globin. It has two sub-types : thalassemia major (Cooley's anemia) and thalassemia intermedia. Thalassemia major is more severe and is expressed when two β-globin gnes are mutated or absent, while thalassemia intermedia is less severe.

6.8.1.1 Symptoms, Diagnosis and Treatment :

The common symptoms are : feeling of tireness, pale skin with severe anemia, enlarged spleen, yellowish skin and dark urine. The disease is diagnosed by blood test and genetic analysis. There are two treatment options : blood transfusion and bone marrow transplantation. However, genetic counseling of the affected person makes him / her conscious about the consequences. He / she advised to take recourse to an appropriate treatment option.

6.8.2 Down sysndrom :

The most common and best characterized genetic disorder in human population is Down sysndrome. It was previously indentified as **mongolism** due to a short stature of the affected persons. **John Langdon Down** first described the clinical symptoms in 1866. In his honour, the syndrome has been named as Down syndrome. The estimated frequency at birth is 1/700.



(a) **(b)**

Fig. 6.12 : (a) A person with Down syndrome phenotypes, (b) Down syndrome chromosome complement (the arrow shows three doses of 21st chromosome).

6.8.2.1 Genetic Basis :

It is caused by a **chromosomal aberration**, known as **aneuploidy (trisomy)**. The twenty first chromosomes is present in three doses, instead of two in normal persons [Fig. 6.12(b)]. Thus the diploid chromosome number becomes 47, instead of normal 46. It is the result of **primary nondisjuction** (failure of separation of homologous chromosomes), which may occur at first meiosis or second meiosis of the maturation phase of gametogenesis. The consequence is that the egg or sperm receives an extra 21st chromosome. If this egg is fertilized by a normal sperm and vice versa, the zygote nucleus will have 47 chromosomes with an extra

21st chromosome. This zygote develops into a baby expressing the sysmptoms of Down syndrome. Through the investigations of **J. Lejeune in 1959**, Down sysndrome was recongnized as the first genetic disorder in human. It is also identified as trisomy 21.

6.8.2.2 Clinical symptoms [Fig. 6.12(a)] :

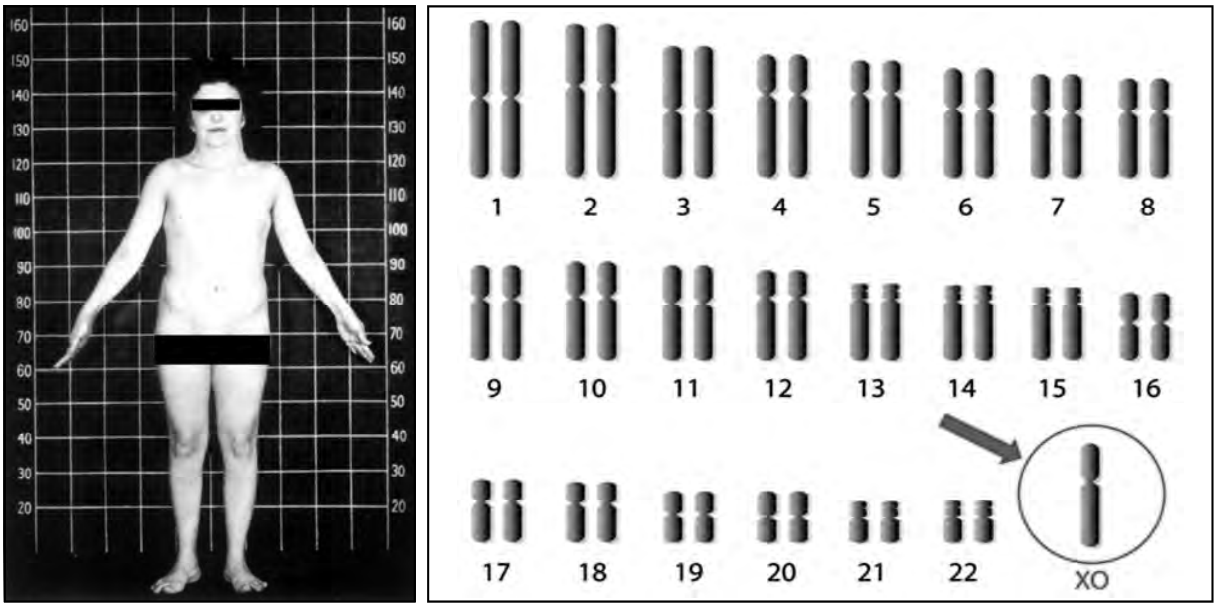
- Short stature with an epicanthal fold.
- Broad head with round face.
- Wide nostril, open mouth and large tongue with distinct furrows.
- Stubby hands with simian crease on the palm.
- Hyperflexible joints.
- Mental retardation.

6.8.2.3 Diagnosis, Treatment and Prevention :

Prenatal screening of the pregnant women is undertaken by ultrasonography and amniocentesis sampling to make sure about the contraction of this disorder. There is no treatment available as yet. However, counseling through education support and creation of sheltered work environment works encouragingly. Life expectany is 50-60 years.

6.8.3 Turner Syndrome :

Turner syndrome is a condition, in which a female is missing one of the two X chromosomes, such that the complment becomes 45, XO [Fig. 6.13(b)]. This condition is associated with many abnormal phenotypes, first described by H.H. Turner in 1938. It occurs in



(a) **(b)**

Fig. 6.13 : (a) A female with Turner syndrome phenotypes, (b)Turner syndrome chromosome complement (the arrow shows one X chromosome, there being no Y chromosome).

about 1 per 2500 live female births. More than 90% of the women bearing fetus affected by Truner syndrome abort spontaneously. An approximate frequency estimate in the human population is 1/5000.

6.8.3.1 Ganetic Basis :

It is caused by a chromosomal aberration, known as **aneuploidy (monosomy)**. In the female, one out of two X chromosomes is missing. Thus, the chromosomal number is 45 instead of normal 46 [Fig. 6.13(b)]. It is the result of primary nondisjunction, which may occur in one of the two meiotic divisions of the maturation phase of gametogenesis. The consequence is the formation of an egg with two X chromosomes and another with no X crhomosome. If the later one is fertilized by a normal sperm bearing an X chromosome, the complement becomes 45, X and Turner syndrome expresses.

6.8.3.2 Clinical Symptoms (Fig. 6.13(a)] :

- Short stature with low-set ears.
- Webbed neck
- Shield-like chest
- Swollen hands and feet
- Virtually no ovaries
- Limited secondary sexual characters.

6.8.3.3 Diagnosis, Treatment and Prevention :

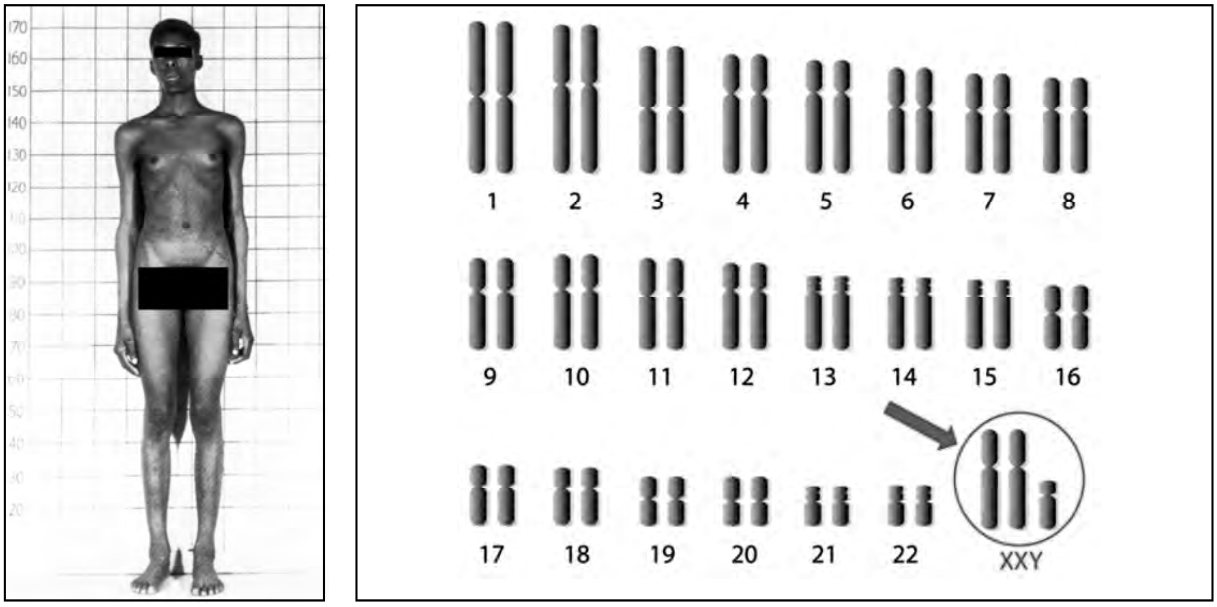
Diagnosis is done by physical examination and genetic analysis Turner syndrome affected subjects undergo hormonal therapy. Growth hormone injection in early childhood may increase the height by few inches. Estrogen replacement therapy is undertaken at puberty to start the breast development. Estrogen and progesterone are administered together, a little later to initiate the monthly cycle. Turner syndrome affected persons have a shorter life expectancy.

6.8.4 Klinefelter Syndrome :

Klinefelter syndrome is an abnormal genetic condition, caused by the presence of an extra X chromosome in addition to the usual male sex chromosome complement of XY [Fig. 6.13(b)]. Thus the diploid chromosome number becomes 47 with XXY sex chromosome complement. This condition was first described by H.F. Klinefelter in 1942. It is estimated to occur in 1 in 500 live male births.

6.8.4.1 Genetic Basis :

The condition is due to the presence of an extra X chromosome in the male. The XXY condition pressumably arises at fertilization of an exceptional egg (XX) by a Y-sperm or an X-egg by an exceptional XY sperm. The exceptional eggs and sperms are the outcome of primary nondisjuction of X and Y chromosomes during maturation phase of gametogenesis. Studies in



(a) **(b)**

Fig. 6.13 : (a) A male showing Klinefelter syndrome phenotypes and (b) Chromosome complement (the arrow shows the presence of an additional X chromosome).

Turner syndrome and Klinefelter syndrome indicate that, the Y chromosome is essential for the expression of maleness. The usual karyotype is 47, XXY, while more complex karyotypes, such as XXXY, XXXXY, XXXXXY, XXYY, XXXXYY are also associated with Klinefelter syndrome. The frequency of occurrence is 1 in 500 male births.

The syndrome is diagnosed by a chromosome complement examination. There is no treatment option. It can be prevented through education and genetic counseling by a trained clinical geneticist.

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer :

(i) A cross between F_1 hybrid and a recessive parent gives the ratio of

(a) 3:1 (c) 1:1

(b) 2:1 (d) 4:1

(ii) A cross of F_1 with the recessive parent is known as

(a) back cross (c) hyrbid cross

(b) test cross (d) double cross

(iii) A woman with albinic father marries and albinic man. The proportion of her progeny is

(a) 2 normal : 1 albinic (c) all albinic

(d) all normal (d) 1 normal : 1 albinic

(iv) Y cromosome is called

(a) sex cromosome (c) androsome

(b) autosome (d) gynaesome

(v) Which one is a sex-linked disorder ?

(a) leukemia (c) night blindness

(b) cancer (d) colour blindness

(vi) A haemophilic man marries a normal homozygous woman. What is the probability that their son will be haemophilic?

(a) 100% (c) 50%

(b) 75% (d) 0%

(vii) What is the probability that their daughter will be haemophilic?

(a) 100% (c) 50%

(b) 75% (d) 0%

(viii) A furitfly exhibiting both male and female trait is

(a) heterozygous (c) hemizygous

(b) gynandromorph (d) Gynandev

(ix) Genes located in Y chromosome are

(a) mutant genes (c) holandric genes

(b) autosomal genes (d) sex-linked genes

(x) A colourblind person cannot distinguish

(a) all colours (c) green colour

(b) red colour (d) red and green colours

(xi) The gene responsible for haemophilia is linked to which chromosome ?

(a) X (c) Y

(b) both X and Y (d) Autosome

(xii) Red-green colourblindness in man is :

(a) sex-linked character (c) sex influenced character

(b) sex-limited character (d) sexual character

(xiii) Sex-linked characters are

(a) dominant (c) recessive

(b) lethal (d) not inherited

(xiv) Which gene is present in the Y chromosome that codes for the protein TDF?

(a) Cry (c) Sry

(b) Try (d) tra

(xv) In birds, which type of chromosomal basis of sex determination is present?

(a) XX - XY (c) ZW - ZZ

(b) XX - XO (d) ZZ - ZO

(xvi) When the ratio of X / A = 0.67 in genic balance theory, which type of sex is expressed ?

(a) super female (c) super male

(b) intersex (d) triploid female

(xvii) Which type of sex determination is found Bonellia

(a) temperature dependent (c) holandric

(b) chemotacic (d) pseudoautosomal

(xviii) In a person with Turner syndrome, the number of X chromosome is

(a) 1 (c) 3

(b) 2 (d) 0

(xix) A Down syndrome will be

(a) 45 + XX (c) 44 + XXY

(b) 44 + XY (d) 22 + XY

(xx) Number of Barr bodies present in Turner syndrome is

(a) 0 (c) 2

(b) 1 (d) b or c

2. Answer each of the following in one or two words :

(i) Name two sex-linked diseases of human being.

(ii) How Down syndrome is caused?

(iii) In which chromosome is the gene for haemophilia located?

(iv) What is the chromosomal formula for Truner syndrome?

(v) Which sex is usually a carrier?

(vi) Who proposed the 'Genic balance theory'?

(vii) What are holandric genes?

(viii) In which chromosome, the factors for haemophilia and colourblindness is found?

(ix) What is the other name of Bleeders disease?

(x) Which protein is in 'Sry' gene of Y chromosome?

(xi) What is 'Gynandromorph'?

(xii) What is 'Free martin'?

(xiii) What 'Criss-cross inheritance'?

(xiv) Which type of defect is found in 'Thalassemia'?

(xv) Who first described 'Klinefelter's syndrome'?

GROUP - B
(Short Answer-type Questions)

1. Difference between :

(i) Phenotype and Genotype

(ii) Autosome and Allosome

(iii) X chromosome and Y chromosome

(iv) Supermale and superfemale

(v) Sex differentiation and Sex reversal

(vi) Gynadromaph and Free martin

(vii) Down syndrome and Turner syndrome

2. Write brief notes on the following (within 50 words each) :

(i) Criss-cross inheritance

(ii) Holandric gene

(iii) Haplo-diploidy mechanism of sex determination

(iv) Genic balance theory

(v) Free martin

(vi) Gynamdromorph
(vii) Single gene effect
(viii) Sex reversal
(ix) Temperature-dependant sex determination.
(x) Chemotactic sex determination
(xi) Thalassemia
(xii) Down syndrome
(xiii) Turner syndrome
(xiv) Klinefelter syndrome

GROUP - C
(Long Answer-type Questions)

1. Discuss the chromosomal theory of sex determination.
2. What is genic balance theory and explain its role in sex determination.
3. Explain sex-linked inheritance. Discuss the phenomenon with the example of colour blindness.
4. Give an account of sex-linkage in Drasophila and Man.

❑ ❑ ❑

CHAPTER 7

MOLECULAR BASIS OF INHERITANCE

After the rediscovery of Mendelism and the acceptance of chromosome theory of inheritance by **Sutton** and **Bovery** in 1902, the pattern of heredity could be explained by the segregation of chromosomes during meiosis. **Johannsen** coined the term **gene** for Mendelian factor in 1909. But, the question that kept the scientific community occupied for the next fifty years was about the exact connection between the hereditary traits and chromosomes. During the same time, in 1902, **Archibald Garrod**, a British physician working with one of the early Mendelian geneticist, his countryman **William Bateson** made an interesting observation. They observed, in the case of inherited disorder like **alkaptonuria,** the patient excreted black urine. It was due to the absence of an enzyme capable of breaking down **homogentisic acid** (alkapton) into simpler substances. In the absence of this enzyme, the urine contains alkapton that is rapidly oxidized in air to turn black. Garrod observed that this disease was inherited in Mendelian pattern. This was the first pointer towards a relation between gene and enzyme.

Further, the nature of the genetic materials also came under investigations. Prior to the rediscovery of Mendelism, **Meischer** discovered DNA in 1869, though he could not assign any role of DNA. With the advancement of molecular biology, it was established that chromosome chemically consisted of proteins and DNA. Gradually, it became clear that protein consisted of twenty amino acids and DNA is made from four nucleotides. Initially it appeared to the scientific community that proteins having a more varied and complex structures could be the possible genetic material. The British microbiologist, **Frederick Griffith** provided the first evidence for DNA as the genetic material.

7.1 DNA AS GENETIC MATERIAL :

In 1928, **F. Griffith** made a series of unexpected observations while experimenting with a pathogenic (disease causing) bacterium, ***Diplococcus pneumoniae*** (then known as ***Pneumococcus)***. This bacterium causes pneumonia in man and most mammals and has two phenotypes. One is the **virulent/ pathogenic** form and possesses a polysaccharide coat that protect the bacterium from phagocytic attack of the host. Because of the coat, the virulent bacteria form smooth edged colonies in culture. The other is **avirulent/ non-pathogenic** and lacks the coat. They form rough edged colonies in cultures. The virulent forms are, therefore

called Smooth-type or **S-type** and the avirulent forms are called Rough-type or **R-**type. There can be several strains of S-type and R-type, like S-I, S-II, S-III and R-I, R-II, R-III etc. Griffith selected mouse as the host and S-III and R-II bacteria for his experiment. It was apparent that mouse injected with S-III bacteria suffered from the disease and died while those injected with R-II did not suffer and survived. But Griffith made some surprising observations when he injected mice with different combinations of bacteria. His experimental findings can be summarized as:

(i)	Mouse injected with live S-III	Died
(ii)	Mouse injected with live R-II	Survived
(iii)	Mouse injected with heat killed S -III	Survived
(iv)	Mouse injected with heat killed S -III + live R-II	Died

↓

Extract from dead mouse

↓ (cultured)

Live R-II+Live S-III

This indicated that live S-III extracted from the dead mouse initially injected with heat killed S-III and live R-II must have arose from R-II. It could not have been due to mutation in R-II; in that case live S-II not live S-III would have been formed. The dead S-III and live R-II would have interacted in some way so that some of the live R-II would have been transformed to live S-III. From the heat killed cells of S-III "something" would have escaped and transformed R-II to S-III. This "something: was referred to as **transforming principle**. Griffith was unaware of the nature of the transforming principle.

Subsequent proof for the chemical nature of Griffith's transforming principle was provided by **Oswald T. Avery** and his co-workers **Maclyn McCarty** and **Colin M. Macleod** of **Rockfeller Institute**, New York, U.S.A. in 1944. They performed in vitro experiments with highly purified DNA extract of heat killed S-III bacterium. They used the extracted DNA along with combinations of different enzymes to transform the R-II type bacteria. This DNA extract retained its transforming ability when subjected to protease (that digests protein) or ribonuclease (that digests RNA), but lost the transforming ability when subjected to deoxyribonuclease (that digests DNA). This experiment can be summarized as below:

(i) R-II +DNA extract of S-III + no enzyme = R-II colonies + S-III colonies

(ii) R-II + DNA extract of S-III +Ribonuclease = R-II colonies + S-III colonies

(iii) R-II + DNA extract of S-III + Protease = R-II colonies + S-III colonies

(iv) R-II + DNA extract of S-III + Deoxyribonuclease = Only R-II colonies.

This experiment showed that the preparation when treated with DNA digesting enzyme deoxyribonuclease, no transformation of R-II strains to S-III strains occurred. This provided the first evidence for DNA as the transforming principle or the genetic material.

7.2 STRUCTURE OF NUCLEIC ACIDS (DNA AND RNA) :

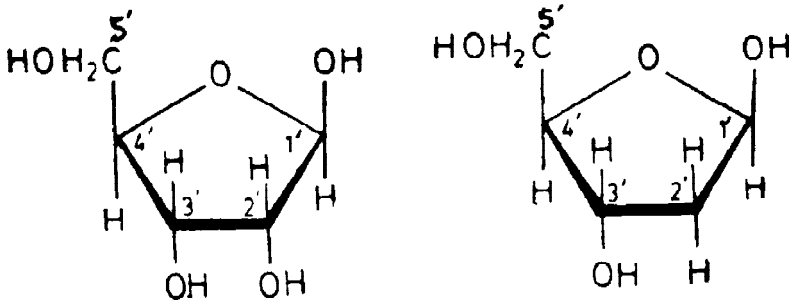
7.2.1 Nucleic Acids :

In every living cell, there are two types of nucleic acids - DNA - Deoxynucleic Acid and RNA - Ribonucleic Acid.

DNAs are found in the chromosomes in the nucleus of plant and animal cells. In prokaryotes also DNA, forms the chromosomes. Some viruses, especially animal viruses have it as their genetic material. Furthermore, it is also found in mitochondria of plant and animal cells and in chloroplasts of photosynthetic organisms.

Ribonucleic Acid (RNA): mainly found in the cytoplasm of cells. There are various types of RNAs (rRNA, tRNAs, mRNA) involved in the expression of genetic information.

In ribonucleic acids, the sugar is ribose; in deoxyribonucleic acids, it is deoxyribose. These two sugars differ in their chemical nature on carbon 2 as shown below.

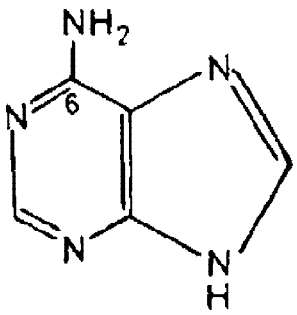


Ribose **Deoxyribose**

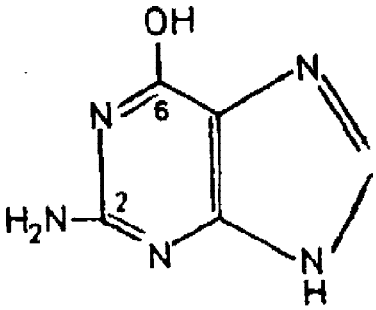
Nitrogenous base: All nitrogenous bases found in DNA and RNA are derive from two heterocyclic bases, purine and pyrimidine.

1. Purine base

Two principal purine bases found in deoxyribonucleic acids as well as ribonucleic acids are adenine and guanine.



Adenine

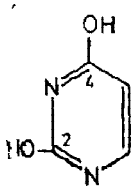


Guanine

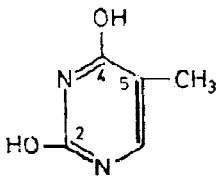
Purines

2. Pyrimidine bases:

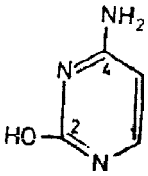
Cytosine and uracil are found in ribonucleic acids; cytosine and thymine, in deoxyribonucleic acids.



Uracil



Thymine



Cytosine

Pyrimidines

Nucleosides

Nucleosides are formed from the linkage of a purine or pyrimidine base with ribose or deoxyribose. This linkage joins nitrogen 9 of the purine base, or nitrogen 1 of the pyrimidine base with carbon 1' of pentose. With ribose ribonucleosides are formed and with deoxyribose, deoxyribonucleosides. The following table indicates the nomenclature of the main nucleosides.

Base	Ribonucleoside	Deoxyribonucleoside
Adenine	Adenosine	Deoxyadenosine
Guanine	Guanosine	Deoxyguanosine
Uracil	Uridine	Deoxyuridine
Cytosine	Cytidine	Deoxycytidine
Thymine	ribothymidine	Deoxythymidine

Nucleotides

Nucleotides are the phosphoric esters of nucleosides. Depending on the nature of the pentose one will have ribonucleotides and deoxyribonucleotides. A ribonucleoside has 3 positions, which can be phosphorylated (2', 3' and 5') while a deoxyribonucleoside can be phosphorylated only in two places (3' and 5'). This results in the formation of nucleoside monophosphate.

A second phosphate group can be bound to the phosphate of a nucleoside monophosphate to form a nucleoside-di-phosphate. Likewise a third phosphate group can also be attached to the second forming nucleosides tri-phosphate.

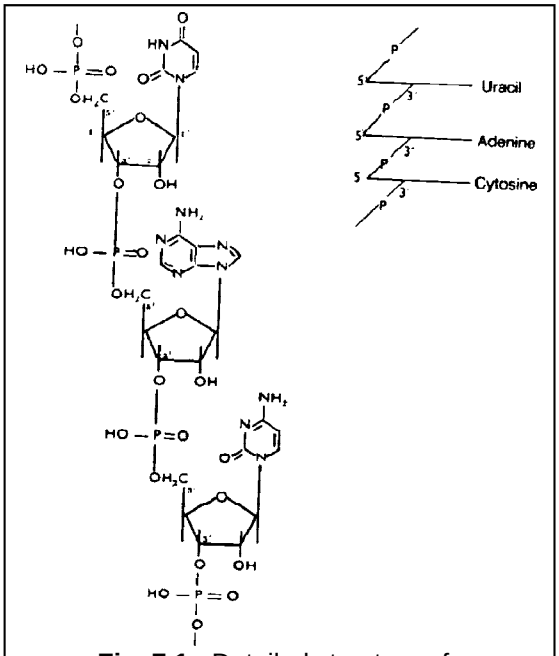


Fig. 7.1 : Detailed structure of a segment of nucleotide

7.2.2 Primary structure of DNA :

In deoxyribonucleic acids, the nucleotides are joined by 3'-5' phosphodiester bonds; in other words each phosphate group (except those present at the end of chains) esterifies to the 3' hydroxyl group of a pentose and to the 5' hydroxyl group of the next pentose. Therefore, the polydeoxyribonucleotide chain consists of alternating deoxyribose and phosphate residues (Fig. 7.1).

7.2.3 Secondary structure of DNA :

The observations in solution state, however, predicted the existence of secondary structure in DNA. Taking these facts into consideration, Watson and Crick in 1953 proposed the secondary structure in the form of the famous double helix model -

(a) It was known through base analyses that there is as much adenine as thymine and as much guanine as cytosine (A/T and G/C = 1). Therefore, the sum of purines is equal to the sum of pyrimidines (A+G = C+T). It is known as Chargaff's rule. Also, experimental results suggested the polydeoxyribonucleotide chains were held together by hydrogen bond.

(b) X-ray diffraction studies (Wilkins, 1952), suggested a helicoidal configuration of DNA.

According to this model, DNA has a double stranded structure where two polydeoxyribonucleotide chains twisted around one another in a double helix. Both the helices are held together by means of hydrogen bonds existing between the nitrogen bases. The diameter of the DNA molecule is 20 $\overset{o}{A}$ (2nm). The length of the DNA in one complete turn is 34 $\overset{o}{A}$ (3.4nm), which incorporates 10 base pairs. Therefore, the distance between two adjacent base pairs is 3.4 $\overset{o}{A}$.

Both the strands have sugar phosphate backbone and are antiparallel to one another. The antiparallel nature is given by orientation of the deoxyribose sugar which is opposite in both the strands. Therefore, the 5th carbon atom of the sugar molecule, which is exposed at one end of a strand (5' end), faces the 3rd carbon atom of the sugar in the opposite strand (3' end). The strands are also complementary to each other. This nature is based on the purine-pyrimidine

links i.e. if a strand is having a purine base (adenine or guanine), the opposite must be its pyrimidine counterpart (thymine or cytosine) e.g. A = T and G = C. in DNA; the nucleosides are joined by means of phosphodiester bonds. (Fig. 7.2)

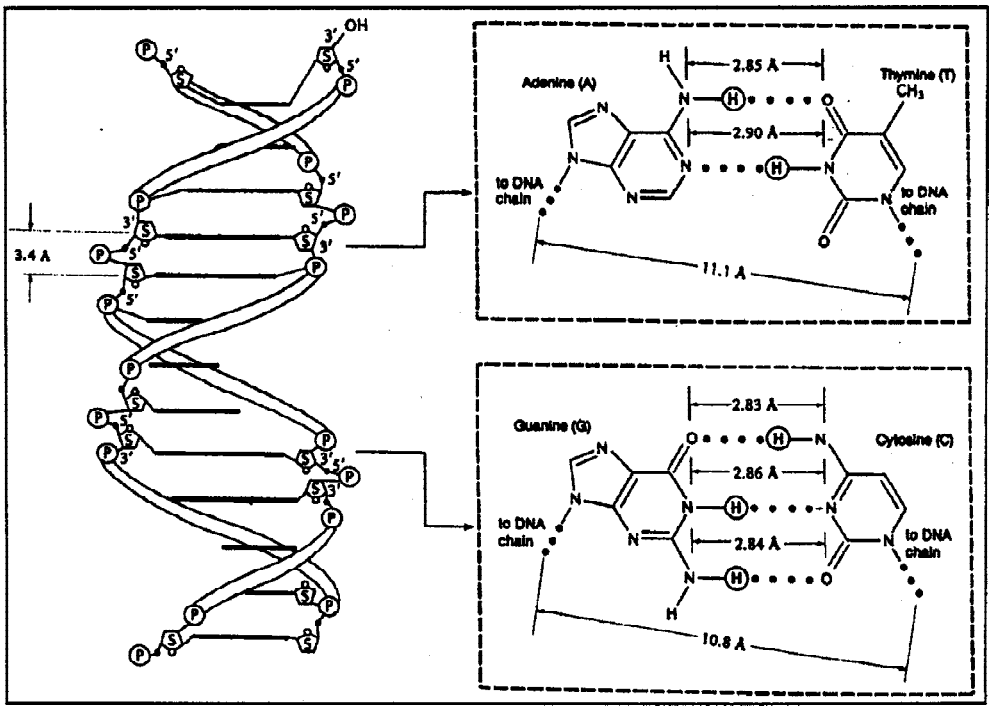


Fig. 7.2 : The Watson-Crick model of B-DNA

Structural forms of the double helix :

There are three major structural forms of double helical DNA. The 'B'-form (Fig. 7.3), described by Watson and Crick, the 'A'-form and the Z-form. (Fig. 7.3)

The B-form is a right-handed helix with ten residues per 360^0 turn and with planes of bases perpendicular to the helix axis. The chromosomal DNA primarily consists of B-DNA.

If B-DNA is moderately dehydrated the A-DNA is produced. A-form is also a right-handed helix, but with eleven base pairs per 360^0 turn and the planes of the bases are tilted 20^0 away from the perpendicular to helical axis. The DNA regions found in DNA-RNA hybrid or RNA-RNA double stranded regions are very close to A-form. The Z-DNA have 'Zigzag' backbone and hence has the name. It is a left-handed helix containing twelve base pairs per turn. The Z-

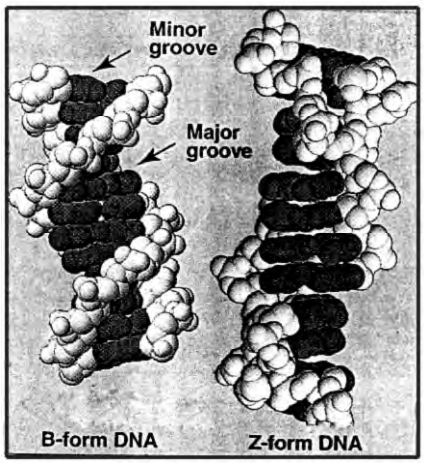


Fig. 7.3 : Different forms of DNA

DNA stretches occurring naturally in DNA have a sequence of alternating purines and pyrimidines, (i.e. Poly GC regions). The transitions among the three forms of DNA may play an important role in regulating gene expression.

Besides these 3 major forms there are two more right-handed forms; C-DNA with nine base pairs per turn and D-DNA with eight base pairs per turn. There are many forms of DNA molecule viruses. (Table 7.1)

Table - 7.1

Various Forms of DNA molecules found in a variety of viruses.

Types of viral DNA molecules	Examples
1. Linear single strand	Parvo viruses
2. Circular single strand	ϕ x174 and other bacteriophages
3. Linear double strand	T7 ; many phages and animal viruses
4. Linear double strand with single chain breaks	T_5
5. Linear double strand with closed ends	Vaccinia
6. Closed circular double strand with or without super coils	Papoviruses, bacteriophage PM_2 and cauliflower mosaic virus.

7.2.4 RNA Structure :

RNA mainly comprises of genetic and nongenetic RNAs.

RNA is a polynucleotide, made of ribonucleotide units having ribose sugar, phosphoric acid and nitrogen bases (Adenine or Guanine or Cytosine or Uracil). It is single stranded. Cellular RNAs are non-genetic and are of three types.Regarding the role of genetic RNAs,it has been mentioned in the central dogma.

Messenger RNA (mRNA) :

It is the RNA formed during the protein synthesis. Five to ten percent of cellular RNA is of this type. The molecular weight of mRNA varies from 30000-1000000. It is short lived. DNA transfers the genetic information to ribosome through this type of RNA during the protein synthesis.

Ribosomal RNA (rRNA) :

The most stable form of RNA in the cell is the r-RNA. About 80% of cellular RNA is of this type. The molecular weight of rRNA ranges from 40000-1000000. It may have some folds to have a complex structure. rRNA units along with protein constitute the protein synthesizing factory or the ribosome.

Transfer RNA (tRNA) :

It is smallest form of RNA made of only 75 to 100 nucleotides. It is also known as the soluble RNA. It forms about 10-15% of total cellular RNA. The molecular weight of tRNA varies from 25,000-30,000. It transfers the amino acids from the cytoplasm to the ribosome, during protein synthesis.

In 1964, Holley gave the detailed structure of tRNA through the 'Clover leaf model'. In that model, it was proposed that tRNA has three loops and a lump. The anticodon loop has the complementary base sequence with respect to a codon of mRNA facilitating the attachment of tRNA with the later. Other two loops are TΨC loop or ribosomal binding loop and DHU loop or amino acyl synthetase binding loop. The 3' end of tRNA ends with CCA-OH, which acts as the amino acid attachment site. The other end ends with G. (Fig. 7.4)

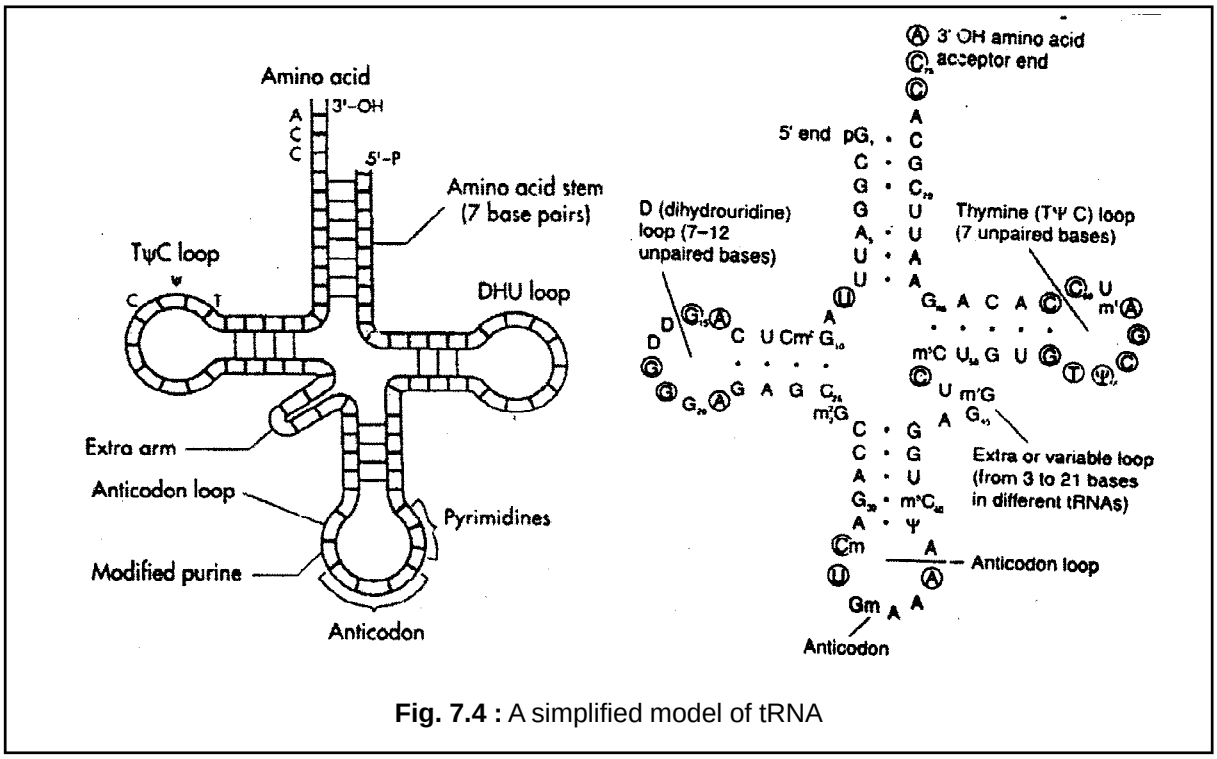


Fig. 7.4 : A simplified model of tRNA

In eukaryotes a variety of other RNAs make RNA world. These are small heterogenous nuclear RNA (snRNA), the precursor of mRNA or heterogenous RNA (hnRNA) or snRNA, concerned with mRNA processing and small nucleolar RNA (snoRNA) concerned with ribosomal RNA processing in nucleolus.There are many other minor RNAs named on basis of their sedimentation co-efficient like 28SRNA,16SRNA,5SRNA etc.

7.3 PACKAGING OF DNA :

DNAs is eukaryotic chromosomed are large molecules. These are precisely packaged by compacting DNA with histone proteins into repeating nucleosome (Chapter-7 of Biology-I, Page-341, 2016). In prokaryotes lacking a defined nucleus the DNA is held with some proteins.

7.4 DNA REPLICATION :

In a multicellular organism, all the cells possess the same quantity and quality of DNA as all the cells are derived from the successive divisions of a single cell. It is a fact that whether a cell has only one chromosome (as in prokaryote) or many chromosomes (as in eukaryote) the entire genome must replicate precisely once per every cell division. The basic principles of DNA replication in relation to cell cycle are:

* Once the DNA replication is initiated, the cell is committed to a division.
* The cell division cannot occur until the replication of entire genomic DNA is complete.

7.4.1 DNA Replication is Semiconservative :

The Watson-Crick double helical model for DNA suggested that the basis for copying genetic material is **base complementarity**. The base sequence of one strand of a DNA molecule determines the sequence on the other strand as the two strands are complementary to each other. The complementarity of two strands provides a means for the accurate replication of the DNA molecule. During replication, the two strands separate into two single strands and then appropriate complementary nucleotides are assembled on each exposed strand to form two DNA molecules. These two new DNA molecules have one strand each from the original molecule replicated and one strand each

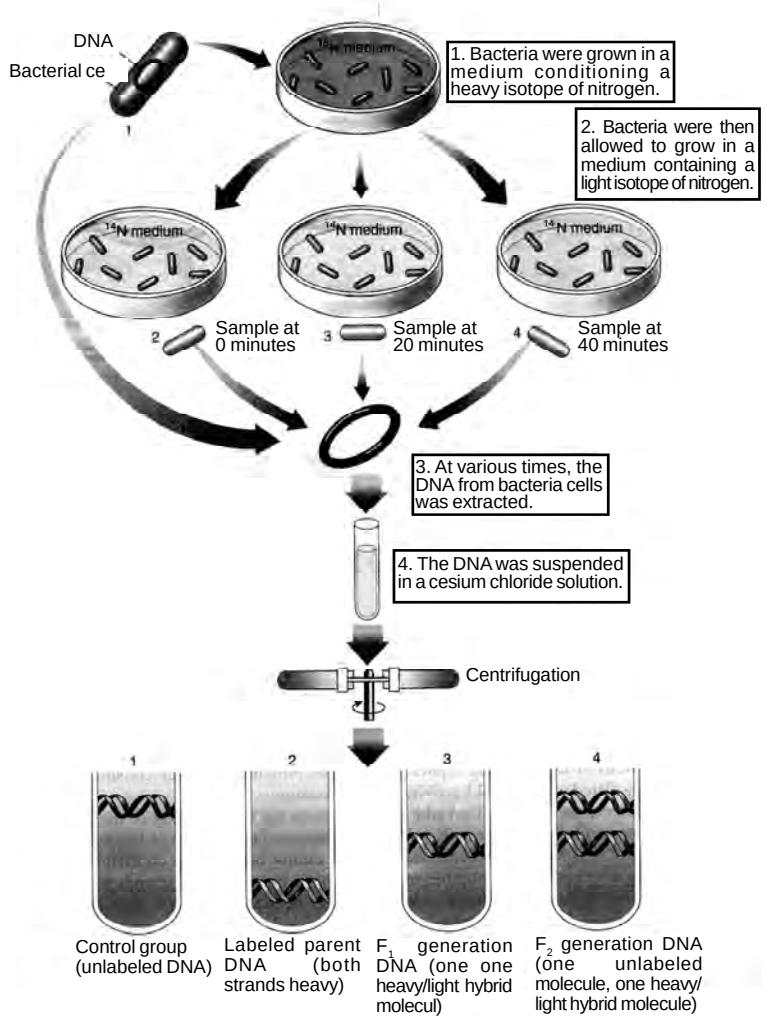


Fig.7.5 : Meselson-Stahl experiment to demonstrate semiconservative replication.

newly synthesized. Thus, as in every DNA, one parental strand is conserved and one new strand is synthesized, this mode of replication is known as semiconservative replication.

The semiconservative mode of replication was experimentally proved by **Mathew Meselson** and **Franklin Stahl** of California Institute of Technology in 1958 with *E.coli*. They grew bacterial strain in a medium containing heavy isotope of Nitrogen (N^{15} Ammonium chloride) as the only source of Nitrogen. After several generations, all the nitrogenous bases of bacterial DNA got labeled with N^{15} Nitrogen. These radio-labeled DNA have greater density than DNA with normal nitrogenous bases. Then the culture of bacteria was washed to make them free from the medium and was transferred to medium containing normal N^{14} ammonium chloride. After one generation the density of DNA extracted from the cultured bacteria was intermediate. This is because during one generation time each DNA double helix had separated and an old strand (with N^{15}) had synthesized a new complementary strand (with N^{15}). The intermediate density is usually referred to as **hybrid density**. After two generations of growth in the normal medium with N^{14}, half of the DNA was of hybrid density and half was light or normal. The density of DNA were compared by density gradient centrifugation in concentrated solution of Cesium chloride. As the growth generations continued in light or normal medium more and more DNA present would be light. This confirmed the semiconservative mode of replication.

Semiconservative mode of DNA replication in bacteria was also demonstrated by **J.Cairns** using autoradiography.**J.H.Taylor** and his co-workers established semiconservative mode of replication in *Vicia faba* (eukaryote). Semiconservative mode of replication of chromosome can be visualized through an examination of chromosomes that are allowed two rounds of replication in medium containing **bromodeoxy-uridine** and staining replicated chromosomes with **fluorescent dye** and **Giemsa.**The newly synthesized strand of each DNA stain differently from old strand. Such chromosomes where the two strands of DNA are stained differently are called **harlequin chromosomes.** Presence of harlequin chromosomes confirm semiconservative mode of replication.

7.4.2 Pre-requisites and steps of DNA replication :

The process of DNA replication is fast, accurate and complex requiring several enzymes and protein factors. This process has been worked out in detail in *E.coli* and its viruses as a result of over last 40 years of extensive research. DNA replication in prokaryotes as well as eukaryotes involves some basic steps which can be outlined as follows:

- Unwinding and separation of the two parental strands of DNA.
- Each parental strand then serves as a template for the synthesis of a new strand basing on the complementarity of the sequence of nucleotide of the old template strand.
- One parental strand and one new strand wind together into a double helix.

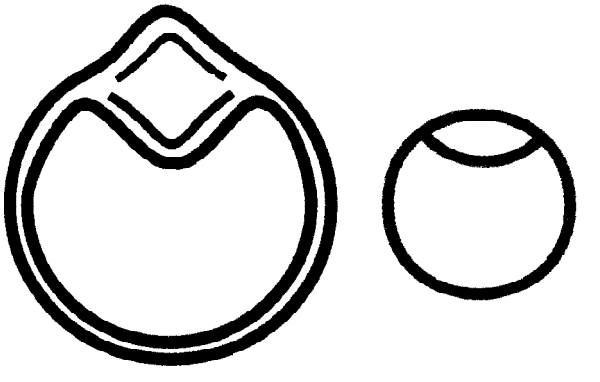


Fig.7.6 : Replication eye or q-replication (Theta) in prokaryote.

DNA replication does not begin just at any where on a DNA. It originates at specific site called replication **origin** and then proceeds in one or both the directions. A DNA segment specifying an origin has been isolated from *E.coli* and several Coli phages and plasmids as well as from Yeast and a number of eukaryotic viruses. In *E.coli,* the origin is a unique sequence of DNA of about 245 base pair long and known as **Ori C**. It is **A-T rich** so that the two strands easily separate at the origin. The origin is specifically recognized by a **replication initiator protein** which binds to the origin to begin replication. In Yeast, the origin is known as **Autonomous Replicating sequence** (ARS) and is 150 base pair long. ARS is the binding site for **Origin Recognition Complex** (ORC). The replication initiated from the origin proceeds along replication forks. So, each origin has two terminii. One origin with its two unique termini is called a **replicon**.In prokaryote like *E.coli* the entire circular DNA is a single replicon. But eukaryotes with larger DNA have several origins per DNA.

The DNA replication can be unidirectional or bidirectional. At the origin when the two strands separate it forms a **replication eye** (Fig. 7.6)**. In unidirectional replication,** one of the two ends of the "eye" remains stationary while the other end moves along the replication fork. In **bidirectional replication** both ends move along the replication (Fig. 7.7). An example of unidirectional replication is replication of mitochondrial DNA (mt DNA) in vertebrates.

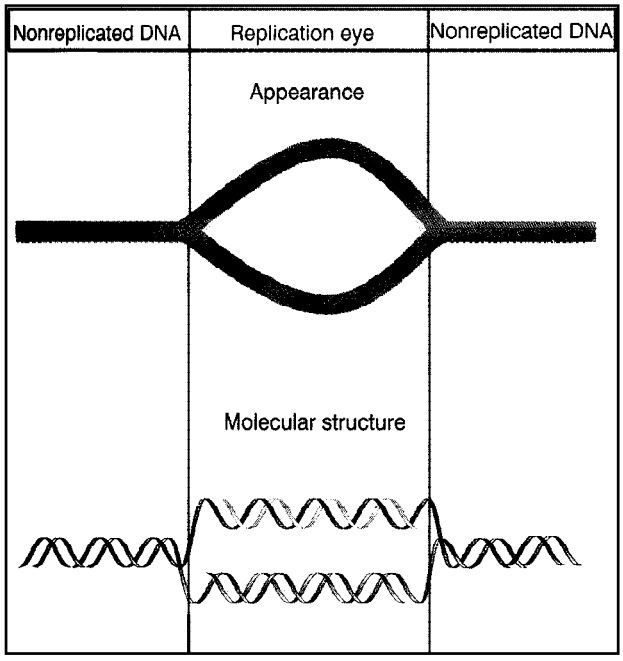


Fig. 7.7 : Bidirectional DNA replication.

DNA polymerase

This is the enzyme which polymerises deoxyribonucleotides. It adds deoxyribonucleotides to the 3′OH end of a growing polynucleotide. The new nucleotide comes as nucleoside-triphosphate and is joined to the open –OH of polynucleotide by the removal of pyrophosphate.

Polynucleotide (n) + d NTP $\xrightarrow{\text{Polymerase}}$ Polynucleotide (n+1) + PPi

Arthur Kornberg and his colleagues in Washington University in 1956 isolated the first DNA polymerase from *E.coli.* It was then known as **Kornberg enzyme**. But later named as DNA polymerase I due to the discoveries of other polymerases like Polymerase II and polymerase III from the same strain of *E. coli.* Polymerase III is the major polymerase involved in DNA replication. Polymerase I and II are involved in DNA repair and proof reading in prokaryotes. DNA polymerase (Pol) requires a template for synthesis of a new strand. They can synthesize only in the 5′-3′ direction. This enzyme cannot start DNA synthesis, rather can only add to an existing **primer** strand. A primer is a small DNA or RNA strand hydrogen bonded to the template. During DNA synthesis new nucleotides are added to the open 3' OH end of the primer or growing polynucleotide so that the synthesis is always in the 5′-3′ direction. Polymerase III has got exonuclease property, i.e. it can remove nucleotides from the 3′ end of the growing DNA strand (3′-5′ exonuclease). It helps in proof reading so that any wrong nucleotide added at 3′ end can be removed. Pol I has 5′-3′ exonuclease function, it can remove short RNA primers from RNA-DNA hybrid. (Table 7.2)

Table 7.2

Properties of different DNA polymerases

Enzyme Activity	DNA Polymerase I	DNA Polymerase II	DNA Polymerase III
1. 5′ to 3′ polymerase	Yes	Yes	Yes
2. 3′ to 5′ exonuclease	Yes	Yes	Yes
3. 5′ to 3′ exonuclease	Yes	No	No

7.4.3 Mechanism of DNA Replication :

The entire set of enzymes and protein factors involved in DNA replication is known as **replicase system** or **replisome** (Table 7.3). The initiator protein recognizes the unique sequence of origin and bind to it.DNA **helicase** enzyme unwinds the double stranded DNA by breaking the hydrogen bonds between the nitrogenous bases. Then **single strand binding proteins** (SSB proteins) bind to the separated strands to keep them in extended position and also to prevent rewinding and attack by single straind nuclease. As a result of the combined action of the enzyme helicase and the protein factors SSB a "V"-shaped fork is created at the origin known as **replication fork**. One must understand that in a bidirectional replication two replication forks are created in opposite direction at the origin. The open ends of the two forks meet at the origin and appears like two "V"s facing each other. As the replication fork moves through the

unwinding of the DNA strands a positive **super coil** is created in the unreplicated portion of DNA ahead of the fork. This is like a knot ahead of fork so that further movement of the fork is hindered. This super coiling is removed by an enzyme called as **topoisomerase II** or **gyrase** in *E.coli* and topoisomerase I in eukaryotes.In *E.coli* the enzyme makes cut on both the strands of the circular DNA and then one segment of DNA passes through other to relieve the super coil and then the cut is sealed. DNA polymerase requires a primer strand for the addition of nucleotides. Enzyme **primase** synthesizes a short primer complementary to the 3′ end of the templates. The replication fork moves by unwinding the double stranded DNA. As a result, one template strand is continuous with the replication fork, i.e. the direction of movement of fork is along the 3′ to 5′ direction of the template strand. In the same replication fork, the other strand is not continuous with the movement of the fork as the fork opens behind the 3′ end of this template strand. The template strand whose 3′-5′ direction coincides with the movement of fork is known as leading template strand or **leading strand**. This strand requires a single initiation event at the start of the replication and then the new DNA synthesis takes place continuously. The other strand whose 3′-5′ direction is opposite to the direction of the movement of replication fork is known as **lagging strand**. On the lagging strand, the direction of DNA synthesis (always in 5′-3′ direction) and movement of fork are in opposite direction. In this case, continous DNA synthesis is not possible rather short DNA strands are synthesized discontinuously which are later joined. Synthesis of each strand coincides a single movement of the fork and necessitates an initiation event each. The small DNA strands on the lagging strand are called **Okazaki fragments** after the Japanese Scientist **Reiji Okazaki** who first observed those fragments. He observed fragments of 1000-2000 nucleotides long in prokaryotes and 100-200 nucleotides long in eukaryotes. One must understand that in a bidirectional replication, from the origin of replication a particular template strand is leading strand along the movement of one replication fork and lagging strand along the opposite replication fork. (Fig. 7.9)

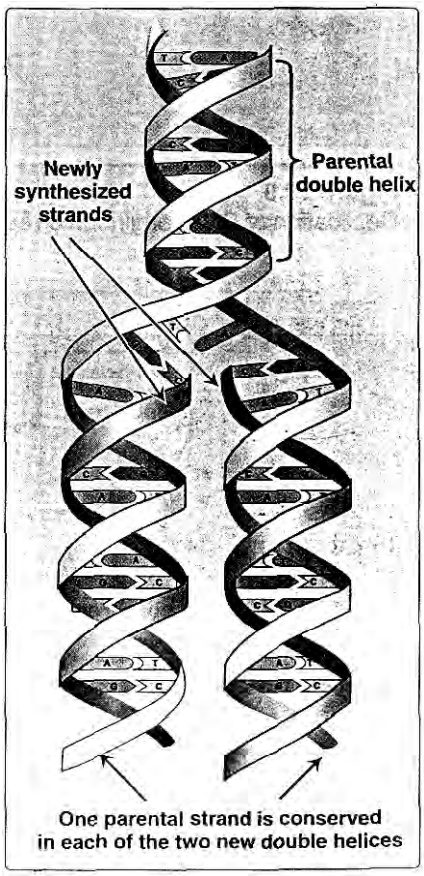


Fig. 7.8 : Semiconservative DNA replication.

Each Okazaki fragment on the lagging strand has its own primer. The **primosome** protein complex moves along the lagging strand and forms RNA primer at intervals on which Okazaki fragments are synthesized. DNA polymerase I enzyme removes the RNA primers from the lagging strand through its 5′-3′ exonuclease activity and fills the resulting gaps by adding nucleotides complementary to those portions of lagging strand.

Finally **ligase** enzyme joins the Okazaki fragments to give a continuous DNA strand complementary to lagging strand. (Fig. 7.8)

Table 7.3

Enzymes and protein factors associated with DNA replication in *E.coli.*

Proteins/ Enzymes	Roles in Replication	Appx. molecules per cell	Remarks
Helicase	Unwinds double helix	20 to produce single strand templates	size-300kd, moves along lagging strand it is a DNA B-protein
Primase	Synthesizes RNA Primer	50	Size-60kd, a subunit of Primosome complex, synthesizes RNA Primer.
SSB Proteins	Stabilize single strand	300 template preventing rewinding	size-74kd. Tetrameric protein, its positive charges interact with negatively charged phosphates of DNA
DNA gyrase	Relieves Super-coiling	250 or torque	Size-400kd. It is Topoisomerase II requires ATP hydrolysis effects double stranded cuts.
DNA Polymerase II	DNA synthesis on both	20 strands	Size-900kd. Exists as repliosome. Fast Polymerising enzyme.
DNA Polymerase I	Removes RNA Primers	300 by 5'-3' exonuclease activity and gap filling	Size-103kd, slow polymerising enzyme; hence required in large number.
DNA ligase	Joins Okazaki fragments	300 on lagging strand; DNA repair	Size-74 kd. Seal open ends of DNA, through 3'-5' phosphodiester formation.

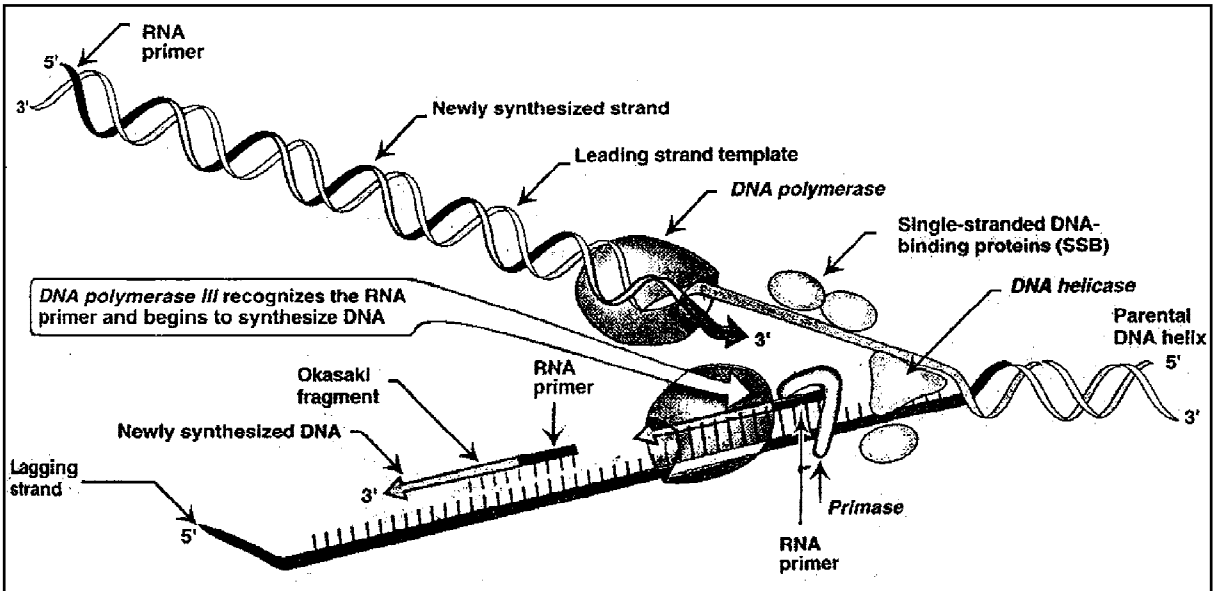


Fig. 7.9 : Replication Fork showing association of different enzymes & Proteins

7.4.3 Eukaryotic DNA Replication :

The replication events at the replication fork are much the same in eukaryotes as in prokaryotes except that the enzymes and protein factors are different. The main polymerizing enzyme is polymerase **α, β, γ, δ & ε** (Table 7.4). This polymerase enzyme is much slower in comparison to that of prokaryotes. DNA pol III adds about 1000 nucleotides per second whereas DNA pol α adds about 50 nucleotides per second. The SSB protein is known as Replication factor A in eukaryote and the topoisomerase is Type I topoisomerase.

Table 7.4
Activities of eukaryotic DNA polymerase

Polymerase	Function	Proof-reading
Pol α	Contains primase Initiates DNA synthesis	Absent
Pol β	Repair	Absent
Pol γ	Replicates mitochondrial DNA	Present
Pol δ	Elongates leading strands and Okazaki fragments	Present
Pol ε	Repair	Present

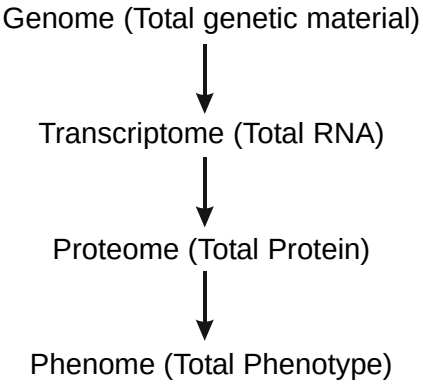
Another big difference is the sheer amount of DNA and the larger size of DNA. Eukaryotes have more than one chromosomes and each chromosome has DNA larger than the genome of

a bacterium. For example, the total length of human DNA of one cell is about 2 meter whereas that of *E.coli* is only 1 mm. So for larger DNA to replicate in quick time, eukaryotic DNA have multiple origin and each eukaryotic DNA is a multiple replicon. The yeast cell chromosomes have about 400 origins and each human DNA with about 1000 origins. Imagine a situation of human genome with $4x10^9$ base pairs to replicate as a single replicon: it will take several weeks. But the cell cycle is completed in 24 hours and for that cycle to operate in time, the DNA replication in human is completed in 6-8 hours of S-phase. This is achieved due to the presence of multiple origins.

During the S-phase of the cell cycle, the DNA replicates only once and then the cell divides. Hence the amount of total DNA is first doubled in S-phase and then equally divided between the two daughter cells during the cell division. Thus the DNA level (and the chromosome number) is kept constant after successive cell divisions. The accurate replication of DNA and their equal distribution among the daughter cells form the basis of transmission of hereditary characters. Any error in DNA replication is taken care of by DNA repair mechanism available in the cell. But, imagine a situation where DNA divides not once but many times before a cell division. In such a situation the total DNA will increase two times, four times or many more times and the subsequent cell division will produce polyploid cells (cells with more than the normal number of chromosomes).This does not happen as the cells have a replication licensing system. During the cell division, in the anaphase stage the replication origins are licensed by a nondiffusible **Replication Licensing Factors** or **RLF.** After the anaphase, no further licensing can occur due to the presence of nuclear membrane. The RLF allows DNA to replicate once in the S-phase and the RLF get destroyed during replication. Further round of replication will require further licensing. Unless the cell undertakes division cycle, it cannot come to anaphase and licensing of origins cannot occur. This mechanism ensures that a cell must divide after a single round of DNA replication.

7.5 TRANSCRIPTION :

Chemically genes can be DNA or RNA but in most life forms DNA is the genetic material. The genetic substances not only controls the inheritance of traits or characters from one generation to the next, but is also able to express its effect through the formation and functioning of traits. The basic concept of heredity is the ability of cells to use the coded information in their genetic materials to produce particular proteins, which thereby determine the behavior of the cell. In fact, proteins are the tools of heredity. It is important to note that a gene can be responsible for the synthesis of a polypeptide or for the synthesis of a tRNA or rRNA. All these three functions together determine the characters of the organism. The relationship between the genes and the characters can be summarized as: **Genome to phenome concept.**



It is evident that cells use RNA to make proteins. Hence gene expression involves two steps: transcription (RNA making) and translation (Protein making). In the first step (transcription) the coded message present in the DNA as the nucleotide sequence is passed on to the mRNA (messenger RNA). In the second step of translation, the coded message in mRNA is translated into the language of polypeptides. The mRNA simply act as a messenger recruited by DNA to carry the genetic information to the site of protein synthesis,i.e, the ribosomes. Initially most of the information about gene expression were gathered from prokaryote *(E.coli)* and gradually with developed techniques information about eukaryotic gene expression were obtained from Yeast, *Arabidopsis thaliana* and other eukaryotes.

7.5.1 Central Dogma :

The flow of genetic informations from DNA through RNA to protein and the transmission of characters through replication of DNA is known as central dogma in molecular biology. (Fig.7.10A)

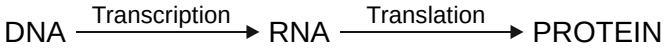


Fig. 7.10A : The unidirectional flow of genetic information from DNA to protein, the central dogma.

This central dogma holds good for most of the cellular genes. But some viruses like Tobacco Mosaic Virus have RNA instead of DNA as the genetic material. Further two other information pathways have been discovered. These are: (1) RNA dependant RNA synthesis or RNA replication and (2) RNA dependant DNA synthesis or reverse transcription. These discoveries led to the modification of the central dogma, (Fig.7.10B)

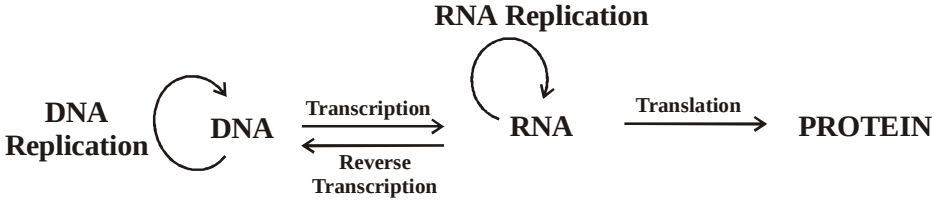


Fig. 7.10B : Modified central dogma

The first step in the genetic information pathway or the central dogma is the transcription. In this step the genetic information from DNA is passed to RNA through enzymatic synthesis of a RNA molecule on a DNA template. Generally we mean the synthesis of mRNA as transcription but the synthesis of rRNA and tRNA is also transcription. For a particular RNA (either mRNA or rRNA or tRNA) in a defined sequence of DNA acting as gene only one of the two strands of the defined DNA sequence act as the template strand. This template strand is known as **template / sense / plus (+) strand**. On the template strand, RNA is synthesized basing on the principle of base complementarity except for Uracil (U) in place of Thymine (T). The other strand in that particular DNA segment is known as **coding / nonsense / minus (-)** strand for that particular RNA. It may be noted that for any other RNA for which the gene is read in reverse direction this template strand becomes coding strand and the coding strand becomes the template strand. The synthesized RNA is a copy of the coding strand except for the presence of Uracil in place of Thymine of coding DNA strand. Two types of genes are transcribed; (1) RNA genes and (2) structural genes. The RNA genes transcribe tRNA and rRNA and the structural genes are transcribed into mRNA which are translated into polypeptides. The segment of DNA that takes part in transcription is known as transcription unit. This unit has three components; (1)the promoter sequence, (2) the structural genes or the RNA genes and (3) the terminator sequence. The eukaryotic transcription may require other sequences like enhancer, silencer etc. The promoter sequence **precedes** the structural gene on the **coding strand**,i.e, towards the 5'-end of structural gene or **upstream side** of structural gene (which is 3'-end of template strand)

The promoter is the sequence to which the transcribing enzyme RNA polymerase (RNA Pol) binds. This sequence is known as **TATA box** or **Pribnow box** in prokaryotes and TATA or **Hogness** box in eukaryotes.

The enzyme that carries out transcription is known as **DNA dependant RNA polymerase** or simply **RNA polymerase** (RNA pol). Like the DNA pol, RNA pol catalyzes polymerization of ribonucleotides basing on complementarity of the template strand. The new ribonucleotides are added to the open 3'-end so that the RNA is always synthesized in 5'-end to 3'-end. The only difference is Uracil incorporated to growing RNA instead of Thymine. The RNA pol does not require a primer unlike DNA polymerase.

RNA Pol. is purified and its structure and function is known in detail. It consists of five polypeptide chains: 2 chains of α-polypeptides, one chain each of β, β_2 and δ. Thus the enzyme can be represented as $\alpha_2\beta_1\beta_2$ (Table 7.5). The association of ó factor is not very firm and it dissociates from the core enzyme, i.e, $\alpha_2\beta_1\beta_2$. The core enzyme associated with σ factor is known as holoenzyme. The core enzyme alone is capable of polymerization of ribonucleotides but sigma factor is required to initiate the RNA transcription at the correct site. The core enzyme has two ribonucleotides binding sites: (1) Initiation site and (2) Elongation site.

Table 7.5
Different sub-units of RNA polymerase

Subunit	Mass in Daltons	Location	Possible function
α_2	40,000 each	Core enzyme	Promoter binding
β_1	155,000	Core enzyme	Nucleotide (Substrate) binding
β_2	160,000	Core enzyme	Template binding
σ	85,000	Sigma factor	Initiation

7.5.2 Transcription In Prokaryotes :

Prokaryotes have only one type of RNA polymerase for the transcription of all types of genes (structural as well as RNA genes). But different sigma factors may associate with the same core enzyme at different times for expression of different genes. In *E.coli*, σ^{70} is used in normal condition σ^{32} / σ^{H} under heat shock, σ^{54} / σ^{N} under Nitrogen starvation and σ^{28} for chemotaxis.

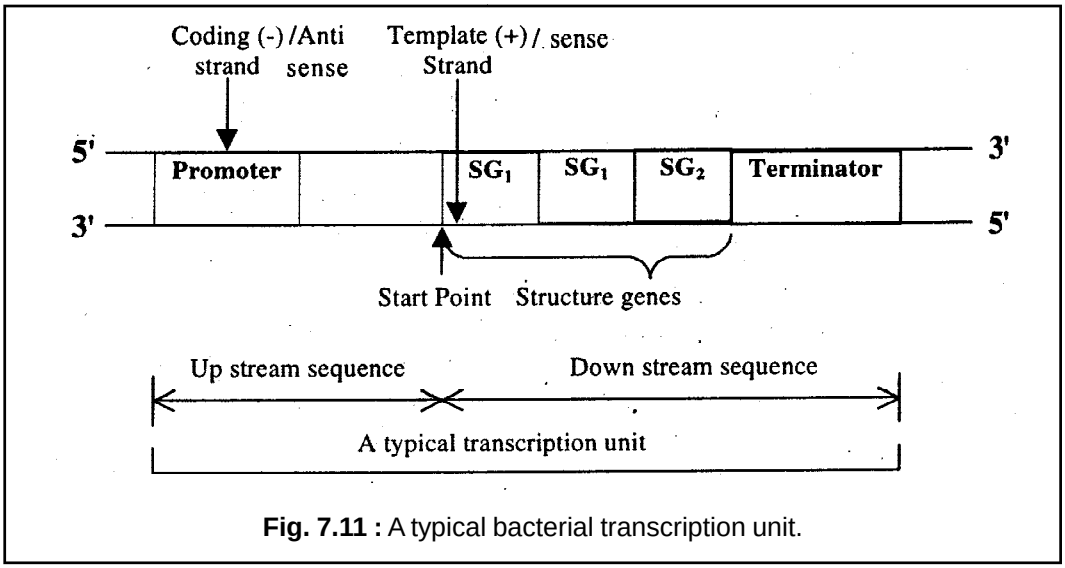


Fig. 7.11 : A typical bacterial transcription unit.

I. Initiation :

The transcription is initiated by the binding of the holoenzyme to the promoter. The ó (sigma) polypeptide of the holoenzyme binds loosely to a sequence of the promoter even before the opening of the DNA double helix. Thus a **loose/ closed binary complex** is formed. After this complex is formed the adjacent sequence of the DNA denatures forming a **transcription eye** or **bubble.** The denaturing facilitated as the promoter region is **AT** rich. This transcription bubble along with the bound holoenzyme is called an **open binary complex.** The transcription

start point is a purine in 90% of cases. The first and the second nucleotide complementary to the first two nucleotides of the template strand binds at the elongation site of the enzyme. A phosphodiester bond is formed between these two ribonucleotides by a hydrophilic attack of the 3/ -OH group of the first ribonucleotide triphosphate on the first phosphate bond of the second nucleotide so that a **pyrophosphate** (P-P) is released in this reaction. Now the complex consists of a partly denatured DNA bound with the holoenzyme having a di-ribonucleotide. This complex is known as a **ternary complex**.More ribonucleotides are added without any movement of the holoenzyme so that a RNA chain of about nine nucleotides is synthesized. During the incorporation of the nucleotides in the initial stage, there is the possibility for the release of small RNA chains, a process described as **abortive initiation**. A cycle of such abortive initiation occurs before the definitive initiation begins. Once the initiation succeeds, the sigma factor dissociates from RNA polymerase, leaving the core enzyme for the elongation of RNA chain. The dissociation of sigma facilitates promoter clearance of core enzyme so that another holoenzyme may bind to promoter for another round of transcription to begin (Fig. 7.12).

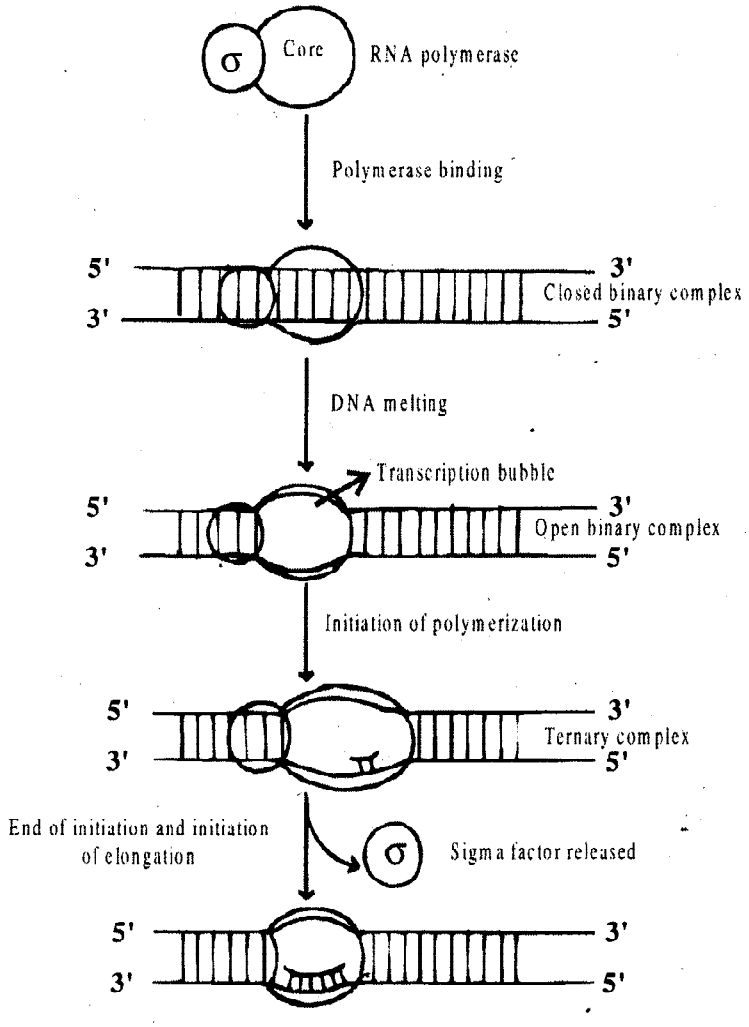


Fig. 7.12 : Binding of RNA polymerase and initiation of RNA synthesis.

II. Elongation :

Elongation of RNA chain takes place by the addition of ribonucleotides to the 3'-end of the RNA so that the RNA chain grows in 5'-3' direction. For this to happen the transcription bubble moves in the 3'end to 5' end of the template strand. When the holoenzyme moves along the bubble the DNA duplex unwinds (denature) in the growing point and rewinds at the opposite end. The new ribonucleotides are added to the 3'-end by the same mechanism of hydrophilic attack, based on the complementarity of the template strand. In each successive elongation cycle, the

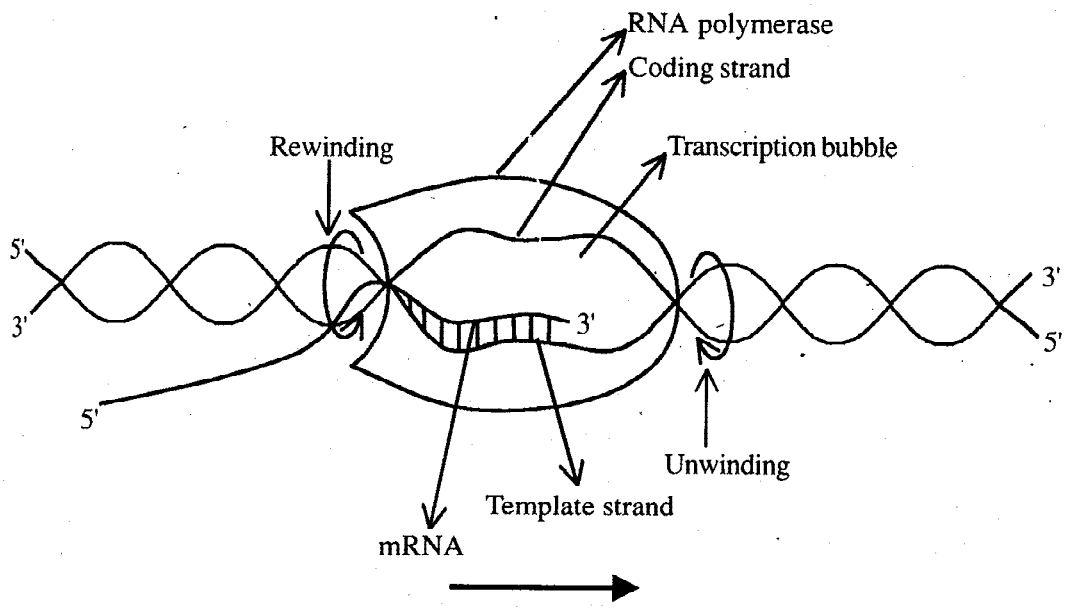


Fig. 7.13 : Elongation of RNA chain by the movement of RNA polymerase and transcription bubble in 5' to 3' direction.

growing site or **leading product** site of the enzyme is filled with ten newly added nucleotides and the opposite point or **lagging product** site contains the previous segment of the RNA. About forty nucleotides are added per second at 37^{0}C. RNA pol. in some phages like T_3, T_4 etc. synthesize RNA at a much rapid rate of about 200 nucleotides/second at 37^{0}C. (Fig. 7.13)

III. Termination :

In prokaryotes, termination of transcription is brought about by certain termination signals on DNA called terminators (these are DNA sequences).In *E.coli* the termination signals fall under two categories, such as:

1. **Intrinsic terminators** or protein factor rho (r) independent**.**
2. **Extrinsic terminator** or rho dependent.

In the intrinsic termination, the RNA at its 3'-end contains a long stretch of U residues hydrogen bonded to the long stretch of A residues of the template. In the stem of the RNA, there is a stretch of G-C rich segment. The G-C rich segment results in a hair-pin loop formation in the RNA stem. As a result the weak association between A-U in the long stretch of termination sequence break and the RNA is released.This happens because the formation of hair-pin loop in the stem of RNA before the termination signal slows down transcription and as a result dA-rU bonds break at any one point releasing RNA from RNA-DNA hybrid.

In extrinsic termination rho protein is required. It is an important protein factor responsible for termination of transcription of many genes in *E.coli.* This protein is active as an hexamer (having six identical subunits). It has a molecular weight of 46,000 and also has ATP hydrolyzing activity. Rho factor binds to the 5'-end of nascent m-RNA and scans down along the length of m-RNA until it reaches the termination point. At termination point when the transcription slows down rho breaks ATP and utilizes that energy to denature the RNA-DNA hybrid so that the RNA is released from the bubble.

In prokaryotes, as many structural genes are contiguously present and are transcribed together the transcribed mRNA is polycistronic.

7.5.3 Transcription In Eukaryotes :

The basic mechanism of transcription in eukaryotes remains the same as in prokaryotes. However, many trnanscription factors (TF) are involved in eukaryotes and also there are other controlling sequences like enhancer and silencer. Further in eukaryotes, there are different polymerases for the transcription of different genes, such as:

(i) **RNA pol.I** Found in nucleolus, catalyzes transcription of 28S, 18S and 5.8S rRNA as a single long precursor RNA which is then spliced to different constituent RNAs.

(ii) **RNA pol.II** Present in nucleoplasm,catalyzes the transcription of all mRNA from all protein coding genes.

(iii) **RNA pol.III** Found in the nucleoplasm, responsible for the transcription of all tRNAs and 5S rRNA.

(iv) **Organellar RNA pol.** These are responsible for transcription of mitochondrial and chloroplast genes. The RNA pols. are organelle specific.

Interrupted Genes and RNA Splicing in Eukaryotes :

Most eukaryotic genes are interrupted by non-coding or non-translatable sequences known as **introns.** The coding or translatable sequences are known as **exons**.Such genes are called **split genes/interrupted genes.** Both intron and exon sequences are transcribed to produce a **primary transcript/precursor RNA/pre RNA**. The precursor RNA for mRNA is known as **heterogenous RNA/hn RNA**. Following the transcription, the intron sequences are removed and exons are joined to form a mature or functional RNA. This process is known as **RNA splicing**. For this reason, the RNA transcript in nucleus differ from the mRNA found in the cytoplasm for translation. For each split gene, only about 25% of hn RNA take part in splicing leading to mRNA formation. The rest undergoes degradation. (Fig. 7.14)

The hn RNA(hetrogenous nuclear RNA) gets modified by the addition of **7-methyl Guanosine residue (m7G**) to the 5'-end even before the transcription is completed. This is

called **5'-capping**. The 5'-cap of m7G helps in recognition of ribosome. The capping is initiated by addition of GTP in 5'-end in reverse orientation of 5'-5' phosphodiester (not the usual 5'-3' diester) and then a methyl group is transferred to N-7 by methyl transferase enzyme. However, some eukaryotic mRNA like those for histone proteins, lack 5'-cap.

The 3'-end of most eukaryotic mRNA has a long stretch "A" residues added after transcription. This stretch of "A" residues is called **Poly-A-tail** and the process of its addition is called **Polyadenylation. Poly-A polymerase** enzyme adds a poly-A tail of about 200 Adenine nucleotide to the 3'-end of the primary transcript.

Some of the hn RNAs are spliced in large complexes called **spliceosomes**. Spliceosomes are complexes of proteins and five types of small nuclear RNAs (S_n RNA). These small nuclear RNAs are U1,U2,U4, U5 and U6. Some hn RNA molecules are also auto spliced without the involvement of spliceosome.

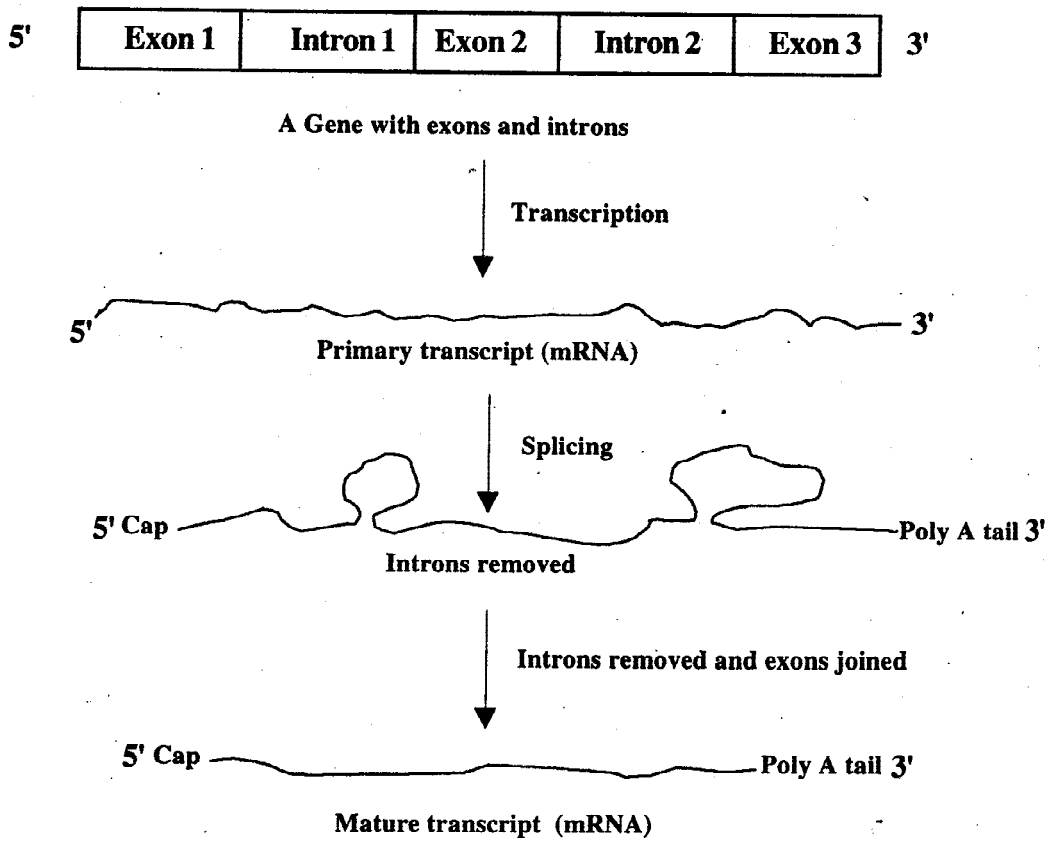


Fig. 7.14 : Expression of an interrupted / split gene and RNA splicing.

7.6 TRANSLATION :

Translation is the second step in the Central dogma where the genetic information contained in mRNA transcript is transferred to proteins or polypeptides. Precisely speaking **the genetic information as the nucleotide sequence of mRNA is translated into amino acid sequence of protein or polypeptide.** Translation takes place in complex cytoplasmic machinery made up of tRNAS, ribosomes and enzymes. It is important to note that all the three types of RNAs are involved in translation. The mRNA and tRNA participate directly in the process whereas rRNA participates as a component of ribosome, the site of translation.

Translation starts when an rRNA molecule in ribosome recognizes and binds specifically to a "start" site on mRNA. The ribosome then moves along mRNA three nucleotides at a time. Each group of three nucleotides is thus a code defining the next amino acid in the polypeptide chain.

7.6.1 The Genetic Code :

Francis Crick and his colleagues in 1961 through an experiment in viral DNA concluded that the genetic code is triplet (read in increments consisting of three nucleotides) and that reading occurs continuously without punctuation between the three-nucleotide units. A triplet of nucleotides specifying a particular amino acid is known as a **codon** and thus, there should be 64 possible codons made out of four nucleotides taking three at a time (4^3)

Once the triplet nature of the codon was established different scientists then tried to establish the codons for twenty different amino acids found in proteins**. Marshall Nirenberg** in 1961 used a synthetic mRNA of **Uracil** only and found that the translated polypeptide was composed of **Phenylalanine** amino acids only. Thus it was established that **UUU** is the codon of phenylalanine.

Then in 1964 **Nirenberg** and **Philip Leder** by employing the technique of triplet binding assay found out some **47** of the 64 possible codons. **Har Gobind Khorana** worked out the remaining 17 codons by employing artificial mRNA (Table 7.6). For this work **Nirenberg and Khorana shared the Nobel prize in 1968 with R.W.Holley who gave details of tRNA structure.**

Table 7.6
Genetic Code Dictionary

……. Second Base …….					
First Base	U	C	A	G	Third Base
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	ACC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met, Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGc Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

7.6.1.1 Properties of the genetic code :

1. **The codon is triplet, ie, :** the code for each amino acid consists of three nucleotides.
2. **The code is degenerate :** There are 64 codes for only 20 amino acids which means more than one codon for each amino acid. This is the degeneracy of the code.
3. **The code is commaless :** The reading of the codons on a mRNA occurs continuously without any punctuation between successive codons.
4. **The code is practically universal :** The genetic code is same in almost all organisms. For example the codon AGG specifies amino acid Arginine in bacteria, animals and plants. But there are some exceptions to it. In mitochondrial genomes, “stop codon” UGA is read as amino acid Tryptophan, AUA as Methionine rather than Isoleucine and AGA and AGG as “stop codon” rather than Arginine.

7.6.1.2 The Start and stop codons :

The start or initiation codon in most cases is **AUG** which normally codes for amino acid methionine. But when AUG is present as initiation codon in prokaryotes, it codes for **formylated methionine.** Rarely **GUG** serves as initiation or start codon. **As start codon GUG codes for methionine where as it normally codes for valine**. Three codons namely, **ochre (UAA**), **amber (UAG**) and **opal (UGA**) are stop codons or **non-sense** codons as they do not code for any amino acid. The translation is terminated at that point where on mRNA any one of these three codons is encountered.

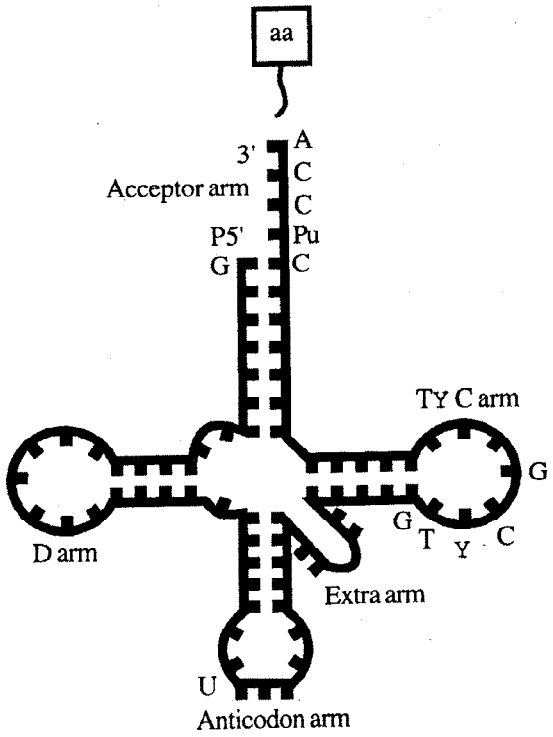


Fig. 7.15 : Adaptor Molecule or tRNA

UGA or Opal has several roles; some of which are, **1**. termination codon in universal genetic code, **2**. tryptophan codon in mitochondria, **3**. an efficiently read tryptophan codon in *B.subtilus* and *E.coli*, **4**. cysteine codon in *Euplotes octocarinatus* and selenocysteine (SeCys) codon in *E.coli*, mammals, higher plants and fungi.

The specification of an amino acid by a codon is mediated by a tRNA (Fig. 7.15). It may be recalled that tRNA has anticodon which matches in base complementarity to a particular codon.So a particular tRNA is meant for a particular codon and that tRNA is specific to a particular amino acid. Hence it is obvious that for twenty different amino acids there must be atleast twenty different tRNAs. In fact there are about forty five different tRNAs for twenty different amino acids. (more than one for each amino acid). These tRNAs function as **adapter** molecules. It is important to note that, excluding the three non-sense codons there are 61 valid amino acid codons. Then about 45 anticodons on 45 tRNA molecules can match 61 codons. This is possible, because some tRNA molecules can recognize more than one codons as the third base on tRNA anticodon allows some "**wobble**".

Crick in 1965 proposed the wobble hypothesis. He discovered that if U is present at the first position of anticodon, it can pair with either A or G present at third position of codon. Similar is the case with G or I (Ionosine, a modified base in tRNA) found in anticodon. Thus the wobbling allows economy of the number of tRNA molecules and several codons meant for same amino acid are recognised by the same tRNA. In wobbling U as first base in anticodon pairs with A/G as third base in codon, G pairs with U/C and I with U,C,and A.

7.6.2 The Process :

Translation is executed in six steps: (i) binding of mRNA to ribosome, (ii) aminoacylation, (iii) initiation, (iv) elongation, (v) termination and (vi) post-translational modification.

(i) Binding of mRNA to ribosome :

Ribosomes occur in the cytoplasm in dissociated condition, i.e., their smaller and larger subunits separated. In prokaryotes, the transcription factor IF3 helps in dissociation of the two subunits of ribosome and then binds to the 30S subunit to prevent premature association of the two subunits. First, the mRNA binds to the smaller subunit. The smaller subunit has two binding sites: **A site or aminoacyl site** and **P site or peptidyl site**. The incoming tRNA with its specific amino acid binds to the A site and the peptidyl tRNA carrying the elongating polypeptide binds to P site. The bacterial ribosome contains another site, the **E site or Exit** site to which the discharged tRNA or tRNA whose peptidyl has already been transferred binds before its release from ribosome. The prokaryotic mRNA has a leader sequence at its beginning just prior to the initiation codon AUG. This sequence is known as **Shine-Delgarno Sequence (SD region),** which has homology with the 3'-end of the 16S rRNA (**ASD** region) found in 30S subunit. This complementarity ensures that the 30S subunit binds at the correct position of mRNA and the translation process starts from the beginning of mRNA. In eukaryote the 40S subunit enters at capped 5'-end of the mRNA and then advances to the start codon by linear scanning.

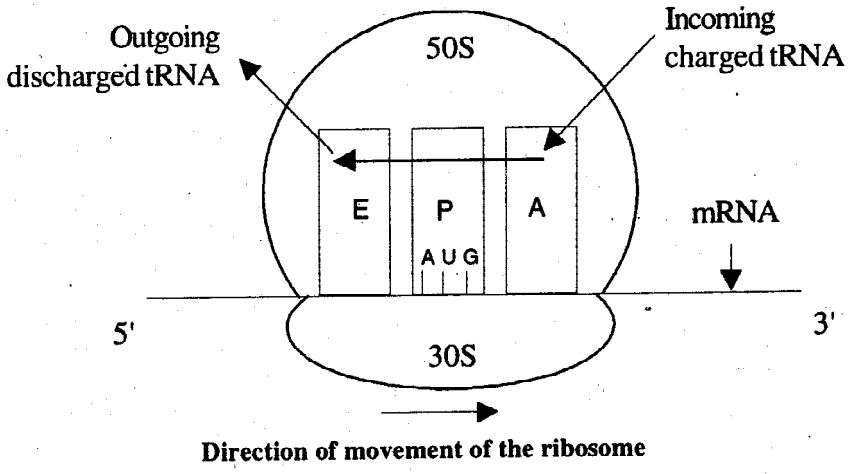


Fig. 7.16 : A bacterial ribosome with three sites ; E, exit site, P, peptidyl site and A, aminoacyl site

(ii) Aminoacylation :

Aminoacylation or activation of amino acid is the step in which all the twenty amino acids are linked to their specific tRNA in the cytoplasm. This reaction is catalyzed by the enzyme **aminoacyl-tRNA sythetase. There are 20 different types of synthetases for 20 different**

amino acids. The amino acids recognized by two or more tRNA molecules are linked by the same enzyme. In the first step of aminoacylation, aminoacyl adenylate enzyme complex or aminoacyl-AMP-Enzyme is formed with the release of pyrophosphate.

AA_1 + ATP + Enzyme ——————— AA_1-AMP-Enzyme + PP

Amino aminoacyl aminoacyl-adenylate

Acid synthetase - enzyme complex

In the second step this complex gets associated with the 3'-OH end (with unpaired CCA sequence) of specific tRNA molecule. The AMP is now hydrolyzed to form an ester bond between the amino acid and its specific tRNA, and the enzyme is also released.

AA_1-AMP-Enzyme + $tRNA_1$——————— AA_1-$tRNA_1$ + AMP + Enzyme

Aminoacyl-tRNA

(iii) Initiation (Fig. 7.17) :

The protein synthesis begins from the amino terminal end of the polypeptide, proceeds by the addition of amino acids through peptide bond formation and ends at the carboxyl terminal end. In prokaryote, the initiation amino acid is formylated methionine while in eukaryote it is methionine. So in the prokaryote, there are **two types of tRNA for methionine**. One is **$tRNAf^{met}$** for initiation carrying formyl methionine and the other one is **$tRNA^{met}$** for carrying normal metheonine to growing polypeptide.The initiation of polypeptide synthesis in prokaryote requires the following:

1. mRNA**, 2.** 30S subunit of ribosome**, 3.** formylmethionyl-tRNA (fmet-$tRNAf^{met}$), **4.** initiation factors IF-1, IF-2 and IF-3**, 5**. GTP, **6.** 50S ribosomal subunit and **7**. $Mg^{+2.}$ The sequence of events that occurs during initiation are:

1. The smaller 30S subunit of ribosome binds to the transcription factor **IF-3** that prevents premature association of the two ribosomal subunits.
2. The mRNA binds to 30S subunit through the interaction of **SD region** of mRNA and **ASD region** of ribosome so that the initiation codon **AUG** is correctly positioned at the **P site** of the ribosome.
3. The fMet-$tRNAf^{Met}$ (the specific tRNA aminoacylated to formyl methionine) binds to the AUG codon at the P site.The **$tRNAf^{Met}$ is the only tRNA that binds to its codon present on the P site. All other tRNA along with their respective amino acids bind to their codon present at the A site.** That is why AUG codon present as initiation codon codes for formylmethionine and when present at other position codes for normal methionine. It can be recalled that there are two types of tRNA molecules recognizing the same AUG codon but carrying two different forms of methionine.

4. The initiation factor IF-1 binds to the A site and prevents binding of any other aminoacyl tRNA to the codon at the A site during initiation.

5. Now the GTP bound IF-2 (**GTP-IF-2**) and the initiating fMet-tRNAf^{Met} join the complex of 30S subunit-IF3-IF1-mRNA.

6. Then 50S subunit joins the complex formed in the previous step. The GTP bound to IF-2 is hydrolysed to GDP and Pi. All the three initiation factors leave ribosome. This complex of 70S ribosome, mRNA and fMet-tRNAf^{Met} bound to initiation codon at P site is known as **initiation complex.**

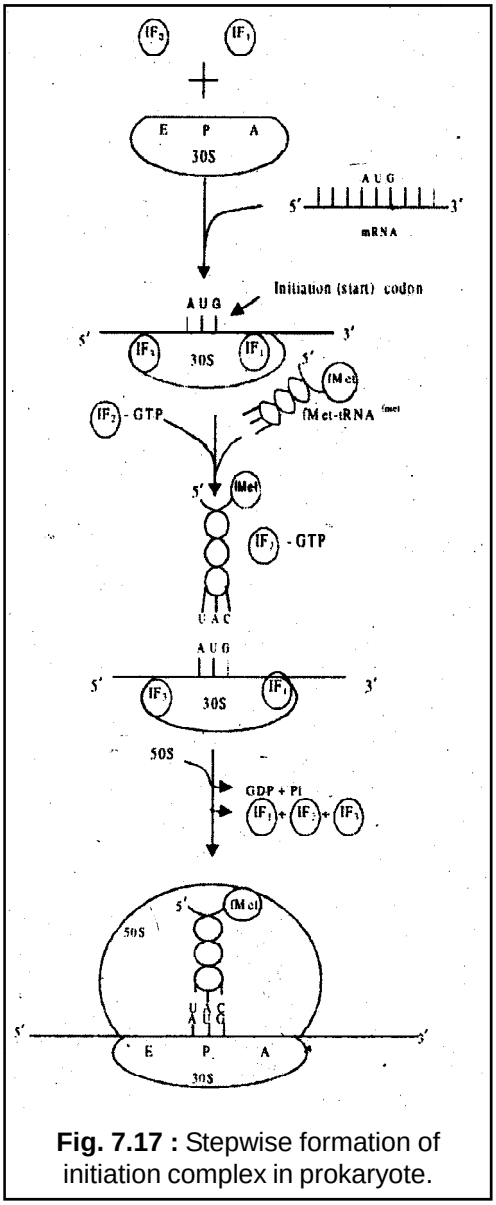


Fig. 7.17 : Stepwise formation of initiation complex in prokaryote.

(iv) Elongation (Fig. 7.18 and 7.19) :

Elongation step involves the addition of further amino acids so that the polypeptide chain would grow. This step requires the following: **1**. the initiation complex, **2**. different aminoacyl-tRNAs, **3.** two elongation factors (**EF-Tu and EF-Ts**) and **4**.GTP. The elongation process takes place in three steps:

Step I : Binding of incoming aminoacyl tRNA

The incoming aminoacyl-tRNA binds to a complex of EF-Tu-GTP to result in a **aminoacyl-tRNA-Tu-GTP** complex. This complex binds to the A site of 70S initiation complex. Then GTP is hydrolysed to GDP and Pi and EF-Tu-GDP complex is released from the ribosome.The EF-Tu-GTP complex is regenerated and recycled by EF-Ts and GTP as follows:

EF-Tu-GDP + EF-Ts =EF-Tu -Ts +GDP

EF-Tu-Ts + GTP = EF-Tu-GTP + EF-Ts

Step II : Peptide bond formation

This is a catalytic process during which a peptide bond is formed between two amino acids bound by their tRNA molecules to the A site and P site. This peptide bond is formed between the free carboxylic group of the N-formylmethionine group attached by its tRNA to the P site and the second amino acid bound by its tRNA to A site. The N-formyl group is transferred

to the amino group of the second amino acid bound to its tRNA at A site. As a result, the tRNA at the A site contains a dipeptide and that at the P site becomes empty. The enzyme responsible for the peptide bond formation is **peptidyl transferase**. **In bacteria the 23S rRNA a component of the 50S ribosomal subunit is thought to carry out peptidyl transferase function.**

Step III : Translocation (Fig. 7.20)

In this step the peptidyl tRNA bound to the A site comes to the P site of ribosome, the empty tRNA at P site comes to the E site and the A site is occupied by a new codon for the next incoming aminoacyl tRNA. This is accomplished by the movement of ribosome by one codon more in 5' to 3' direction of mRNA. The translocation of ribosome requires **EF-G (translocase)** and GTP. The deacylated tRNA interacts with the E site mainly located on 50S subunit through its CCA sequence at the 3'-end. The two step transfer of tRNA molecules from A site to P site and from P site to E site could result from the reciprocating motions of the two subunits of ribosome. This means that 50S and 30S subunits move alternately not simultaneously. Finally the deacylated (empty) tRNA is released to cytosol from the E site.

(v) Termination (Fig. 7.21) :

Termination of the synthesis of polypeptide is brought about by the presence of any one of the three termination codons on the mRNA. These termination codons are recognized by any one of the

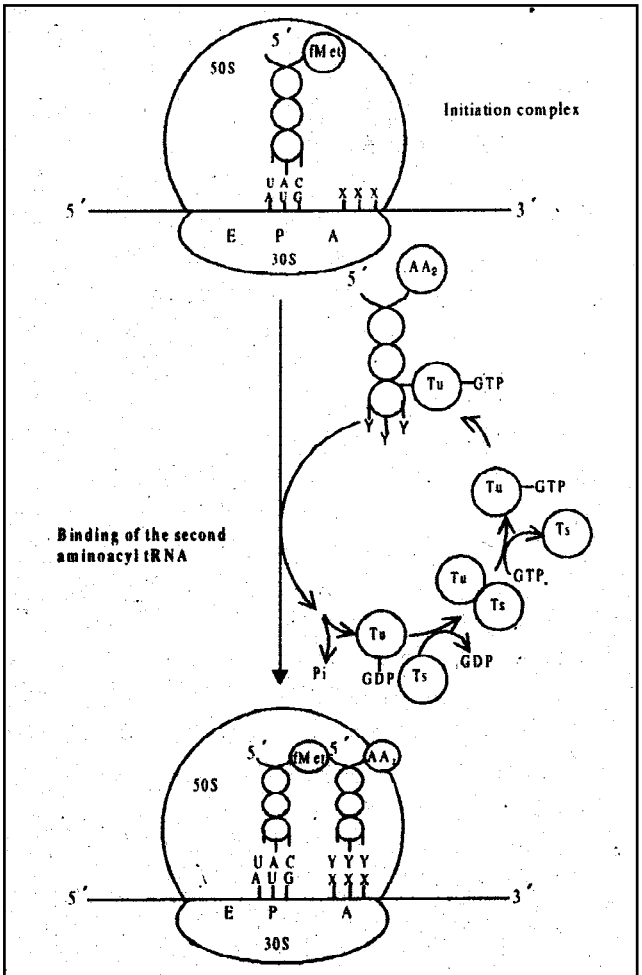


Fig.7.18 : Binding of the second aminoacyl tRNA to the A site of ribosome

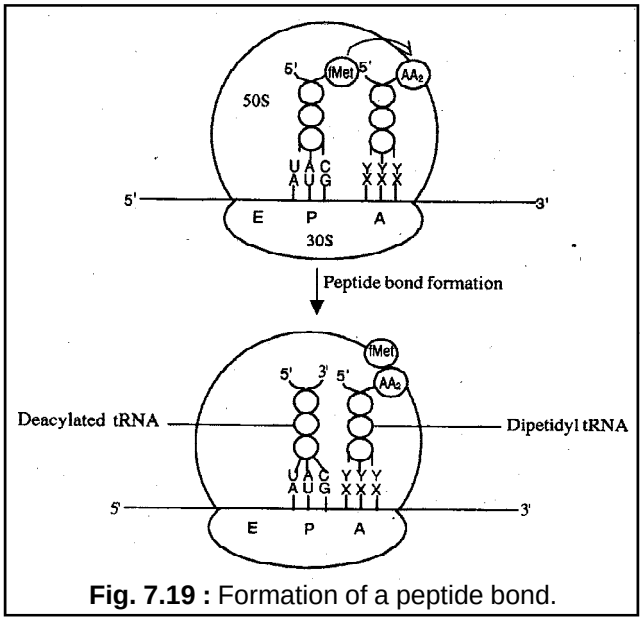


Fig. 7.19 : Formation of a peptide bond.

three **release factors/ termination factors**, **RF1, RF2 and RF3**. RF1 and RF2 resemble the structure of tRNA and compete with it for binding to any one of the termination codon at the A site of ribosome. This phenomenon is known as **molecular mimicry**.RF1 recognises UAG and RF2, UGA. Both recognize UAA. When the A site of the ribosome encounters a termination codon occupied by a release factor instead of an aminoacyl tRNA, elongation of polypeptide stops. At the P site one tRNA with the polypeptide chain is bound to another codon. The release factor RF3 coupled with GTP splits the peptidyl-tRNA bond. The polypeptide is thus released and the discharged or empty last tRNA is also released from the P site. The ribosome dissociates into 50S and 30S subunits. In eukaryote only one release factor **eRFI** is known.

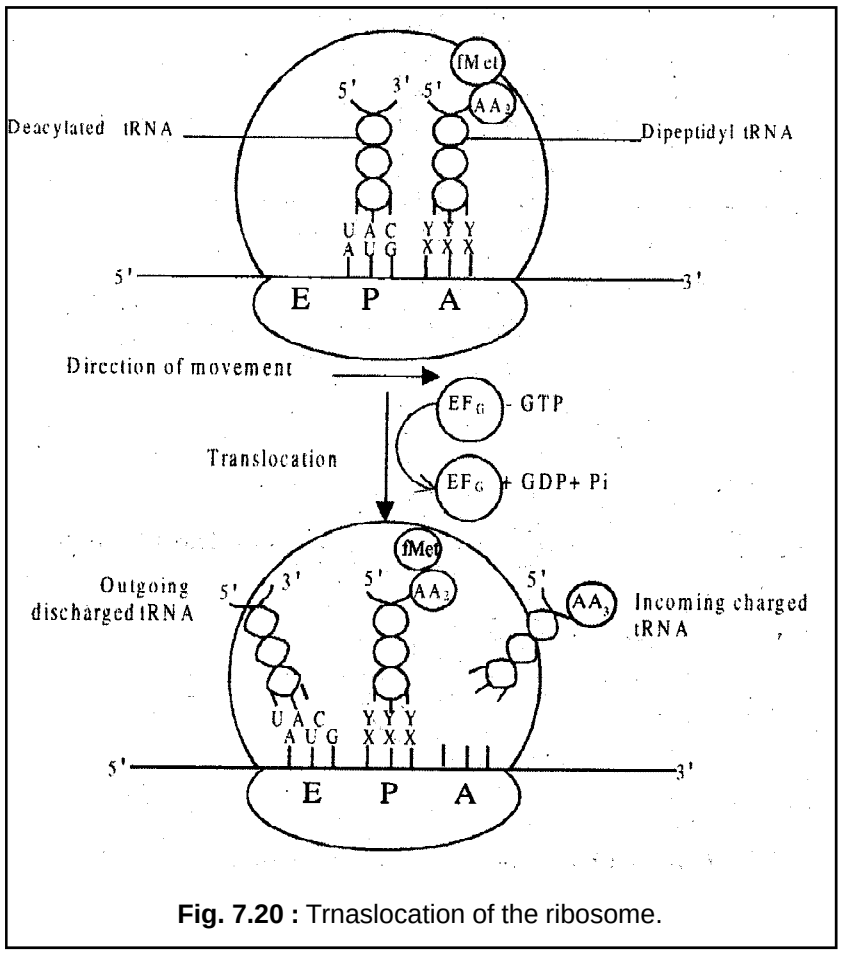


Fig. 7.20 : Trnaslocation of the ribosome.

7.7 GENE EXPRESSION AND REGULATIONS :

In any organism, all the proteins or enzymes are not required always, which means all the genes are not required to express at a given time. Further in multicellular organisms different cells of the same individual organism differ in their structures and functions though all these cells are derived from a single cell, the zygote, and thus contain the same set of genetic complements. For example, the liver cells and muscle cells though derived from the same zygote and have the same complements of genes, liver cells produce biles while muscle cells cannot. Similarly, the young plants cannot bear flowers and fruits but the matured plants can, though the gene complements are the same in both cases. This implies that genes are expressed, only whenever and wherever they are required to do so. Not all genes present in a cell are expressed at the same time; some are expressed and some are switched off. There is some sort of regulation of gene expression so that genes are expressed in appropriate time and also

in specific cell types. This allows the cell to conserve its resource and overcome wastage. However, there are certain genes which are expressed in all cell types continuously, like the genes for respiratory enzymes, RNA polymerase,DNA polymerase etc. These genes are called **house keeping genes** or **constitutive genes.**

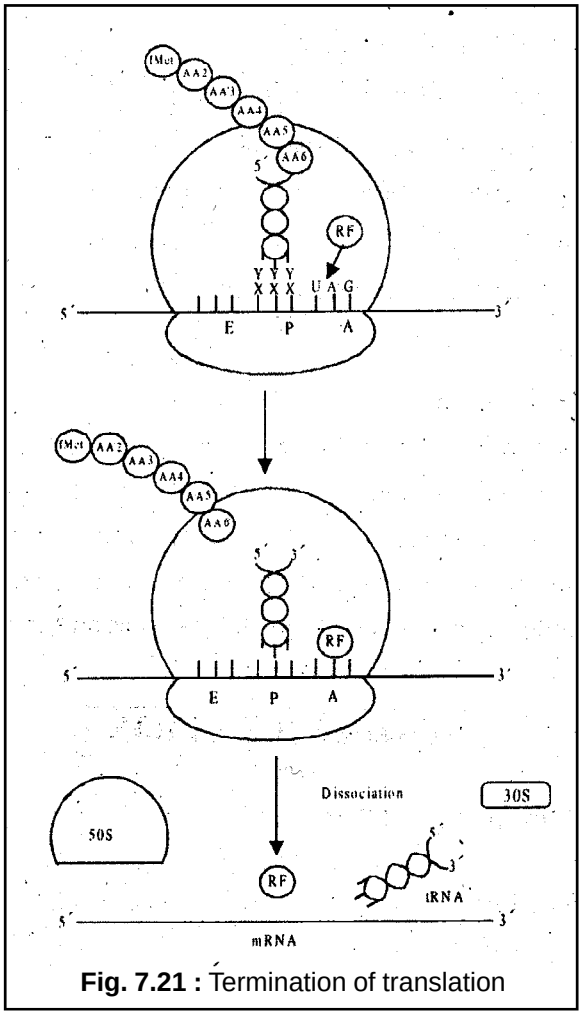


Fig. 7.21 : Termination of translation

7.7.1 Induction And Repression of Gene Expression :

Some genes are normally switched off and the presence of certain substances, switch on the genes. Such type of gene regulation is called induction. Synthesis of an enzyme in response to the presence of its substrate is called **induction** and the gene is called inducible gene, and, the substrate itself is known as **inducer.** Generally the enzymes of catabolic pathways are inducible. One should bear in mind that this induction allows the cell to conserve its resource and avoid wastage. When the substrate is not available, the enzyme has no role to play and the cell does not synthesize the enzyme, thereby conserve the huge resource that would have been utilized in the transcription and translation of the enzyme gene.

Conversely when the expression of a gene is turned off in response to a substance, the process is called **repression** (Fig. 7.22) and the gene is called repressible gene. In this case, the genes are normally on and producing their proteins (enzymes) responsible for synthesis of some other products. But when the end product itself is available to the cell the gene expression is switched off. The repressing substance is known as **co-repressor** and is usually the end product of an enzymatic pathway. Usually the enzymes of anabolic pathways are repressible.

7.7.2 The Operon concept / model :

Francois Jacob and Jacques Monod in 1961 on the basis of their study on the inducible system for the synthesis of β-galactosidase enzyme in *E.coli* proposed the operon model to explain the induction and repression. They were awarded with Nobel prize in physiology and medicine in 1965. **An operon is a unit of coordinated control of gene expression in bacteria (prokaryote) including the structural genes and the controlling sequences on DNA**

recognized by regulator gene product. An operon thus consists of, (i) **an operator gene / sequence** which control the activities of a number of contiguous **structural genes** that take part in synthesis of protein (s). The structural genes synthesize mRNA molecules under the operational control of operator. The operator is under the control of a repressor molecule synthesized by **regulator gene** which is not a part of operon.

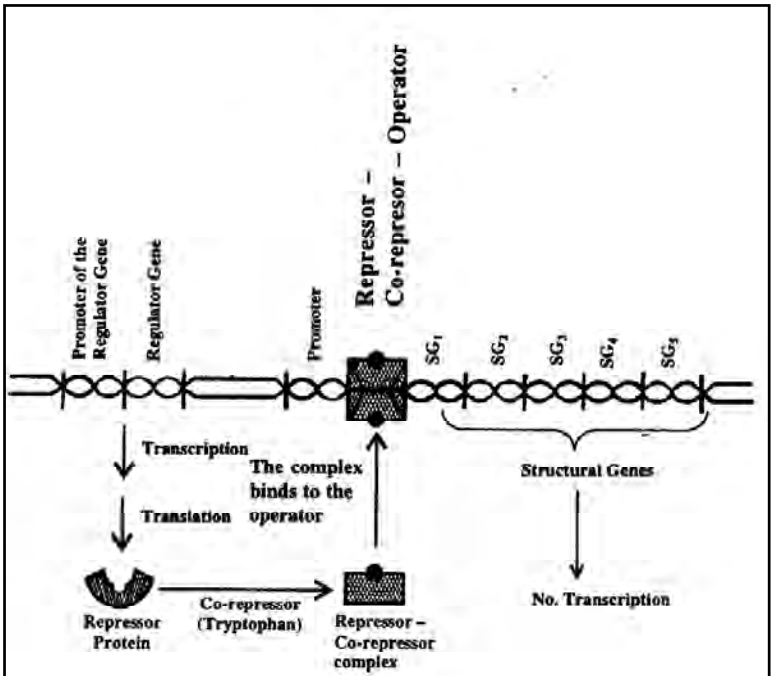


Fig. 7.22 : The repressible operon

In the inducible system (Fig. 7.23) the regulator synthesize active repressor protein which as an active dimer binds to the operator inhibitng the binding of RNA polymerase to the promoter, thereby inhibiting the expression (transcription) of all the contiguously present structural genes. In the presence of inducer the repressor instead of forming an active dimer forms iducer-repressor complex which does not bind to the operator. As a result, RNA polymerase binds to the promoter and all the structural genes are transcribed into a common mRNA (polycistronic).

In the repressible system, the regulator synthesizes inactive repressor that cannot bind to the operator, so that the operon is turned on normally. When the corepressor is present, the inactive repressor forms a complex with the co-repressor which binds to the operator and prevents gene expression.

Lac Operon in *E.coli*

The lac operon found in *E.coli* is an inducible system responsible for the synthesis of enzymes involved in lactose (the milk sugar). It has an operator sequence of 26 base pairs and **three structural genes**. The first structural gene (SG) is **lac z** of 3063 base pair and is responsible for the synthesis of the enzyme **β-galactosidase**. The operator is a part of lac z. The other two genes are **lac y** for sythesis of **β-galactoside permease** and **lac a** for β-**galactoside transacetylase. β-Galactoside permease** is a transmembrane protein that pumps galactose into the cell and B-galacto- sidase breaks lactose to galactose and glucose. The function of galactoside transacetylase is not clear. When lactose is available to the bacterium, the active repressor produced by the regulator forms an inactive dimer with lactose, as lactose, the substrate

of the enzyme galactosidase acts as an inducer. This inactive dimer cannot bind to the operator and the three contiguous structural genes are transcribed into a polycistronic or polygenic mRNA. This polycistronic mRNA is translated into three proteins (enzymes). In the absence of lactose, the inducer, the product of regulator forms active inhibitor dimer, that binds to the operator and prevents transcription.

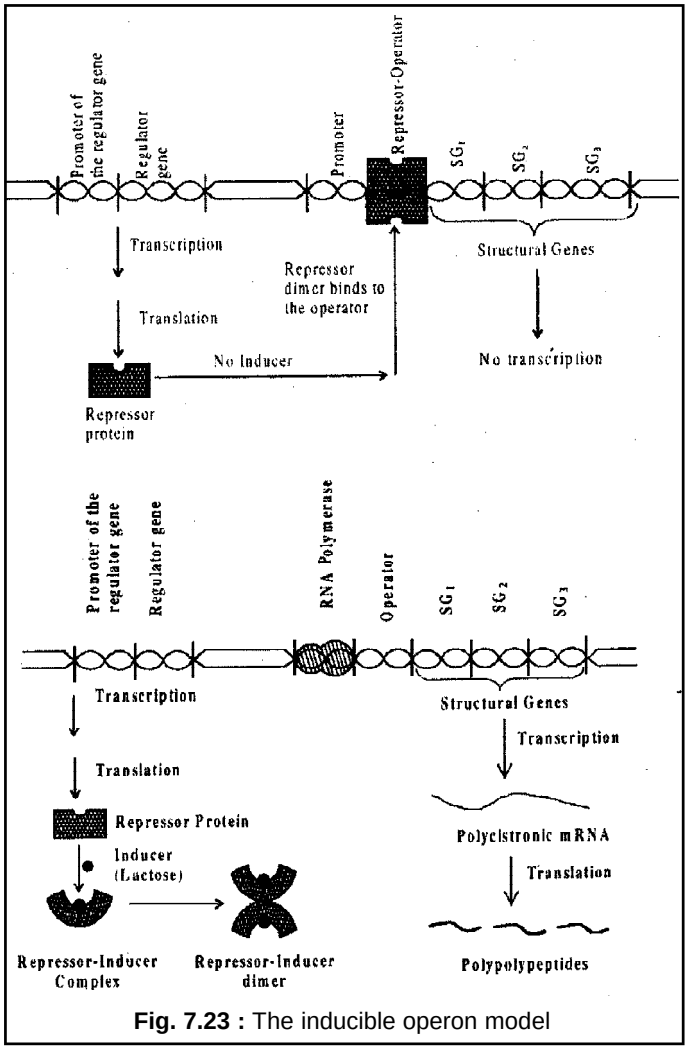


Fig. 7.23 : The inducible operon model

7.8 GENOME AND HUMAN GENOME PROJECT :

Genome ordinarily means the haploid set of chromosomes in a gamate or microorganism or each cell of multicellular organism. It consists of DNA (or RNA in RNA viruses)

Each genome contains all of the information needed to build and maintain an organism. The human genome project was formally launched in 1990 with goal of determining the sequence of base pairs which make up human DNA and, also of indentifying and mapping all of the genes of human genome. In May 2016, scientists considered extending the project to include creating a synthetic human genome. This $3 billion project was formally founded by US Dpeartment of Energy and the National Institute of Health and was expected to take 15 years. In addition to United States, the international consortium comprised geneticists from UK, France, Australia, China and many other countries. A 'rough draft' of the genome was finished in 2000 (announced jointly by US President Bill Clinton and British Prime Minister Tony Blair on 26th June 2000). The announcement of essentially complete genome was made on 14th April 2003; two years earlier than planned. In May 2006, the sequence of last chromosome was published.

Findings :

The key findings of the draft (2001) and complete (2004) genome project include :

1. There are approximately 20,500 genes in human genome, the same as mice.
2. The genome has more indentical or repeated sequences of DNA than previously suspected.

3. At the time the draft sequence was published fewer than 7% of protein families appeared to be vertebrate specific.

Applications and proposed benefits :

It has applications ranging from molecular medicines to evolution. It can help us understanding diseases including

1. genotyping of specific viruses to direct appropriate treatment,
2. identificatin of mutations linked to different forms of cancer,
3. the design of medication and more accurate prediction of their effects,
4. advancement in forensic science,
5. biofuels and other energy applications,
6. agriculture, animal husbandry, bioprocessing, risk assessment and human evolution.

7.9 DNA FINGERPRINTING :

The technique of DNA fingerprinting was developed and established by British geneticist Dr. Alec Jeffreys. Every individual organism is unique in its fingerprints. Similarly every individual differs from other in his DNA pattern or design. Fingerprints can be altered by surgery but there is no known procedure available to alter the DNA design of an individual. For obtaining the DNA fingerprints of an individual, one should look for genes that is highly polymorphic or occur in multiple forms in different individuals. (In other words, genes which are multi allelic in a population).

Principle of DNA finger printing (Fig. 7.24)

First of all, for DNA fingerprinting or profiling or typing short nucleotide sequences having variable number of repeats are identified. These repeats are called variable Number Tandem Repeats or VNTRS. These VNTRS of two individuals may be of same length and same sequence at certain sites of DNA but vary at others. This will be clear if we consider the following example.

Suppose the mother has six VNTRS on one chromosome (two chromatids) and father has four VNTR on the same chromosome (two chromatids). The child will in herit a chromosome with six repeats from mother and its homologous chromosome with

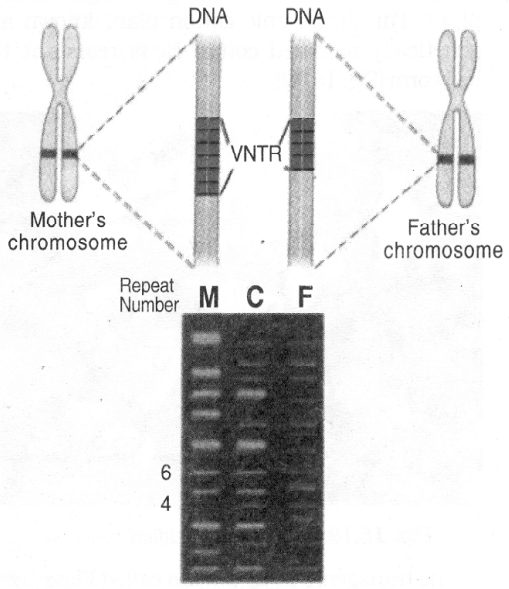


Fig. 7.24 : Variable Number Tandem Repeats (M=mother, F=father, C=child)

four repeats from father. The DNAS/chromosomes samples of father, mother and child collected separately can be compared on gel electrophoresis.

Applications of DNA fingerprinting

This technique can be applied in various fields such as :

(i) In forensic Science to identify the criminals.

(ii) To establish the parentage of a child i.e. to establish the biological father or mother of a child in case of a dispute.

(iii) To identify an ethnic group or to deduce the evolution of a racial group.

_____

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the blanks with correct answers from the choices given in the brackets of each bet.

(i) In split genes, the coding sequences are _____.

(introns, operons, exons, cistrons)

(ii) The smallest part of the gene is called _____.

(recon, muton, exon, cistron)

(iii) The enzyme referred to as Kornberg enzyme is _____.

(DNA polymerase I, DNA polymerase II, RNA polymerase, ligase)

(iv) The polymerase that has 5' to 3' exonuclease property is known as _____.

(DNA pol I, DNA pol II, RNA pol, DNA ligase)

(v) The termination factor that recognises the termination codon UAG is _____.

(Only RF, only RF_2, both RF_1 & RF_2, neither RF_1 and RF_2)

(vi) The enzyme that removes formyl group from the first amino acid methiomine of a newly synthesized polypeptide is _____.

(RF_3, translocase, deformylase, exoaminopeptidase)

(vii) The word gene was coined by _____.

(Garrod, Johannsen, Meischer, Griffith)

(viii) In 1869, _____ discovered DNA

(Garrod, Meischer, Griffith, Wilkins)

(ix) The virulent, Pneumococcus possessed a _____ coat for its protection.

(Protein, Lipid, phospholipid, Polysaccharide)

(x) Complete sequence of amino acids in _____ was proposed by sanger.

(Insulin, haemoglobin, kinetin, polymerase)

(xi) RNAs lack _____ as nitrogenous base.

(Adenine, guanine, cytosine, Thymine)

(xii) One complete turn of B-DNA Contains _____ number of nitrogenous bases.

(10,11,9,12)

(xiii) The most stable form of RNA is _____ RNA.

(messenger, transfer, ribosomal, small nuclear)

(xiv) When a codon codes for more than one amino acid, it is called _____ code.

(Commaless, degenerate, nonsense, universal)

(xv) The start codon is _____.

(UAA, UGA, AUG, UGA)

2. Express in one word only :

(i) If in a double standed DNA, there is 25 per cent of thymine, then calculate the per cent of guanine.

(ii) What is the complementary base of Adenine in RNA?

(iii) In a double helix, if one stand is on 5' ⟶ 3', what will be arangement of other strand?

(iv) What are the basic proteins called in eukaryotic DNA?

(v) What is called to amino acids with more than one codon ?

(vi) What type of genes do express continuously?

(vii) What type of RNAs do carry amino acids to the site of protein syntheses?

3. Correct the sentences in each bit without changing the underlined word/words :

(i) Watson and Griffith <u>proposed double helical structure of DNA</u>.

(ii) A nucleoprotein is building block of all <u>nucleic acids</u>.

(iii) The strand of the <u>DNA double helix</u> represent uncleotide phosphate backbone and are antiparallel.

(iv) The helical turns are right handed is <u>Z</u> DNA.

(v) Avery, McCarty and Macleod experimentally proved that the <u>transforming principle</u> is a protein.

(vi) Meischer proposed the <u>transforming principle.</u>

(vii) The enzyme, ligase is responsible for <u>transcription.</u>

(viii) The operator is under the control of a <u>repressor molecule</u> synthesized by structural gene which is not a part of operon.

(ix) The example of regulatory gene is genes of <u>respiratory enzymes</u>.

(x) <u>P-site</u> in prokaryotes only accepts $tRNA^{met}$.

(xi) The coding or translatable sequences are <u>introns.</u>

(xii) The structural genes transcribe <u>tRNA and rRNA.</u>

(xiii) A Primer is a small DNA or RNA strand hydrogen bonded to a template.

(xiv) In DNA replication, as per semiconservative model, two new strands synthesized, form new DNA molecules.

4. Fill in the blanks :

(i) The enzyme _____ hydrolyses DNA molecules.

(ii) Clover leaf model of tRNA was proposed by _____.

(iii) The segment of DNA that expresses specific character is called _____.

(iv) The enzyme _____ helps to join nucleotides.

(v) The DNA strand which takes part in transcription is called _____.

(vi) VAG is a _____ codon.

(vii) The gene which becomes active due to the presence of specific substance is called _____ gene.

(viii) To identify criminals DNA _____ is done.

GROUP - B
(Short Answer-type Questions)

1. Write notes on the following with atleast 2 valid points :

(i) Inducible operon

(ii) Repressible operon

(iii) House keeping genes

(iv) Adaptor molecules

(v) Split genes

(vi) RNA splicing

(vii) Termination of translation

(viii) Okazaki fragments

(ix) Central dogma

2. Differntiate with atleast 2 valid points :

(i) Genes and chromosomes

(ii) DNA and RNA

(iii) Purines and pyrimidines

(iv) Exons and Introns

(v) B-DNA and Z-DNA

(vi) Replication and Transcription

(vii) Transcription and Translation

(viii) House keeping gene and Inducible gene

(ix) Degenerate codon and nonsense codon

GROUP - C
(Long Answer-type Questions)

1. Give the structure of DNA. Add a note on different forms of DNA.
2. Describe the semiconservative model of DNA replication.
3. Give evidences of DNA as genetic material.
4. Explain the mechanism of translation in Prokaryotes.
5. Describe transcription in prokaryotes.
6. Give an account of the operon model.

❑ ❑ ❑

CHAPTER 8
EVOLUTION

Evolution literally means change or gradual development with ticking time. Everything in the universe and so on our planet changes with time. The evolution that is being discussed in this chapter relates to **organic evolution** or **biological evolution**. Organic evolution is the process by which changes in the genetic composition of populations of organisms occur in response to environmental modifications in course of time. The plants and the animals that we see today are considered as modified descendants of those living in the past, the ancestors. The ancestors were again the modified descendants of their predecessors and so on until we go back to the beginning of the mystery. An analogy to explain this sort of change over time is the evolution of automobiles. It started from bullock cart to horse-dragged carriage, then to bicycle and presently to various forms of automobiles with a great deal of modification. The same principle applies to the organisms in the process of evolution.

The organisms live in a given space and a set of physical and chemical conditions, which together constitute the environment. The environment is considered as the home of the organisms. The organisms always tend to adapt themselves to the environment they live in to guarantee that their existence continues. Like every other thing, the environment also changes with time. The organisms do possess an inherent tendency to adapt themselves to the continuously changing environment. In doing so, necessary changes are made in their structure and physiology. These changes are known as **adaptive characters**, which ensure not only their existence, but their continuation through generations as well. The changes occur in very minute doses that are not visible. Over a long period of time, the cumulative changes give us a visible appearance that can be marked. The consequence is the descent of a different-looking organism from its predecessor. Thus, the **diversity** of organisms changes on earth over time. The organisms, which fail to accumulate the adaptive characters, are eliminated from the race. Alternately speaking, they become extinct, just as that happened to the dinosaurs.

From the foregoing discussion, it is evident that the environment plays a crucial role in bringing about adaptive changes in organisms. The changes in the adaptive characters modifies the diversity of organisms. **Thus, organic evolution may be defined as a change in diversity and adaptations in populations of organisms.** Here again, we emphasize on populations of organisms. A population is an interbreeding group of animals or plants occupying a given space

at a given point of time. All members of a population have the same genetic material and are said to belong to the same species. They are influenced by the same environment and hence undergo nearly the same kind of change. In summary, it can be said that the product of organic evolution is a diverse group of better adapted organisms. Organic evolution in a condensed form can be understood as a two-step process. The first step is the **production of variations among populations** and the second step is the **ordering of these variations by natural selection**. The causes of variation and the working mechanism of natural selection will be discussed in details in a later part.

8.1 ORIGIN OF LIFE :

The **Big Bang** theory explains the creation of the present universe as the consequence of a single huge explosion that happened about 13.7 billion years ago. The universe has expanded from a very high density and high temperature state to the present condition that comprises of huge clusters of galaxies. The galaxies, on the other hand, consist of stars and clouds of gas and dust. The solar system is a part of the milky way galaxy and our planet earth was formed from a mass of cosmic cloud of dust and gases about **4.5 billion years ago**. The present earth consists of three parts- core (central part), mantle (middle part) and crust (surface part). Though it appears large to us, the size of earth is insignificant in comparison to the magnitude of the universe.

8.1.1 Theories of Origin of Life :

The presence of organisms on earth is considered as a very unique phenomenon since the existence of life in other parts of the universe has not yet been discovered. Several theories have been proposed on the origin of life. Out of these **theory of special creation** (creation of living forms by The Almighty), **theory of catastrophism** (creation of new life forms after each catastrophe on earth) and **cosmozoic theory** or **theory of panspermia** (life coming to earth in the form of spores from other parts of the universe) have been discarded due to lack of logical explanations.

Francesco Redi (1668), Spallanzani (1767) and Louis Pasteur (1862) proved that life originated from prexisting life and thus developed a concept that life originated from life only. However, a question was asked as to where did life originate from, when there was no life on earth? The answer is that life originated from non-living matter and thus the **theory of abiogenesis** gained ground. This idea was strenthened by A.I. Oparin (1923) and J.B.S. Haldane (1928) by proposing the chemical origin of life or chemical evolution.

8.1.2 Chemical Evolution :

(i) The primitive earth did not have an atmosphere. It was **anaerobic**.

(ii) **Early molecules :** The earth was a hot gaseous mass at its origin, which gradually cooled down into a solid form. While the heavy elements like nickel and iron settled at the centre, elements like aluminium and silicon remained in the middle and lighter elements such as oxygen, nitrogen, hydrogen and carbon formed the early atmosphere. With further cooling, hydrogen, nitrogen, water, ammonia, methane, carbon dioxide and possibly hydrogen cyanide were formed. However, **free molecular oxygen (O_2) was not present** for which the primary atmosphere was reducing in nature. Torrential rains gave rise to hot oceans, seas and lakes.

(iii) **Simple organic molecules :** At a higher temperature, radiations and lightening, molecules and minerals dissolved in water bodies reacted to produce many simple organic molecules such as simple sugars, amino acids, glycerol, fatty acids, purines and pyrimidines. This fact was established by **Stanley Miller** and **Harold Urey** (1953). They conducted an experiment (Fig. 8.1) by heating ammonia, methane,

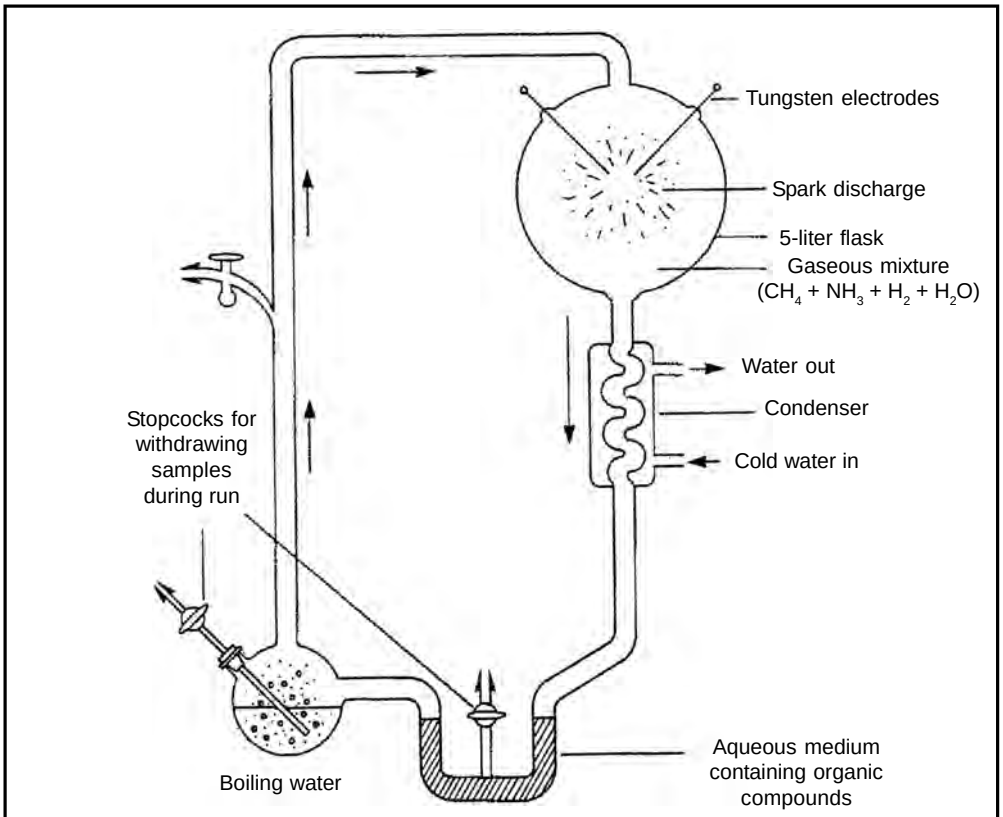


Fig.8.1 : Miller-Urey experiment explaining formation of organic molecules

hydrogen and water vapour at 800^0C in a glass apparatus designed by them. Simple organic molecules were identified in the aqueous medium that collected at the bottom. This experiment demonstrated that more complex organic compounds could have been formed spontaneously from simpler inorganic precursors, which could mark the bgining of life on earth.

(iv) **Complex organic molecules**: Further reactions occurred in the aqueous medium of oceans resulting in the formation of complex organic molecules such as polysaccharides, fats, proteins, nucleotides and later nucleic acids (DNA and RNA).

(v) **Molecular aggregates and cell-like structures**: Aggregates of complex organic molecules were formed later in the sea water. The sea water, rich in the soluble organic matter was termed as **prebiotic** or **primordial soup**. Colloidal particles forming droplets originated from such aggregates, which could grow and divide. Oparin termed these as **coacervates**. Sydney Fox (1965) used the term **microspheres** to describe small spheres of complex molecules covered by external membranes, which could also grow and divide. It is believed that first non-cellular forms of life originated about 3.5 billion years ago. They would have contained various macromolecules such as polypeptides, polysaccharides, lipids and nucleic acids. Such first cell-like structures are termed as **protobionts**, **protocells** or **eobionts**. Probably, **viruses** also evolved at the same time. However, first cellular forms of life could have originated close to 2 billion years back. It is accepted that first form of living matter evolved by the aggregation of various non-living molecules and once created the cellular forms evolved to diverse species of living organisms in course of time.

8.1.3 Biological Evolution :

After creation of living matter from the pre-biotic soup in the sea, evolution of living forms occurred in the following manner.

(i) **Prokaryotes**, such as bacteria were created in sea water as the first cellular organisms. They were single celled with naked DNA as the genetic material and covered by external membranes. They were first **chemoheterotrophs** and utilized the organic molecules available in plenty in their environment. Respiration was anaerobic as free oxygen was not available.

(ii) The mode of nutrition then changed to chemosynthesis (synthesis of organic molecules from inorganic substances). Prokaryotes with such ability are termed as **chemoautoprophs,** which derived energy from reducing the inorganic materials.

(iii) Later **photoautotrophs,** (bacteria-like organisms) evolved about 3.5-3.8 billion years ago, which possessed chlorophyll pigment. They could absorb solar energy

and execute **photosynthesis** and synthesize the required organic compounds. First photosynthesis was anoxygenic, but later it became oxygenic producing free oxygen (as in **cyanobacteria** that appeared at least 3 billion years ago). Gradually the atmosphere became rich in oxygen. Ozone layer was formed protecting the earth from UV radiation. Respiration gradually became **aerobic** as free oxygen was available.

(iv) In the course of biological evolution, **eukaryotes** then evolved around 1.6 billion years ago (some suggest it as far back as 2.6 billion years) that possessed well-defined nuclei. Single-celled algae, fungi, protozoa, etc were formed in seawater. Evolution of eukaryotes occurred in the form of two distinct forms: plants and animals. Several invertebrates and then vertebrates evolved constituting the animal kingdom, while groups such as algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms formed the plant kingdom.

(v) There is a gradual change in the characters of many life forms. Some forms that existed earlier on earth, such as dinosaurs, Indian cheetah and pink-headed duck, are extinct now and others which are present on earth currently did not exist earlier. Some organisms develop improved characters to survive better in natural conditions than others. Those having better fitness in a particular environment leave more offsprings and thus are selected by nature. Charles Darwin termed this as **natural selection** that forms the basis of the mechanism of evolution.

8.2 EVIDENCES OF BIOLOGICAL EVOLUTION :

Now we have a gross idea that the existing complex forms of organisms have arisen from very simple forms of life through millions of years of evolution. Evolutionary changes are steady and very slow to be detected by the human eye. The changes accumulate over a long period of time giving rise to structurally different organisms. Thus, the diverse species of animals and plants existing on earth at present have descended from their ancestors. To establish the authenticity of the evolutionary process, scientists have documented many evidences from different branches of biology, like (1) palaeontology, (2) morphology and comparative anatomy, (3) embryology (4) genetics and molecular biology, (5) biochemistry and comparative physiology, (7) taxonomy, and (8) geographical distribution. Though many evidences are circumstantial, those from palaeontology (fossil record) provide direct evidence of the evolutionary process.

8.2.1 Evidences from Palaeontology :

Palaeontology deals with the study of fossils of both animals and plants. **Leonardo da Vinci** is known as the 'Father of Palaeontology', while **Georges Cuvier** as the 'Father of Modern Palaeontology'. Fossils of organisms of remote past recovered from the earth crust provide direct evidences in support of organic evolution. Fossil records of some animals, such as horse, elephant and man, are so complete that they clearly explain gradual evolutionary changes. Extinction of dinosaurs about 66 million years ago is also explained by their fossil record.

8.2.1.1 Fossils :

A fossil is any remain or impression of the entire body or parts of an animal or plant living in the remote past that has been preserved in the sedimentary rock deposits of earth's crust. Fossils include hard parts like bones, teeth and shell; impressions or imprints of relatively softer parts like feathers and leaves; and casts or moulds of entire organisms or parts.

Fossilization is the process of formation of fossils. Entire bodies or parts of dead organisms are washed away to the sea through rivers that settle down at the bottom. These are then covered by mud and sand, though most are decomposed leaving no trace of their existence. However, harder parts like bones, teeth, scales, shells, and wood and leaf skeletons are sometimes preserved after decomposition of the soft parts. The hard parts in some cases may be slowly replaced by minerals from the surrounding mud or sediment, which form casts and moulds. As additional layers are deposited in course of time, the lower layers harden into rock under pressure. This type of rock is known as sedimentary rock.

8.2.1.2 Types of Fossils :

(a) **Unaltered :** Whole bodies of some extinct organisms have been preserved in the ice in polar regions. Woolly mammoth of 25,000 years old is recovered in Siberia and insects trapped in the amber of plants are two outstanding examples of unaltered or whole body fossils [Fig. 8.2(c)].

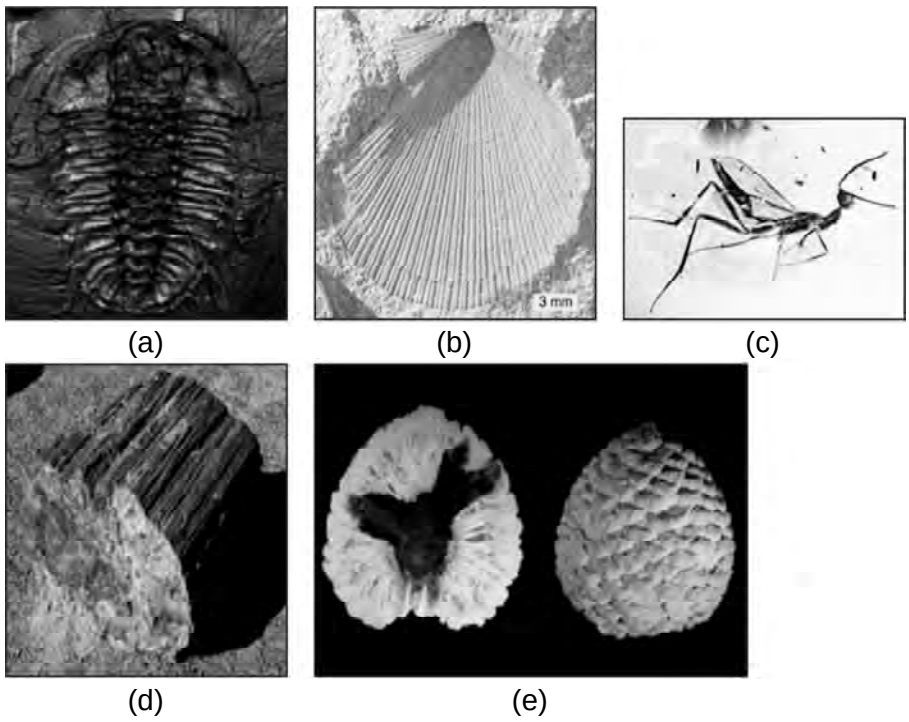


Fig. 8.2 : (a) Imprint of a crustacean, (b) Mould of a bivalve, (c) Insect trapped in amber, (d) Petrified softwood, (e) Petrified cone of *Araucaria mirabilis* (a coniferous tree)

(b) **Petrified :** Mineral substances from the surrounding replace the hard body parts of the extinct organisms preserved within sediments of the sea bed. By this process, called **petrification** or petrifaction, the orginal structures of the entire plant or animal or parts thereof are preserved [Fig. 8.2 (d) & (e)].

(c) **Moulds and Casts :** Sometimes a dead organism burried in the muddy sediment is decayed, but the mud covering it hardens retaining true copy of its shape and forms a fossil called mould. When the cavity within the mould is filled with minerals, the fossil is a petrified fossil, called a cast [Fig. 8.2(b)].

(d) **Imprints or Impressions :** Prints of hard parts such as stems, leaves, wings, feathers, shells, etc or footprints of animals made on soft mud later on harden to form imprints or impression fossils [Fig. 8.2(a)].

8.2.1.3 Important characteristics of fossils :

The fossil record constitutes direct evidence in support of organic evolution due to the following reasons.

1. Fossils of different ages are mostly found in **sedimentary rocks** present in different layers or strata in an ascending order.
2. The lower layers contain early fossils of simple nature, while upper layers with more recent fossils, which are more complex in structure. Fossils are not found in the rocks of the first era, the Archaeozoic Era.
3. Less numbers of fossils are found in the second era (e.g. Proterozoic). These are simple, soft-bodied organisms, such as marine invertebrates. Fossils are more in number in the upper strata of rocks, which belong to organisms of later ages.
4. Differences are observed between the fossils of two consequitive strata indicating the occurrence of progressive changes in course of time.
5. Fossil records of some mammals, such as horse, elephant, camel and man, are so complete that they clearly explain the gradual evolution of these species.
6. Fossils of some **transitional forms**, also called **connecting links** or **missing links**, have been recovered, which make clear about the emergence of a new species from its ancestor. Fossil of ***Archaepteryx*** (Fig. 8.3) discovered from the rocks of Jurassic Period at Bavaria, Germany in 1861 is a good example of a connecting link that explains the evolution of birds from reptiles. As a transitional form it possessed characters of both reptiles and birds.
7. A **living fossil** is a living species which closely resembles the features of a recorded fossil. Such organisms have undergone very slow changes over a long span of time [e.g. *Latimeria* (a coelacanth fish)].
8. The approximate ages of fossils have been worked out by different radioactive dating methods.

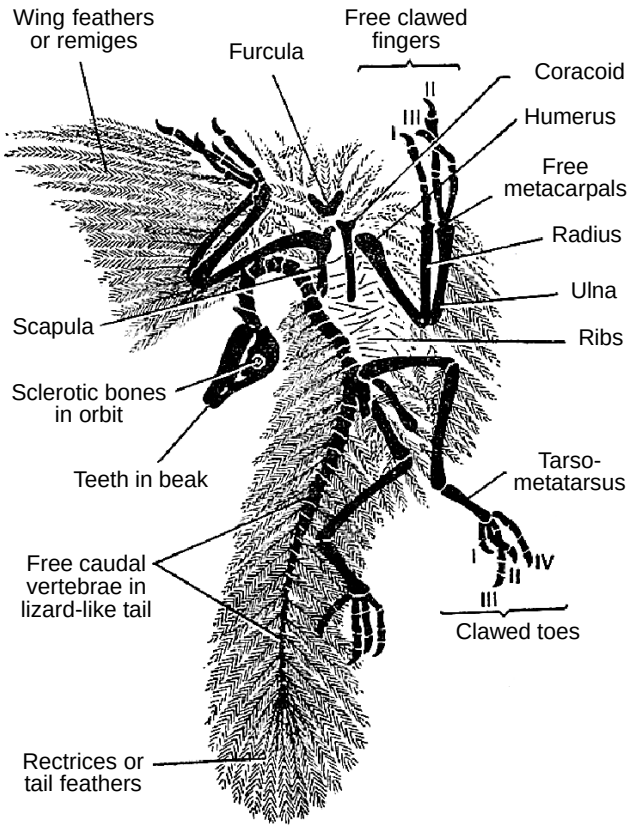


Fig.8.3 : Fossil of *Archaeopteryx* after restoration

8.2.1.4 Geological Time Scale :

It has been prepared by the scientists to describe the gradual evolutionary changes in the ascending order of time. The time scale is divided into five divisions known as **eras**: **Archaeozoic**, **Proterozoic**, **Paleozoic**, **Mesozoic** and **Cenozoic**. Each era is further divided into several **periods** and each period into **epochs** (Table : 8.1). The most primitive era, Archaeozoic is placed at the bottom, while the most recent, Cenozoic is positioned at the top. The dominant life forms, plants and animals, of the eras when written from bottom to top in a column constitutes an ascending order of evolution of diverse groups.

8.2.1.5 Calculating the Age of Fossils :

The **radioactive dating** method is used to determine the age of the fossils. Among the best-known techniques are Uranium-Lead dating, Potassium-Argon dating and radiocarbon dating. Radioactive element Uranium is decayed into Lead over a period of time through many intermediate stages. Considering that one million gram of Uranium is converted into 17,600 gram of Lead in one year (rate of decay), the age of a rock and that of a fossil contained in it, is estimated approximately from the amount of Lead present in the rock.

Table : 8.1

Geological timescale (starts at the bottom) indicating origin and evolution of important groups of organisms.

Ascending order of time ↑

Era	Period	Epoch	Dominant forms of life
Cenozoic (63 million years old)	Quaternary	Recent	Human
		Pleistocene	
	Tertiary	Pliocene	Mammals
		Miocene	
		Oligocene	
		Eocene	
		Paleocene	
Mesozoic (230 million years old)	Cretaceous		Birds and Reptiles
	Jurassic		
	Triassic		
Paleozoic (600 million years old)	Permian		Invertebrates, Fishes and Amphibians
	Carboniferous		
	Devonian		
	Silurian		
	Ordovician		
	Cambrian		
Proterozoic			Lower invertebrates
Archeozoic			Bacteria

8.2.1.6 Evolution of Horse :

The fossils of modern horse, recovered from rocks when arranged in an ascending order clearly demonstrate its evolution through time (Fig. 8.4). The earliest horse was ***Eohippus*** or ***Hyracotherium*** living in the plains of North America. It had four toes (II – V) in each of the fore legs, while three (II – IV) in the hind legs. There was a gradual reduction in the number of toes in both the fore and hind legs. Simultaneously, the **grinding surface** (**cusp**) of the molar teeth was also modified. In the **modern horse** (***Equus***), the digit III in both the fore and hind legs persists as the functional toe. Digits II and IV persist as reduced structures known as **splint bones**. The evolution of horse is a unique instance of paleontological evidence in favour of organic evolution.

8.2.2 Evidences from Comparative Anatomy and Morphology :

All members of a population or species exhibit morphological and anatomical resemblances among themselves. Members of closely related groups of organisms also exhibit similarities in many structures. The degree of similarity decreases with an increase in the distance of relationship between two groups. Comparison of such morphological and anatomical features or organs of different groups throws light on the occurrence of organic evolution. It explains how the two-chambered heart in fish has gradually evolved into the four-chambered heart in birds and mammals, and how the simple brain of fish has evolved into the most complex brain in human.

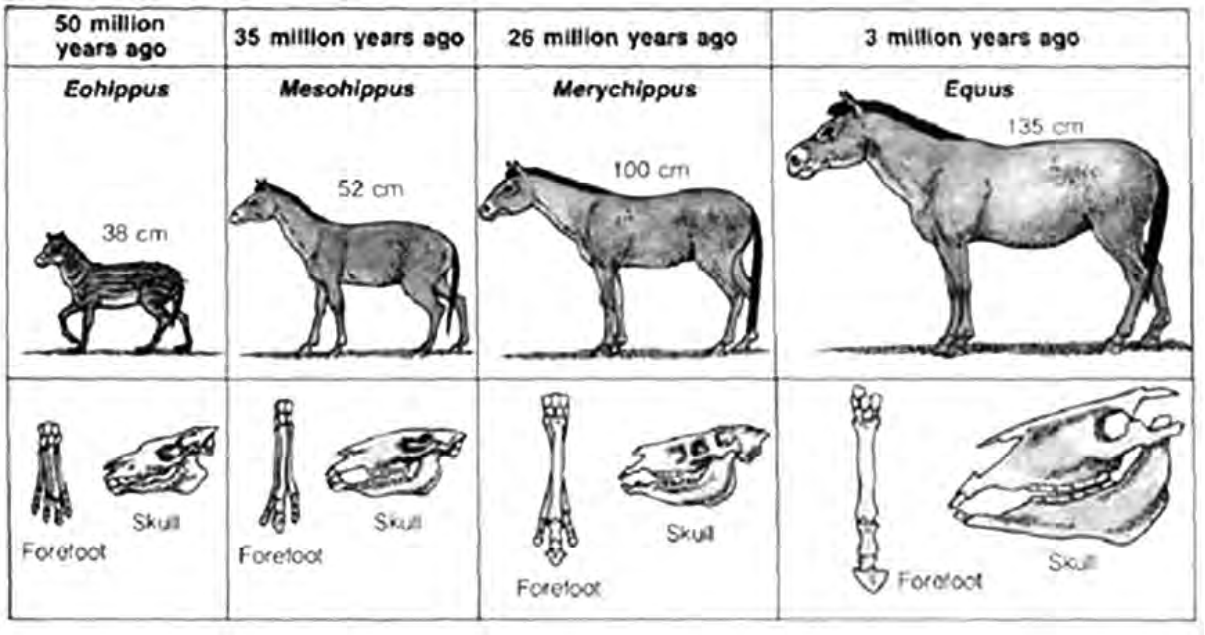


Fig. 8.4 : Evolution of horse from *Eohippus* to *Equus*

8.2.2.1 Homologous organs and Homology :

The organs which have the same fundamental structure and embryological origin, but appear different externally and carry out different functions are called **homologous organs**. Such a similarity is called **homology,** which points at common ancestry. These organs belong to animals of the same groups. Fig. 8.5 depicts fore limbs of diverse groups of vertebrates (tetrapods). All have **pentadactyl** (five digit) fore **limbs**, possessing the same number of skeletal elements arranged in the same order (proximal to distal), same muscle, nerve fibres, and blood vessels. However, these limbs have undergone adaptive modifications, with the basic plan remaining similar, to adapt to their environment and to perform the required functions. For example, the fore limbs of whale, horse, mole, bat and man are modified for swimming (aquatic), running (cursorial), digging burrow (fussorial), flying (aerial) and grasping, respectively.

It is logical to say that all mammals have originated from an ancestral terrestrial mammal through adaptive modifications of the basic pentadactyl limb plan. This is known as **adaptive radiation**, also called **divergent evolution.** In the same principle, it may be inferred that all vertebrates have originated from a common ancestor that may have possessed pentadactyl limbs. Homology is also observed in the structure of different mouth parts of some insects, and structural organization of skull, heart, brain, kidney, muscles, etc of vertebrates.

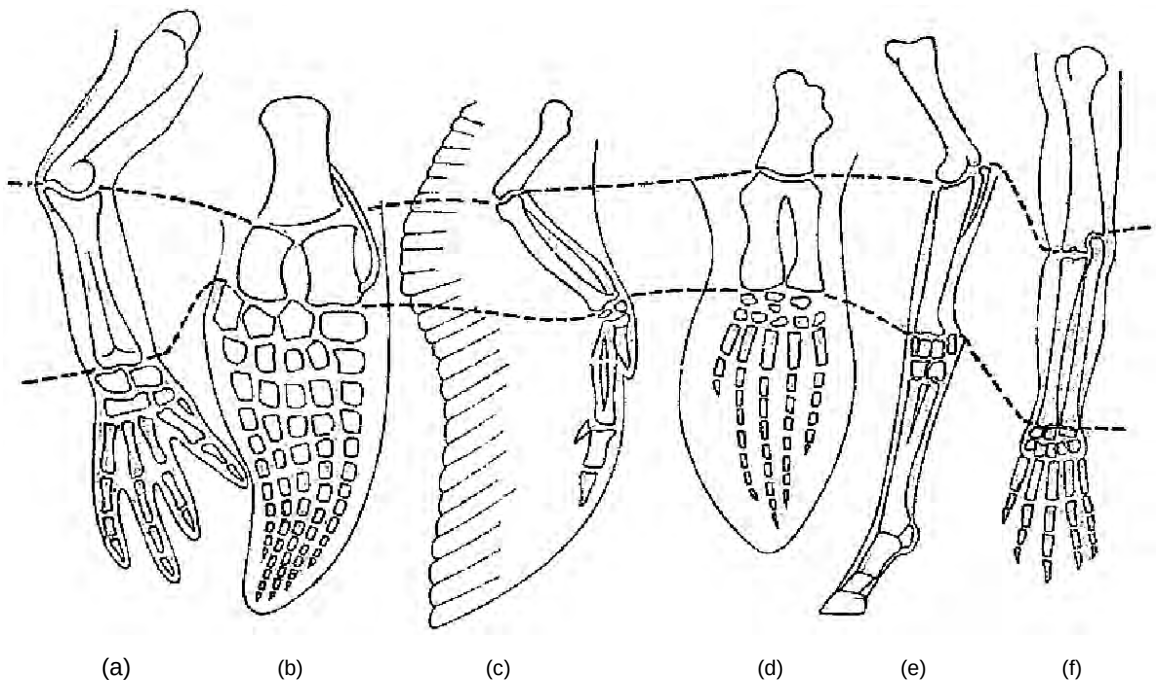


Fig. 8.5 : Homologous organs as exhibited by the fore limbs of vertebrates.
(a) frog; (b) Icthyosaur (aquatic reptile); (c) bird; (d) whale; (e) horse; and (f) human.

8.2.2.2 Analogous organs and Analogy :

The organs, which appear similar externally, carry out similar functions, but have different structure and embryological origin, are called **analogous organs** (Fig. 8.6). Such organs are present in unrelated groups of organisms. The phenomenon of such similarity is called **analogy**. It indicates different ancestry. This also explains **convergent evolution**. The wings of an insect, *Pterosaur* (extinct flying reptile), a bird and a bat (flying mammal) are considered as analogous organs. Except the insect, the rest three are all vertebrates and their wings are modified fore limbs adapted for flight. All three have similar internal organization possessing muscles and bones, while wings of an insect have completely different structure having no bone and muscle. Insect wings are thin membranous extensions of exoskeleton made up of chitin, supported by veins (modified tracheae). However, wings of all these animals are adapted for flight to meet

their need. The wings of an insect are, therefore, said to be analogous to the wings of the flying vertebrates. Such similarities in analogous structures are superficial. Fins of fishes and flippers of whales are also analogous organs.

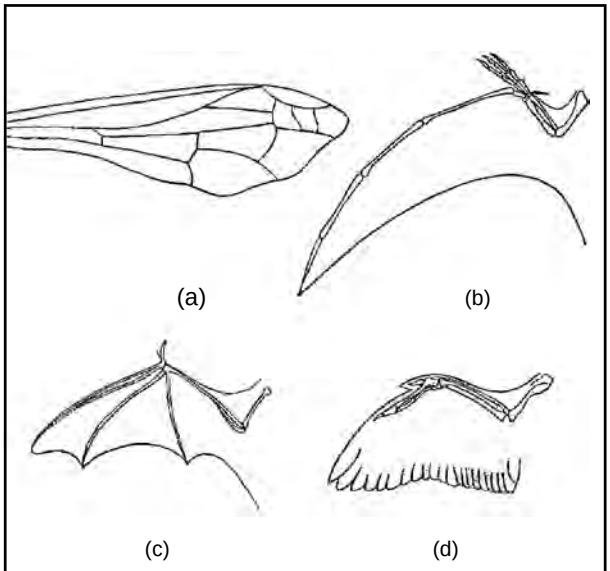


Fig. 8.6 : Analogous organs as exhibited by (a) wing of an insect; (b) wing of an extinct reptile (*Pterosaur*); (c) wing of bat; and (d) wing of a bird.

8.2.2.3 Vestigial Organs :

Some structures in animals are present in reduced form and these apparently do not perform any function. However, these correspond to well developed and functional organs or structures of related animals. Such organs are known as **vestigial organs**. They are considered as remnants of well developed and useful structures, earlier present in their ancestors that were essential for them.

Vestigial organs in human body : There are about a hundred vestigial structures present in human. The most common of these is the **vermiform** (**worm-like**) **appendix**. It is a small and blind finger shaped process arising from a short pouch known as caecum, located at the junction of ileum and colon (Fig. 8.7). In herbivorous mammals (e.g., cattle) the caecum and the appendix are well developed, which harbour cellulose digesting bacteria for digestion of cellulose. As cellulose is very less in human diet, these structures are not used. In carnivores, these are further reduced. Another example is **semilunar fold** or **plica semilunaris** (a small fold of flesh in the inner angle of each eye). It is a vestige of the 3rd eye lid or **nictitating membrane**. Other common vestigial organs in human are **coccyx** (degenerate tail bone), **rudimentary muscles in the ear pinna**, **nipples in male**, **wisdom teeth** and **tonsils.**

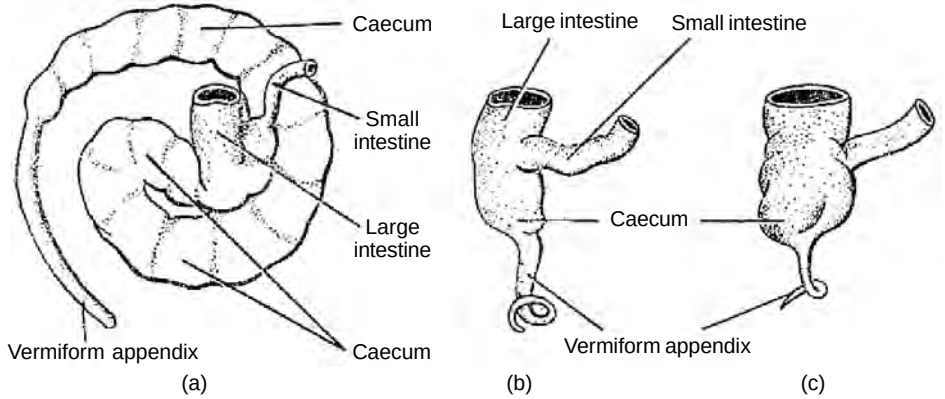


Fig. 8.7 : Vestigial organs. (a) well developed vermiform appendix in a ruminant mammal; (b) & (c) rudimentary vermiform appendix in non-ruminant mammals.

Vestigial organs in other animals: Good examples of such structures are small bones representing the pelvic girdle and hind limb bones in boas and pythons, splint bones representing the metacarpals of digits II and IV in horse and rudimentary wings supported by muscle in Kiwi, the flight-less bird of New Zealand.

Vestigial parts in plants: Dandelions and other asexually reproducing plants retain flowers and produce pollengrains, which are necessary for sexual reproduction.

8.2.2.4 Connecting Links :

These are organisms possessing characters of two different groups indicating evolution of one from the other. They are also called **transitional forms**. Some examples are as follows.

(i) *Euglena* and other chlorophyll-bearing protozoa are considered as connecting links between animal kingdom and plant kingdom.

(ii) *Peripatus* (belonging to *Onychophora*) is a link between *Annelida* and *Arthropoda*.

(iii) *Archaeopteryx* is a connecting link between reptiles and birds.

(iv) Platypus and Echidna (egg-laying mammals) are connecting links between reptiles and mammals.

8.2.2.5 Atavism :

The process of reappearance of some ancestral characters in the present day organisms is known as **atavism**. It indicates their evolution from ancestors possessing these characters on an equal basis. Examples of atavism in human include **moving ear pinna**; **short tail in babies**; **elongated canine teeth**; and **long and dense hairs on body**.

8.2.3 Evidences from Embryology :

The similarities in the process of embryonic development of various groups of animals and their embryos provide most conclusive evidences in support of organic evolution. Such similarities prove that evolution of organisms has occurred through a common pathway. These also establish the degree of intimacy in the relationships among different groups of animals. The following conclusions are derived from the study of comparative embryology.

8.2.3.1 Common pattern of development :

In the process of sexual reproduction, all multicellular animals produce a diploid zygote after fertilization, which undergoes cleavage to form a solid ball of cells called **morula**. The morula then forms a single-layered embryo called **blastula** (with a hollow central cavity termed blastocoel), which then develops into a two-layered or three-layered **gastrula**. Differentiation of tissues and organogenesis occur following the gastrula stage resulting in the formation of various organs. This common pattern of development in the animal kingdom with similar stages justifies common ancestry.

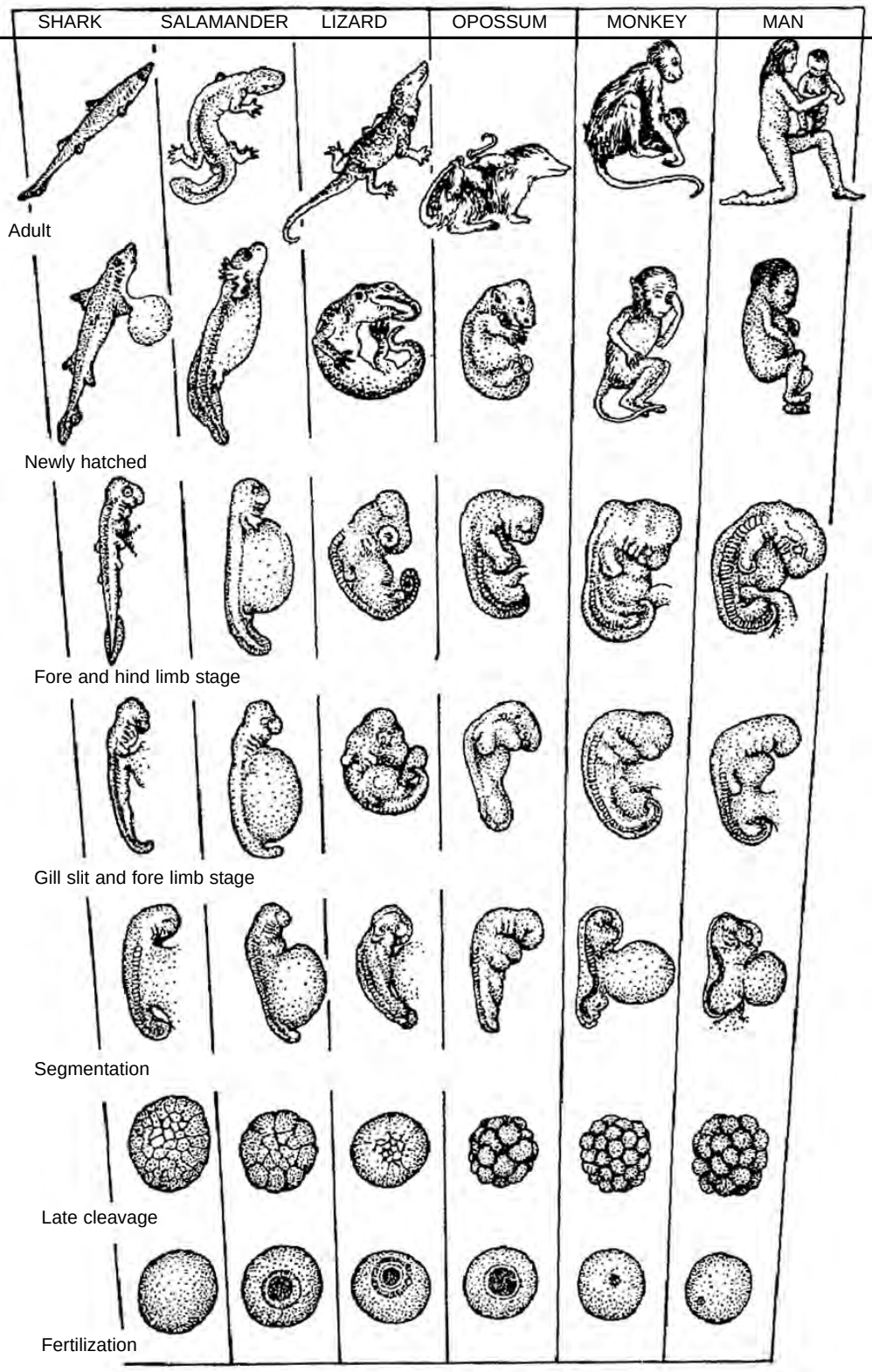


Fig.8.8 : Comparative embryology from fish to human showing homology in embryos.

8.2.3.2 Similarity in early embryos of vertebrates :

A comparative study of the early embryos of different vertebrates, such as fish, salamander, lizard, Opposum, monkey and human, provides striking similarities in the early embryos and it even becomes difficult to distinguish them from each other (Fig. 8.8). They all possess external gills, notochord, tail-like structures and other similar organs. Development of organs like heart, brain, lungs, ear, alimentary canal, etc. in the vertebrates occurs essentially in the same manner. As development progresses, the embryos develop specialized characters and they gradually appear different. Such similarities in early embryos suggest that all these animals have a common ancestry.

8.2.3.3 Recapitulation in embryos :

Karl E von Baer stated that during the development, distantly related animals depart more and more than do closely related animals. von Baer's concept was strongly supported by Ernst Haeckel (1905), who stated that **embryos of higher animals repeated the adult stages of their ancestors**. The thought is restated as "**ontogeny recapitulates phylogeny**". Ontogeny refers to the sequence of developing stages of an animal and phylogeny its racial history i.e. the sequence through which an animal has evolved. The development of an animal reflects its evolutionary history. The most outstanding example in this respect is the development of an anuran amphibian (a toad). An adult toad is terrestrial. However, like all amphibians, it goes back to its ancestral habitat, water, to lay eggs. The eggs develop and hatch into a tadpole larva, much similar in habit and structure of a fish. This type of development strengthens the fact that amphibians have evolved from fish ancestors.

8.2.3.4 Embryological evidences in plants :

Protonema a moss is similar to certain green algae, which indicates that the mosses might have evolved from the later. Bryophytes and pteridophytes, close to each other in the taxonomic hiearchy, develop ciliated sperms and need water for fertilization. Primitive gymnosperms like *Cycas* and *Ginkgo* possess ciliated sperms like those of pteridophytes. It is therefore, believed that gymosperms have evolved from pteridophytes.

8.2.4 Evidences from Biochemistry, Physiology and Molecular Biology :

The molecular evidences derived from the study of cell biology, physiology, biochemistry and molecular biology also explains about the process of organic evolution. The following points clearly explain a close relationship among living organisms and their evolution through a common pathway.

(a) **Protoplasm :** The cells of all organisms, from bacterium to human, contain a mass of the living substance called **protoplasm**. Its composition also remains essentially similar.

(b) **Organelles** : The molecular structure of various cellular organelles and membranes remains largely similar.

(c) **Universal hereditary material:** Deoxyribonucleic acid (**DNA**) is the genetic material in all organisms. However in a few viruses, it is RNA. While in prokaryotic cells, the DNA occurs naked in the protoplasm, in eukaryotes it is present within the chromosomes and the chromosomes in a nucleus. The DNA in all organisms is built on the same structural plan and chemical organization.

(d) **RNAs:** Different types of ribonucleic acids (mRNA, tRNA and rRNA) in diverse organisms are similar in their structure and function.

(e) **Nucleotides:** Both DNA and RNA are composed of **nucleotides,** which are made up of nitrogenous bases (adenine, guanine, cytosine and thymine / uracil), pentose sugar and phosphate. All these constituents are structurally similar in diverse groups of organisms.

(f) **Genetic code**: The genetic information encoded within DNA is translated into proteins by all cells. There exist 64 triplet codons (formed from of A, G, C, T) which code for 20 different amino acids during protein synthesis. In essence, the genetic code is universal.

(g) **Genes**: A gene is a sequence of nucleotides, present in DNA. This sequence directs the synthesis of a polypeptide. Other mechanisms of its functioning also remain similar.

(h) **Central dogma**: The transfer of genetic information from DNA occurs in two steps: **transcription** and **translation**. This is known as the **central dogma** (Fig. 8.9), which is nearly universal. However, in some viruses the pathway is reversed, i.e. from RNA to DNA and then to polypeptide as usual.

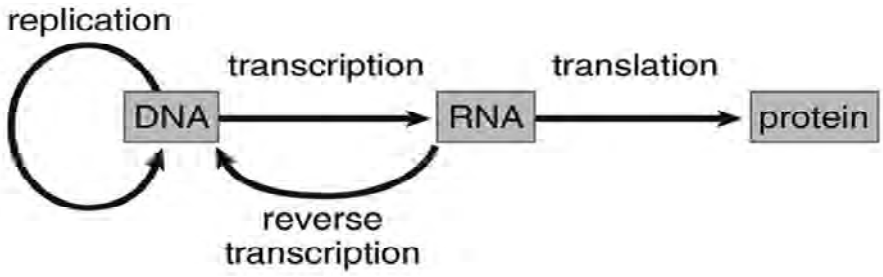


Fig. 8.9 : Genetic information pathway (Central Dogma)

(i) **Nucleotide sequences :** Although the DNA is built on a common fundamental plan, it differs in its sequence of nucleotides from organism to organism. However, there are sequence similarities among closely related species. For example, human and chimpanzee share more similarities in the nucleotide sequences than do human and other animals. Therefore, human and chimpanzee are considered as very close relatives.

(j) **Protein structure :** Proteins are macromolecules consisting of linear sequences of amino acids joined by peptide bonds. Some proteins in diverse organisms like yeast, *Neurospora* (a mould), insects, fishes, reptiles, birds and mammals are similar in their amino acid sequences to a considerable extent and carry out similar functions. **Cytochrome C**, present in the mitochondria, constitutes a component of the electron transport system and is composed of 100 amino acids, and this sequence is mostly similar in all organisms. The molecule also performs the same function in all.

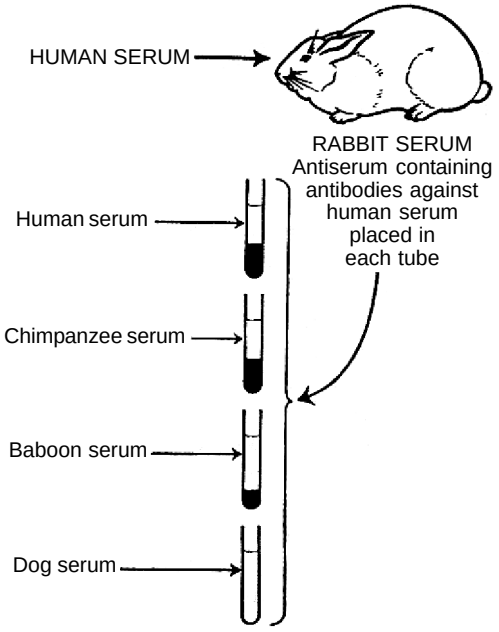


Fig. 8.10 : Precipitin test establishing closeness among different species.

(k) **Haemoglobin :** Haemoglobin, the red colour pigment present in RBC of blood, is found to have similar structure and function (transport of O_2), despite minor variations in its structure, in all vertebrates and some invertebrates.

(l) **Serum proteins :** Serum, the clear part of the blood plasma, is unique in having some soluble proteins. A fraction of the serum proteins function as **imunoglobulins** or **antibodies**. Serum proteins of closely related mammals bear similarity. By the **precipitin test** or **serological test** (antigen-antibody precipitation) (Fig. 8.10), closeness among different species is established. From this test, it is evident that human is more close related to great apes (chimpanzee, gorilla and orangutan) than to the dog.

(m) **Blood groups :** Human blood is classified in to four groups: A, B, AB and O based on the presence or absence of A and / or B **antigens** on the membrane of RBCs. Similarly, the serum of each person has non-reacting **antibodies** (a and / or b). Such blood group antigens have also been found in great apes. Thus, the presence of the blood group antigens is another line of evidence establishing relationship of human with the great apes.

(n) **Metabolism :** Metabolic processes such as digestion, respiration, biosynthesis, etc are more or less similar in most animals. For example, the biochemical steps in glycolysis, Krebs cycle and electron transport system of cellular respiration are similar in all organisms.

(o) **Enzymes** : The biocatalysts that increase the rates of biochemical reactions are proteins in nature in all organisms. They exhibit specificity and a specific enzyme carries out

similar functions in many organisms. For example, starch is digested by amylase, proteins by pepsin and trypsin, and lipids by lipase in all animals.

(p) **Hormones** : Secreted by endocrine glands, hormones show similarity in their structure and function in different animals. For example, cattle insulin also works in human body if injected in case of its deficiency (diabetes). Mammalian thyroxine if injected into tadpole larva of frog (its thyroid gland having been removed), brings about early metamorphosis of the larva.

(q) **Nitrogenous wastes** : Most of the aquatic animals excrete **ammonia** as nitrogenous waste, while the land animals have **urea**, except insects, reptiles and birds which excrete **uric acid**.

In addition to the above discussed evidences, other branches of biology, such as **taxonomy** and **zoogeography** also provide ample of evidences explaining the origin of diverse species of organisms through the process of organic evolution.

8.3 THEORIES OF EVOLUTION :

8.3.1 Lamarckism :

Jean-Baptiste de Lamarck (1744–1829)

Jean-Baptiste de Lamarck (**1744 - 1829**), a French naturalist, proposed the first complete theory of organic evolution that is known as Lamarckism or the **Theory of Inheritance of Acquired Characters** (published in his book *Philosophie Zoologique*). The essence of the theory is that the environment influences the organisms and consequently, the organisms undergo some modifications. The modifications acquired by the organisms during their lifetime are inherited by their offsprings in the next generation. Lamarck was impressed by the long neck of the giraffe (Fig. 8.11), which formed the basis of his theory. His theory may be explained by considering the following elements: (1) Internal vital force; (2) Changing environment and new needs; (3) Use and disuse; and (4) Inheritance of acquired modifications (characters).

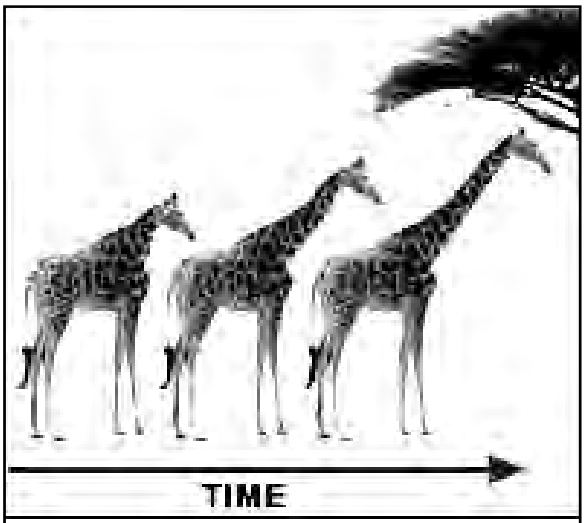
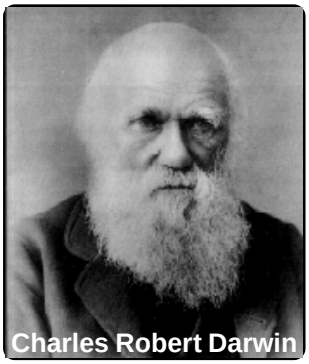


Fig.8.11 : Diagrammatic presentation of Lamark's giraffes acquiring long fore legs and necks

Criticism : The concept of inheritance of acquired characters has been subjected to severe criticism. **Georges Cuvier** and **August**

Weismann were great critics of Lamarck. Weismann's **theory of germplasm** gave a setback to Lamarckism. He proposed that any change in the germ cells (germplasm) will only be passed on to the next generation, but not the changes in the somatic cells (somatoplasm). Lamarck didn't distinguish between somatic and germinal changes.

8.3.2 Darwinism (Natural Selection Theory) :


Charles Robert Darwin
(1809 - 1882)

Although evolutionary thought gained root long back in the history, the mechanism of the origin of new species was not clearly explained by any naturalist before **Charles Robert Darwin** (**1809-1882**), a British naturalist. He went on a world voyage in 1831 for five years on board the ship **HMS Beagle** and chose to settle in the **Galapagos Archipelago** (situated in the Pacific Ocean about 950 km west of South America) to study the animal and plant diversity therein. After a careful and thorough observation he proposed natural selection as the main driving force of organic evolution.

Darwin was particularly impressed by the varied adaptations exhibited by the finches (birds), now called **Darwin's finches**, which were distinctly different from the finches of the mainland of South America, particularly in their beak pattern. He observed different types of beaks in the same population. He termed this phenomenon as adaptive radiation, i.e. the changes in beak structure were the result of adaptations to the available food to the native finches. Over the years, the ancestral beak evolved into diverse types of beaks. Thus, Darwin understood the importance of competition and adaptation in the evolution of finches.

After his return, Darwin developed his concept of organic evolution in the form of a theory. He was also influenced by a paper published by **Robert Malthus** (1838) on populations, which states that the population increases in a geometric progression, while the food supply increases more slowly. Therefore, the food supply becomes a limiting factor. Subsequently, Darwin received a write-up from **Alfred Russel Wallace** (1823-1913). Wallace was also a British naturalist, who was studying flora and fauna of earstwhile East Indies (Now Indonesia). Darwin found the content of Wallace's write-up similar to that of his thinking. The papers of both Darwin and Wallace were published in the 'Journal of the Proceedings of The Linnaean Society of London' in 1858. Darwin published a book entitled '**On the Origin of Species by Means of Natural Selection**' (later changed to '**Origin of Species**' in its 6th edition in 1872), embodying his observations and conclusions in 1859. It not only changed the idea of people on organic evolution, but also attracted the attention of the scientists across the world in the succeeding years.

The elements / propositions of Darwin's theory of natural selection are as follows: (1) prodigality in reproduction, (2) limiting factors, (3) struggle for existence, (4) variations and heredity, (5) survival of the fittest, (6) natural selection and origin of new species.

8.3.2.1 Prodigality of reproduction (Overproduction) :

Organisms have an inherent tendency to reproduce and increase their number rapidly. A female salmon fish lays 28 million eggs in a breeding season. Frogs lay 20 thousand eggs annually. An oyster releases 114 million eggs at a spawning. House flies and mosquitoes also lay thousands of eggs. If all the eggs hatch and all the embryos change into full grown mature adults and these adults again reproduce, the situation will be hard to imagine. The elephant is considered as the slowest breeder among the animals. It remains sexually active between 30 – 90 years in its lifespan of about 100 years. Each elephant can give birth to 6 young elephants during this period. At this rate after 750 years, there would be 19 million elephants descending from a single couple. But the total elephant population of the world at present is about 7 lacs only. Thus, the question arises as to how the populations of different species are held in equilibrium.

8.3.2.2 Limiting factors :

There are some limiting factors, which put a check on the enormous number, growth and distribution of animals and plants. These are food supply, predatory animals, diseases, space restriction and harsh physical conditions of the environment.

8.3.2.3 Struggle for existence :

The overproduction of organisms leads to a severe struggle among the offsprings to survive and propagate through generations. Three types of struggles or competitions are faced by organisms: (1) **intra-specific struggle**: It operates among organisms of same species as their requirements, like food, shelter, reproductive partner and other essential elements of life, are in a common resource; (2) **inter-specific struggle**: It works among the organisms of different species where food and shelter place too are common; and (3) **environmental struggle**: This is against unfavourable climatic conditions, such as extreme low and high temperature, flood, drought, cyclone, heavy rain, earthquake, etc. Animals and plants often struggle to cope up with the inanimate environmental conditions. In doing so, they develop heritable adaptive features, which help them to survive.

8.3.2.4 Variations and Heredity :

Individuals of the same species are not alike. Similarly, different species also differ from each other in their characteristics. These differences constitute **variations**. As the limiting factors operate and the organisms face three-fold competitions, they try to be better suited to the changing environment by developing variations. Such adaptive variations work to the advantage of the organisms. Individuals or populations with harmful (non-adaptive) variations lag behind in the struggle and are eliminated in course of time. Darwin believed that variations are continuous and he had no idea about discontinuous variations. Heritable variations provide essential raw materials for evolution. Thus, variations, which are not inherited are meaningless. However, Darwin had no idea about inheritance of characters.

8.3.2.5 Survival of the fittest and Natural selection :

Those organisms, which have inherited useful variations, become successful in the struggle and adjust with the changing environment most effectively and survive. This is termed as **'survival of the fittest'** (the phrase being originally used by Herbert Spencer). Organisms without useful variations appear unfit and are eliminated. Nature plays a decisive role in selecting the fit organisms. **Natural selection** is based on merit and is without any prejudice or bias. It eliminates the unfit ones and selects the fit ones that adapt better with the environment and produce offsprings in large number. Survival alone does not make any sense from evolution point of view. The fit organisms must reproduce to contribute to the next generation. Lerner (1959) says, "Individuals having more offspring are the fit ones."

8.3.2.6 Origin of new species :

Having been selected by the nature, organisms possessing favourable heritable variations go on accumulating the variations or new characters from generation to generation. Each successive generation tends to become better adapted, but with a few differences from the parental generation. After a long period of time, the offsprings of the new generation become visibly distinct from the ancestor by possessing varied characters and a **new species** thus originates. Organisms of the same species living under different environmental conditions in different parts of the earth may differ from each other in course of time. This is termed as **speciation**.

8.3.3 Criticism to Darwinism :

Darwin's theory of natural selection has gained much acceptance as the only explicit theory on organic evolution. But, neverthless, it has been subjected to criticism. A few objections are discussed below :

1. Darwin didn't explain the use and disuse of organs, and the presence of vestigial organs.
2. He didn't distinguish between somatic and germinal variations for his ignorance on heredity. He considered all variations as heritable.
3. His natural selection was based on the mistaken concept of **artificial selection**. He believed that changes imposed on animals by domestication are also heritable.
4. He believed in the occurrence of small continuous variations. He didn't recognize large fluctuating variations or sports (variations caused by mutation). This fact is explained by considering the **dried leaf butterfly**, ***Kallima***. The dried leaf appearance is an adaptive feature of the butterfly. But what value would be the first small change in the direction of resemblance with a dried leaf?
5. Natural selection explained about the survival of the fittest, but didn't explain about the **arrival of the fittest**.

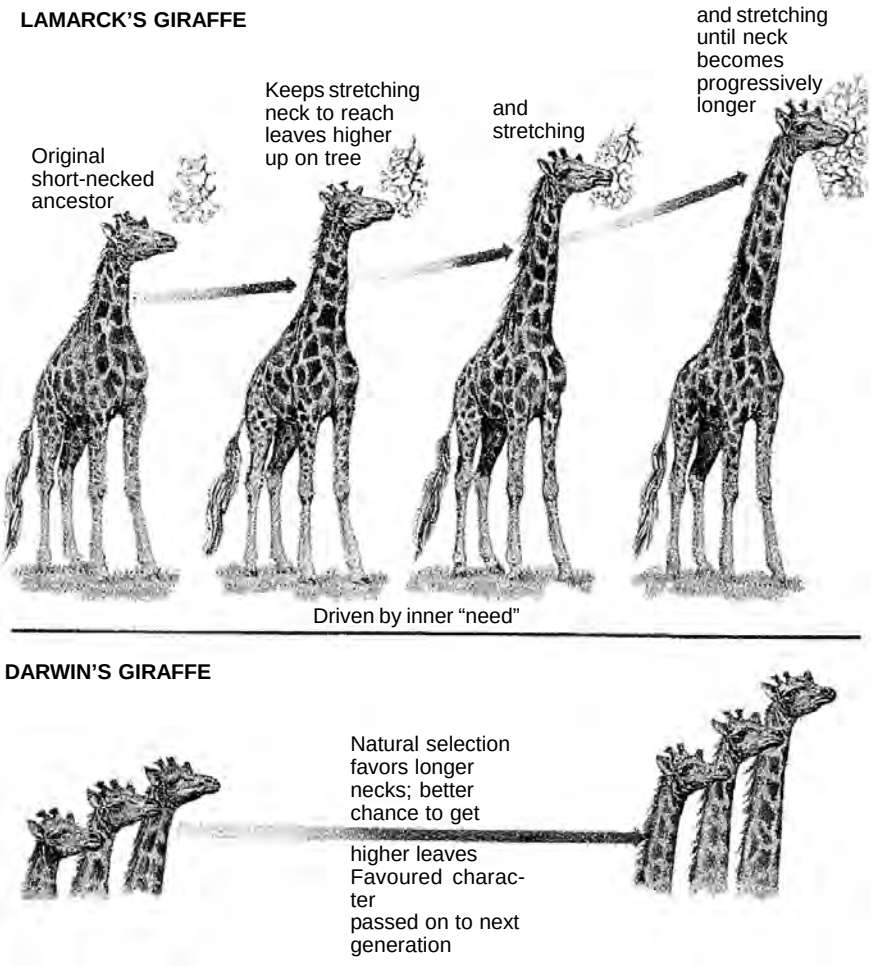


Fig. 8.12 : Diagrammatic comparison between Lamarckism and Darwinism taking giraffe's example

6. Darwin used the expression 'survival of the fittest', which actually means one. But indeed many fit ones survive in the struggle. Therefore, it would have been better if the expression '**survival of the fit**' were used.

7. The **Theory of Pangenesis** advocated by him stating that hereditary particles called pangenes or gemmules are transmitted from parents to offspring was also not accepted.

8.3.4 Lamarckism vs Darwinism :

The fundamental difference between Lamarckism and Dawinism can be diagrammatically explained by considering giraffe as an example (Fig. 8.12).

1. Lamarckism : Lamarck supposed that there were only short-necked giraffes, when the vegetation consisted of grass, herbs and shrubs. There was hardly any tree. These giraffes could eat the foliage with ease. However, there was a

drastic change in the environment and this change had an impact on the flora and fauna. As a result, trees evolved with foliage at a considerable height. Short-necked giraffes kept their necks stretching to reach the foliage of tall trees. The neck was overused and consequently, it became relatively longer than ever before. The longer neck was an acquired modification and this feature passed on through generations.

2. **Darwinism :** Conversely, Darwin supposed that there were short-necked as well as long-necked giraffes at the beginning. The long neck was a beneficial or adaptive character and hence, it was favoured by nature, while short neck was a nonadaptive character. The giraffes, possessing this character were unfit and hence, were eliminated in the struggle for existence. The encouragement of the adaptive character over the nonadaptive one was referred to as natural selection by Darwin.

8.4 MODERN SYNTHETIC THEORY OF ORGANIC EVOLUTION :

Darwin and his contemporaries studied, understood and explained the fundamental mechanism of organic evolution at a time when a very little was known about Mendel's laws of inheritance. Gregor Johann Mendel proposed the laws of inheritance in approximation with that of Darwin's natural selection. The facts, however, remained in obscurity until 1900. The validity of the laws were verified and brought to limelight by Correns, Tschermak and DeVries. The science of genetics expanded and homologous genetic recombination and mutation were known as the causes of genetic variation in organisms. Variation was proposed as the raw material for natural selection to act upon. Homologous recombination was small and continuous, while mutation was large and discontinuous i.e. appeared once in a while.

Later, **Hardy** and **Weinberg** proposed that evolution was a population character and not an individual one. The population remains in a state of genetic equilibrium as long as no external force acts on its gene pool. Stated in another way, "evolutionary changes over successive generations occur by changes in gene frequency of the population". Thus emerged a branch of genetics i.e. Population Genetics. In the midst of new upcomings, Sewall Wright, R. A. Fischer, J. B. S. Haldane, Jullian Huxley, G. L, Stebbins, Ernst Meyer, T. Dobzhansky etc. proposed a synthetic theory of evolution that is also known as the post-Darwinian synthesis. This theory is a collective explanation of the fundamental mechanism of evolution. **Homologous recombination**, **mutation**, **natural selection**, **isolation**, **genetic drift** and **migration** are the bases for the mechanism of evolution.

8.4.1 Genetic Recombinations :

Homologous combinations between genes present on paternal and maternal chromosomes during gametogenesis is known as genetic recombination. These may occur

at three levels: (i) production of **new gene combinations** due to mutual exchange of genes (two different alleles) present on non-sister chromatids of homologous chromosomes by the process **crossing over** during Meiosis I. By this way numerous recombinations can be found in a population. The large number of gametes thus formed at the end of meiosis differ genetically from each other causing variation; (ii) **independent assortment of chromosomes** that occurs during meiosis results in the formation of many genetically different haploid gametes. For example, two pairs of chromosomes can assort in $2^2 = 4$ ways, five pairs in $2^5 = 32$ ways, ten pairs in $2^{10} = 1024$ ways and 23 pairs can assort in $2^{23} = 8.4$ million (8.4×10^6) ways to form sperms or eggs; (iii) random fusion of male and female gametes from two parents during fertilization, i.e. any sperm can fuse with any egg during sexual reproduction, to produce a new individual. Thus, in human, $(8.4 \times 10^6) \times (8.4 \times 10^6) = 70 \times 10^{12}$ combinations can be formed, whereas the total human population is much less than this.

8.4.2 Changes in chromosome number and structure :

Chromosomal mutations, also known as **chromosomal aberrations** arise by changes in the number and structure of chromosomes identifying a species. A change in the number may involve the entire set (euploidy) or one or both chromosomes of a homologous pair (aneuploidy). Strucural changes are classed as deletion (loss of a part of a chromosome), duplication (addition of a chromosomal fragment to an existing chromosome), inversion (reversal of chromosomal fragment) and translocation (mutual exchange of chromosomal fragments between two non-homologous chromosomes).

8.4.3 Gene Mutations :

Hugo de Vries (1901) first coined the term 'mutation' and proposed the '**Mutation Theory**' of evolution based on his work on evening primrose (*Oenothera lamarckiana*). Mutations are sudden, heritable and discontinuous variations which arise due to a change in the genetic material for various reasons. Whenever a change occurs in the chemical structure of a gene, its phenotypic effect is modified and a new character appears. A gene mutation involving only one nucleotide is called **point mutation** and more than one nucleotide or base pair is called **gross mutation**. Such modifications can produce drastic changes, which may be harmful and lethal or can remain insignificant or may have some positive impacts. There is also an equal chance for a gene to mutate back to its normal form. In many cases, the mutant alleles are recessive to their normal alleles and are able to express phenotypically only in homozygous condition. Thus, gene mutations tend to produce discontinuous variations in organisms; as opposed to gradual, small variations of Darwin. Both variations constitute raw materials for evolution. Accumulation of mutations in a population brings about a large scale change in a species in the long run.

8.4.4 Natural selection :

Natural selection is the main driving force of evolution. It acts on variations produced by genetic recombination and mutation. It encourages favourable or beneficial variations and allows

organisms possessing these to reproduce and form the next generation. On the other hand, it discourages harmful or non-adpative variations and eliminates the organisms from the population by not allowing them to reproduce. Thus, it acts on variations as raw materials and decides on whether these will continue through generations or not.

8.4.5 Isolation :

Isolation is the segregation of a population into two sub-populations by a geographical barrier, such that each sub-population influences a different environment. This segregation is termed as **geographical isolation**. Geographically isolated population are known **allopatric populations**. In due course of time each sub-population undergoes changes in accordance with its environmental influence. In a long run, if individuals of both the sub-populations are brought together they fail to reproduce. This is the consequence of the fact that, each sub-population has its own direction of evolutionary changes. This isolation has been termed as **reproductive isolation**. Reproductively isolated population are known as **sympatric populations**.

8.4.6 Random Genetic Drift :

Natural selection has been recognized as the main driving force of evolution in **large randomly breeding populations**. It acts upon variations to encourage favourable gene combinations and eliminate unfavourable ones. This results in the development of a more efficient and adaptive relationship between a population and its environment. However, experiments indicate that selection is not the only force producing changes in populations. In a **small population**, many genetically regulated characters exhibit random variations from population to population without an apparent correlation with the changing environmental factors. Some of these characters have a neutral selective value i.e. these continue to exist despite having no adaptive value, while other characters are non-adaptive and hence, are fixed or eliminated from the population. **Sewall Wright** recognized this evolutionary force as **genetic drift** or more specifically as **random genetic drift**. It simply refers to a random change in the gene frequency i.e. the percentage of the gene, in question, in the population. In his honour genetic drift has been named as **Sewall Wright effect**. This is explained by considering the underlying example.

Two islands are assumed to have similar environment. A heterozygous self-fertilizing plant is placed on each island. The plant is heterozygous for one pair of alleles, A^1 and A^2. The genotype of the plant is A^1A^2. Each plant reproduces to form F1 offsprings. It is further assumed that only one plant survives among these offsprings and this plant reproduces to form the F2 offsprings. Two questions are asked at this juncture. What will be genotype of the surviving F1 offspring and what will be the genotypes of the plants in the subsequent generation on each of the islands ? The genotypes of the parental and F1 generations are outlined as follows.

$$A^1A^2 \times A^1A^2 \longrightarrow \text{(Parental generation)}$$

$$\downarrow$$

$$A^1A^1 \quad 2A^1A^2 \quad A^2A^2 \longrightarrow \text{(F1 generation)}$$

As per the assumption, only one out of the three possible genotypes will survive to continue to the F2. Which out of these three will survive is a pure chance. Now suppose, the genotype that survives on the island 1 is A^1A^1 and that on island 2 A^2A^2. If this random process continues for generations, different genotypes are produced on each of the islands despite having similar environmental conditions. Alternately stated, if the population size is small, there will be a random fluctuation in the gene frequencies due to chance alone. This chance fixation of genotype is known as **genetic drift**.

8.4.7 Migration :

Migration of individuals both into and out of the original population changes its structure and reproductive pattern. Thus, gene pool of the population changes and is subjected to action of natural selection.

8.4.8 Speciation (Origin of new species) :

The consequence of the action of natural selection is the accumulation of invisible adaptive changes in the structure of organisms of a population. Over a long period of time the cumulative structural changes give a visible appearance. Organisms possessing these structures appear different from those of their predecessors and are considered as belonging to a new species.

8.5 MECHANISM OF EVOLUTION :

Evolution is a population character, i.e. it occurs in a population and not in a few individuals of the population. A **population** is an interbreeding group of individuals of one species living in a given geographic area at an instance of time with a collection of genes called the **gene pool.** As changes in the gene pool occur, a population gradually evolves. Speaking in a simple term, evolution is a two-step process: (1) origin of variations and (2) selecting these variations by natural selection.

8.5.1 Variations :

Variations or in a better sence genetic variations contistute raw materials for natural selection to act on. There are two prime causes of origin of genetic variation: (1) genetic recombination and (2) mutation. These have been discussed in details in a preceding section (sections 8.4.1, 8.4.2 & 8.4.3).

8.5.2 Natural Selection :

Natural selection can occur with or without environmental change. Thus, three possibilities are seen in nature :

(i) In a constant environment, natural selection will keep a population stable and essentially maintain an equilibrium.

(ii) In a constant environment, if a new beneficial or adaptive variation arises, it will be encouraged and the species will evolve.

(iii) In a changing environment, natural selection will favour variations that result in a better fitness in the new environment, resulting in adaptation and evolution.

8.5.3 Natural selection in action :

8.5.3.1 Industrial melanism :

That the natural selection has been operating is established by a change in the morphology (pigmentation) of the **peppered moth** (***Biston betularia***) in the Manchester city, at a time, when industrial revolution was picking up. Prior to the onset of the revolution the moths were not pigmented i.e. they were light coloured. In being so, they could not be easily identified and thus could escape their predators. But in 1845, a black coloured moth of the same species was identified in the atmosphere of Manchester city. This event was synchronous with the growing industries in the city. Thick black smoke bellowing from the chimneys made the atmospheric background dark and sooty. As time passed on, more and more black coloured moths were identified and by 1895, 99% of the Manchester population was black coloured. In the present day, there are only a few white coloured individuals. The change from white colur to black colour morphology was induced by the environment and was an adaptive feature. Natural selection eliminated the white phenotypes over a period of time. The change was not just morphological, but occurred at the gene level. There was a change in the gene frequency from the white phenotype to the black phenotype. All present day black moths are homozygous for the black allele. This phenomenon has been termed as **industrial melanism** (Fig. 8.13).

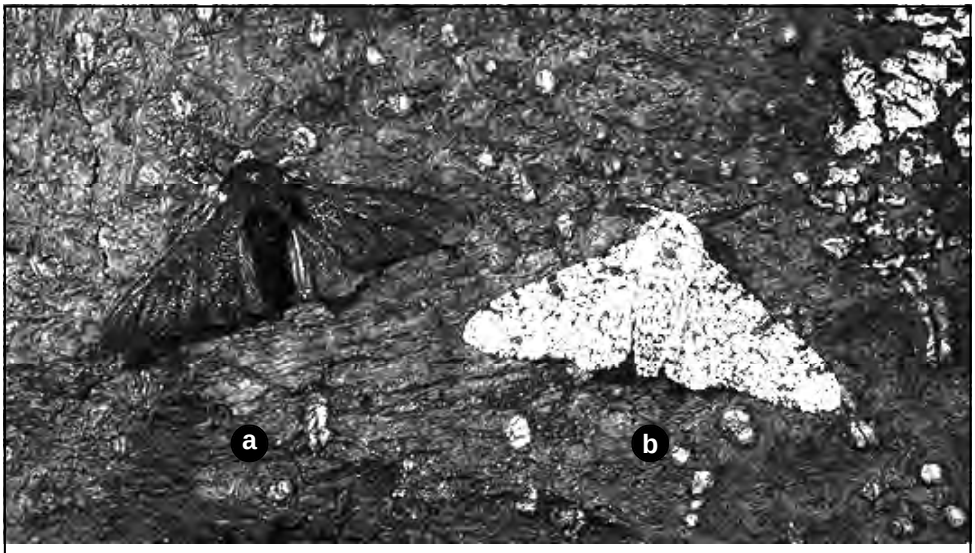


Fig. 8.13 : Peppered Moth, (a) melanistic form and (b) normal white coloured

8.5.3.2 Pesticide resistance by Mosquitoes :

After the introduction of pesticides, such as DDT and malathion, pests like mosquitoes, flies, body lice, etc developed resistance within a few years. Mutant strains with DDT resistance gradually became well established in the population and they replaced the original DDT-sensitive insects.

8.6 TYPES OF NATURAL SELECTION :

Three types of natural selections are observed.

8.6.1 Directional Selection :

It is a type of selection in which individuals of a population are eliminated from the population with non-adaptive variations. The action of natural selection discourages individuals with non-adaptive variations and encourages or favours individuals with adapative or beneficial variations to survive and reproduce. Thus, the action is in one direction. For example, if thicker-shelled oysters are more resistant to breakage than thinner-shelled oysters, crabs will be less able to prey upon them, and thicker-shelled oysters will be more likely to survive to reproduce.

8.6.2 Stabilizing Selection :

This type of selection eliminates individuals from both ends of a phenotypic distribution and hence it maintains the same distribution average. It occurs when natural selection favours individuals with intermediate phenotypic characters expressed by continuous variations. In course of time, such individuals become dominant compared to those with extreme variation. Consequently individuals with extreme variations are eliminated from the population. In the oyster example, very light-coloured or very dark-coloured oysters might be more frequently preyed upon by shore birds, simply because they are more easily noticeable. As a result, the intermediate colours become more frequent.

8.6.3 Disruptive or Diversifying Selection :

In this type of selection, individuals with intermediate phenotopic characters are eliminated from the population and thus, the distribution becomes bimodal. Here natural selection favours both extremes of continuous variation. Over the time, the two extreme variations of the phenotypic character will become more common and the intermediate forms will be less common or lost. Disruptive selection can lead to two new species. In oyster for example, this might happen in shallow water of the rocky bed. Light-coloured oysters are more cryptic (less easy for a predator to see), because they match the rock colour. Dark-coloured oysters blend into the shadows cast by the rocks. In this case, intermediate coloured oysters would be most heavily preyed upon by the crabs, and very light and very dark coloured oysters would survive to reproduce.

8.7 GENE FLOW AND GENETIC DRIFT :

The sumtotal of all genes in a population of a species at a given time is called its **gene pool**. When individuals migrate from one population to another, some alleles of genes move

with them. If the migrating individual of one population is reproductively fit and reproduces with another individual of the other population, there is a transfer of alleles form one to the other. This process of allele transfer from one population to another is called **gene flow**. The gene pool of a small randomly breeding population is small. Hence, random breeding among individuals and migration changes the frequencies of some genes and hence, the structure of the gene pool. In this situation, random genetic drift operates as the main driving force of evolution. Genetic drift and its consequences have been discussed in section 8.4.6.

Two effects caused by random genetic drift are discussed below :

8.7.1 Bottleneck effect :

The Bottleneck effect occurs when there is a dissaster of some sort that reduces the size of a large population to an insignificance. This leaves smaller variation among the small number of surviving individuals, which disables natural selection to operate. In this situation random genetic drift becomes main driving force of evolution.

8.7.2 Founder effect :

The Founder effect occurs when a new colony is started by a few members being separated physically from the original population. Here, reduced genetic variation and changes in the allele frequency are observed. The new population gradually appears distinctly different from the original population in the absence of interbreeding. This drifted population becomes the founder and the effect is called **founder effect**. This was first outlined by Ernst Mayr (1942). In extreme cases, the founder effect is thought to lead to speciation and subsequent evolution of a new species. This effect is easily recognized in case of genetic diseases.

8.8 HARDY-WEINBERG'S PRINCIPLE :

The Hardy–Weinberg principle, also known as the **Hardy–Weinberg law of genetic equilibrium,** proposes that evolution is a **population character** and not an individual based process. It states that allele and genotype frequencies in a population will remain constant from generation to generation, if evolutionary forces, such as mate choice, genetic recombination, mutation, natural selection, genetic drift and migration, don't operate. As one or more of these factors operate on populations, the Hardy–Weinberg principle describes an ideal condition against which the effects of these influences can be analyzed.

Here we consider the simplest case of a single gene locus with two alleles denoted A and a **(dominant and recessive)**, respectively with frequencies $f(A) = p$ and $f(a) = q$, respectively (Table-8.2). The expected genotype frequencies are $f(AA) = p^2$ for the AA homozygotes, $f(aa) = q^2$ for the aa homozygotes, and $f(Aa) = 2pq$ for the heterozygotes. The genotype proportions p^2, $2pq$, and q^2 are called the Hardy–Weinberg proportions. The sum of all genotype frequencies of this case is the binomial expansion of the square of the sum of p and q, and this sum is equal to 1 that represents the total of all possibilities. Thus, $(p + q)^2 = p^2 + 2pq + q^2 = 1$. G H Hardy and W Weinberg first demonstrated it mathematically. If union of gametes to produce

the next generation is random, it can be shown that the new frequency $f2$ satisfies $f2$ (A) = f(A) and $f2$ (a) = f (a). That is, allele frequencies are constant between generations, so equilibrium is reached.

Table : 8.2
Punnett square for Hardy-Weinberg Genetic Equilibrium

		Female	
		A (p)	A (q)
Male	A (p)	AA (p^2)	Aa (pq)
	a (q)	Aa (pq)	aa (q^2)

8.9 ADAPTIVE RADIATION :

It is the diversification of the organisms of a population into a number of new groups with adaptive characters suiting their need for survival. This has been termed as divergent evolution.

As we know that the basic pattern of the pentadactyl limb has undergone adaptive modifications in vertebrates (see section 8.2.2.1), the same adaptive modification rule applies to mammals also. In Fig. 8.14, a typical pentadactyl limb is seen in a **terrestrial mammal**. This

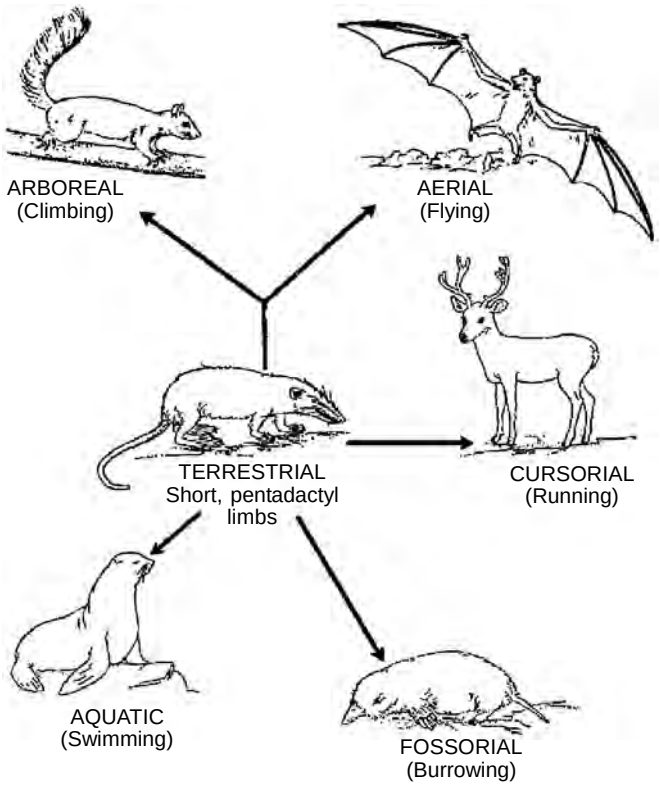


Fig.8.14 : Adaptive radiation in the limb structure of mammals.

pattern has been modified to perform different functions such as running (cursorial), swimming (aquatic), flying (aerial), climbing (arboreal) and burrowing (fussorial). It is, logical to say that all mammals have originated from an ancestral terrestrial mammal through adaptive modifications of the basic pentadactyl limb plan.

8.10 HUMAN EVOLUTION :

Evolution of human, *Homo sapiens*, has occurred through a lengthy process of changes over a long period of time, approximately six million years. Two main trends, such as bipedalism and cranial capacity (brain volume) characterize human evolution (Fig. 8.15 & 8.16). One of the outstanding human traits, bipedalism i.e., the ability to walk on two legs, evolved over 4 million years ago. Other important human characters, such as a large and complex brain, the ability to make and use tools, and the ability for language are more recent. Many advanced traits including complex symbolic expression, art, and elaborate cultural diversity emerged mainly during the past 100,000 years. Human have a very close relationship with another group of primate species, the apes. Scientific evidences show that the physical and behavioural traits possessed by present-day human originated from ape-like ancestors. They first originated in Africa, and the progress of evolution continued there. Then they migrated into Asia and later to Europe and other parts of the world.

Table : 8.3

Evolution of human with age, height and cranial capacity

Stage	Years ago	Height (cm)	Weight (kg)	Cranial capacity (cc)
Homo sapiens sapiens (Modern man)	25,000	150-190	50–100	1300-1800
Homo sapiens neanderthalensis (Neanderthal man)	100,000	150-170	55–70	1200-1900
Homo erectus (Erect man)	1.9 million	165-180	60	850-1100
Homo habilis (The tool Maker)	2.8 million	110-140	33-55	510-660
Australopithecus africanus (First ape man)	5 million	110	40	600

Although scientists have recognize about 15 to 20 different early human species, many of them left no living descendants. The factors that influenced the evolution of *H. sapiens* and extinction of the other species remains a mystery. Two early primate species, ***Dryopithecus*** and ***Ramapithecus***, with hairy body and walking like gorillas and chimpanzees, were surviving

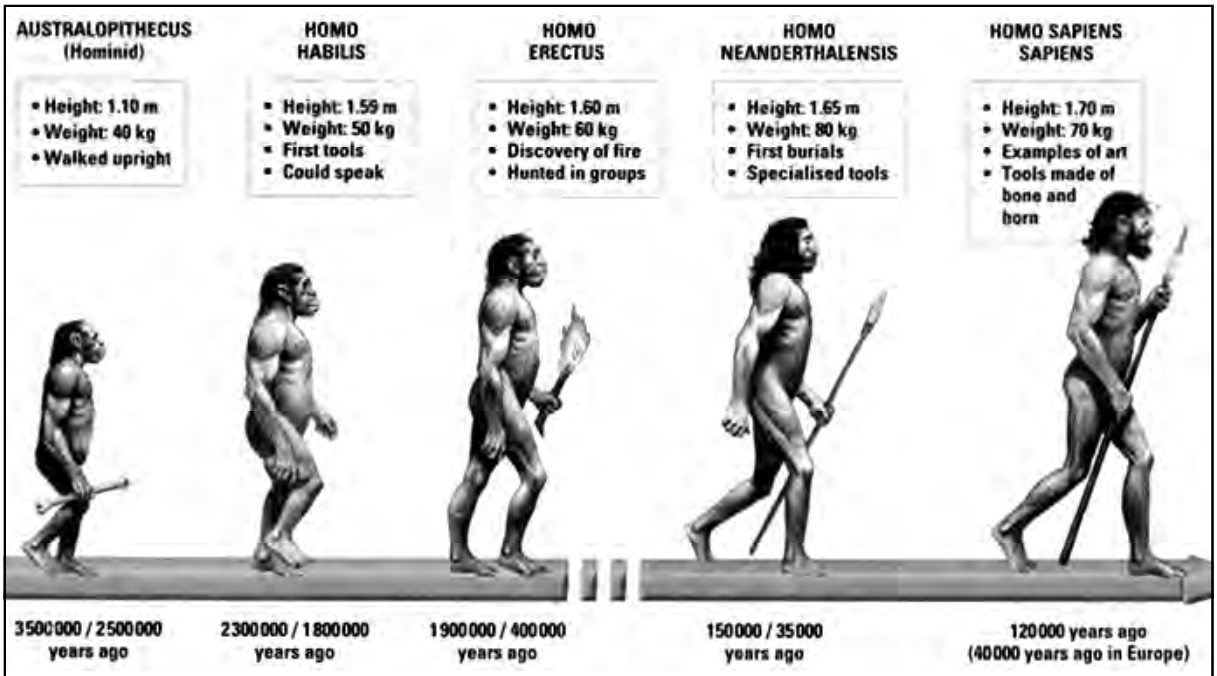


Fig. 8.15 : From *Australopithecus* to present-day human

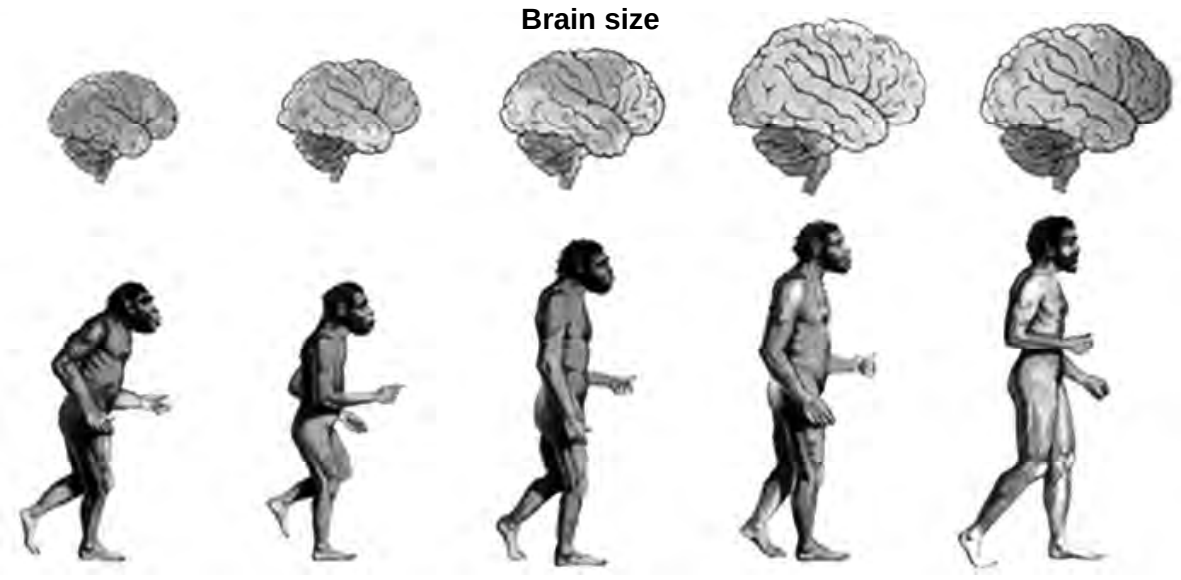


Fig. 8.16 : Gradual increase in cranial capacity in human evolution

some 15-20 million years ago in the **Miocene Epoch**. While the former was more ape-like, the later was more human-like. Fossils recovered from Ethiopia and Tanzania suggest that man-like primates, ***Australopithecus***, lived in East African grasslands about 3 million years ago. Alhough they were fruit eaters, they hunted with stone weapons. ***Homo habilis***, more closer to human, eveloved later who were probably vegetarian in food habit.

Homo erectus, the next stage in human evolution, originated about 1.9 million years ago, as has been revealed by the fossils discovered from Java in 1891. This was the first hominid to have emigrated from Africa to Asia and Europe about 1.8 to 1.3 million years ago. Archaic ***Homo sapiens***, the forerunner of modern human, evolved in the Middle paleolithic period between 400,000 to 250,000 years ago. ***Homo sapiens neanderthalensis***, the Neanderthal man, were living primarily in east and central Asia. They were using hides to protect themselves and burying the bodies when dead. Ultimately, the modern man, ***Homo sapiens sapiens***, evolved during 75,000 to 20,000 years ago in the ice age. The migration of modern human from Africa is estimated to have begun about 70,000 years ago and they subsequently spread globally, replacing earlier hominids through either competition evolution. Evidences of pre-historic cave art by man dates back to 18,000 years ago, and development of agriculture and civilization dates part to about 10,000 years ago. The comprehensive human evolution tree is presented in Fig. 8.17.

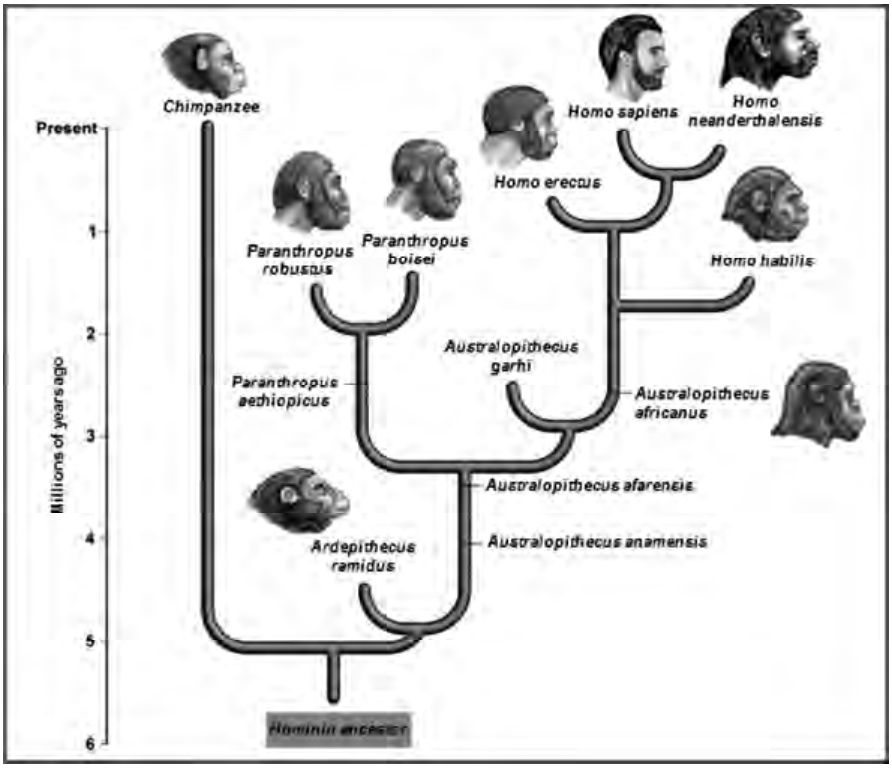


Fig. 8.17 : Human evolution tree

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Multiple choice questions: Choose the correct answer.

(i) Life originated on earth about:

(a) 2.5 billion years ago (c) 4.5 billion years ago

(b) 3.5 billion years ago (d) 5.5 billion years ago

(ii) Which theory proposes the formation of living beings from non-living things?

(a) Theory of Panspermia (c) Theory of Biogenesis

(b) Theory of Abiogenesis (d) Theory of Special Creation

(iii) Who proposed the chemical origin of life?

(a) AI Oparin - JBS Haldane (c) Francesco Redi - JBS Haldane

(b) Louis Pasteur - AI Oparin (d) Spallanzani - Louis Pasteur

(iv) Which of the following compounds Miller-Urey used in the experimental synthesis of amino acids?

(a) CH_4, NH_3, CO_2 and H_2O (c) CH_4, CO_2, H_2 and H_2O

(b) CH_4, NH_3, H_2 and H_2O (d) CH_4, N_2, H_2 and H_2O

(v) Hot ocean water containing concentrated of prebiotic organic compounds was known as:

(a) Colloid (c) Gelatinous mixture

(b) Crystalloid (d) Primordial soup

(vi) Which of the following was formed first?

(a) Virus (c) Coacervate

(b) Prokaryote (d) Eukaryote

(vii) A paper on natural selection and origin of species was presented in the Linnaean Society of London in 1858 by:

(a) Charles Darwin - Robert Malthus

(b) Charles Darwin - Alfred R Wallace

(c) Hugo de Vries - Robert Malthus

(d) Alfred R Wallace - August Weismann

(viii) Analogous organs have:

(a) Different origin and similar function

(b) Similar origin and similar function

(c) Similar origin and different function

(d) Different origin and different function

(ix) Find out the odd match:

(a) Aerial-Flying (c) Cursorial-Running

(b) Fussorial-Burrowing (d) Arboreal-Swimming

(x) One of the following sets of organs constitutes vestigial organs:

(a) Appendix, Coccyx and Plica semilunaris

(b) Appendix, Pectoral girdle and Caecum

(c) Large intestine, Coccyx and Ear muscle

(d) Appendix, Coccyx and Rectum

(xi) What is the correct ascending order:

(a) Mesozoic, Cenozoic and Paleozoic

(b) Cenozoic, Mesozoic and Paleozoic

(c) Paleozoic, Mesozoic and Cenozoic

(d) Paleozoic, Cenozoic and Mesozoic

(xii) Who is known as the 'Father of Modern Palaeontology'?

(a) Leonardo da Vinci (c) Ernst Haeckel

(b) Karl Ernst von Baer (d) Georges Cuvier

(xiii) Find the incorrect match.

(a) Blood group A-Antigen A (c) Blood group O-Antigen A and B

(b) Blood group AB-No antibody (d) Blood group B-Antibody anti-A

(xiv) Which is not a case of chromosomal aberration?

(a) Recombination (c) Inversion

(b) Duplication (d) Translocation

(xv) Which type of natural selection removes individuals from both ends of a phenotypic distribution?

(a) Directional (c) Stabilizing

(b) Disruptive (d) None of these

(xvi) Which is not a great ape?

(a) Gorilla (c) Orangutan

(b) Chimpanzee (d) Macaque

(xvii) What is the correct sequence in human evolution?

(a) *Homo habilis, H erectus, H neanderthalensis, H sapiens*

(b) *Homo erectus, H habilis, H neanderthalensis, H sapiens*

(c) *Homo habilis, H neanderthalensis, H sapiens, H erectus*

(d) *Homo erectus, H neanderthalensis, H habilis, H sapiens*

2. Fill in the blanks with appropriate words.

(i) Organic evolution refers to a change in diversity and _____in populations of organisms.

(ii) The concept of chemical evolution was proposed by J.B.S. Haldane and a Russian scientist, _____.

(iii) Charles Robert Darwin hailed from _____.

(iv) Charles Robert Darwin went on a voyage on board the ship _____.

(v) Jean Baptiste de Lamarck wrote a book, entitled _____, which embodied his theory of inheritance of acquired characters.

(vi) Charles Darwin was inspired by the population theory proposed by _____.

(vii) Darwin's contemporary _____ was studying population diversity in the erstwhile East Indies.

(viii) The mutation theory was proposed by _____.

(ix) All the present day life has originated from a single ancestral form, designated as _____.

(x) A gene mutation involving only one nucleotide is called as _____.

(xi) Abiogenesis of simple organic molecules was experimentally proved by _____ and _____ .

(xii) The theory of inheritance of acquired characters was proposed by _____, who hailed from _____.

(xiii) August Weismann's _____ theory gave a thunder blow to Lamarckism.

(xiv) Charles Darwin studied the diversity of a class of birds, commonly known as _____, in the Galapagos Archipelago.

(xv) The original title of Darwin's book was _____.

(xvi) Natural selection in action was demonstrated by _____ moth.

(xvii) The earliest form of horse was _____ that was living in the plains of North America.

(xviii) The fossil of _____ discovered from the sedimentary rocks of Bavaria, Germany is the missing link between reptiles and birds.

(xix) Digits II and IV persist in modern horse as reduced structures, known as _____ bones.

(xx) Modifications of the basic pentadactyl limb plan in vertebrates to meet their needs is known as _____.

(xxi) The arrangement of different eras, periods and epochs in their ascending order of time constitutes the _____.

(xxii) Peripatus is a connecting link between _____ and _____.

(xxiii) _____ era is known as the era of reptiles.

(xxiv) Sudden reappearance of some ancestral characters in the present organisms is called as _____.

(xxv) The effect of _____ is larger in small populations and smaller in large populations.

3. Answer each of the following in one word or more words, wherever necessary :

(i) The theory that explains that life originated on this planet from non-living chemical constituents.

(ii) The ocean water that contained concentrated amount of prebiotic organic compounds.

(ii) The droplets formed by the separation of high molecular weight organic compounds in a colloidal solution.

(iv) Protenoids, when dissolved in water by boiling and then cooling, organized structures are formed.

(v) Buffon, Erasmus Darwin and Lamarck proposed theories on organic evolution, which had one thing in common.

(vi) Name the naturalist, who proposed that ontogeny recapitulates phylogeny.

(vii) Name the theory, which explains about the origin of amphibians from aquatic fish-like ancestors.

(viii) DNA $\longrightarrow$ RNA $\longrightarrow$ Protein concept.

(ix) Genetic recombination occurs in cell division. Name the cell division.

(x) Hugo de Vries proposed mutation theory on his observations on the morphological features of a plant. Name the plant.

(xi) Breakage, exchange and rejoining of homologous chromosomal segments.

(xii) A single nucleotide substitution in the nucleotide sequence of a gene.

(xiii) The collection of all genes of a population of species.

(xiv) A sudden change in the genetic make-up that ends up in a new expression.

4. Write whether the following statements are 'True' or 'False' :

(i) The primitive atmosphere was reducing.

(ii) Heterotrophic organisms with aerobic respiration evolved prior to anaerobic organisms.

(iii) Continuous genetic variation originates through mutation.

(iv) Serum proteins of closely related animals are similar in their amino acid sequences to a greater extent.

(v) Reptiles flourished in the Paleozoic era.

(vi) Close similarity in the nucleotide sequence between two organisms depicts close relationship between them.

(vii) Numerical changes, involving one or both chromosomes of a homologous pair, are known as euploidy.

(viii) Genetic drift is the main driving force of evolution in a large randomly breeding population.

(ix) Discontinuous variation is the product of mutation.

GROUP - B
(Short Answer-type Questions)

1. Answer each within 50 words.

(i) Explain the theory of spontaneous generation.

(ii) What do you mean by chemical evolution?

(iii) Describe Miller-Urey experiment.

(iv) Explain prebiotic or primordial soup.

(v) What is prodigality of reproduction? Give an example.

(vi) Write three criticisms on Darwinism.

(vii) Explain how homologous organs reflect organic evolution.

(viii) Describe homology in early embryonic development.

(ix) Explain the theory of recapitulation.

(x) How do fossils support organic evolution?

(xi) Why do you call *Archaeopteryx* as a connecting link between reptiles and birds?

(xii) What do you mean by a geological time scale?

(xiii) Explain serological test.

(xiv) What is industrial melanism?

(xv) Explain genetic drift.

(xvi) What is speciation?

(xvii) What is bottleneck effect?

(xviii) What is Hardy-Weinberg's Principle?

(xix) What is adaptive radiation?

2. Differentiate between :

(i) Abiogenesis and Biogenesis

(ii) Chemoautotrophs and Photoautotrophs

(iii) Chemical evolution and Biological evolution

(iv) Homologous organs and Analogous organs

(v) Moulds and Casts

(vi) Genetic recombination and Mutation

(vii) Somatic variation and Germinal variation

(viii) Chromosomal aberration and Gene mutation

(ix) Euploidy and Aneuploidy

(x) Natural selection and Genetic drift

(xi) Convergent evolution and Divergent evolution

GROUP - C
(Long Answer-type Questions)

1. Give an account of the chemical basis of origin of life.
2. Discuss the evidences of organic evolution from comparative anatomy and morphology.
5. Give an account of the embryological evidences of organic evolution.
6. Describe palaeontological evidences of organic evolution.
7. Describe Darwin's theory of natural selection and origin of species and discuss about the criticisms.
8. Discuss about the synthetic theory of organic evolution.

❑ ❑ ❑

UNIT - III : BIOLOGY AND HUMAN WELFARE

CHAPTER 9

HEALTH AND DISEASE

There is proverbial saying that health is wealth. It is more precious than money or any material beloging. A good health is maintened by balanced diet, good routine habit, a sense of good hygine, physical exercise and mental wellbeing. However, the state of good health is disturbed by several agents. One of these is pathogenic or disease causing organisms or simply pathogens. These organisms enter into the body and interfere with and destabilize the normal physiological functions of the body. Consequently, the subject falls ill and expresses several symptoms. In the event of this, a correct diognosis and treatment becomes essential to get rid of the pathogens from the body and restore the physiological state of equilibrium.

9.1 PATHOGENS :

A pathogen is an infectious agent that causes a disease or illness to its host. It disrupts the normal physiology of organisms, either plants or animals and expresses a number of symptoms. The human body contains many natural defence mechanisms against some common pathogens. Some pathogens have been found to be responsible for massive casualties. Despite many medical advances for safeguarding human beings from infections by pathogens through the use of vaccines, antibiotics and fungicides, pathogens continue to threaten human lives.

Typically, the term pathogen is used to describe infectious agents such as viruses, bacteria, fungi, prion and parasites of various forms. A pathogen may be described in terms of its ability to produce toxins, enter tissues, colonize and share nutrients and its ability to induce immunosuppression in the host. Major classes of pathogenes are described below :

Viruses - adenovirus, picorna virus, retro virus, papovavirus,polyoma virus etc.

Bacteria - mycobacterium, streptococcus, shigella and salmonella.

Fungi - saprophytic pathogenic fungi.

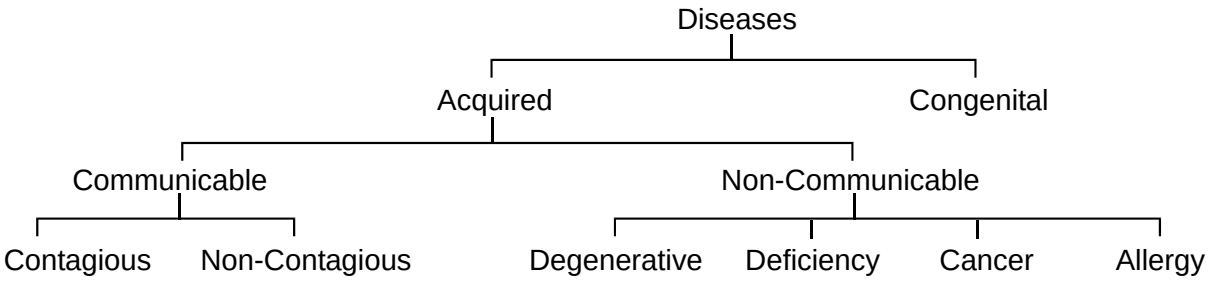
Prions - protein pathogens that do not contain nucleic acids.

Parasites - protozoon parasites and helminth parasites.

Pathogenicity is the disease causing property of pathogens. This property is also known as virulence.

9.2 PARASITES CAUSING HUMAN DISEASES :

Diseases - A disease is defined as the condition of the body or a part of the body in which normal body functions are disrupted leading to abnormal functions in an organ or system. Diseases can be classified as below :



1. **Acquired diseases -** Acquired diseases occur only after birth. These may be either communicable or non-communicable.

   (a) **Communicable diseases -** These are infectious and spread from infected persons to healthy persons through pathogens. Communicable diseases may be contagious or non-contagious. Contagious diseases are transmitted through contact. e.g. syphilis, chicken pox, measles and leprosy. Non-contagious diseases are transmitted through agencies like water, air**,** food and vector organisms.

   (b) **Non communicable diseases -** It doesn't spread from person to person. These are of four types:

      (i) **Degenerative diseases -** These occur due to degenerative changes in some vital organs. (e.g., Cardiovascular diseases, Brain diseases, arthritis etc.).

      (ii) **Deficiency diseases -** These are caused due to deficiencies in food or hormone. [e.g., Kwashiorkor (Protein deficiency), Pellagra (Vitamin-B5 deficiency), Goitre (Iodine deficiency), Diabetes mellitus (Insulin deficiency)].

      (iii) **Cancer -** These are caused by several physical and chemical agents, collectively known as cancer causing agnets or carcinogens.

      (iv) **Allergy -** These are caused by several allergens (foreign substances). (e.g., asthma, hay fever).

2. **Congenital diseases -** These are inherited genetic disorders. (e.g., colour blindness, Down's syndrome and haemophilia).

9.2.1 Typhoid :

Typhoid fever (enteric fever) is a life-threatening disease caused by the bacterium, *Salmonella typhii*. This fever is contracted by the ingestion of the bacteria-contaminated food or

water. Typhoid fever is still common in the developing world, where it affects about 21.5 million persons every year (1 million in India). Patients with acute typhoid fever may contaminate the surrounding water through defaecation. The bacterium multiplies in the gall bladder, bile ducts and liver and passes into the bowel. The bacteria can survive for weeks in water or dried sewage.

9.2.1.1 Symptoms :

The incubation period is usually 1 to 2 weeks and the duration of illness is about 4 to 6 weeks. The patient experiences :

- Poor appetite
- Abdominal pain
- Headache
- Generalised ache and pain
- Lethargy
- Intestinal bleeding or perforation after two to three weeks of infection
- Diarrhoea or constipation

People with typhoid fever usually have a sustained fever as high as 103^0F – 104^0F. Rose spots on the abdomen of a person with typhoid fever may appear. Chest congestion develops in many patients, and abdominal pain and discomfort are common. Improvement occurs in the third or fourth week in those without complications. Around 10% of the patients have recurrence (relapse) after feeling better for one to two weeks. Relapses are actually more common in individuals treated with antibiotics. Typhoid is diagnosed by **WIDAL TEST**.

9.2.1.2 Infection and Transmission :

After the ingestion of the contaminated food or water, the *Salmonella* bacteria invade the small intestine and enter the blood stream temporarily. *Salmonella typhii* lives only in the human host. The bacteria are then carried by the white blood cells to the liver, spleen and bone marrow. They multiply in the cells of these organs and reenter the bloodstream. Following this, the patient develops symptoms, including fever. They invade the gallbladder, biliary system and the lymphatic tissues of the bowel. Here they multiply in a high number. The bacteria pass into the intestinal tract and can be identified in cultures of the stool.

A number of persons recover from typhoid fever but continue to carry the bacteria. They are known as carriers.

Transmission takes place by eating contaminated food or drinking contaminated water and beverages. Therefore, typhoid fever is more prevalent in areas of the world, where sanitation is poor.

9.2.1.3 Treatment :

Typhoid fever is treated with antibiotics. Resistance to multiple antibiotics is gradually increasing in *Salmonella*. Reduced susceptibility to fluroquinolones (e.g. ciprofloxacin) and the emergence of multi-drug resistance has complicated the treatment procedure, especially in those who have acquired infection from South Asia. Antibiotic susceptibility testing may help in deciding an appropriate therapy. The choice of antibiotic therapy includes fluroquinolones for susceptible infections, ceftriaxone and azythromycin for established infections. Persons who donot get treatment may continue to have fever for weeks or months and as many as 20% may die from complications of the infection.

9.2.1.4 Prevention and control :

There are several ways one can practice to avoid typhoid bacteria contraction.

- Avoid apparently contaminated food and drink.
- Get vaccinated against typhoid fever.
- Drink packaged water with statutory quality marking on the bottle or boiled water brought to room temperature. Bottled carbonated water is proved to be safer than uncarbonated water.
- Ask for drinks without ice unless the ice is made from bottled or boiled water. Avoid popsicles and flavoured ice that may have been made with contaminated water.
- Eat food that has been thoroughly cooked and that is still hot and steaming.
- Avoid raw vegetables and fruits. These may be washed properly and then peeled.
- Avoid food and beverages from street vendors. It is difficult to keep the food clean alongside the street and many travellers get sick from food bought from the street vendors.

Typhoid fever vaccines have been successfully developed and commercialized. There are two types of vaccines :

1. **Vi antigen vaccine -** It is an inactivated vaccine available in injectable form. Vi antigen vaccine is given in a dose of 0.5 ml. intramuscularly either on thigh or arm. It is given as a single dose.
2. **Oral ty21a vaccine -** This is an oral live vaccine. It is a course of three capsules given orally on alternate days. The capsule should be swallowed intact and not opened or chewed. In most, the pack contains four capsules and these capsules are given on alternate days. Liquid form of oral ty21a vaccine in sachet form is more effective than the capsule form. But the sachet form is not available in India.

The protective efficacy lasts for 2-3 years in most of the vaccines. For a long-term protection one has to revaccinate every 3 years.

9.2.2 Pneumonia :

Pneumonia is a lung infection accompanied by cough, fever and difficulty in breathing. Following infection, there may be inflammation in the air sacs and fluid may accumulate. For most people, pneumonia can be treated at home. It often clears up in 2 to 3 weeks. But older adults, babies and people with other diseases may become seriously ill. They may need intensive care in the hospital. More than 10 million cases have been documented every year in India.

Pneumonia may be contracted in the daily life, such as at school or work. This is called community-associated pneumonia. The desease may also be contracted, while in a hospital or nursing home. This is called healthcare-associated pneumonia.

9.2.2.1 Causes of Pneumonia :

Pathogens like bacteria and viruses usually cause pneumonia. It usually starts when someone breaths the pathogens into the lungs. One may be more likely to be infected following a cold or flu. These illnesses make it difficult for the lungs to fight infection, so it is easier to contract pneumonia. Having a long-term or chronic, disease like asthma, heart disease, cancer or diabetes also makes one likely to get pneumonia.

9.2.2.2 Symptoms :

1. Cough - One is likely to secrete much mucus (sputum) from the lungs. Accumulating mucus causes irritation leading to severe coughing. Mucus may be rusty or green or tinged with blood.
2. Fever.
3. Fast breathing and feeling of breathlessness.
4. Severing as though there is a chilled ambience.
5. Chest pain that often feels worse when one coughs or breaths.
6. Fast heart beat
7. Feeling extremely tired and weak
8. Nausea and vomiting
9. Diarrhoea

9.2.2.3 Diagnosis :

Primarily, a physical examination is conducted. If necessary, a patient undergo chest **x-ray** and a **blood test**. This is sufficient to diagnose pneumonia. In an extreme case, the mucus from the lungs may be pathologically examined to findout if causative pathogens are present.

9.2.2.4 Treatment :

If pneumonia is diagnosed to be caused by bacteria, antibiotics are prescribed. These almost always cure pneumonia caused by bacteria. One needs to take the full course of the prescribed antibiotics. Plenty of rest, sleep and intake of rehydration drink are required. Smoking is totally prohibited.

Pneumonia, caused by a virus usually is not treated with antibiotics. Sometimes antibiotics may be used to prevent complications. But more often rest and treating cough with conventional medicines work.

9.2.2.5 Prevention :

People of 65 years of age or more, having smoking habit and with cardiovascular and lung problems need to have a pneumococcal vaccine. The vaccine does not keep pneumonia away. However, if pneumonia occurs, complications may not occur.

9.2.3 Common Cold / Rhinitis :

It is one of the most common infectious diseases of human, which is caused by some 200 types of Rhino viruses and a small bacterium, *Dialister pneumosintes*. The pathogens donot reach the lungs. They infect nose and upper respiratory passage causing inflamation of mucus membranes. There is inflammation of the nasal tract, nasal congestion, flow of mucus, sneezing, sore throat, hoarseness, cough, tireness, headache and slow fever. Some persons also suffer from allergic rhinitis. Common cold spreads through oozing droplets from talking and sneezing, direct contact, hand shake and using common articles like pen, pencil, books, cups, door handles, computer key boards, computer mouse etc. It cures automatically after 3-7 days. Medicines are taken to reduce severity of nasal irritation and clearing the nasal tract.

9.2.4 Malaria :

Millions of people die of malaria every year, especially in tropical and subtropical regions. In india alone, it annually costs about half a million lives. Malaria is caused by a protozoon parasite, *Plasmodium*, which is transmitted by a vector; female anopheles mosquito. The parasite was first discovered by Charles Laveran (1880). Sir Ronald Ross, a doctor in the India army, first observed oocysts of *Plasmodium* in female Anopheles.

9.2.4.1 Symptoms :

Clinical symptoms of the disease are chill, fever with period of latency, enlargment of spleen and secondary anaemia. A typical attack of malaria comprises three successive stages.

(a) Cold stage or Rigor stage : The fever comes with rigor and sensation of extreme cold, which lasts from 15 minutes to an hour.

(b) Hot stage or Febrile stage : The temperature of the body increases to 106° F, which lasts for about 2 to 6 hours, associated with intense headache.

(c) Sweating stage or Defervescent stage : Fever comes down with profuse sweating, which lasts for 2 to 4 hours.

Malaria in man is caused by four different species of malaria parasite. They are *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale*. *P.vivax* has the widest geographic distribution in India. 70% of the total infection is due to *P. vivax*, 20-30% due to *P.falciparum* and only 4.8% due to mixed infection. *P.ovale is* a very rare in human population and mostly confined to tropical Africa.

9.2.4.2 Life Cycle :

Malarial parasite is a digenetic parasite and has two cycles of development. Its asexual cycle takes place in human, which is the primary host and sexual cycle takes place in the intermediate host, female anopheles mosquito.

Human Cycle : The infective stage of malarial parasite is **sporozoite**, which is injected into the body of a healthy person by the bite of an infected mosquito. The sporozoites disappear within 60 minutes from the peripheral circulation and reach the liver cells.

After one to two weeks of development they form **hepatic schizonts** in the liver cells and then burst to produce numerous **merozoites**. The process continues for many cycles and a stage comes when the parasites enter the R.B.C. The cycles in which liver schizogony takes place are referred to as **pre**-and **exo-erythrocytic schizogony**.

The next cycle is **erythrocytic schizogony**, where the merozoites enter the R.B.C and pass through the stages of **trophozoite** and **schizont.** Trophozoite feeds on haemoglobin. The byproduct from the feeding process is a toxic substance, known as **hemozoin**. After every cycle of erythrocytic schizogony, hemozoin concentration in the blood increases. Erythrocytic phase ends in liberation of merozoites which infect fresh R.B.C. R.B.C. merozoites and hermozoin granules are liberated into the blood plasma. Increased concentration of hemozoin granules causes severing fever. The cycle is repeated for many times and a stage comes when the parasite develops in the R.B.C, but does not divide. These are called as **male and female gametocytes.** At this stage there is no further development of the parasite as it needs a cold blooded animal, i.e., female anopheles for further development.

Mosquito cycle : The mosquito cycle begins while the gametocytes are ingested by the vector mosquito when sucking blood from the infected person. In the stomach of the vector, the male gametocyte undergoes a process of **exflagellation** forming 4-8 threadlike **microgametes.** The female gametocyte undergoes the process of maturation and becomes female gamete or **macrogamete**. Fusion of male and female gametes produce the zygote which becomes active within 18-24 hours and is called an **ookinete.** This cycle is referred to as **gamogony**. The ookinete penetrates through the stomach wall and encysts as an **oocyst**.

Then starts the **sporogony cycle**, where the oocyst grows and divides to form numerous sporozoites which are liberated into the haemocoel or body cavity of the mosquito. From here, the sporozoites migrate into the salivary glands of the vector making the vector infected. Such an infected mosquito, when bites a healthy person, transfers the sporozoites into the blood along with saliva. Thus, the lifecycle completes in two hosts.

9.2.4.3 Diagnosis :

- Malaria can be diagnosed by a rapid diagnostic test (RDT). This test is used in the field by ASHA workers and primary health centers, especially in remote areas.
- Malaria can also be diagnosed by a laboratory blood test. It involves examining a drop of patient's blood under a microscope for ascertaining the presence of the parasite.

9.2.4.4 Treatment :

The following drugs are recommended for treating malaria fever.

1. Chloroquin
2. Primaquin
3. Atovaquon - Proguanil combination
4. Artemether - Lumefantrine combination
5. Mefloquine
6. Quinine
7. Quinidine
8. Doxycycline in combination with quinine
9. Clindamycin in combination with quinine
10. WHO reccomends Artemisinin - based combination therapies (ACTs)

A COMPARISON OF HUMAN INFECTING SPECIES OF PLASMODIUM

Parasite	Types of Malaria	Incubation Period	Recurrence of fever
P. vivax	Benign tertian	10 days	48 hours
P. malariae	Quartan	28 days	72 hours
P. ovale	Mild tertian	15 days	48 hours
P. falciparum	Pernicious, cerebral subtertian, aestivo autumnal or Tropical malaria, Black water fever.	12 days	48 hours

9.2.4.5 Prevention and control of Malaria :

In 1979, the WHO Expert Committee on Malaria summarised antimalaria measures, as outlined below.

(a) Use of mosquito repellents, bed-nets and screening of houses;

(b) Use of domestic space spray including aerosol;

(c) Destruction of mosquito larvae by larvicides or by introducing larvivorous fishes like *Gambusia*;

(d) Filling of small scale drainage and other forms of water management;

(e) **Chemoprophylaxis** or taking little dose of quinine in malaria prone area and chemotherapy by taking medicines like Quinine, Paluidrine, Camoquin, Resochin, Mepacrine, Lavagnin, Daraprim etc.

In April, 1953 National Malaria Control Programme (NMCP) was launched, which was changed to National Malaria Eradication Programme (NMEP) later in 1958.

9.2.5 Filariasis :

It is a disease causd by a digenetic nematode parasite *Wuchereria bancrofti* which is transmitted to human by mosquitoes like Culex, Aedes or Anopheles. The disease causes lymphoedema (lymphatic obstruction of lymphatic vessels and glands), lymphadenitis (infection of lymph nodes), lymphangitis (infection of lymph vessels), elephantiasis (enormous enlargement of scrotom, feet, hands, legs, etc.)

The life cylce is completed in two hosts. The primary host is human and the secondary or intermediate host is a female mosquito (usually *Culex pipiens*). There is a distinct sexual dimorphism in the parasite. The male worm measures 25 to 40 mm in length and 0.1 mm diameter having a curved tail. The female worm measures 80-100 mm in length and 0.2 to 0.3 mm in diameter. The female is viviparous and gives birth to as many 50,000 microfilariae per day. The microfilariae find their way into the blood stream where they live upto 70 days without any developmental changes. Due to their nocturnal periodicity they are sucked up by the secondary host, which is prevalent in night hours. The life cycle completes in 10 to 14 days through the following stages.

(a) Exsheathing : The micro-filariae comes out of the sheath within 1-2 hours of ingestion in the stomach of the mosquito.

(b) First stage larva : After exsheathment, the larva penetrates the stomach wall of the mosquito in 6-12 hours and migrates into the thoracic muscles where it grows and develops into a sausage-shaped form.

(c) Second stage larva : The larva grows in size and develops in the alimentary canal but remains inactive.

(d) Third stage larva : In this stage, the larva becomes active and infective. It migrates to the proboscis of the mosquito and is ready to be transmitted to a new host. In the human host, the infective stage larvae grow into the adult males and females.

9.2.5.1 Treatment :

Detection and treatment of human carrier : The present strategy is to detect and treat the human carriers by the use of drugs like diethyl carbamazine (DEC) in 12 doses at the rate of 6 mg per kg body-weight daily to be completed in two weeks.

9.2.5.2 Prevention and control :

Antimosquito Measures : The main objective of this method is the elimination of breeding places of the vectors. The vector controlling methods are:

(i) Recurrent anti larval measures in endemic urban areas;

(ii) Use of larvicides like pyrosene oil;

(iii) Removal of aquatic plants like pistia:

(iv) Destruction of derelict water bodies and swamps;

(v) Anti-adult vector measures like spraying pyrethrins;

(vi) Avoidance of mosquito bite by using mosquito net; and

(vii) Using larvivorous fishes like Gambusia.

The National Filarial Control Programme was initiated in 1955-56, but since 1978 the programme has been merged with Malaria Control Programme.

9.2.6 Amoebiasis :

The term amoebiasis is a condition of harbouring *Entamoeba histolytica* with or without clinical manifestations. Amoebiasis may be intestinal or extra-intestinal. The intestinal amoebiasis manifests symptoms of **amoebic dysentery**, non-dysenteric colitis, amoeboma and amoebic appendicitis bringing about many complications like intestinal perforation, peritonitis and haemorrhage. The extra-intestinal amoebiasis occurs in the liver, lungs, brain, spleen and skin etc. The more common among these is hepatic amoebiasis. Amoebiasis is estimated to affect 10% of the world's population and 15% of Indian population.

The causative agent, *E. histolytica* is a lumen-dwelling protozoon parasite and exists in two forms : (i) **trophozoite** or **magna** or **feeding stage** (ii) **Cystic** or **minuta** or **infective**

stage. The cysts, which are the infective forms, are resistant to marked changes in the external environment. *E. histolytica* is a monogenetic parasite and its only host is human. Its feeding stage; the trophozoite is monopodial, i.e., with one pseudopodium and no contractile vacuole. It has two forms: the pathogenic **magna** form and nonpathogenic **minuta** form. Magna form affects the mucosa and submucosa causing ulcers. The minuta form is found in the lumen of the intestine and forms the tetra-nucleate encysted mature cyst. The mature cyst bears two chromatoid bodies and four nuclei. This is the infective state of *Entamoeba* and is released from the body of the host through faecal matter. The source of infection is the faecal matter which contains the cysts of *E. histolytica*. The primary causes of prevalence of amoebiasis in India are defaecation in the open air leading to contamination of the soil, lack of pure drinking water supply and low standard of living.

9.2.6.1 Transmission :

Transmission of amoebasis is mainly by the oral route. It is due to intake of cyst contaminated water and food. The infection may also be caused by flies, cockroaches and rodents which carry the cysts and contaminate food and drink. The incubation period is 3 to 4 weeks but shorter in massive infection.

9.2.6.2 Symptoms :

Amoebasis causes 6-10 loose motions per day with blood stained mucous. Loose motion may be altered by constipation and massive infection leads to ulceration of the gut, liver, lungs and brain.

9.2.6.3 Treatment :

There is no single drug which can eliminate all stages of *E. histolytica*. Infections usually can be treated effectively by oral dose of Metronidazole at the rate of 400-800 mg t.d.s. for ten days. The other drugs are Mexaform, Enteroquinol, Diodoguin, Tinidazole, Enterovioform and Tinidafylplus.

9.2.6.4 Prevention and control :

Primarily, prevention is aimed at discouraging defaecation in open air, which leads to contamination of water, food, vegetables and fruits. Water filtration and boiling are effective measures against amoebiasis. Vegetables should be properly washed before use. Safe disposal of human excreta is crucial to controlling amoibiasis. Educating ignorant people with personal hygiene and proper toilet habits also proves fruitful.

Secondary prevention aims at early diagnosis of the disease. Intestinal amoebasis is diagnosed by examining the faces of the patient to identify the trophozoites and cysts. Extra intestinal amoebiasis is diagnosed by the serological tests, counter immuno-electrophoresis (CIE) and ELISA.

9.2.7 Ascariasis :

It is the infection of the intestinal tract by the adult nematode parasite, *Ascaris, lumbricoides*. Adult female worms can grow over 12 inches in length, while adult males are smaller.

Ascariasis is the most common human nematode parasite. Infection occurs world wide and is most common in tropical and subtropical areas where sanitation and hygiene are poor. Children are infected more often than adults. In our country, infection is more common in rural areas of the south-eastern part.

9.2.7.1 Symptoms :

Most people infected with ascaris have no symptoms. Moderate to heavy infections express symptoms that may vary depending on which part of the body is infected.

In the lungs : After the ingestion of ascaris eggs, they hatch in the small intestine and the larvae migrate through the blood or lymph into the lungs. At this stage, symptoms similar to asthma or pneumonia with persistent cough, shortness of breath and wheezing are expressed.

After spending 6 to 10 days in the lungs, the larvae travel to the throat, where these are coughed up and swallowed.

In the intestine : The larvae mature into adult worms in the small intestine, where they live until they die. In mild or moderate ascariasis, there is mild abdominal pain, nausea and vomitting and diarrhoea or bloody stool.

If the person has a heavy infection, a large number of worms may be present and may cause severe abdominal pain, fatigue, vomitting and weight loss.

9.2.7.2 Epidemiology :

Ascaris lives in the lumen of small intestine. Around 2,40,000 fertilized eggs are laid by the female per day and are passed out with the faecal matter of the host. In the external environment they become embryonated. The first larval stage is called **rhabditiform** larva, which undergoes first moulting to produce second larval stage within 2-3 weeks and this is the infective stage of *Ascaris*. When human ingests these embryonated eggs they hatch in the intestine. They larvae penetrate the gut wall and are carried to the liver. From liver they invade lungs through the blood supply. They are coughed up through the trachea and then swallowed to reach the intestine. They take about 60-80 days to become mature adults. Each adult has a lifespan of 6-12 months. Copulation takes place in the intestine of the host. Fertilization is internal i.e. in the oviduct of the female.

Ascaris is a soil transmitted helminth and its eggs remain viable for months and years. Contamination of the soil by *Ascaris* eggs is due to human habit of open air defaecation.

9.2.7.3 Diagnosis :

Infection with *Ascaris* is confirmed by a pathological examination of the stool. Sometimes a whole worm is passed in the stool or is coughed up. If this happens, it is identified by the physician.

9.2.7.4 Treatment :

Effective drugs like Piperazine, Mebendazole, Levamisole, Pyrantel, and oil of Chinopodium are administered. Mass treatment of periodic deworming at intervals of 2-3 months may be undertaken in areas, where hygin is poor and protein malnutrition is prevalent. Sanitation improvement and treatment go hand in hand to eliminate ascariasis.

9.2.7.5 Prevention and Control :

- Avoid contact with the soil that may have been contaminated with human faeces.
- Donot defaecate outdoors
- Dispose off diapers properly
- Wash hands with soap and water before handling food
- When travelling to countries where sanitation and hygiene are poor, avoid water or food that may be contaminated
- Wash, peel off or cook all raw vegetables and fruits before eating.

9.2.8 Ringworm :

Ringworms are pathogenic microscopic fungi called **dermatophytes**. They cause superficial skin infections, also known as **tinea**. They live and grow on parts of the skin, hair and nails, much like a mushroom grows on the bark of a tree.

Ringworm infection is characterised by a red ring of small blisters or a red ring of scaly skin that grows outword as the infection spreads. Although children are more susceptible to catching ringworm, it can infect adults as well.

9.2.8.1 Symptoms :

The following are the types of ringworms or tinea :

1. Tinea barbae : Ringworm of the bearded area of the face and neck, with swelling and marked crusting.
2. Tinea capitis : Ringworm of the scalp, commonly affects children, mostly in late childhood or adolescence.
3. Tinea corporis : Rignworm of the general skin of the body. It often produces round spots of classic ringworms. Sometimes, these spots have an active outer border as they slowly grow and advance.

4. Tinea cruris : Ringworm of the groin tends to have a reddish-brown colour and extends from the folds of the groin down onto one or both thighs.
5. Tinea faciei : Ringworm on the face except in the area of the beard. It causes red, scaly patches with indistinct edges.
6. Tinea manus : Ringworm involving the hands particularly the palms and the spaces between the fingers.
7. Tinea pedis : Ringworm in the athlete's feet may cause scaling and inflamation in the toe webs.
8. Tinea unguium : Fungal infection of the fingernails and more often, the toe nails.

9.2.8.2 Treatment :

Home remedies cannot cure ringworms, it is necessary to apply antifungal medications. Ringworm can be treated topically with extenal applications or systemically with oral medications.

Topical treatment : When the fungus affects the skin of the body or the groin, antifungal creams can clean the condition in around two weeks. Examples of such preparations include those that contain Clotrimazole [e.g., Cruex, Desenex and Lotrimin (cream and lotion)], Miconazole (e.g., Monistat cream), Ketoconazole (e.g., Nizoral cream), Econazole (Spectazole), Naftifine (Naftin) and Terbinafine (Lamisil cream and solution). These treatments are effective for many cases of foot fungus as well. It is usually necessary to use topical medications for at least two weeks.

Systemic treatment : Some fungal infections do not respond well to external applications (e.g., scalp fungus of the nails). Oral medications are essential for penetrating into depper areas of injection.

9.3 IMMUNITY :

In simplest term, immunity is defined as the response of the body to infections. It refers to the sum total reactions expressed by an organism to inhibit, inactivate or destroy the invading microorganisms and their toxic products or other foreign substances that enter into the body. Immunity is broadly classified into two types, namely (i) **innate (inborn or non-specific** or **non-adaptive) immunity** and (ii) **acquired (specific or adaptive) immunity** (Fig. 9.1).

9.3.1 Innate immunity :

The defense mechanism is active right from the time, a child is born (hence, innate or inborn). The specificity of innate immunity is low as it lacks the ability to distinguish one microbe from another. Hence, it is also known as non-specific immunity. As this immune response is naturally gifted since birth, it is also called as **natural immunity**.

Innate immunity provides the early lines of defense against pathogens, The principal components of innate immunity are:

(a) Mechanical barriers

(b) Chemical barriers

(c) Phagocytosis

(d) Fever

(e) Inflammation

(f) Acute-phase proteins

(g) Natural killer cells (NK cells)

(a) Mechanical barriers - A mechanical or physical barrier refers to various barriers blocking the entry of the microbes into the host body. This is the **first line of defense** which includes skin and mucous membranes.

Skin - The outer layer of epidermis, coated with tough and insoluble protein called, keratin, does not support viral replication and penetration by bacteria. The epidermis of skin is periodically shed off, resulting in the continual removal of any clinging pathogen.

Mucous membrane - The gastrointestinal tract, urinogenital tract and conjunctiva are all lined by mucous membranes. In the respiratory tract, goblet cells secrete mucous that entraps dust and microbes, which are propelled out by coughing and sneezing. The mucous membrane of gastrointestinal tract offers the same protection. Tear and saliva flush out entrapped microbes.

(b) Chemical barrier - The host body has several chemical / physiological barriers that contribute to innate immunity. These are as follows :

1. Acidic gastric secretion from the stomach (pH-1.5 to 2.0) is extremely inhospitable.
2. Low pH of sebum secreted by sebaceous glands of the skin containing organic acids (pH 3.0 to 5.0), inhibits or retards growth of most microorganisms.
3. Lysozyme is a hydrolytic enzyme, present in all mucous secretions, including tear, saliva and nasal secretion. It lyses Gram-positive bacteria.
4. Gastric and duodenal enzymes like proteases and lipases digest a variety of structural and chemical constituents of pathogens. For example, Rhino viruses are easily inactivated by gastric acids.
5. Human milk is rich in antibacterial substances namely Lactoferritin and Neuraminic acid, which fight against staphylococci.
6. The interferon refers to a group of proteins produced by virus infected cells that induce a generalized activated state in neighbouring uninfected cells.

7. Antimicrobial peptides : All insects and mammals including human, secrete a number of antimicrobial peptides, such as **defensins**, for their protection. The human body is protected by one micrometre thick biofilm of defensins that protects the skin from microbial assault.

(c) Phagocytosis - When bacteria or other invading parasites penetrate the skin or mucous membrane the phagocytes, such as neutrophils, monocytes and tissue macrophages, surge towards the site of infection. The phagocytes engulf the pathogens to form a large intracellular vesicle called phagosome. This fuses with the lysosome to form a phagolysosome. The release of lysosomal enzymes digests the bacteria. The useful products are absorbed back into the cell while the waste is egested out of the cell. It is also regarded as **second line of defense.**

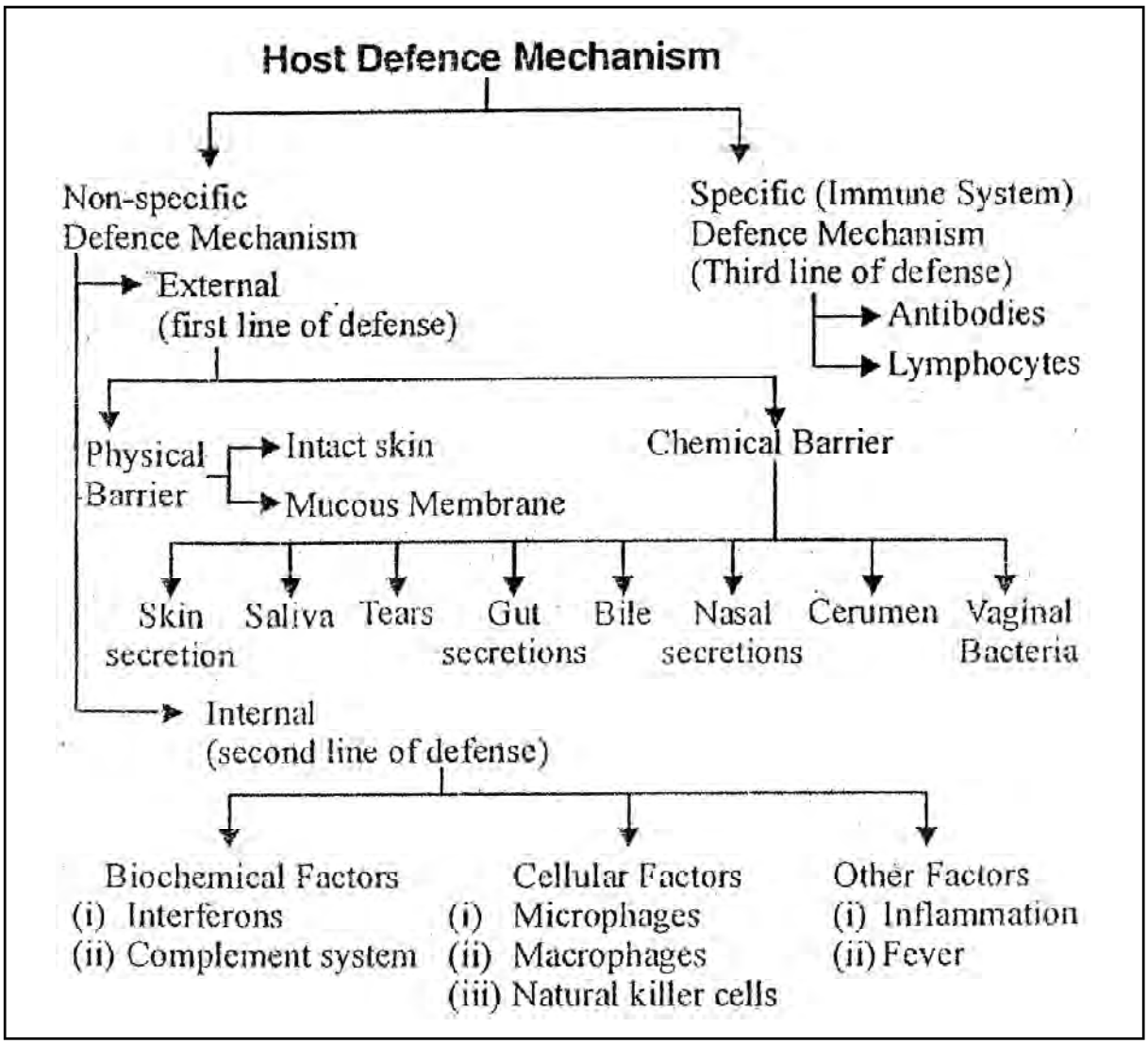


Fig. 9.1 : Types of host defence mechanism

(d) Fever - fever is a condition of rise in the body temperature which is caused due to endogenous pyrogens (cytokines) or endotoxins produced by various pathogens. Rise in the temperature helps to destroy temperature sensitive pathogens. Fever is also indicative of an internal infection by pathogens. The symptoms helps diognose the cause.

(e) Inflammation - inflammation is the reaction of the living tissue to either an injury or an infection. Inflammation is characterised by heat, redness, swelling and pain. It is a non-specific response of the body to injury. Injury maybe caused by either mechanical agents (cut, prick) or chemical agents (bee venom, acid or alkali) or infectious agents (bacteria or other pathogens). In the case of inflammation, the macrophages proceed to the site of injury. The blood vessels dilate due to the action of chemicals like histamin, bradykinin, etc., secreted by mast cells. Due to vasodilation blood accumulation and redness takes place at the site of inflamation. The accumulation of fluid results in tissue swelling (oedema). After a few days, due to phagocytosis, a cavity containing necrotic tissue, dead bacteria and dead phagocytes is formed. This fluid mixture is often called pus. Pus formation continues until all infection is suppressed.

(f) Acute phase proteins - It is a group of heterogenous plasma proteins which are important in the innate defense against microorganisms. In response to tissue invasion, cells circulating in the blood, such as macrophages and neutrophils secrete a variety of cytokines that stimulate the liver to produce acute phase proteins. These proteens inhibit various viral infections.

(g) Natural Killer (NK) cells - The natural killer (NK) cells are non-phagocytic granular lymphocytes. They can kill a range of tumour cells or cells infected by viruses without any antigen specificity. NK cells release substances called **perforin** or **cytolysin**, which lyses the virus infected cell.

9.3.2 Acquired immunity :

The resistance developed by human during his life through exposures to pathogens is known as acquired, specific or adaptive immunity. This is different from innate immunity in that it is due to specific antibodies or sensitized lymphocytes produced in responce to specific antigens. Hence, this immunity is also known a specific immunity. Acquired immunity is conferred by lymphocytes. Lymphocytes are derived from pluripotent haemopoietic mesodermal stem cells in yolk sack of the embryo. These cells later migrate to the bone marrow. After birth, these cells transform into thymus derived T-lymphocytes and bursa of Facbricius derived B-lymphosytes. Acquired immunity is of two types : **active immunity** and **passive immunity**. Both active and passive immunity may be natural or artificial.

9.3.2.1 Active Acquired Immunity :

Acquired immunity that is induced by natural exposure to a pathogen or by vaccination. The body learns the kind of mechanism to be employed through exposures. This is a long lasting process. It may be natural or artificial.

- **Natural Active Acquired Immunity :** It is acquired through continuous infections caused by bacteria or viruses which largely remain unnoticed.
- **Artificial Active Acquired Immunity :** This type of immunity is usually obtained through vaccination or through administration of toxoids.

9.3.2.2 Passive Acquired Immunity :

The immunity acquired by administering antibodies or sensitized WBCs from an immune individual is known as passive acquired immunity. It is again of the following types :

- **Natural Passive Acquired Immunity :** This can be acquired through trans-placental transfer of immunoglobulins (IgG) from mother to the fetus. This immunity lasts for about six months after birth. These antibodies of maternal origin protect the fetus and the infant from diphtheria, streptococci, tetanus, mumps, polio, etc. The secretory immunoglobulin (IgA) present in the mother's first milk (colostrum) provides immunity in the gastrointestinal tract of the infant. Moreover, The colostrum is rich in macrophages and lymphocytes.
- **Artificial Passive Acquired Immunity :** It is achieved by administering specific antibodies or antiserum from one individual to another non-immunized individual. Antibodies against a microbe or its antigen or toxin can be raised in a suitable animal through repeated injection of a suitable antigen. Injection of polyvenin causes passive immunity against snake venom. Anti Tetanus Serum (ATS) also creates passive immunity against tetanus.

9.3.2.3 Mechanism of active acquired immunity :

Active immunity is more effective and superior than passive immunity. Active acquired immune response takes two distinct forms called **cell mediated** and **humoral immune responses**.

(i) Cell Mediated Immune Response : The immunity conferred by the sensitized T-lymphocytes is called cell mediated immunity. Here antibodies are not produced. T-lymphocytes or T-cells respond to cells infected by pathogens, such as viruses and bacteria. Activated T-lymphocytes undergo proliferation and differentiate into various types of effector cells, such as T-helper (T_H) and T-cytrotoxic or killer (T_C / T_K) lymphocytes and memory T-lymphocytes (T_M). T_M confers a long term memory against the invading pathogen. T_C / T_K cells directly kill or destroy antigens or antigen bearing pathogens. T_H cooperates with B-lymphocyte and triggers its transformation into a plasma cell.

(ii) Humoral Immune Response : It is confered by B-lymphocytes or B-cells. When B-lymphocytes are sensitized by toxins or antigens, multiply in number and

transform into larger cells called plasma cells or plasmocytes. This transformation into plasma cells is assisted by T_H. The plasma cells are potential antibody secreting cells. The antibodies destroy antigens by species-specific antigen-antibody interaction.

Antibodies are glycoproteins called immunoglobulins. They fall in five classes, such as IgM, IgG, IgA, IgD, and IgE.

9.3.2.4 Structure of Immunoglobulin G (IgG) (Fig. 9.2) :

Of all the five classes of immunoglobulins, IgM is the first antibody synthesized by the new-born infant's immune system. Soon, IgM is replaced by IgG, which becomes the principal antibody in the serum, thereafter. Its structure is as follows :

1. It is a monomeric structure of four interacting polypeptiles, two large polypeptiles, reffered to as heavy or 'H' chains and two small polypeptiles, reffered to as light or 'L' chains.
2. A heavy chain is joined to a light chain of its side by a disulfide (S-S) bond.
3. Two heavy chains are joined to each other by two disulfide bonds.
4. Three fourth (3/4) part of each heavy chain from the C-terminous is made by constant aminoacid sequence. These regions are known as CH_1, CH_2 and CH_3 (C for constant). Remaining onefourth (1/4) part is made by variable aminoacid sequence. This region is known as VH (V for variable).
5. Similarly a half of each light chain from the C-terminus is made by constant aminoacid sequence (CL) and other half by variable sequence (VL).
6. Each heavy chain has a flexible region between CH_2 and CH_3, so that during cirulation, IgG become 'Y' shaped. This flexible point is known as the hinge.
7. Oligosaccharide residues are joined to the aminoacids of the heavy chain constant region below the hinge, thus making the antibody a glycoprotein structure.
8. At the N-terminus the variable aminoacids of each heavy chain and each light chain of its side, together constitute a site for recognition of a complementary antigen and binding to it. This site is known as the **antigen binding site (Fab)**. Thus, IgG has two antigen binding sites.
9. Heavy chains in the constant region have effector functions.

IgG is the most abundant antibody present in the serum. Two each of heavy and light interacting polypeptides constitute a complete unitary structure of IgG. Alternately, its structure has been referred to as a monomeric structure.

IgM is the largest antibody having a pentameric structure i.e. five monomeric units, like that of the IgG, interact and form the structure of IgM. It is the first antibody to be synthesized in neonatal babies. However, it is replaced by IgG thereafter.

IgA is the second most abundant antibody of the serum. It is also present in extracellular secretions, like saliva and first milk of the lactating mothers (colostrum). It has a dimeric structure.

IgD has a monomeric structure, bound to B-lymphocyte membrane, IgE also has a monomeric structure, bound to membrane of basophils and mast cells, where it mediates in the release of histamin.

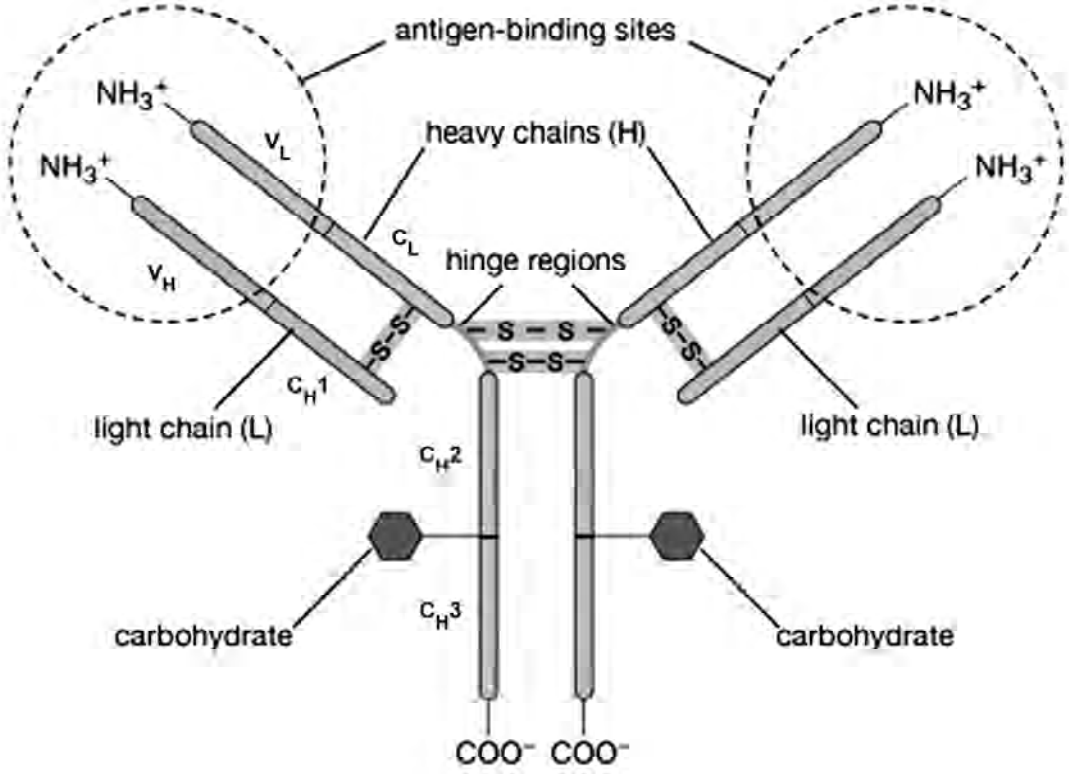


Fig. 9.2 : The molecular structure of an immunoglobulin G (IgG)

9.3.3 Antigen antibody interaction :

Antigens (Ag) or immunogens are whole organisms or molecules or foreign origin that can evoke immune responses and can bind to antibodies (Ab) in a species-specific manner. Normally an antibody does not interact with the whole antigen but reacts specifically with a part of it called an **antigenic determinant** or **epitope** (Fig. 9.3). Epitopes are immunologically specific and active sites of an antigen, which bind to complementary part of an antibody called **paratope**, present on the antibody. Small foreign molecules do not stimulate antibody formation. They do so, when taged to macromolecules. The small molecule is known as a **hapten**. This

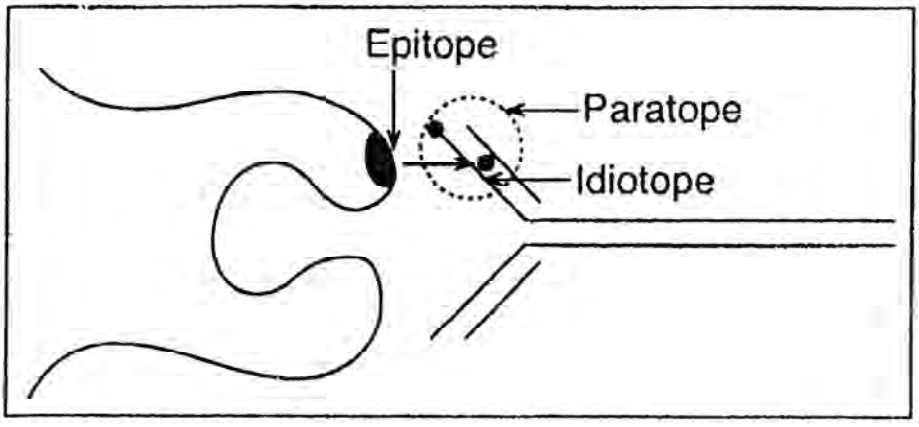


Fig. 9.3 : Antigen recognition by antibody

combination leads to the formation of antigen-antibody complex. The formation of these complxes is known as **precipitation** or **agglutination**. The consequence is the immobilization and precipitation of the antigen. The complexes are engulfed by macrophages and phagocytes and digested. Thus antigens are eliminated from the body.

9.3.4 Vaccines :

A vaccine can be defined as a preparation of bacterial, viral or other pathogenic agents or their isolated antigens, which is administered with the objective of stimulating a recipient's protective immunity. Thus, a vaccine is basically an antigen or its component that induces acquired immunity in the host, producing T and B-lymphocytes.

Types of Vaccines : There are several types of vaccines, like natural live, live attenuated, inactivated toxoid, polysaccharide, recombinant antigen, live vector and DNA vaccines.

- **Natural live vaccines :** This vaccine includes natural non-pathogenic organisms. Cow pox virus vaccine, simian and bovine retrovirus vaccines have been used in vaccination but with moderate success. Currently these vaccines are not used.
- **Live attenuated vaccines :** Attenuation refers to the weakening of a pathogenic bacterium or virus by making it less virulent. Microorganisms are attenuated or weakened so that they do not cause diseases. BCG (Bacillus Calmette-Guerin) is a commonly used vaccine of this kind against tuberculosis. Attenuated viruses are also used as vaccines for polio, yellow fever and measles.
- **Inactivated vaccines :** This is another useful vaccine achieved by inactivating the whole pathogen or antigen. The inactivation of pathogen is done by modifying it chemically by formaldehyde treatment or physically by heat treatment. Salk polio vaccine, whooping cough vaccine are included in this category. One of the greatest advantages of using inactivated or killed pathogen in a vaccine is that there is no danger of mutation or reversion to the pathogenic form.

- **Toxoid vaccine :** Some bacterial pathogens such as diphtheria and tetanus bacilli produce exotoxins (cytotoxic microbial poison) that induce several characteristic symptoms associated with these diseases. These exotoxins are isolated and chemically modified so that their toxicity is lost. These non-toxic immunogenic derivatives of exotoxin or toxoid are commonly used in vaccines. Examples are diphtheria and tetanus vaccines.
- **Polysaccharide vaccines :** The capsular polysaccharide of bacteria serves as an excellent vaccine because it resists immune action. The polysaccharide capsule of *Hoemophilus influenzae*, *Streptococcus pneumoniae* act as good antigens.
- **Live Vector vaccines :** In this type, the desired gene coding for the target antigen of the virulent pathogen is joined to a suitable vector (attenuated bacteria or virus) and then this transformed vector is inoculated into an individual, where the vector slowly replicates and serves as a source of the said antigen. The most commonly used viral vectors are small pox virus, adenovirus, and bacterial vectors include attenuated *salmonella typhi,* BCG strain of *Mycobacterium bovis*, *Vibrio cholerae*, etc. These vectors act as a source of antigens inside the host.
- **Recombinant antigen vaccines :** A gene coding antigenic proteins can be introduced and expressed in yeast, bacterial or even mammalian cells using recombinant DNA technology. These cells are then cultured in the laboratory and the protein produced is harvested. The gene that is selected for making a recombinant antigen usually expresses surface antigens (glycoproteins).
- **DNA vaccine :** The DNA vaccine represents a recent type of vaccine in which there is a deliberate introduction of DNA plasmid into the muscle cell of the recipient. The plasmid contains a protein coding gene which acts as an antigen. This antigen is expressed in the cell leading to both humoral and cell-mediated immune responses.

9.4 CANCER :

Cancer is regarded as a group of diseases characterized by:

(i) an abnormal growth of cells,

(ii) an ability to invade other tissues, and / or organs,

(iii) eventual necrosis of the tissues or organs leading to the death of the affected person.

An abnormal and uncontrolled growth of cells develops a tumour which consists of mass of cells termed as **neoplasm**. If the neoplasm remains confined to the affected organ without spreading into other tissues, it is called a **benign tumour** or nonmalignant tumour.

If the neoplasm spreads into adjoining tissues, it is called a malignant tumour. Benign tumours have well differentiated cells and are not fatal, while malignant tumours are very dangerous as they invade other parts of the body. Spreading of cancerous cells to other tissues and organs at distant sites is known as **metastasis** (Fig. 9.4).

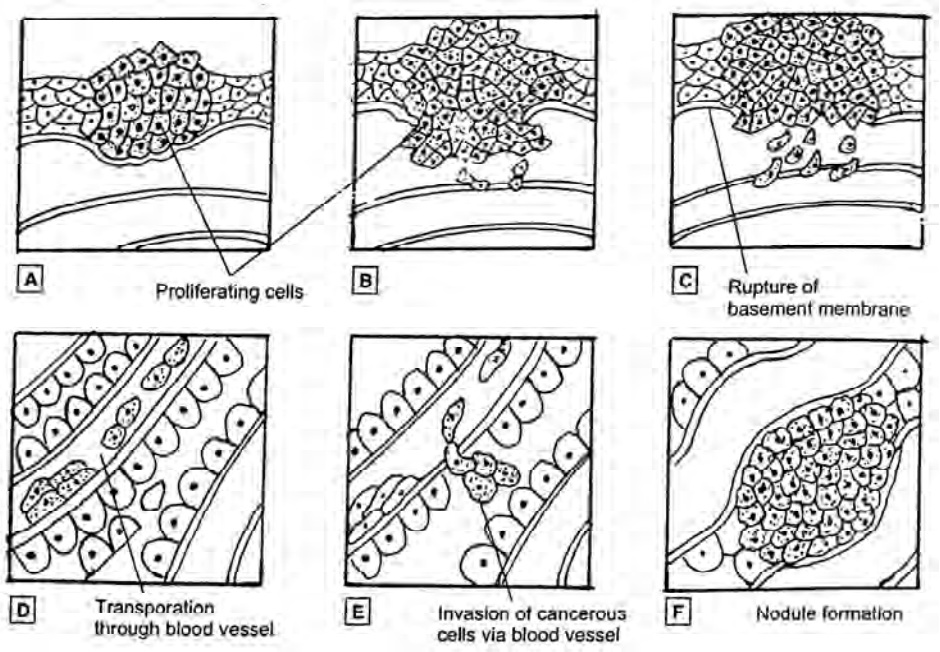


Fig. 9.4 : Growth, invasion and metastasis of malignant cells.

9.4.1 Major types of cancer :

On the basis of pathology, cancer can be classified into four major types :

(i) Carcinoma : This is the malignant growth of epithelial tissue which covers or lines the body organs. The common carcinomas are skin cancer, breast cancer, lung cancer, stomach cancer and pancreas cancer.

(ii) Sarcoma : It is the malignant growth of connective tissue, derived from primitive mesoderm. It includes bone cancer and muscle cancer.

(iii) Leukaemia : It arises due to the uncontrolled proliferation of blood corpuscles and their precursors in the bone-marrow, resulting in the formation of increased number of leucocyte.

(iv) Lymphoma : It is the cancer of lymphatic system.

9.4.2 Agents / Factors causing cancer :

Epidemiological, clinical and pathological studies have revealed a variety of factors associated with the initiation of cancer. These are :

(i) **Physical Agents :** The agent which induces cancer is carcinogenic or oncogenic. The physical carcinogenic agents are ultraviolet rays, ionizing radiation, solar radiation and continued heat.

(ii) **Chemical Agents :** The carcinogenic chemical agents are tar; dyes; aromatic amines; urethane; various metals like Nickel, Beryllium, Arsenic and Chromium; asbestos; hormones and aflatoxin. In the cigarette smoke there are no less than 15 chemical carcinogens.

(iii) **Nutritional Agents :** Deficiency of proteins, vitamins and minerals are also known to cause cancer. Alcohol and food contaminants do also have carcinogenic effects.

(iv) **Biological Agents :** Many types of cancers are known to be causd by viruses. Association of Hepatitis B with primary liver cancer has been established.

(v) **Mechanical Factors :** Severe friction, trauma and irritation have also been identified to cause malignancy.

(vi) **Other Factors :** Other factors responsible for cancer are the host and the environmental factors. The host factors include age, sex, martial status, race, socio-economic status, customs and habits. The environmental factors includes radiation, air pollution, diet, drugs and social environment.

9.4.3 Genetic basis of cancer :

All cancers have a genetic basis and are caused by genetic transformations of cells. The genes that have been implicated in carcinogenesis (causation of cancer) are divided into two broad categories, **oncogenes** and **tumour- suppressor genes**.

9.4.3.1 Oncogenes :

Oncogenes encode oncoproteins that promote the loss of growth control and the transformation of a cell to a malignant state. Some viruses are the source of the oncogenes. These viruses are called **oncoviruses** and the genes as ***v-onc*** (viral protooncogenes). These viral oncogenes have homologous regions in the human genome. These homologous genes are called **cellular protooncogenes (*c-onc*)**. Cellular protooncogenes are involved in normal cell functions and about 100 protooncogenes are known. In some cases mutation in proto-oncogenes causes their abnormal functioning and tumour formation. Among the other factors, viral protooncogenes constitute a class of transforming factors, which transform cellular protooncogenes into expression-ready cellular oncogenes. The protooncogenes are mutated or transformed and encode abnormal proteins called oncoproteins. There oncoproteins bring about the loss of growth control in cells. The names of oncogenes have usually been derived from the names of the viruses, in which they are discovered. (e.g., *v-src*, *v-myc* etc.)

9.4.3.2 Tumour Suppressor Genes :

Tumour Suppressor genes or anti-oncogenes encode proteins that restrain abnormal cell growth and prevent cells from becoming malignant. Several tumor suppressor genes have so for been characterised. More common among these are : retinoblastoma protein coding gene (***rb***) and ***p53*** gene. If the tumour suppressor genes on both the homologous chromosomes undergo mutation, it loses the control over the cell growth which results in development of cancer.

9.4.4 Diagnosis :

Several modern methods of detection and dignosis of cancer are in practice. Some of these are enlisted below :

(a) Fine Needle Aspiration Cytology (FNAC)

(b) Biopsy of tissues (Histopathological examination)

(c) PAP test (Cytological staining) used for detection of cervix cancer.

(d) X-rays, CT scans, MRI scans detect cancers of internal organs.

(e) Mammography for detection of breast cancer.

(f) Abnormal count of WBCs in Leukemia.

(f) Monoclonal antibodies along with radio-isotopes can detect cancer specific antigens as those in prostate cancer and thyroid cancer.

9.4.5 Prevention :

According to World Health Organization (WHO), the definition of cancer prevention is "the elimination of, or protection against, factors known or believed to be involved in carcinogenesis and the treatment of precancerous conditions". Prevention is better than cure. When everybody knows that late-stage cancer can not be cured, its better we adopt some possible preventive measures for its initiation and progression. These preventive measures are :

Some Other Types of Cancer

ADENOMA	:	Cancer of glands.
LIPOMA	:	Cancer of Adipose tissue.
GLIOMA	:	Cancer of glial cells of central nervous system.
MYOMA	:	Cancer of muscular tissue
MELANOMA	:	Cancer of pigmented epithelium of skin.

(1) Cancer Education :

It is to motivate people to go for early diagnosis and early treatment for better chance of survival. The possible symptoms of the initiation and progression of cancer are:

(a) a lump or hard area in the breast;

(b) a change in wart or mole;

(c) a persistent change in digestive and bowel habit;

(d) a persistent cough or hoarseness;

(e) excess loss of blood at the monthly period or loss of blood outside the usual dates;

(f) blood loss from any natural orifice;

(g) a swelling or sore that does not get better; and

(h) unexplained loss of weight.

It is important to educate people about the oncogenic effects of tabacco and prohibited drugs through advertisements.

(2) Other Measures

People be made conscious about the following :

(a) personal hygiene;

(b) control of air pollution;

(c) testing of drugs and cosmetics;

(d) reducing the amount of radiation;

(e) organizing occupational health programmes;

(f) treatment of precancerous lesions; and

(g) legislation to control known environmental carcinogens.

(h) legislation to display pictorial hazards of cancer on all packets selling tobacco or its products.

9.4.5 Treatment :

Treatment of cancer is undertaken taking the type of cancer into consideration. The primary approach is the surgical removal of the tumour. This is followed by a histopathological examination of the affected organ for identifying the malignant cells and their metastasis. If the result is positive, then radiotherapy by exposing the cancerous tissue to ionising raditon; radio isotope therapy; immunotherapy and chemotherapy treatments are undertaken as per the advice of the oncologist.

9.5 AIDS AND HIV :

AIDS or Acquired Immuno Deficiency Syndorme is an immunodeficient condition of the body. It is caused by a virus known as **Human Immunodeficiency Virus** or **HIV**. It belongs to the **retrovirus family** (RNA virus) and **Lentivirus subfamily**, which infects and kills $CD4^+$ cells (helper T lymphocytes), macrophages and dendritic cells in a progressive manner and weakens the immune response. This condition leads to many opportunistic infections by several pathogenic organisms. This condition continues for 10-12 years after infection and finally the subject dies. As on date, there is no effective treatment and therefore, 'prevention is better than cure' action is practiced to contain it from spreading in the population.

AIDS was clinically diagnosed in 1981 in USA in male homosexuals and injection drug users. They showed symptoms of pneumonia, an opportunistic infection compromised by the immune system. Later, other male homosexuals were identified havint Kposi's Sarcoma, a rare skin cancer. Following this, the US Centre for Disease Control and Prevention (CDC) set up a take force to monitor the outbreak. It conducted a scientific investigation and in a meeting in July, 1982, the name, AIDS was introduced.

The naming of HIV also follows a history. In 1983, CDC named the causative virus as **lymphoadenopathy virus** (**LAV**). Another investigating school named it as **human T-lymphotrophic virus III** (**HTLV III**). There are four types of HTLVs : HTLV I, II, III and IV. Out of these, HTLV III expresses the symptoms of AIDS. Since, LAV and HTLV III were one and the same, the virus was identified in the name of HIV in 1986.

Two strains of HIV, namely HIV-1 and HIV-2 have been discovered. These viruses have originated from non-human primates in West-Central Africa and transferred to human in early part of the twentieth century. HIV-1 is worldwide in distribution and is more pathogenic. HIV-2 is less prevalent and less pathogenic, distributed in Western Africa only.

9.5.1 Structure (Fig. 9.5) :

It is a virus belong to retro-virus family and lentivirus subfamily. It is roughly spherical and measures around 60 nm in diameter. The core has two singlestranded RNA, enveloped by a conical capsid, made up of viral proteins, P24, typical to lentiviruses. Each RNA is bound to nucleo-capsid proteins and enzymes, like reverse transcriptase and integrase. A matrix of other viral proteins surrounds the capsid. Proteases and other proteins are present in the virus between the capsid and matrix. The matrix is surrounded by a lipid bilayer envelop of host cell origin. The envelop is formed, when the viral particle buds off from the host cell. Two glycoproteins, namely, **gp 120** and **gp 41** are anchorded to the lipid bilayer envelop. These are required for anchoring to the host cell and entering into it.

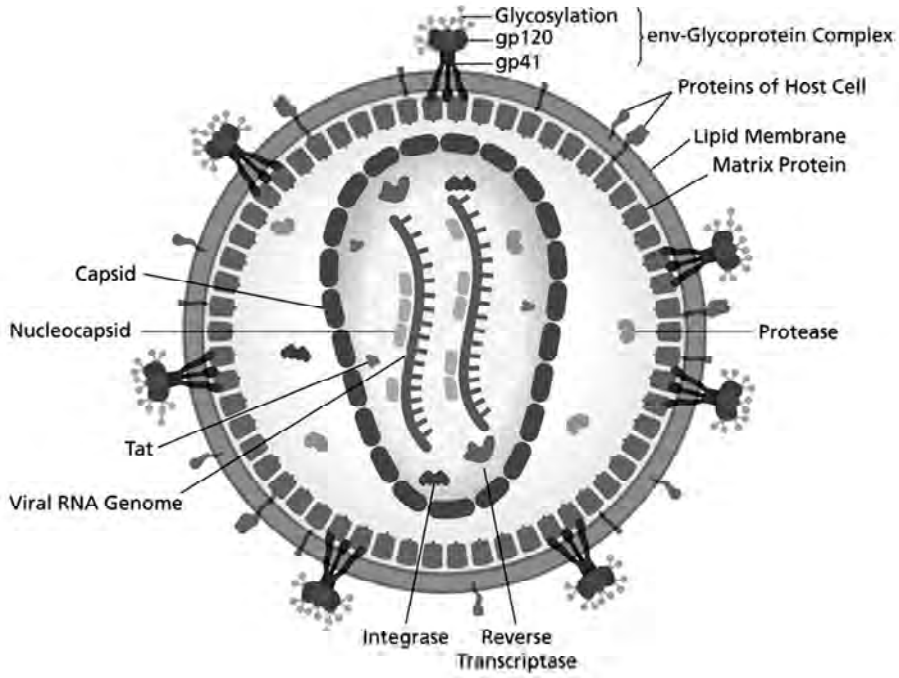


Fig. 9.5 : Structure of Human Immunodeficiency Virus

9.5.2 Infection :

The HIV infects $CD4^+$ T lymphocytes macrophages and dendric cells. Prior to entering into these cells, it anchors to the suface of the host cell body by adsoption assisted by the glycoproteins, the capsid is releasd and then a replication cycle onsets ending in the formation of viral particles like that of a retrovirus. If left untreated; HIV progresses in three stages :

1. Acute infection
2. Clinical latency (Chronic HIV infection)
3. AIDS

9.5.2.1 Acute infection stage :

Within 2-4 weeks after HIV infection, people develop flu-like symptoms like fever, swollen glands, soar throat, rash, muscle and joint pain and headache. During this stage, one is at higher risk of transmitting the virus through sexual intercourse and injectable drug abuse using a contaminated needle.

9.5.2.2 Clinical latency stage :

Latency literally means, a virus is replicating in a host cell without expressing the symptoms If an infected person undergoes anti-retroviral therapy, he may live for decades. For people, who are not on this therapy, the latency stage lasts on an average for ten years.

9.5.2.3 AIDS :

This stage of HIV infection occurs, when the immune system is badly damaged. The $CD4^+$ lymphocytes fall below 200 per cubic mililiter of blood. The infected person contracts many bacterial and fungal diseases, which he would have otherwise been able to fight off. These infections are referred to as opportunistic infections. Som of the prevalent symptoms are as follows :

- Weight loss and unexplained tireness
- Chronic diarrhoea
- Pneumonia
- Prolonged swelling of the lymph glands of armpit, groin and neck.
- Recurring fever with night sweats.
- Persistent cough
- Mouth and skin problems
- Recurrent infections
- Sores of the mouth, anus and genitals

Without treatment, people who progress to AIDS survive about three years.

9.5.3 Treatment :

HIV is treated using a combination of medicines. This is called **antiretroviral therapy**. It involves taking a combination of HIV medicines (called HIV regimen) everyday as prescribed Antiretroviral drugs fall under six classes. It is beyond the scope of describing these in details. However, some effective drugs are mentioned below :

Dextran sulfate	-	Inhibits viral binding to the host cell.
Azidothymidine	-	Acts as a reverse transcriptase inhibitor.
Dioxycytosine	-	Inhibits reverse transcription.
Phosphonoformate	-	Inhibits reverse transcription.

9.5.4 Prevention and Control :

So far, no concrete treatment method has been developed to treat AIDS. Therefore, prevention is the best way to keep away from AIDS. The following steps are recommended to prevent AIDS infection:

(i) Sterlisation of all surgical instruments is essential before use.

(ii) Blood for transfuision be subjected to HIV test and such blood pouches be properly labeled and passed for transfusion.

(iii) HIV positive women should avoid pregnancy, otherwise, the child would contract HIV infection.

(iv) Ilicit heterosexual activities be prohibited and polygamous men be advised to use condoms during sexual intercourse.

(v) Disposable vans be used for every injection and used hypodermic needles and vans be disposed off after single use.

(vi) Government of India has constituted National AIDS Control Board, National AIDS Committee, National AIDS Control Organisation etc. These organizations are creating awareness among people about HIV contraction and progression of AIDS.

9.6 ADOLESCENCE :

9.6.1 Common Problems of Adolescence (Drug, Alcohol and Tobacco)

Adolescence is an important phase in human life between childhood and adulthood between 10 and 19 years of age, characterised by several distinct physical, physiological and psychological changes in the body. It is an important transition which can be the best or the worst period in ones life.

9.6.1.1 Physical changes :

Since it is a period of active growth and sexual maturity, growth becomes apparent with an increase in the body size, height and weight due to continued secretion of grwoth hormone. Because of increased secretion of gonadotropic hormones (FSH and LH), gonads mature and start functioning. Under the influence of the sex hormones from the testis and ovary, several secondary sexual characters appear in the body both in the male and female, which result in sexual dimorphism. In the male, such characters include growth of beard, growth of hair on the body, change in the voice, increased muscularity, while in the female, these include an increase in the breast mass and beginning of menstruation. Pimples on the face appear in both the male and the female under the influence of male hormones like testosterone in the male and male hormones from the adrenal cortex of the female.

9.6.1.2 Psychological changes :

Several noticeable changes in the behaviour, emotion and attitude are associated with this phase, which can not be assigned any specific reason thereof. Tendency to differ from parents, difficulty to cope up with pressure of studies at school or college, need for money for increased expenditure often drive these teen aged adolescents to take to bad company, drugs, alcohol, tobacco and stealing and robbery.

9.6.1.3 Behavioural changes :

(i) Changes in eating and sleeping habits

(ii) Changes in emotions, mood and tendency to find male or female company

(iii) Lack of interest in activities like studies

(iv) Tendency to appear more handsome or beautiful

(v) Low self-esteem, lower confidence, poor performance in studies, depression

(vi) Tendency to become hostile, irritable and non-cooperative

(vii) Lying, cheating and often stealing money

(ix) Tendency to take to drugs, tobacco and alcohol

(x) Indulging in criminal activities

9.6.2 Alcohol abuse or alcoholism :

Alcoholism is a very common social problem that has affected both the poor as well as affluent sections of the society. In affluent societies, it has become a fashion or means of socialisation. The habitual drinker always begins as a casual or occasional drinker in the youth and later it falls into a habit. Several reasons may be attributed to it. It may be due to bad company or a desire for enjoying the excitement, out of curiosity, a desire to escape from failures and dissappointments or to overcome hardships of the daily life.

9.6.2.1 Effects of Alcohol :

1. A high dose of alcohol i.e., more than 30 ml. acts as an intoxicant and affects the functioning of the CNS as a depressant. Drowsiness affects driving making it accident prone. Judgement, co-ordination, alertness, vision, responsiveness, behaviour are also affected.

2. Alcohol damages one of the most important internal organs, the liver. In the liver, alcohol is converted to acetaldehyde and then into fat. Hence, the liver becomes a fat depot or turns into a fatty liver. Liver cells degenerate and cause cirrhosis, in which the glycogen and protein synthetic activities are affected. Alcohol may also cause hepatitis and liver cancer.

3. Alcohol has a widening effect on blood vessels. Any quantity of alcohol taken in excess of 30 ml. / day makes the blood vessels hard and brittle leading to bradycardia and moyocardiopathy.

4. Alcohol decreases ADH secretion which controls diuresis. Hence, there may be loss of water from the body.

5. Excessive intake of alcohol makes a person unusually aggressive and makes his talk and behaviour irrelevant and a laughing stock before others.

6. Habitual drinking by any member in the family affects the social status of that member as well as that of the whole family, since drinking is considered as a social evil.

9.6.3 Drug abuse and addictive disorders :

The term drug refers to a chemical which is used in the treatment of a disease under the supervision of a physician and is withdrawn after the desired effects are achieved. But the prolonged and unnecessary use of a drug makes a person dependent and an addict to that drug. This is called drug addiction and the disorders they express, are called addictive disorders.

There can be several reasons for developping a drug addiction. Some may take it out of curiosity or under pressure from friends in order to experience excitement and adventure, to overcome depression and frustration, for enhancing mental and physical activity or to get relief from pain. Use of drugs starts casually or out of curiosity and then its uncontrolled use leads to an addiction.

Several types of drugs are known depending on their chemical nature and effects on the body or more specifically on the brain. These include sedatives and tranquillizers, stimulants, hallucinogens and opiate narcotics. The drugs can be psychotropic or psychedelic depending on their effects.

(i) Sedatives and Tranquillizers : These depress the activities of the central nervous system particularly the brain, imparting a feeling of relaxation and calmness accompanied by drowsiness. They lower tension and anxiety and in larger doses induce sleep. Examples of such drugs are benzodiazephines and barbiturates.

(ii) Stimulants : These cause a stimulation or excitement of the CNS, affect the release of adrenalin from the adrenal gland and make a person alert, wakeful, active and reduce apetite. Caffeine contained in tea, coffee and cocoa belongs to this category. Excessive and prolonged use may cause acidity, loss of appetite and can also cause cancers. Cocaine from the plant *Erythroxylon coca* has stimulatory and anaesthetic effects. Amphetamines are synthetic drugs, act as strong stimulants of the CNS and are often used by night workers and truck drivers as anti-sleep pills. They are also used by athletes to increase their performance and hence this is included in the Dope Test scheduled for the athletes.

(iii) Hallucinogens : These drugs change a persons thoughts, feelings and perceptions, cause hallucinations, illusions of sounds and objects not actually

seen. Such drugs include LSD (Lysergic acid diethyl amide) that is derived from the ergot fungus (*Classceps purpurea*). It is one of the most dangerous drugs that damages the CNS. It may cause chromosomal abnormalities in the fetus, if taken by a pregnant woman.

Serval hemp plant products from the plant *Cannabis indica* and C*annabis sativa* are used to obtain charas (hashish), bhang and ganja, while marijuana is obtained farm *Cannabis sativa*.

(iv) Opiate narcotics : These drugs suppress pain, reduce anxiety and tension and produce a feeling of well being. Drugs like opium or afim, derived from the latex of the poppy plant, *Papaver somniferum*, morphine and codeine which are opium derivatives, heroin or brown sugar, which is also a refined product of morphine belong to this category. Opium, if taken regularly, causes addiction and in large amounts even death. Terrible withdrawl symptons are seen when the drug is stopped. Morphine is a mild analgesic and also causes addiction. Codeine does not cause addiction and hence, it is commonly used in cough syrups and other medicines of the respiratory tract. Heroin or brown sugar is sniffed, smoked or injected and is much more potent than other drugs. Carelessness in using syringes and needles helps to spread dangerous diseases like AIDS and hepatitis.

Combination of several drugs are often used for a 'kick' which can even be more dangerous. Some drugs are taken in combination with alcohol for a desired effect.

9.6.4 Withdrawl Symptons, Treatment and Rehabilitation :

Each kind of addiction may show a characteristic set of withdrawl symptons when it is withdrawn. Withdrawl symptons of alcohol addiction may be seen in the form hallucinations, fits, tremors which need treatment with detoxifying drugs like diazepam, vitamin B, chlordizepoxide, apomorphine. Antioxidants like disulfiram, cephalosporin, metronidazole can be helpful for reducing alcohol dependence.

For deaddiction of drug addicts, deaddiction centres are established where both outdoor and indoor facilities for counselling and treatment are available. Severe physiological and psychological disturbances are seen. Nausea, vomitting, diarrhoea, perspiration, shearing muscle cramps, insomnia, loss of apetite and epilepsy may be observed which need symptom-based treatments. Pharmacotherapy, psychological treatment and supportive care by friends and family members help in preventing the relapse. Care must be taken to see that the supply of drugs is stopped.

9.6.5 Social and Moral Implications :

Apart from health hazards, any kind of addiction particularly drug and alcohol addiction have many important social and moral implications. Addiction to alcohol or drugs is considered as a social stigma, a mark of disgrace for the family in the society. They are not easily accepted by friends, colleagues and relatives. People avoid and ignore them. Family income is unnecessarily squandered depriving other members in the family of their basic needs. There is quarrel and unhappiness in the family over it. Drug addicts resort to taking loans without thinking of their ability to repay, even to stealing, robbing in order to obtain money somehow for the purchase of drugs which are often very expensive.

Alcohol addicts are quarrelsome, become violent, accident prone in their driving and riding, often are involved in crimes and corruption in offices, remain absent from duty. Often they manhandle the family members, if money is not made available for the purchase of alcohol. Friends and relatives avoid them for foul smell, misbehaviour and antisocial activities.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer :

(i) Which of the following diseases are communicable ?
- (a) Deficiency diseases
- (b) allergies
- (c) Degenerative diseases
- (e) Infectious diseases

(ii) The nature of the spread of communicable diseases is termed as :
- (a) Parasitology
- (b) Immunology
- (c) Epidemiology
- (d) None of these

(iii) Which of the following is a sexually transmitted disease ?
- (a) Q fever
- (b) Leprosy
- (c) Whooping cough
- (d) Gonorrhoea

(iv) Gonorrhoea is a :
- (a) Bacterial disease
- (b) Veneral disease
- (c) STD
- (d) All of these

(v) Anthrax is caused by :
- (a) Vibrio
- (b) Bacillus
- (c) Salmonella
- (d) Virus

(vi) Some common diseases caused by bacteria are :
- (a) Measles, mumps and malaria
- (b) Tetanus, typhoid and tuberculosis
- (c) Syphilis, smallpox and sleeping sickness
- (d) Pneumonia, polomyelitis and psittacosis

(vii) Which of the following disease is spread through wounds ?
- (a) Tetanus
- (b) Cholera
- (c) Plague
- (d) Tuberculosis

(viii) Which of the following is a bacterial disease ?
- (a) Measles
- (b) Smallpox
- (c) Rabies
- (d) Tuberculosis

(ix) Causative agent of TB is :
- (a) Salmonella
- (b) Streptococcus
- (c) Mycobacterium
- (d) Pneumococcus

(x) BCG vaccine is a preventive measure against :
- (a) Tuberculosis
- (b) Typhoid
- (c) AIDS
- (d) Cholera

(xi) Which one is not a bacterial disease ?
- (a) Tuberculosis
- (b) Typhoid
- (c) AIDS
- (d) Cholera

(xii) Mantoux test is for :
(a) Scarlet fever (b) Diphtheria
(c) Rheumatoid fever (d) Tuberculosis

(xiii) Chickenpox is caused by
(a) Varicella virus (b) Adeno virus
(c) SV 40 virus (d) Bacteriophage T_2

(xiv) Smallpox is due to
(a) Virus (b) Bacterium
(c) Protozoan (d) Helminth

(xv) The disease caused by virus is
(a) Pneumonia (b) Tuberculosis
(c) Smallpox (d) Typhoid

(xvi) Polio is caused by :
(a) Virus with double stranded DNA
(b) Virus with double stranded RNA
(c) Virus with single stranded DNA
(d) Virus with single stranded RNA

(xvii) Mumps is a :
(a) Protozoan disease (b) Viral disease
(c) Fungal disease (d) Bacterial disease

(xviii) Which one is a viral disease ?
(a) Measles (b) Rickets
(c) Syphilis (d) Congenital night blindness

(xix) Amoebiasis is caused by :
(a) Plasmodium vivax (b) Entamoeba gingivalis
(c) Trypanosoma gambiense (d) Entamoeba histolytica

(xx) Entamoeba histolytica infection ocurs thrugh :
(a) Mosquito bite (b) Bird droppings
(c) Sweat (d) Contaminated food and water

(xxi) The infective stage of Entamoeba histolytica is :
(a) Binucleate form (b) Tetranucleate form
(c) Minute form (d) Sporozoite stage

(xxii) Malaria is transmitted by :
(a) Male Anopheles (b) Female Anopheles
(c) Female Culex (d) Female Aedes

(xxiii) Select the incorrect pair :
(a) Pediculus - Typhoid (b) Xenopsylla - Plague
(c) Culex - Malaria (d) Aedes - Yellow fever

(xxiv) Filaria is transmitted by :

(a) Tsetse fly (b) Sand fly

(c) anopheles (d) Culex

(xxv) Culex causes the disease :

(a) Malaria (b) Filariasis

(c) Yellow fever (d) Sleeping sickness

(xxvi) The disease elephantiasis is caused by :

(a) Culex mosquito (b) Anopheles mosquito

(c) Housefly (d) Tsetse fly

(xxvii) Microfilariae are found in the peripheral blood of man during

(a) Day time (b) Day and night time

(c) Night time (d) None

(xxviii) Infection of Ascaris occurs due to :

(a) Tsetse fly (b) Mosquito bite

(c) Imperfectly cooked pork (d) Contaminated food and water

(xxix) A disease caused by nematode parasite

(a) Filariasis (b) Leprosy

(c) Amoebiasis (d) Poliomyelitis

(xxx) AIDs is caused by

(a) HTLV-III (b) Herpes virus

(c) Rota virus (d) Orthomyxo virus

(xxxi) Cerebral malaria is caused by plasmodium

(a) vivax (b) ovale

(c) falciparum (d) all of the above

(xxxii) Which of the glands is often referred in relation with AIDS ?

(a) Thyroid (b) Adrenal

(c) Thymus (d) Pancreas

(xxxiii) AIDS is caused by :

(a) Virus (b) Fungus

(c) Helminth (d) Bacterium

(xxxiv) AIDS is due to :

(a) Reduction in number of helper T-cells

(b) Lack of interferon

(c) Reduction is number of killer T-cells

(d) Auto-immunity

(xxxv) AIDS virus has

(a) Double stranded DNA (b) Single stranded DNA

(c) Single stranded RNA (d) Double stranded RNA

(xxxvi) AIDS spreads through
(a) Immoral way of life (b) Infected needles and syringes
(c) Homosexuality (d) All the above

(xxxvii) Cancer is
(a) non-malignant tumour (b) controlled division of cells
(c) unrestrained division of cells (d) microbial infection

(xxxviii) Cancer cells are damaged by radiations while othres are not :
(a) being different in nature (b) being starved
(c) undergoing rapid division (d) none of the above

(xxxix) Sarcoma is the cancer of
(a) Epithelial tissues (b) Connective tissues
(c) Blood (d) Endodermal tissues

(xl) Blood cancer is called :
(a) Leukaemia (b) Hemophilia
(c) Thrombosis (d) Hemolysis

(xli) The cells affected by leukaemia are :
(a) Plasma cells (b) Erythrocytes
(c) Thrombocytes (d) Leucocytes

(xlii) Genes involved in cancer are
(a) Tumour genes (b) Oncogenes
(c) Cancer genes (d) Regulator genes

(xliii) Oncology is the study of
(a) Living cells (b) Cancer cells
(c) Dead cells (d) Dividing cells

(xliv) The most common cancer in women is :
(a) Breast cancer (b) Skin cancer
(c) Cervix cancer (d) Leukaemia

(xlv) Breast cancer is an examle of
(a) Adenoma (b) Lymphoma
(c) Carcinoma (d) Sarcoma

(xlvi) Cancer treatment includes :
(a) Surgery (b) Radiotherapy
(c) Treatment with anticancer drugs (d) All of these

(xlvii) The most common type of cancer is man is
(a) Skin cancer (b) Lung cancer
(c) Cancer of prostate (d) Cancer of bladder

(xlviii) Which of the following is a cancer causing agent ?
(a) Tobacco (b) Radiation
(c) Smoking (d) All of these

(xlix) Which of the following is an oncogenic virus ?
(a) Herpes Simplex II (b) Papilloma
(c) Epstin-Bar (d) All of these

(l) The spread of cancerous cells to distant sites is termed :
(a) Metamorphosis (b) Metagenesis
(c) Metastasis (d) Metachrosis

(li) Adenoma refers to the cencer of
(a) Glands (b) Lymph nodes
(c) Blood (d) Muscles

(lii) Which one of the following is an anticancer drug ?
(a) Aspirin (b) Flagyl
(c) Streptomycin (d) Vineristin

(liii) Which of the following scientists got Noble Prize in 1989 for the studies on the genetic basis of cancer ?
(a) Philip Sharp and Richard Roberts
(b) David Baltimore and Howard Temin
(c) Michacl Bishop and Harold Varmus
(d) Stanley B. Prusiner

(liv) HIV attacks which of the following ?
(a) B-cells (b) T- cells
(c) Antigen Preventing cell (d) T-helpev cells

(lv) Which of the following is not a component of innate immunity ?
(a) Antibodies (b) Interferons
(c) Complement proteins (d) Phagocytes

(lvi) Which of the following is involved in defense mechanism of the body ?
(a) Lymphocytes (b) Neutrophils
(c) Macrophages (d) All

(lvii) During allergic reactions, which of the following is secreted ?
(a) Allergens (b) Histamines
(c) Immunoglobulins (d) Pyrogens

(lviii) Immunoglobulins are
(a) Antigen (b) Antibodies
(c) Antiseptics (d) Antibiotics

(lix) B-Lymphocytes are prouced by
(a) Liver (b) Thymus
(c) Spleen (d) Bone marrow

(lx) Cell-mediated immunity is due to
(a) B-cells (b) T-cells
(c) T-helper cells (d) All

(lxi) The cells which release the antibodies are
(a) Helper T- cells (b) B-cells
(c) Plasma cells (d) T-cells

(lxii) Antiviral substances are
(a) Antibodies (b) Antibiotics
(c) Interferons (d) Vaccines

(lxiii) The major phagoeytic cells are
(a) Lymphocytes (b) Mast cells
(c) Macrophages (d) Plasma cells

(lxiv) Which Immunoglobulin is the largest in size ?
(a) I_gA (b) I_gD
(c) I_gE (d) I_gM

(lxv) Vaccine for rabies was first produced by
(a) Louis pasteur (b) Edward Jenner
(c) Paur berg (d) None

(lxvi) Vaccination means introduction in our body of
(a) Weakened germs (b) WBCs from other animals
(c) Antibodies (d) All

(lxvii) The biochemical basis of vaccination was given by
(a) Louis paster (b) Salk
(c) Kohler (d) Malaria

(lxviii) Against which foreign organism (antigen) antibiotic is effective ?
(a) Virus (b) Bacteria
(c) Fungal infection (d) Protozoan

2. Fill in the blanks with suitable words :

(i) The immunity, present right from birth is known as _____ immunity.

(ii) The immunity generated on exposure to foreign antigens is known as _____ immunity.

(iii) Anti tetanus serum (ATS) administration generates _____ immunity in the body.

(iv) Toxoid is an example of _____ immunity.

(v) A part of an antigen that evokes an immune response is called _____.

(vi) Antibodies segregate with _____ class of serum proteins.

(vii) The stem of the 'Y' shaped immunoglobulin molecule carries out _____ functions.

(viii) Among all immunoglobulins _____ can cross the placental barrier.

(ix) During primary immune response, _____ immunogloulin is predominant.

(x) Immunoglobulin _____ is present in the mother's milk, tear and saliva.

(xi) Formation of antibodies against self antigens leads to an _____ disorder.

(xii) _____ released by mast cells causes inflammatory response.

(xiii) Humans get AIDS virus from _____

(xiv) The tests conducted for determining AIDS and typhoid are _____ test and _____ test respectively.

3. Answer the following in one or a few sentences :

(i) What is passive acquired immunity ? Explain.

(ii) What is an antigenic determinant (epitope) ?

(iii) Explain humoral immunity.

(iv) Explain about the antigen binding sites of an antibody.

(v) Mention about the effector functions of an antibody.

(vi) How do antigens inteact with their antibodies ?

(vii) What is a toxoid ? Name the bacterial diseases against which toxoids are used as vaceines.

(viii) What is an oral polio vaccine ?

(ix) What is immunosuppression ?

(x) Explain autoimmune haemolytic anemia.

(xi) What is an immune deficiency ?

(xii) Explain reticular dysgenesis.

GROUP - B
(Short Answer-type Questions)

1. Distinguish between :

(i) Amoeba and Entamoeba

(ii) Filaria and Malaria

(iii) Communicable and Non-communicable disease

(iv) Magna and Minuta Stage

(v) Infection and Infestation

(vi) Carcinoma and Sarcoma

(vii) Benign tumour and Malignant tumour

(viii) Sporogony and Gamogony

(ix) Innate immunity and Acquired immunity

(x) Cell mediated immunity and Humoural Immunity

(x) Vaccination and Immunization

2. Short answer types questions :

(i) What is the causative organism of filariasis and write a note on its prevention and control.

(ii) Write the names of five drugs to control malaria.

(iii) What are the different species of malarial parasite ?

(iv) What are the causes of non-communicable diseases ?

(v) What are the measures taken to control malaria ?

(vi) Write a short note on tumour and their types.

(vii) What is ascariasis and how it is controlled ?

(viii) Write a short note on amoebiasis.

(ix) What is AIDS ? How can it be prevented ?

(x) What is diabetes mellitus ? How can it be controlled ?

(xi) What are carcinogen ?

(xii) What STDs stand for ? Explain with examples.

(xiii) What is cancer ? Give its causes.

(xiv) Write down diferent types of cancer.

(xv) What is the causative agent of gonorrhoea ? What are its symptoms and treatment ?

(xvi) Explain Oncogenes.

(xvii) Explain tumour suppressor gene or antioncogene.

(xviii) Write a note on parasite.

(xix) Explain incubation period of malaria parasite.

(xx) What kind of physical changes are characterstic of adolescence ?

(xxi) What kind of psychological changes characterise adolescence ?

(xxii) Which is the most common skin problem that affects the youth in adolescence ? What are its causes ?

(xxiii) What is the cause of alcoholism ?

(xxiv) What are the effects of alcoholism in the body ?

(xxv) What are the moral and social implications of drinking ?

(xxvi) What are the reasons of drug abuse by the youth ?

(xxvii) Write briefly on the main classes of drugs in use.

(xxviii) What are the withdrawl symptoms that are seen after drug abuse ?

(xxix) What are the social and moral implications of drug abuse ?

(xxx) What are the effects of tobacco use in the body ?

(xxxi) What kind of diseases affect the body in smoking ?

(xxxii) What is mental illness ?

(xxxiii) What are the causes of mental illness ?

(xxxiv) What are the different types of mental disorders seen in man ?

GROUP - C
(Long Answer-type Questions)

1. What are pathogens ? Classify diseases and give a note on this.
2. Give the symptoms, infection, prevention and control of typhoid.
3. Describe the symptoms, diagnosis, treatment and control of malaria.
4. What are acquired and innate immunity ? Discuss the mechanical and chemical barriers of innate immunity.
5. Mention the factors causing cancer. Add a note on diagnosis and prevention of cancer.
6. Give the structure of HIV. Give an account of infection, control and prevention of AIDS.

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IMPROVEMENT IN FOOD PRODUCTION

CHAPTER 10

10.1 PLANT BREEDING :

We need to increase our food production in order to provide adequate nutrition to our ever increasing population. There are many different approaches to solve this problem like increasing area of land under cultivation and implementation of modern agricultural practices etc. The first approach has severe limitations because of many obvious reasons. However, the other approach provides enormous scope. The modern agricultural technology for improvement in food production needs seeds having better genetic make up so that it can grow under varied climatic conditions and be resistant to deseases. The net result will be better crop production in less space. Here, comes the role of plant breeding. It is the purposeful manipulation of varieties so as to evolve plants which can grow better, have superior quality so far as the yield is concerned and are resistant to many deseases. The technique of plant breeding have evolved from simple method of remote past to present in the form of selection, hybridization, introduction, mutation breeding and breeding for resistance. With the advanced knowledge in the fields of genetics, molecular biology and tissue culture etc., the plant breeding is now increasingly been carried out using tools of molecular genetics.

Plant breeders always strive to incorporate into the crop plants, the characters for improved crop yield, increased tolerance to enviromental stess factors like salinity. drought; resistance to pathogens and insect pests. Plant breeding programmes are implemented in an orderly manner all over the world in the institutions dealing with crop plants. The main steps in breeding new genetic variety of crop plant are-

10.1.1 Collection of germplasm :

Germplasm is the sum total of all the alleles of genes present in a particular crop and its related cultivated and wild specis. It consists of-

(a) Cultivated improved varieties

(b) Varieties that are no longer in cultivation

(c) Pure lines development by plant breeders

(d) Wild related species and such other types.

This helps to effective exploitation of natural genes. Hence, germ plasm collection is an essential requirement for plant breeding.

10.1.2 Evaluation and selection of parents :

The germ plasm is thoroughly evaluated by plant breeders to identity the desired characters. After completion of evaluation of traits, only those parents are selected which possess such desired characters. Then, the seeds were obtained and sown to verify the characters are successfully transmitted to subsequent generations.

10.1.3 Cross Hybridization among selected parents :

The selected parents may have limited extent of genetic variability. To obtain wide range of variability, cross breeding between genetically diverse parents are done. This is called hybridization.

The objectives of hybridization are :

(i) To evolve a variety with high yielding, high resistance to diseases, drought or water logging, higher nutrient value and better taste etc.

(ii) To produce useful variations by recombination of characters.

(iii) To produce and utilize hybrid vigour, i.e. the superiority of hybrid over its parents.

Depending on the nature of plants involved in the cross, there may be different types of hybridization such as :

(a) inter-varietal : cross between two varieties of same crop.

(b) intra-varietal : cross between different genotypes of the same variety.

(c) intra-generic : cross between two species of a genus.

(d) inter-generic : cross between two genera.

10.2 TECHNIQUES OF HYBRIDIZATION :

Techniques of hybridization need skilled hands since the process involves successful contact between stigma and pollen of desired plants. Normally, the parents selected for the corss are healthy and vigorous. The first step is hybridization is to ensure no pollination is effected before the intended artificial process. In order to achieve this, the following procedure is normally followed :

(a) **Emasculation :** This is a process where anthers are physically removed in self pollinated plants. In case, the physical removal is not prosible, other methods are adopted. For example, panicle of *Sorghum* is dipped in lukewarm (50°C) water for 10 minutes or in *Triticum*, the flowers are exposed to chemicals like 2, 4 dichloro pheneoxyacetic acid, maleic hydrazide etc.

(b) **Bagging :** After emasculation, flower buds are enclosed with bags to avoid getting pollens from undesired sources. Bagging is done with special papers or polythene bags. Care is taken to provide complete protection to the flowers.

(c) **Tagging :** The emasculated and bagged flowers or floral parts are tagged. Date of emasculation, date of pollination, the name of cross and other relevant details of the parents, if any are written. The name of the female parent is written first followed by a cross(x) sign and then, male parent.

(d) **Artificial pollination :** Pollens from the selected male parents are collected in suitable containers such as paper bags, tubes or dishes and then, taken to the receptive female plant where it is dusted with a fine brush. After that the female plant is securely sealed in a bag till the time of seed production.

(iv) **Selection and Testing of Superior recombinants :** Seeds are collected from the hybrids and F1 plants are raised. The F1 offsprings are allowed to self pollinate and seeds are collected to obtain F_2 generation. The aim is to procduce all the plants nearly homozygous for certain dominant desired genes so that they can breed true with superior traits.

(v) **Testing, release and commercialisation of new varieties :** The new selected hybrids are evaluated in respects of their superior a economic traits like disease rosistance, increased yields etc. Tese are, then, subjected to field testing under the watchful eyes of the breeders in order to establish the reliability. The process is repeated again and again and the findings are shared with scientific community and other stake holders. Once the new variety passes all the stringent testings, it is released for controlled use by farmers. The performance of the crop yield and its other desired results are observed for at least 3 seasons before its general commercial implementation.

Examples of some improved varieties :

(1) Wheat - Kalyan sona, Sonalika - these are semi-dwaf, high yielding and resistant to root desease, introduced to wheat growing belt of India.

(2) Rice - Along with the above wheat varieties, rice varieties such as IR-8 and Taichung and their derivatives Jaya and Ratna varieties introduced around the same time in India. All these contributed to the quantum jump in food production which is called ‘green revolution’.

10.3 PLANT BREEDING FOR DISEASE RESISTANCE :

A large number of fungi, bacteria, viruses, nematodes attack crop plants and cause several types of diseases. As a result of pathogenesis, crop yield is reduced to a great extent, even 20 to 30 per cent of its orginal potential. Hence, it is necessary to breed and develop varieties which are resistant to diseases. This can boost crop production. Again, this biological

preventive method can reduce dependence on fungicides, bactericides etc. In order to develop crop varieties resistant to disease, one has to know the genotype of the host crop plant, pathogen as well as the condition of the environment in which the plant is cultivated and pathogen is transmitted.

Here, breeding is carried out by the conventional breeding methods or by mutation breeding. Since limited number of disease resistant genes are present and indentified in a number of crop plants or their wild relatives, mutation is induced in plants through a variety of ways, then screening is done and desiable genes iddentified. Plants with these changed characters are either multiplied directly or can be used in breeding.

Mutation : It is the only means of variability through change is the genetic make up of the concerned organism. Hence, it results in creation of new character in the offspring which was not seen in the parents. It can be induced artifically through the use of chemicals or radiations. The plants that have described character as source of breeding is called mutation breeding several wild relatives of cultivated species have shown disease resistant genes but have low yield. Such genes are identified introduced into high yielding susceptible varieties in order to develop desease resistance and high yielding traits, in this method.

10.4 BREEDING FOR RESISTANCE TO INSECT PEST :

Like desiease, insects are major causal factor of biotic stress in crop plants. Insects attack all crop plants and cause considerable loss in the production. As a result of insect attack, there may be (i) reduced growth or stunting in plants (2) damages to the vegetable and reproductive parts (3) premature defoliation (4) wilting of the plants. Insects cause approximately 14% loss in crop production.

There are two important methods of insect control (a) chemical (b) Bioligical. Chemicals in from of pesticides may cause damage to environment. But biological methods may be developed by use of biological pesticides like neem cakes, *Datura, Impomoea* parts or by use of resistant varietis. Development of genetic resistance by manipulation of genotypes resistant to insect pests is new, the most preferred method of eliminating pest attack. Here, the breeder has to know th high yielding susceptible varieties to a particular insect pest and he has to replace the susceptible gene here with resistant one.

10.5 PLANT BREEDING FOR IMPROVED FOOD QUALITY :

Aim of the plant breeding is not only the enhancement in the food production but it aims at improvement in the food quality. Quality may include flavours, colours, shape, size, degree of damage, nutrient levels and traits that permit greater perceived food safety or environmental sustainability, superiority for multiple quality traits and yield traits is esential for economic substainability in a successful variety of crop plant.

10.6 BIOFORTIFICATION :

Majority of the world population suffer from deficiencies of vitamins and minerals. There is a need for multiple complementary strategies to address micronutrient deficiencies. Biofortification represents one promising startegy to enhance the availability of vitamins and mineral for people whose diets are dominated by micronutrient deficient the staple food crops. It involves identification or varieties of crop that naturally contain high densities of certain micronutrients. Plant breeders use these varieties to develop new, productive and 'biofortified' crop lines for farmers to grow, maket and consume.

Examples of biofortified crops : Maize with high betacarotene traits has been shown to be an efficacious as suppllements of this orange coloured maize were released in Zambia in 2012. They yield at par with traditional varieties and have been shown to have nutritional impact.

Rice biofortified with zinc was released to farmers in Bangladesh in 2013. The biofortified rice varietes have zinc content, i.e. 30% higher than local varieties. The new rice matures faster than some traditonal ones and have zinc in the endosperm rather than periphery which is usually lost during polishing.

Now-a-days, breeders are striving to develop improved nutritional quality in the following aspects of crop plants, besides vitamins and minerals :

(a) protein content and quality.

(b) Oil content and quality.

10.7 TISSUE CULTURE :

Tissue culture is the process of maintenance and growth of cells and tissues in suitable, aseptic, artificial nutrient medium. Plant tissue culture process involves the culture of plant cells, tissues and organs. This process is based on the unique property of plant cells known as totipotency. Gottilieb Haberlandt (1902) who discovered totipotency is also credited for attempting to cultivate plant leaf cells in simple nutrient medium for the first time. In the recent times, plant tissue culture technique has become a major tool in crop improvement, experimental biology and also in fundamental or applied research.

10.7.1 Tissue culture techniques and steps

Some basic steps are followed in plant tissue culture which are described below.

Table-10.1 : Some commercially, important plant developed by tissue culture

Crops :	*citrus,* lettuce, Banana, Papaya etc.
Horticultural :	*Chrysanthemum*, *Gladiolus,* Rose, Orchids, Gerbera etc.
Vegetables :	*Capsicum,* Cauliflower, Seedless tomato etc.
Silvicultural :	Pine, Red wood etc.

10.7.1.1 Explant selection

Explant is the plant part used for tissue culture. Explant from healthy and young part of the plant is used. Parenchyma from stems, rhizomes, tubers, roots is easily accessible and respond quickly to culture condition.

10.7.1.2 Sterilization :

Sterilization means elimination of all living organisms (particularly microbes). The tissue culture is carried out in completely aseptic condition for which there is a need to properly sterilize the glassware, culture media and explants. The explants are surface sterilized by repeated washings in sterile water and by using disinfectants such as mercuric chloride, hydrogen peroxide etc. The glassware, culture media and other instruments are sterilized in autoclave where sterilization is done in steam under high pressure or in a pressure cooker.

10.7.1.3 Preparation of nutrient or culture medium :

The culture medium is prepared in aseptic condition. The basic constituents of any culture medium are:

Inorganic nutrients: Inorganic nutrients inculde macronutrients as salts of nitrogen, phosphorous, potassium, calcium, magnesium and sulphur and micronutrients like boron, molybdenum, copper, zinc, Iron and chloride.

Source of carbon

Sucrose is used as the source of carbon.

Growth hormones and vitamins

Several growth hormones like 2,4,D, cytokinins-benzylaminopurine, kinetin, myoinositol, IAA, NAA are used. vitamin like pyridoxine-HCL added to the medium.

After the addition of all the ingredients in appropriate proportion, agar-agar is added to prepare a solid medium. In some types of culture (like root culture) liquid medium is used (no agar-agar used).

10.7.1.4 Inoculation :

Inoculation is the process of the transfer of explant to suitable nutrient medium contained in culture vessels. This is done in sterile condition either in an inoculation chamber or under laminar are flow. After the inoculation the culture vessels are maintained in controlled temperature and light. The suitable temperature for tissue culture ranges between 18-25^{0}c.

10.7.1.5 Callus formation and its culture :

A **callus** is an amorphous mass of loosely arranged thin walled parenchyma cells developing from proliferating cells of parent tissue (Dodos & Roberts, 1985). The nutrient medium supplemented with auxins induces cell division and soon the upper surface of explant is covered by callus. The callus has the biological potential to develop normal root, shoots and ultimately forming a plant. Callus is formed through three developmental stages: induction, cell division and differentiation. Callus formation is governed by the source of explant, nutritional composition of the medium and environmental factors. During the induction period the metabolic rate of

cells is stimulated. Owing to increased metabolic rate cells enter to cell division stage. In the third phase, cellular differentiation and expression of certain metabolic pathways start leading to secondary products. When callus are grown on a nutrient medium for a long time, it becomes essential to subculture it in fresh media.

10.7.1.6 Organogenesis :

Organogenesis means the development of organs like root, shoot and leaves (but not embryo). Organogenesis starts with stimulation caused by the chemical of medium, substances carried over from the original explants and endogenous compounds produced by the culture. Organogenesis can be induced with the application of varying proportions of auxin and cytokinin. Skoog and Miller (1957) demonstrated that a high ratio of auxin: cytokinin (3 : 0.02) stimulated root formation in tobacco callus but a low ratio of the same (3 : 0.2) induced shoot formation.

10.7.1.7 Somatic Embryogenesis (Fig. 10.1) :

This is the process of inducing embryo formation from somatic cells of cultured plant tissue. The embryo thus developed is known as embryoids. Two different nutritional media are required to obtain embryoids. First medium contains auxin to initiate embryogenic cells. Second medium lacks auxin or has reduced level of auxins for subsequent development of embryonic cells into embryoids and plantlets. The embryogenic cells pass through three different stages, e.g. globular, heart shaped and torpedo shaped to form embryoids. Some plants in which somatic embryogenesis has been induced in vitro *are Atropa belladona, Brassica oleracea, Carica papaya, Coffea arabica, Citrus cinensis, Daucus carrota, Nicotiana tabacum* etc.

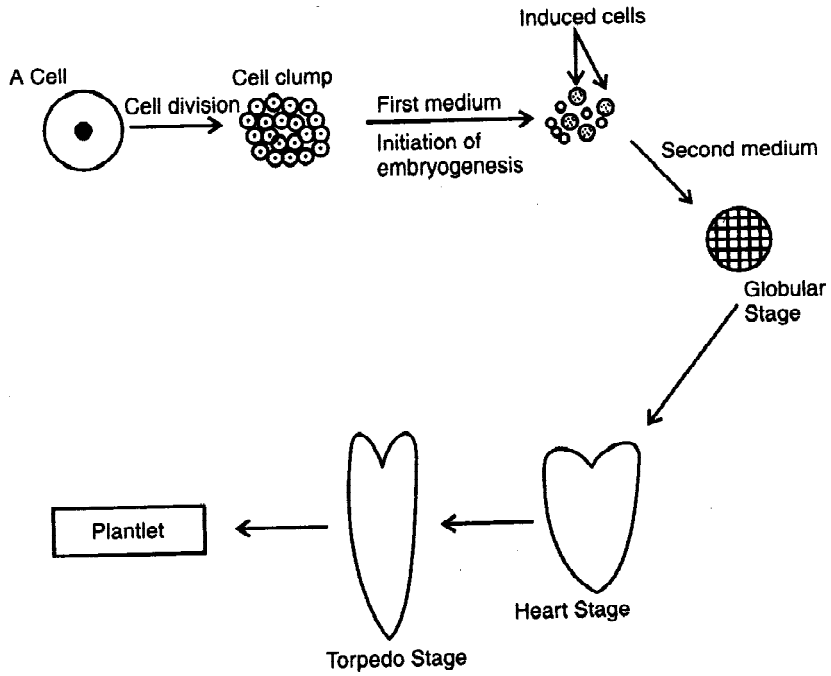

Fig.10.1. Events of somatic embryogenesis.

Somaclonal Variations: In 1981, P.J. Larkins and W. R. Scowcrott at the Division of Plant Industry, C.S.I.R.O. Australia gave the name somaclonal variation to genetic variability generated during tissue culture. Though the cultured tissues are grown from single explant, over a long period of maintenance, genetic variabilities are marked in the cultures. This may be due to (i) reflection of heterogeneity between cells and explant tissues, (ii) a simple representation of spontaneous mutation rate or (iii) activation by culture environment of transposition of genetic materials. Somaclonal variants of leaf callus culture of *Solanum tuberosum* have shown characters like disease resistance, variations in maturity dates of tubers, yield and shape.

Cell suspension cultures: Cell suspension is prepared by transferring a fragment of callus (about 500 mg) to liquid medium (500 ml) and separating them aseptically in a shaker to make the cells free. The suspension then includes single cell, cell aggregates, residual including dead cells. A good suspension contains high proportion of single cells. Cell suspension cultures have many advantages over the callus cultures as below :

(i) The cell suspension can be pipetted.

(ii) They are less heterogeneous.

(iii) They can be cultured in volumes up to 1,500 liters.

(iv) They can be subjected to more stringent environmental controls.

(v) They can be manipulated for production of natural products.

10.7.2 Protoplast culture and Somatic hybridization :

When the cell wall of the plant cell is mechanically or enzymatically (cellulase and pectinase) removed the naked cells are known as protoplast. The protoplast remains biologically active and in tissue culture the somatic cell protoplasts are induced to fuse to produce somatic hybrids and cybrids. The protoplast fusion is induced by polyethylene glycol (PEG) or under high voltage electric current. When protoplasts of two different cell lines are induced to fuse, it results in coalescence of cytoplasms. The nuclei of two protoplasts may or may not fuse even after fusion of cytoplasms. The resulting binucleate cells are known as heterokaryons or heterocytes. When the two nuclei are fused a somatic hybrid is produced. But, when cytoplasm are fused and

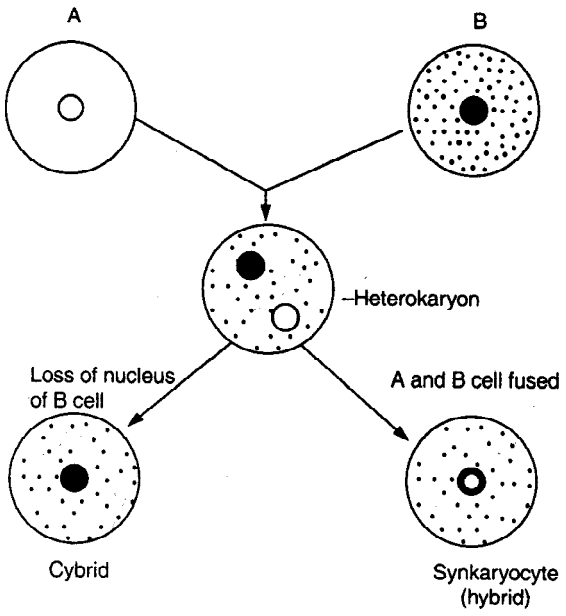

Fig.10.2 : Hybrid/cybrid production through protoplast fusion.

one of the two nuclie is lost a cybrid or cytoplasmic hybrid or heteroplast is produced (Fig. 10.2). The fused protoplasts are grown on suitable nutrients medium. The protoplasts regain cells walls, divide to form callus and finally develop to plantlets.

Both hybrids and cybrids have their utility in crop improvement. Through protoplast fusion somatic hybrids of genetically different cell lines or species can be produced. These genetically different cell lines are otherwise not sexually compatible for sexual hybridization procedures.

In the field of pest and disease resistance and transfer of some qualitative and quantitative characters somatic hybridization through protoplast fusion has shown good results.

Some genetic factors are carried in cytoplasmic inheritance, like male sterility in some plants, susceptibility and resistance to some pathotoxins and drugs etc. Therefore, production of cybrids can help in transfer of cytoplasmic genetic information. Cybrid technology has been successfully applied to Rice, carrot, *Brassica sp. Citrus,* tobacco and sugar beet.

10.7.3 Anther culture and production of haploids :

Anther, the male reproductive organ after microsporogenesis contain pollens or microspores which are haploid. Anthers or pollens are cultured to raise haploid plants. These haploids may not be of any commercial use but can be subjected to colchicine treatment to double their chromosome number so that completely homozygous diploid plants can be obtained. The haploid plants can also be useful in screening of recessive mutation because in diploids or polyploids screening of recessive mutation is not possible.

10.7.4 Application of Plant Tissue Culture :

(1) Micropropagation or clonal propagation :

Through the process of cell, tissue or organ culture large number of plants can be raised in small area and in less time. As all the plants developed in a tissue culture are genetically identical, this procedure is also known as clonal propagation. Banana, Begonia, cardamoms, coffee, bamboo, grapes are some examples of micropropagated plants.

(2) Production of virus free plants

Asexually reproducing crop plants are prone to viral infection and the virus spreads through the vegetative organs for propagation like stem, tuber, rhizome etc. Cambium culture in such plants produce virus free plants.

(3) Synthetic seeds/artificial seeds

The somatic embryos/plantlets encapsulated in protective capsules of calcium alginates to prevent dessication are known as artificial seeds or synthetic seeds. These are used for rapid propagation of crop plants. The farmers can easily use them like normal seeds in their fields.

(4) Secondary metabolites production

Plant tissue culture involving large scale cell suspension cultures are in use for commercial production of secondary metabolites like alkaloids, tannin, resin, latex etc. These would have been difficult to synthesize through chemical synthesis due to huge cost. Some of the examples of secondary metabolites produced in plant tissue cultures are given below:

(i) Taxol, an anticancer drug against ovarian and breast cancer is obtained from cultured cells of *Taxus*. Actually the cultured cell of *Taxus* produce a chemical very similar to taxol which is then chemically converted to taxol.

(ii) Similarly cultured cells of *Digitalis lantana* are used to convert digoxin to digitoxin. This is used as a drug in cardiac treatment.

(5) Embryo Rescue

It has been observed that in some instances of inter specific crosses though pollination and fertilization are successfully completed, the embryo does not develop after an initial divisions.In such cases, immature embryos are dissected out from the fruit (seeds) and grown in nutrient medium to develop into plantlets. This technique of growing immature embryo is known as embryo rescue. At the International Crop Research Institute for Semi Arid Tropics (ICRISAT), Hyderabad this technique has been used to improve pigeon pea, chick-pea, ground nut etc.

(6) Endosperm culture

Endosperm culture is employed to produce triploids. This is used to produce seedless apple, pear, citrus etc, having good commercial values.

10.8 SINGLE CELL PROTEIN :

On an average, the microbial blomass contains about 45 to 55 per cent protein, although in certain bacteria the protein content is as high as 80 percent. The microbial biomass also contains other essential nutrients. Therefore, it can be ideal supplement to our conventional foodstuff and protein requirement. The term 'single cell protein' refers to any microbial biomass obtained from uni or multicelluar microorganisms such as algae, fungi, bacteria which can be the source of food or feed additive. The term is not restricted to only single cells from which protein is obtained.

Large scale production of proteins from bicrobial biomass in place of costly traditional methods has following advantages.

(i) Microorganism multiply at a very fast pace

(ii) They have high protein content

(iii) They can use wide variety of carbon sources even the waste products

(iv) High yielding strains can be produced easily

(v) Production of microbial biomass is independent of seasonal and climatic hazards.

Organisms used :

Algae like *Chlorella, Scendedesmus, Spirulina* etc. are now-a-days used for the production single cell protein. Particularly, *Spirulina* is cultured, dried, powdered and then used in the form of tablets. It contains nearly 60 per cent protein, essential vitamins and unsaturated fatty acids.

Production of edible mushrooms has gained momentum all over the world. These have very high amount of protein and are easily adored by all for its goods taste. The fungus, *Chaetomium cellulyticum* is abo a good source of protein. Besides, some species of bacteum, Pseudomonos methyltrophluns, yeasts such as *sacchromyces lipolytica* are used as the source of single cell protein.

Process :

Depending on the organism, the process of obtaining single cell protein (scp) varies. However the following steps are followed unobtaining SCP

(i) Preparation of suitable medium with definite carbon source

(ii) Prevention of contamination

(iii) Production of the desired microorganisms only

(iv) Separation of microbial biomass and its adequate processing

If the microorganism is autotroph like *Spirulina*, then there is no need of dissolved carbon source is the medium. But heterotrophs like fungi and bacteria need specific carbon sources for the best production of proteins.

Requirement for the organisms for single cell protein source :

The microorganisms used for single all proteins must satisfy following characteristics, they

(i) should be nonpathogenic to plants, animals and humans,

(ii) have good nutritional value,

(iii) free from any toxic compound

(iv) cost of production may be low.

10.9 APICULTURE :

The description of honey being used as food and medicine appears in ancient scriptures like Upanishad, Vedas and Puranas. It is the nectar and pollen of flowers collected and stored in a specially designed honey comb or bee hive by an insect known as honey bee. Honey bees live in a colony. They build a nest, especially on the twigs of trees. The nest, known as a honey comb or bee hive is built in the vicinity of plenty of flowers. This facilitates the collection of nectar with ease. The comb has many hexagonal cells, where the queen bee and the juveniles live and honey is stored. Honey bees have an incredible social organization, sense of discipline and division of labour, which human envies. Considering the commercial importance, honey bees are cultured artificially in specially designed bee hives and honey is harvested. This practice is known as **bee keeping** or **apiculture**.

10.9.1 Species of Honeybee :

Four common species are of commercial importance. They are:

1. ***Apis dorsata*:**
   - Comparatively larger in size, therefore, referred to as giant honey bee.
   - Aggressive and migratory in behavior.
   - Difficult to domesticate.
   - Yeild maximum honey.
2. ***Apis indica*:**
   - Indian bee found both in forests and plains
   - Build nests in secluded places.
   - Docile in nature and hence are domesticated.
3. ***Apis florea*:**
   - Not gregarious in nature
   - Yeild small amount of honey and therefore, are not domesticated.
4. ***Apis mellifera*:**
   - Gregarious and docile.
   - Although yield less honey, it is the most commonly domesticated species.

10.9.2 Social Organization :

(i) Castes of honey bee: 40-50 thousand honey bees live in an organized colony divided into three castes: **(i) queen**; **(ii) drone**; and **(iii) worker**. (Fig. 10.3)The queen and the workers are fertilized (diploid) females, while the drones are haploid males. One among the diploid females is fed with the **royal jelly** and becomes the queen. The rest of the diploid females

become the workers. The queen bee is fertilized by the haploid males in the air following a characteristic dance. Following fertilization, the queen bee lays two types of eggs: diploid and haploid. The diploid eggs develop into diploid females, while the haploid eggs develop into haploid males, which are called drones. This process of development of males without fertilization is termed as **parthenogenesis**.

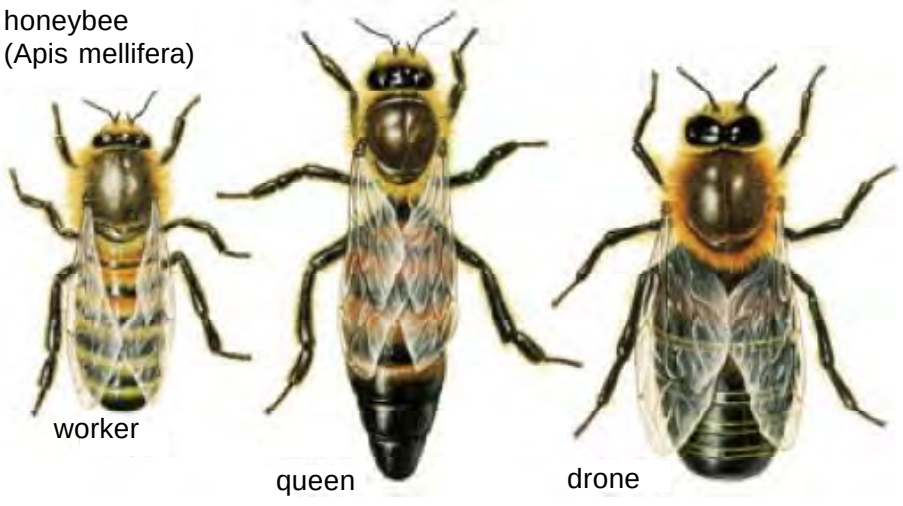

Fig. 10.3: Three castes of honey bee (*Apis mellifera*).

(ii) Life Cycle (Fig. 10.4) : Fertilization in bee is aerial. The queen bee exhibits a characteristic flight, called **nuptial flight**. The virgin queen bee is followed by many drones during flight. This is called **swarming**. One of the drones copulates with the queen. The sperms received are stores in the spermatheca. Soon after copulation, the drone dies. The queen bee lays fertilized or unfertilized eggs at will. The fertilized eggs develop into workers, while the unfertilized eggs develop into drones by parthenogenesis. The queen lays one egg in one brood cell. The development of the egg is indirect i.e. the egg hatches into a larva. The larva then changes into a pupa. The pupa metamorphoses into a miniature honey bee. On feeding, the miniature bee turns into a mature adult.

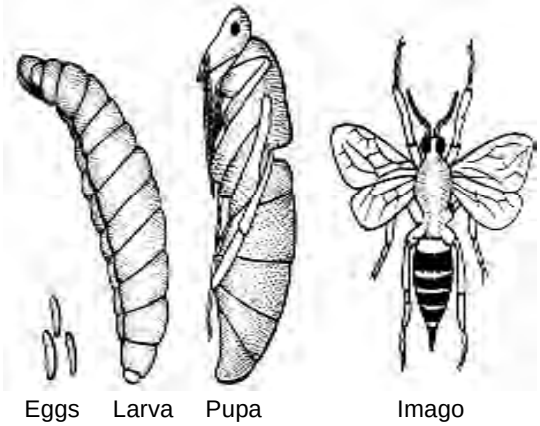

Fig.10.4 : Different stages in the life cycle of honey bee.

In early summer, when the hive is loaded with honey and overcrowded by bees, the queen leaves the hive with some drones

and workers to establish a new colony at some other place. This process is known as **swarming**. In the old hive, a worker bee is fed with royal jelly to become the new queen of the colony.

(iii) Honey Comb: The house of the honey bee is known as comb or hive. It consists of many hexagonal cells made from wax secreted by the worker bee [Fig. 10.5 (a) & (b)].

(a) (b)

Fig.10.5: Honey comb. (a) lower magnification and (b) higher magnification showing juveniles lodged in cells.

The juveniles are reared in the lower and central cells called **brood cells**. There is no cell for adults. The adult bees cluster around the hive. The upper cells are meant for storing honey.

10.9.3 Bee Keeping :

The domestication of honey bee for commercial purpose is known as **bee keeping** or **apiculture**. Two important aspects must be looked into while practicing apiculture. Firstly, the bees should be docile and of gentle temperament. For this reason, *Apis mellifera* is the species of choice. Secondly, suitable flora should be in plenty and in close range of the hive. The rich nectar yielding plants are neem, jamun, soapnut etc. Some pollen yielding plants are maize, rose and sorghum. Plants like plum, cherry, sheesham, coconut, guava and mustard yield both pollen and nectar. There are two methods of bee keeping: **indigenous** and **modern**.

(i) Indigenous Method: Two types of hives are used in the indigenous method: **fixed hive** and **movable hive**. In fixed hive, the bees themselves construct the hive on the wall or on the twig of a tree. In moving type, an empty box or an earthen pot is placed in the shade. There are two openings, one for the entry and the other for exit for the bees. The bees come to the place of their own accord and construct the hive. The honey is extracted by driving away the bees from the hive by exposing burning fire at night. The comb is cut into pieces and squeezed thoroughly to extract honey. The honey extracted by this method is contaminated by unnecessary things.

(ii) Modern Method: In this method, a movable hive is constructed by a wooden box. There is an opening for the entry and exit of the drones and the workers. The queen, once placed inside, does not go out. The hive consists of six parts: stand, bottom board, brood chamber, super, inner cover and top cover (Fig. 10.6).

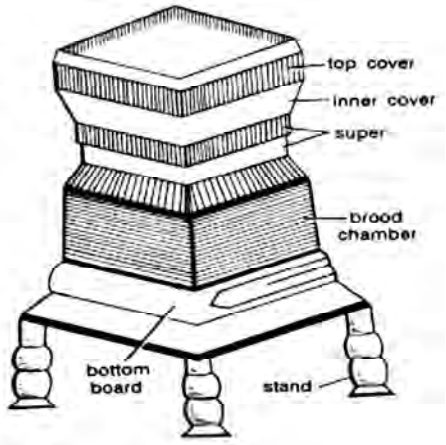

Fig.10.6: A typical movable hive.

The **stand** is the basal part on which rests the hive. The stand is made sloppy so that rain water is drained off quickly. The **bottom board** is situated above the stand. It bears two openings, one for the entry and the other for exit for bees. The **brood chamber** is the most important part of the hive. It is the largest chamber of all and provided with 5 to 10 frames, each frame bearing a wax sheet with hexagonal frames. The sheet is held by two wires in a vertical position. The bees use the hexagonal frames in constructing chambers. Each sheet of wax is known as a **comb foundation**. The **super** is provided with many frames, each containing a comb foundation. This serves as an additional space for the expanding hive. The inner cover is a wooden cover on top of the super. It has many small openings for ventilation. The top cover is mainly protective. It is made from zinc sheet and made sloppy for draining the rain water.

The honey is collected by centrifugation so that the cells in the comb have their structural integrity and can be reused in another cycle of honey harvest. Another important aspect of apiculture is that as long as the queen bee is there, the workers and drones are there and there is honey production. Therefore, care is taken to see that the queen does not escape for several cycles of harvest.

(iii) Chemical Composition of Honey:

Constituent	Percentage
Levulose	38.9
Dextrose	21.8
Maltose and other sugars	8.81
Enzymes and pigments	2.21
Ash	1.0
Water	17.20

(iv) Bee's Wax: Bee's wax is a useful byproduct extracted from the hive. It is yellowish in colour insoluble in water but soluble in organic solvents like ether. It is secreted by the workers and deposited in the form of flakes. It protects the hive from getting drenched in water. It is used in the manufacture of face cream, paints, ointments, insulators, polishes and lubricants.

10.10 ANIMAL HUSBANDRY :

10.10.1 Dairy Farm Management :

Protein is as much important as the other constituents of human diet. Human gets this share of the diet both from plant and animal sources. The plant sources constitute pulses, nuts, etc., while animal sources are fish, egg, meat, milk and milk products. Milk and milk products account for 9.2% and 12.2% of protein intake in rural and urban areas of the country, respectively. Milk is produced through a practice known as **dairy farming**.

Dairy farming integrated with agricultural farming has been the base of Indian economy since time immemorial. It was practiced by the domestication of traditional livestock animals such as cattle, buffaloes and goats. It has served as a means of livelihood earning in millions of rural household. With the vast potential of livestock animals, India has become a leader in milk production in the world with 155.5 million tons of world's 863.0 million tons of milk production in 2015-2016. The targeted production in 2016-20017 is 163.74 million tons. The total estimated production has been 105.42 million tons (summer and rainy season) showing an achievement of 64.38 per cent of the target. At this rate, the milk production is expected to reah 191 million tons in 2020. Gradually, there is a shift from this traditional practice to a scientific method with the raising of improved breeds of cattle, buffaloes and goats and better farm management practice. Consequently, it became an organized sector earning livelihood to millions of landless labourers, small and marginal farmers and medium to large farmers. India emerged as the leader in milk production in 1997 and continues to do so till date.

10.10.1.1 Breeds of Dairy Cattle :

There are 30 breeds of cattle, 10 breeds of buffaloes and 20 breeds of goats in the country. The indigenous breeds of cattle are classified into: (i) **milch breeds**; (ii) **dual purpose breeds**; and (iii) **draught breeds**.

(i) Indigenous Milch Breeds of Cattle: These are high milk producers. These include: **Sahiwal**, **Red Sindhi**, **Gir**, **Tharparkar**, and **Rathi**.

Sahiwal [Fig.10.7(a)]:

(i) Native of Montgomery district and its adjoining places in Pakistan.

(ii) Also native to Ferozpur, Amritsar and Gurudaspur districts of Punjab.

(iii) Red to light brown in colour.

(iv) Yeilds about 1,350 kg of milk in lactation for 305 days.

Red Sindhi [Fig.10.7(b)]:

(i) Native of Karachi and Hyderabad districts of Sindh province of Pakistan and some states of North-Western India.

(ii) It is an important dairy cattle breed in Indian sub-continent.

(iii) Yeilds about 1,800 kg of milk in lactation for 305 days.

Gir [Fig.10.7(c)]:

(i) Found in Junagarh, Bhavnagar and Amreli districts of Gujarat.

(ii) Also found in Rajasthan, Madhya Pradesh and Northern parts of Maharastra.

(iii) Yeilds about 1400 kg of milk in lactation.

Tharparker [Fig.10.7(d)]:

(i) The name is derived from the place of its origin, the Thar Desert.

(ii) Found in Tharparker district of southeast Sindh in Pakistan.

(iii) Yeilds about 1750 kg of milk in lactation for 285 days.

Rathi [Fig.10.7(e)]:

(i) Named after the pastoral tribe Raths of Rajasthan.

(ii) Found in Bikaner district of Rajasthan.

(iii) Have a mixed inheritance of Sahiwal, Red Sindhi and Tharparkar cattle breeds.

(iv) Yeilds about 1500 kg of milk in lactation.

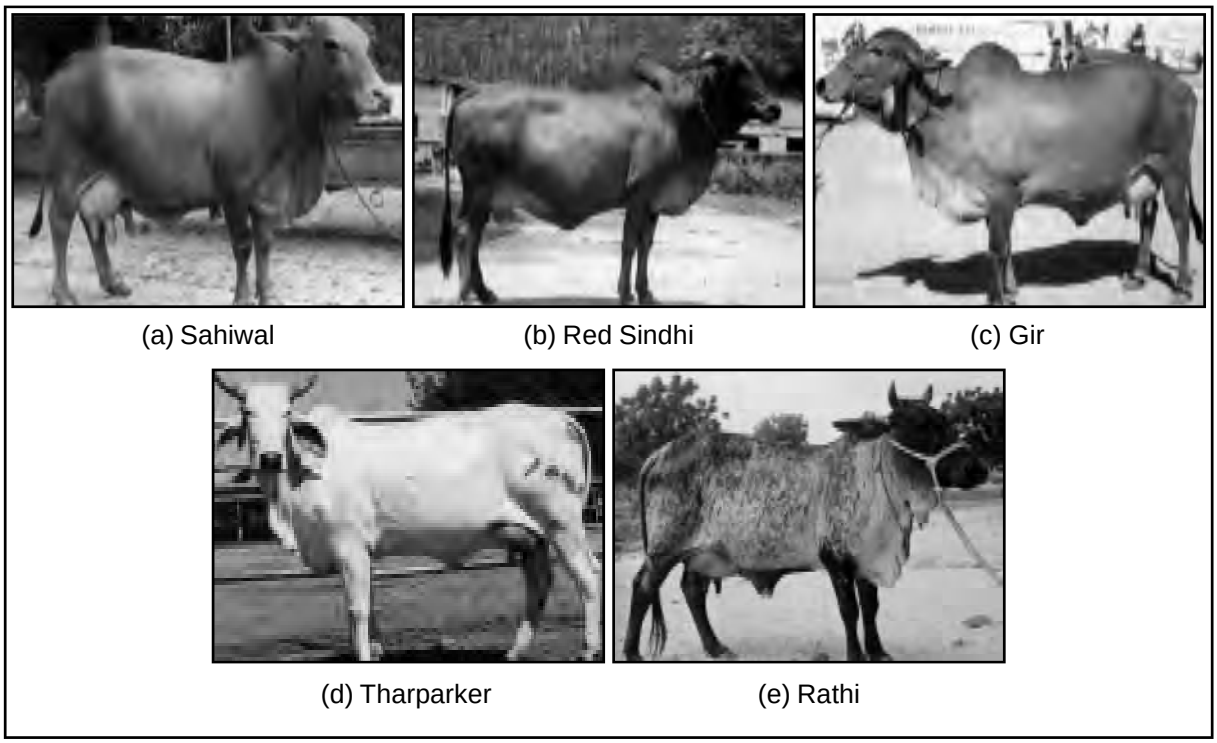

Fig. 10.7 (a-e) : High yielding indigenous breeds of cattle

Dual-purpose breed cows yeild average quantity of milk, while the males are good working bullocks. Some examples are, **Deoni**, **Hariana**, **Kankrej** and **Ongole**.

(ii) Exotic Milch Breeds of Cattle: These are high milk yielding cattle breeds of other countries, which are cross bred with indigenous breeds for producing high yielding hybrids, adapting to Indian conditions.

Some common exotic breeds used in crossbreeding programmes are Holstein-Friesian of Netherlands [Fig. 10.8(a)], Jersy of Europe and America [Fig.10.8(b)], Brown-Swiss of Switzerland and Red Dane of Denmark.

(a) Holstein Friesian

(b) Jersy

Fig.10.8 (a) & (b) : Some exotic milch breeds of cattle.

(a) Karan Swiss

(b) Karan Fries

Fig.10.9 (a) & (b) : Cross-bred strains of cattle.

(iii) Cross-bred Strains of Cattle: Karan Swiss [Fig.10.9(a)] : It is developed by crossing Brown-Swiss bull with Sahiwal or Red Sindhi cows. **Karan Fries [Fig.10.9(b)]:** It is developed by crossing Holstein-Friesian bulls with Tharparkar cows. **Frieswal:** It is developed by crossing Holstein-Friesian bulls with Sahiwal cows. **Sunandini:** It is developed by crossing Brown-Swiss bull with Sahiwal or Red Sindhi domestic household cows of Kerala.

Daught breed cows are poor milkers, while the bullock are superior in quality. Prominent among these are **Nagpuri, Bechaur, Malvi, Khillari, Amritmahar**, etc.

(iv) Milh Breeds of Buffaloes: There are seven indigenous dairy breeds of buffaloes, namely **Murrah**, **Nili-Ravi**, **Bhadawari**, **Jaffarabadi**, **Surti**, **Mehsana** and **Nagpuri**. Besides providing milk, buffaloes are also used for carting, ploughing and other agricultural operations.

(v) Milch Breeds of Goats: Among all milk producing animals, goats can adapt to varying environmental conditions. These are the source of income of a large number of rural people, especially the socially and economically backward classes of the society. The important indigenous milch goat breeds are: **Jamunapuri**, **Beetal**, **Zalawadi**, **Jhakrana**, and **Surti**.

10.10.1.2 Housing :

A house is essential for the dairy animals to provide them a conducive atmosphere for effective growth, reproduction and milk production. The house should be designed keeping in view the number of animals, flooring, ventilation and drainage. There should be sufficient floor area to avoid unnecessary competition for feeding, drinking water and taking rest.

Mainly, there are two housing systems for cattle: **loose housing** and **closed housing**. In loose housing system, the cattle are kept free in an open paddock in a group of 40-50. On the other hand, in closed housing system, the shelter is closed on all sides except ventilation outlets. The closed system should have proper lighting, cooling and aerating arrangements. The floor should be made of RCC or made with concrete flooring tiles having rough anti-skid surface. The drainage should be such that the floor remains dry. There should be a number of feeding and drinking water troughs to avoid competition and infighting. In an open paddock, half of the floor should be sand-bedded and the other half, brick-paved. In both systems, appropriate waste management system should be there for better sanitary conditions. Solid wastes may be dumped into a pit for biodegradation into compost and the liquid waste containing mostly urine may be drained to agricultural field or collected in a pit.

10.10.1.3 Nutrition :

Appropriate nutrition accompanied by proper health care of dairy animals is of prime importance for good yield of milk and hence more profit.

Feeding of rural dairy animals is mainly based on grazing of native pastures of low productivity. In South Asian countries including India, the cattle and buffaloes are fed on **wheat**, **paddy** and **ragi straws**. These are supplemented with small amount of grass. Generally the food is not supplemented with concentrate in growing, working, pregnant and dry animals. Only the lactating animals are given an additional supplement of **byproduct concentrates** such as **oil cakes**, **brans** and **pulses**. Cattle get 60% of the dry matter from crop residues. Due to a large volume of crop residues, such as cereal straw, the cattle are deficient in many essential nutrients such as proteins and minerals. The consequence is many deficiency diseases. Hence, the farmers balance the feed containing crop residues and grass with **leguminous fodder** or with byproduct concentrates like brans and oil cakes and grains or pulses depending on the

availability for meeting the protein requirement. This food should be supplemented with the required amount of common salt and trace element mixture. Common salt and mineral mixture licks are commercially available. Urea, molasses and mineral block licks containing deficient minerals have proved useful across the tropics.

Next question is how much food the animals are to be fed. Body condition scoring (BCS) is an easy to learn method for dairy men to evaluate the status of the animal's nutrition for improved milk production. BCS score is based on the quantity of reserve fat in the body of the animal. A score of 1-6 has been suggested (Table-10.2).

Table - 10.2: Body condition scores and corresponding state of health of cattle.

Score	Condition
1	Very Poor
2	Poor
3	Moderate
4	Good
5	Fat
6	Very Fat

10.10.1.4 Healthcare :

The healthcare of the dairy animals is an important part of dairy farm management for high productivity and hence more profit. The incidence of diseases is minimized by providing the animals with good shelter, balanced diet, good ventilation and improved sanitation. Despite all these facilities, the dairy animals are prone to many diseases caused by pathogenic organisms. Some common diseases of the dairy animals such as cattle and buffaloes are **foot and mouth disease**, **haemorrhagic septicaemia**, **black quarter**, **tuberculosis**, **brucellosis**, **mastitis**, **pneumonia**, etc.

Vaccination Programme: Vaccination is a method of long term induction of active acquired immunity against pathogenic organisms in an animal by injecting the modified organism itself or a part of it. The agent, inducing immunity, is known as a **vaccine**. Several cattle vaccines have been formulated for developing immunity against diseases like foot and mouth disease, haemorrhagic septicaemia, black quarter, tuberculosis and brucellosis. All vaccines except brucellosis vaccine impart short term protections. Brucellosis vaccine, however, imparts a lifelong protection against the disease. The standard vaccination schedule should be strictly adhered to.

10.10.1.5 Reproduction and Propagation :

The successful viability of a dairy farm depends on the successful reproduction management of the animals. The maintenance of high fertility rates in these farm animals is an advantage. Generally speaking, a calf per year is said to be an optimum fertility rate. The first step in this management process is the selection and rearing of a bull. The young bull is selected on the basis of its superior genetic potential and reproductive characters. It is fed with a balanced diet and a routine health check up is conducted. The young bull is vaccinated by following the standard vaccination schedule.

The old method of propagation is substituted by **artificial insemination**. In this method, the semen is collected from a superior bull and the cows are inseminated. Before insemination, the semen undergoes several investigations to prove that the semen is superior and potent. One ejaculate from a bull can inseminate 400-500 cows. At this rate, the semen from one bull can fertilize 50,000 cows per year. This means that the superior 1% bulls can be selected and used on cows. The discovery of **cryopreservation** (preservation at ultra low temperature) has inspired using frozen semen instead of fresh semen.

10.10.1.6 Clean Milk Production :

Clean and high quality milk can only be produced from healthy dairy animals under clean and hygienic conditions. Effective mastitis control programme and proper hygienic conditions are adopted during the milking process. Clean aspects of the milking process include the animals, the environment, milking system, milking practice and storage.

10.10.1.7 Organic Dairy Farming :

In recent years, there has been a slow but gradual shift in the agricultural and dairy farming practice from the present scientific method to the organic system. The current practice uses chemical fertilizers, pesticides, antibiotics and other inorganic products for increasing the harvest. Although the milk production has increased dramatically by using the synthetic chemicals on cattle and buffaloes, the threat of contamination of the harvested milk and milk products has increased. It has often been reported that milk and milk products are contaminated by residues of some of these harmful chemicals. These enter into the body by consumption, bioaccumulate and biologically magnify to express many harmful manifestations.

Organic dairy farming stands as an alternative to the present practice, which forbids the use of synthetic chemicals. The local breeds of cows are hardy and resistant to diseases and do not need veterinary drugs. These animals are fed on crop residues, grass and concentrates like cereals, pulses and oil cakes derived from plants grown without fertilizers, pesticides and other synthetic chemicals.

10.10.1.8 Success Story of Dairy Farming in India :

Agricultural and dairy farming have been playing a pivotal role in the uplift of rural economy and serving as the means of livelihood for millions of rural people in India. Dairy farming practice has grown to such a height that India earned the largest milk producing country status in 1997. The milk production has grown from 55.5 million tons in 1991-1992 to 155.5 million tons in 2015-2016. This is largely due to the success of the **Operation Flood** programme started in 1970 by the National Dairy Development Board (NDDB). Operation Flood is the world's largest integrated dairy development programme. It establishes linkages between rural milk producers and urban consumers through farmer-owned and –managed dairy cooperative societies. The success of this programme is known as **white revolution**. The main architect of this programme was Late Dr. Verghese Kurien. Dr. Kurien quit a government job to join the Kaira District Cooperative Milk Producers Federation, now known in every Indian household as AMUL (Anand Milk Union Limited). AMUL is managed by Gujarat Cooperative Milk Marketing Federation Limited (GCMMF). It is owned by 3 million milk producers in Gujarat. The pattern of cooperative society was so successful that Late Lal Bhadur Shastri, the then Prime minister of India established the National Dairy Development Board (NDDB) to spread the programme nationwide. For this extraordinary leadership, Dr. Kurien was named as the first chairman of NDDB.

10.10.2 Poultry Farm Management :

Another major share of the dietary protein requirement comes from poultry egg and meat. Poultry includes a number of bird species such as **chicken**, **duck**, **turkey**, **quail**, **goose**, **guinea fowl**, **pigeon**, **swans**, **pheasant** and **emu**. These birds have been domesticated and bred for meeting the egg and meat requirements since 5000 BC. This practice is known as **poultry farming**. Among these, chicken is the most favoured in India having commercial value. Poultry farming was mostly under **free-range condition** upto 1960. During the last five decades, the entire scenario of poultry farming has changed. From a free-range condition, these birds are cultivated in confinement in specially designed structures called **poultry farms**. Presently, it is an organized sector with modern technology input. It serves generate earning for the unemployed youth both in rural and urban areas. Along with dairy farming, poultry farming has proved a potential tool in the generation self employment, alleviation of poverty and elevation in the standard of living of the people.

10.10.2.1 Breeds of Poultry :

(i) Multinational Industrial Breeds: Apart from free range domestic birds, extensive animal breeding practice has developed many stocks of birds for egg and meat. The animal breeding laboratories of large multinational companies have developed high yielding stocks of poultry for commercial benefits. These poultry are grouped as **industrial stocks**. This stock includes, **white layers**, **brown layers**, **chicken broilers**. The white egg layers are developed

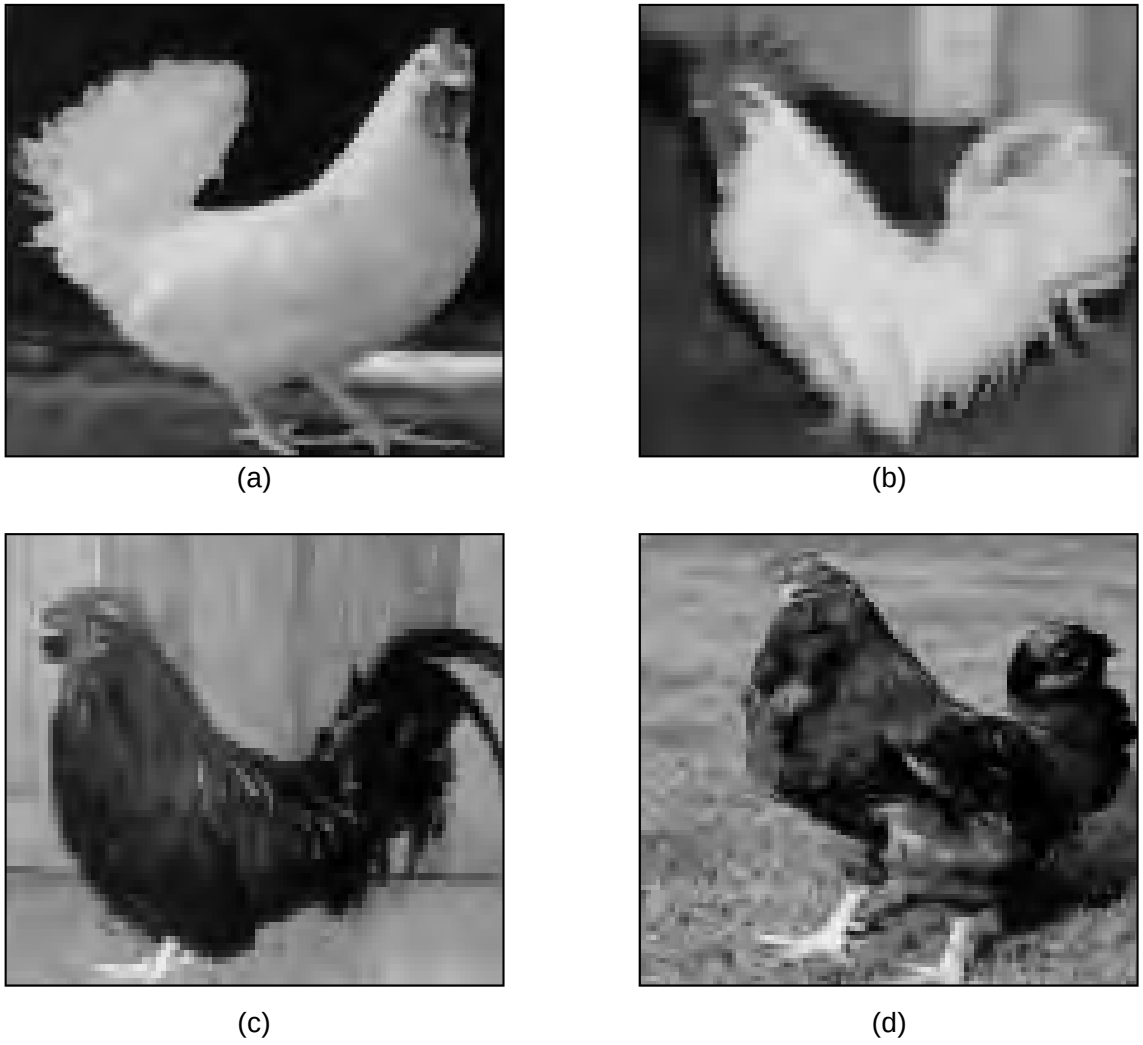

Fig. 10.10 (a-d): Some breeds of chicken. (a) White Leghorn layer; (b) White Nicobari (male); (c) Brown Nicobari (male); and (d) Red Cornish (male).

from **White Leghorns** for commercial egg production. The brown layer comprises of **Rhode Island Red**, **Barred Plymouth Rock**, **Australorp**, **New Hampshire**, **Dahlem Red**, etc. The broilers are developed by crossing White Cornish with White Plymouth Rock stocks. This stock is used for meat.

(ii) Indian Breeds: There are twenty indigenous breeds of chicken in India. These are comparatively hardy varieties and are resistant to some poultry diseases. Some well known breeds are **Chittagong**, **Kadaknath**, **Nicobari**, etc. Most of these are poor layers, but some yet yield good meat.

10.10.2.2 Hatchery Management :

Hatching of eggs is the most important task in commercial layer and broiler production. The breeder birds must be in good state of health and vigour and must be disease free. The eggs are carefully selected, properly fumigated to make these contamination-free, stored in an appropriate place having optimum temperature, humidity and ventilation before being transferred to the incubator for hatching. The eggs are then transferred to an incubator maintained at an optimum temperature, humidity and ventilation. The temperature should be adjusted to 100° F in still air incubator. The temperature should be maintained at 1° less during the last three days. The relative humidity should be at 58-60% during the first 18 days, while it should be elevated to 65-70% during the last three days. Fresh air containing oxygen be supplied continuously, since the developing embryo requires more oxygen. Similarly, the carbon dioxide be removed from the incubator continuously. The eggs must be turned 2-3 times daily for the first 18 days. Turning prevents the embryo from sticking to the shell membrane.

10.10.2.3 Housing Management :

It is an important aspect of poultry farming. It provides the birds protection from sun, rain, wind, cold, predators, etc. In warmer countries, like that of ours, the farm house is open-sided to allow ventilation of fresh air. Four types of housing systems are generally followed depending on the ambient surrounding. These are: **(a) free range or extensive system**; **(b) semi-intensive system**; **(c) folding unit system**; and **(d) intensive system**.

(i) Free Range System: The birds are set free to move outside. In doing so, they collect substantial amount of food. The farmer provides the birds with some supplementary food as required.

(ii) Semi-intensive System: This system is adopted, where the space is limited. There should be space for the birds to run to fresh ground at will.

(iii) Folding Unit System: This system is portable. The birds are confined to an area and next day to another area. In doing so, the birds have an access to new food resource and environment every day.

(a)

(b)

Fig.10.11 (a & b): Poultry housing system. (a) Cage housing; and (b) Three-tier cage housing.

(iv) Intensive System: In this system, the birds are confined to a walled house with no access to outside. This system is adopted in large scale production of meat and eggs by commercial farmers. Two types of intensive housing systems, namely **cage** and **litter systems** are generally adopted. Cage system is the most intensive system, where the birds in flocks are confined to cages. This prevents the spreading of diseases. In the litter system, the birds are housed on a floor covered with litter such as rice husk, saw dust, dried leaf, chopped straw, etc.

Whatever is the intensive housing system, three factors, namely sanitation, temperature and humidity and lighting are mandatorily considered. Beak trimming of the chicks is essential to avoid aggression and cannibalism. Footbath in a disinfectant is recommended before entering into the intensive housing system. The disinfectant is kept in a pit at the entry into the house. This a mandatory practice, especially in broiler farm management.

10.10.2.4 Nutrition :

Balanced nutrition of the poultry is the key to profitable harvest. The essential poultry nutrients are carbohydrate, fat, protein, minerals, vitamins and water.

Fig.10.12: Feeding of commercial broiler.

(i) Carbohydrates: Carbohydrate is supplied through poultry feed containing cereals, cereal byproducts and oil seed cakes. The birds cannot digest non-starch polysaccharides. Therefore, non-starch digesting enzymes are added to the feed as a supplement.

(ii) Fats: Poultry can digest unsaturated fats only. The fat requirement is met through oil seed cakes like groundnut cake, sunflower cake, mustard / rape seed cake, sesame cake etc. Antioxidants are added to the feed containing oil cakes.

(iii) Proteins: Proteins are essential for growth of the body. The feed contains vegetable protein sources like soya bean meal, groundnut cake, sunflower cake, mustard / rape seed cake, cotton seed meal, etc. Dry fish / fish meal and meat and bone meal are added to the feed as animal protein sources.

(iv) Minerals: Minerals are essential for metabolic activities. Deficiency of minerals leads to many deficiency diseases. Therefore, the chicken feed is supplemented with several essential minerals like calcium, sodium, potassium, magnesium, iron, manganese, zinc, copper, selenium, cobalt, fluorine, iodine, chloride, phosphorus, etc. Minerals like calcium, sodium, potassium, phosphorus and chloride are required in large quantity, while some minerals like manganese, zinc, iron, copper, iodine, selenium, and cobalt are required in small quantity. These are known as **trace minerals**.

(v) Vitamins: All the water soluble vitamins (vitamin B and C) and fat soluble vitamins (vitamin A, D, K and E) are essential for normal growth of the chickens. Deficiency of any one of these constituents leads to deficiency diseases.

10.10.2.5 Healthcare Management :

Poultry suffer from many diseases either caused by pathogenic organisms or due to deficiency of minerals and vitamins. The causative organisms are viruses and pathogenic bacteria. Some viral diseases are **Newcastle's disease** or **Ranikhet disease**, **infectious bursal disease**, **fowl pox**, **infectious bronchitis**, **avian diphtheria**, **avian influenza**, **avian encephalomyelitis**, **hepatitis**, **Marek's disease** and **avian leucosis**. Some common bacterial diseases are **pullorum disease** or **bacillary white diarrhoea**, **typhoid**, **paratyphoid**, **colibacillosis**, **fowl cholera**, **infectious coryza**, **chronic respiratory disease** and **avian spirochaetosis**. Poultry are also prone to parasitic infections. Some common parasitic infections are **coccidiosis** and **ascariasis**, Ectoparasites are organisms that live by sucking blood or lymph. Important ones are lice, ticks, mites and fleas.

Vaccination: Prevention is better than cure is followed in its totality in poultry farming practice. Since birds are more prone and susceptible to infections and diseases that we have to take due care of well ahead of time. Secondly, the birds that are reared for more benefit are high yielding varieties and are the descendents of crosses between exotic breeds and indigenous breeds. They are poorly adapted to the local conditions. For their better health, they should be vaccinated as per the standard schedule. Vaccines are now available for Marek's disease, Ranikhet disease, infectious bursal disease, and fowl pox.

10.11 ANIMAL BREEDING :

Revolution**s** in agricultural, dairy and poultry farming practices over the past century have taken place, mainly due the introduction of high yielding varieties of crops, livestock animals and poultry with superior high yielding characters. This has been possible due to a success in animal breeding practice. **Animal breeding** is a branch of science, which deals with the selection of animals having superior characters in terms of growth rate and meat, milk, egg and wool production in livestock animals and poultry. The superior characters are based on estimated breeding value of the animal.

10.11.1 Traditional Method of Animal Breeding :

There are three usual methods of introducing the beneficial traits (characters) in economically important animals. These are: **(1) Inbreeding**; and **(2) Outbreeding**.

(i) Inbreeding: Crossing of closely related animals is known as inbreeding. When inbreeding is repeated it is called upgrading. Inbreeding is beneficial in introducing as many beneficial genes as possible into an animal's genotype keeping the original genetic combination

intact. However, inbreeding may bring the deleterious (harmful) recessive genes together i.e. homozygous recessive condition may result. This may express the harmful effects of the deleterious gene. This leads to a decreased fitness of the population, which is called **inbreeding depression**. Secondly, it has been observed that the hybrid vigor and the fertility rate decrease with repeated inbreeding. If the population does not contain harmful genes, this method is safe to produce improved animals with superior traits.

(ii) Outbreeding: The crossing of distantly related animals is known as outbreeding. The important aspect of this type of breeding is the selection of the breeding stock with superior traits. This is the method of choice to produce livestock animals for more milk and poultry for more meat and large sized eggs. There is less chance of two deleterious recessive alleles coming together in a homozygous condition. This method of breeding is also favoured by nature due to the silencing of the effect of deleterious recessive alleles in heterozygous conditions. The fitness of the population increases and consequently natural selection occurs.

10.11.2 Modern Methods of Animal Breeding :

With advancement in genetics, several modern methods of animal breeding have come up with success. Some of these like (1) **artificial insemination**; (2) ***in vitro* fertilization and embryo transfer**; and (3) **transgenic animals** are discussed in brief hereunder.

(i) Artificial Insemination: This is a method of introduction of the semen of a bull with selective advantageous traits into the vagina of a cow. This results in fertilization, development and birth of a calf with superior traits such as better growth and milk production.

(ii) *In vitro* Fertilization and Embryo Transfer: In this method, the eggs of an ovulating livestock animal e.g. cattle are isolated and fertilized *in vitro* by the semen of a bull with desired beneficial characters. The fertilize egg is placed in a suitable medium and is stimulated to undergo cleavage to a 8-16 cell stage embryo known as a **blastocyst**, also *in vitro*. This embryo is then transferred or implanted into the uterus of a surrogate cow, made **pseudopregnant**. The cow comes to term and gives birth to a calf with selected characters.

(iii) Transgenic Animals: This is a method of transfer of selected beneficial gene(s) into the fertilized egg of cattle *in vitro* by microinjection or by any standard gene transfer technique. The transferred genes undergo homologous recombination with the genome of the fertilized egg. The fertilized egg is selected for the successful recombination of the said gene. Such eggs are transplanted into the uterus of pseudopregnant surrogate mother cattle. The cattle comes to term and gives birth to a calf with desired characters. The cattle is known as a transgenic. This is, by far, the most modern method of animal breeding.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the blanks with correct answers from the choices given in the brackets of each bit.

(i) Physical removal of anthers is done by ——— process.
(introduction, mutation, hybridization, emasculation)

(ii) The cross between two varieties of same crop is called ——— hybridization.
(intervarietal, intravarietal, intrageneric, intergeneric)

(iii) In the process of ——— breeding, genetic makeup of the concerned organism may be changed.
(mutation, interspecific, selection, intraspecific)

(iv) The plant part used in tissue culture is called ———.
(cells, zygote, explant, gamete)

(v) To produce haploid plants, ——— culture can be made.
(anther, embryo, endosperm, zygote)

(vi) The autotroph ——— is cultured to obtain single cell protein.
(*Saccharomyces, Pseudomonas, Spirulina, Chaetomicem*)

2. Choose the correct answer:

(i) The cross breed milch breed is
(a) Red Sindhi (c) Frieswal
(b) Tharparker (d) Sahiwal

(ii) The exotic breed of cattle is
(a) Jersy (c) Gir
(b) Sahiwal (d) Red Sindhi

(iii) An indigenous breed of cattle is
(a) Red Dane (c) Karan Swiss
(b) Jersy (d) Rathi

(iv) Sunandini, a crossbred cattle is produced by crossing
(a) Brown-Swiss bull with Sahiwal cow
(b) Jersy bull with Red sindhi cow
(c) Red Dane bull with Sahiwal cow
(d) Holstein-Friesian bull with Rathi cow.

(v) An indigenous milch breed of buffalo is
(a) Hariana (c) Kankrej
(b) Jaffarabadi (d) Jamunapuri

(vi) The indigenous breed of poultry is

(a) Nicobari (b) Rhode Island Red

(b) Barred Plymouth Rock (d) New Hampshire

(vii) Commercial poultry production is done under

(a) Free range system (c) Semi-intensive system

(b) Intensive system (d) Folding-unit system

(viii) Which of the following is a disease of cattle

(a) Ranikhet disease (c) Bacillary white diarrhea

(b) Marek's disease (d) All of the above

3. Answer in one word only :

(i) What is the nutrient source not required for obtaining single cell protein from autotrophs ?

(ii) What is called to the amorphous mass of loosely aranged thin walled parenchymtous cells developed in the process of tissue culture?

(iii) What is called to the remaining part of plant celly when its wall is mechanically or enzymatically removed?

(iv) What is called to the sum total of all the alleles of gene present in a particular species and its allied wild and cultivated varieties?

(v) What is the process called where flower buds are artificially enclosed to avoid undesired pollination?

(vi) In which process can genetic make up concerned organism changed ?

(vii) The preservation of semen at ultra-low temperature.

(viii) The substance the queen bee is fed with.

(ix) Development of haploid eggs without fertilization.

(x) The important monosaccharide present in honey.

(xi) The repeated breeding between closely related individuals.

(xii) Breeding between unrelated individuals.

4. Correct the sentences in each bit by changing the underlined wood/woods only :

(i) The process of aseptic transfer of explant from nutrient medium to culture vessels is called <u>micropropagation</u>.

(ii) When cytoplasms are fused and one of the two nuclei lost in formation of new organism, it is called a <u>hybrid</u>.

(iii) For nuclear fusion, PEG is used.

(iv) Cross between different genotypes of same variety is called <u>intrageneric</u> hybridization.

(v) When pollens from selected male parents are transferred to stigma, it is called <u>natural</u> pollination.

5. Fill in the blanks :

(i) The cross between two species of a genus is called ——— hybridization.

(ii) In selection and testing of superior recombinants, F, generation offsprings are ——— pollinated.

(iii) Biofortification is done to enrich crops with micronutrients like minerals and ———.

(iv) Explants sterilized by mercuric chloride or hydrogen peroxide etc. are known as ——— sterilization.

(v) Through the process of tissue culture, large number of plants raised in a small area and called micropropagation or ——— propagation.

(vi) Triploids can be raised by ——— culture.

(vii) Milk yielding cattle breeds are known as _____ breeds.

(viii) Foot and mouth disease is a common disease of _____.

(ix) Traditional method of breeding is substituted by artificial _____.

(x) The housing system employed in the commercial poultry farming is known as _____ farming.

(xi) Ranikhet disease is a common disease of _____.

(xii) Culture of honey bee on a commercial basis is known as _____ culture.

(xiii) The drones develop from haploid eggs, which are not fertilized. This development is termed as _____.

(xiv) The deserting of the queen bee is known as _____.

(xv) The characteristic flight of the queen bee during fertilization is known as _____.

(xvi) The juvenile bees are reared in _____ chamber of the honey comb.

(xvii) The worker bees develop from fertilized eggs and hence are diploid _____.

GROUP - B
(Short Answer-type Questions)

1. Answer the following within 50 words each:

(i) Name five indigenous breeds of cattle.

(ii) Name three cross-bred cattle.

(iii) Name three exotic breeds of cattle.

(iv) What is cryopreservation?

(v) Describe organic dairy farming.

(vi) What is free-range poultry farming?

(vii) Name five exotic breeds of poultry.

(viii) What is intensive housing system in poultry?

(ix) Describe the protein source in poultry nutrition.

(x) Why is inbreeding harmful?

(xi) What is artificial insemination?

(xii) What is *in vitro* fertilization?

(xiii) What is a transgenic animal?

(xiv) Enumerate the castes in honey bee.

(xv) Describe swarming.

2. Write notes on with 2/3 valid points :

(i) Germ plasm collection

(ii) Emasculation

(iii) Bagging

(iv) Artificial pollination

(v) Breeding for disease resistance

(vi) Biofortification

(vii) Explant

(viii) Tissue culture medium

(ix) Totipotency

(x) Micropropagation

(xi) Anther culture

(xii) Somaclonal variation

(xiii) Synthetic seeds

(xiv) Secondary metabolites

(xv) Embryo rescue

3. Differentiate with at least 3 valid characteristics :

(i) Bagging and Tagging

(ii) Chemical and Biological method of pest control

(iii) Callus and Protoplast

(iv) Synthetic seeds and embryo

(v) Endosperm culture and anther culture.

(vi) Hybrid & Cybrid

GROUP - C
(Long Answer-type Questions)

1. Describe the main steps of breeding to develop genetic variability in crop plants.
2. Describe the techniques of hybridization.
3. Give an account of techniques and steps of plant tissue culture.
4. Elaborate the application of plant tissue culture.

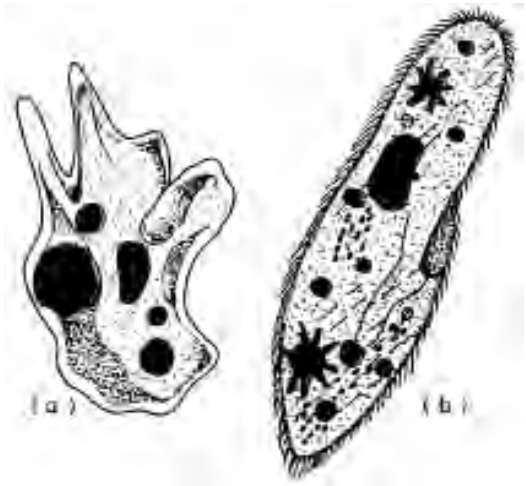
❑ ❑ ❑

MICROBES IN HUMAN WELFARE

CHAPTER 11

Plants, animals and microbes form the living world of this universe. Of these, microbes are tiny, minute organisms, not visible to our naked eyes.But they are the complete living beings since all the functions and manifestations that signify life are seen in them. Prokarytes and primitive eukaryotes together form the world of microbes. Specifically, bacteria, cyanobacteria, majority of algae, fungi, protozoans and many infectious agents like viruses at the border line of life are normally called the microbes (Fig.11.1, 11.2, 11.3, 11.4, 11.5).

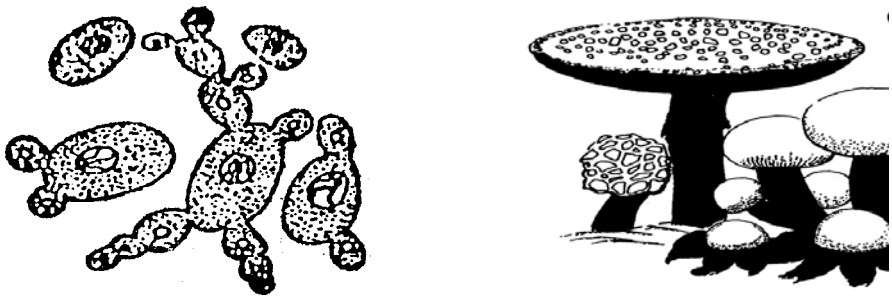
Microbes however, form one of the dominant life forms of our planet , yet most of us are ignorant of their true profile. This is because the organisms are out of sight of the common man due to their microscopic size. Approximately 300 years back, the human being could notice the wide ranging effects of these organisms.



11.1 : Unicellular microbe, protozoa, (a) *Amoeba* (b) *Paramaecium*

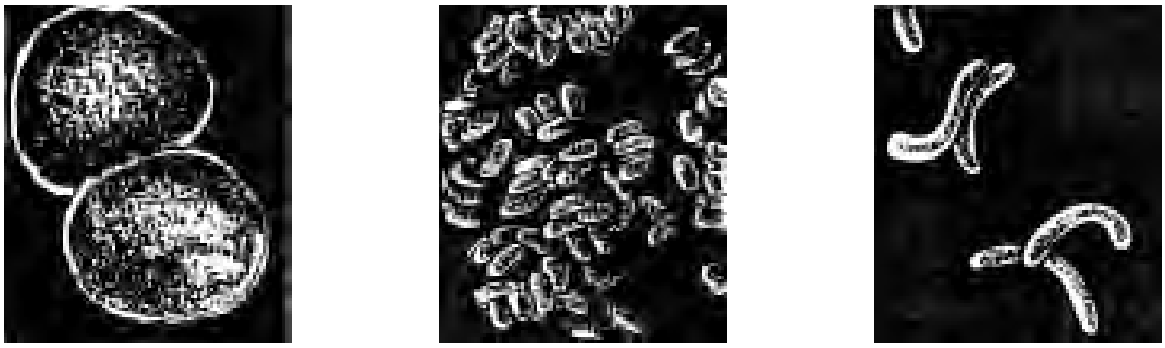
(a) (b) (c)

11.2 : algae (a) Unicellular - *Chlamydomomas,* (b) *Volvox* colony, (c) *Ulothrix* filament



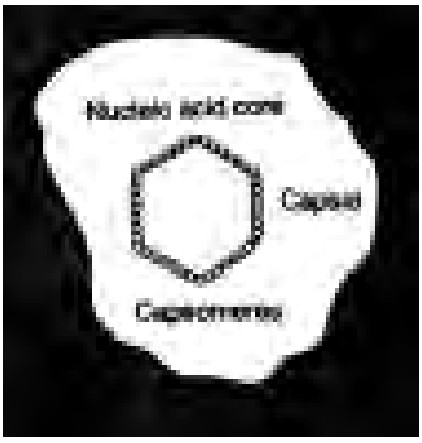
(a) (b)

11.3 : Fungi, (a) unicellular yeast, (b) Penicillium

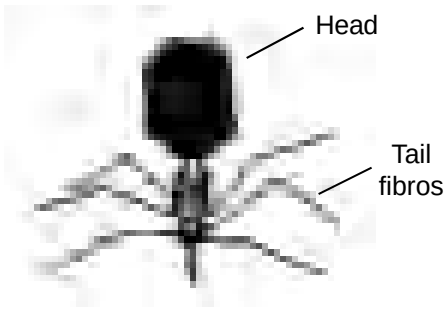


(a) (b) (c)

11.4 : Prokaryotic microbes: Three types of bacteria (a) Coccus, (b) Bacillus, (c) Spirillum



(a)
Envelope with inface projection



(b)
Bacteriophage

10.5 : Microbes as infectious agent (a) Virus, (b) Bacteriophage

These unique organisms are present everywhere in nature, the soil, water and air. They are also present inside and outside the surface of the most of the plants and animals. They are present in the habitats where no other life form exists such as the coldest conditions of polar regions , hot sulphur springs or highly saline water bodies.

The contributions of these interesting living beings have not been properly appreciated yet, although we have reached the zenith of development in the field of science and technology. The only aspect of their myriad actions that get highlighted is their potential to cause misery, disease and injury. On the other hand , the microbes are closely associated with the health and well being of humans. Certain household, domestic and industrial products like curd,cheese,butter etc; manufacture of antibiotics like penicillin, streptomycin; production of organic acids, alcohols and processing of domestic and industrial wastes and many other benefits are obtained from these tiny,little organisms. Rarely a moment passes when we are not influenced by the microbes. Life in this planet would not have been possible if there were no microbes. Microbes have multifarious uses in various fields for the human welfare.

11.1 Household food processing :

There are many microorganisms that can obtain energy in the absence of oxygen called fermentation. We utilize the process for the cultivation and propagation of microbes and its application to obtain the desired product in due course. The process of making idli or curd is being done in all our households. For the preparation of curd, a spoon of mother curd is added to lukewarm containers of milk and kept covered overnight. Similarly for making idli, batter rice and black gram are soaked in water for few hours,then coarsely ground and kept overnight at room temperature.

Microbes like *Lactobacillus* and certain other lactic acid bacteria can only grow in milk and convert it to curd. During their growth, utilizing the milk sugar called, lactose, bacteria in the process of fermentation, produce acids that lead to coagulation of milk protein called casein. If the curd of long stored or aged curd is added to milk, then it develops very sour taste due to greater accumulation of lactic acid. Similarly nice fluffy idlies are obtained if the soaked idli mix is allowed to remain so for only a fixed period. In order to prevent the immediate growth of bacteria and other microbes on milk, the milk is heated at particular temperature for a fixed period of time and then cooled. In this process called Pasteurization, the fermenting microbes can be in activated for a certain period of time and used in food product preparation.

Food is the basic requirement of the human beings. The use of fermented food or rather the use of microbes for food processing is a long story dating back from ancient times.Over the ages, the human civilization has developed a long list of fermented food items.The variation is because of diversity in raw materials, knowledge, perception, taste and eating habits. A number of fermented items are prepared from plant products like traditional drinks, soybean and bamboo shoots.Various types of milk products such as butter milk, cream, yoghurt, ghee, cheese etc are obtained at every home.

11.2 INDUSTRIAL PRODUCTS :

Certain microbes are employed in fermentation industry in the production of various chemicals because of their ability to give consistently high yeild of desired products in a reasonable time. It is done from cheap and readily available raw materials.A microbe should have certain characteristics for its application in industrial fermentation. It should be nonpathogenic,have the ability to grow rapidly on suitable nutrients and should possess high levels of enzymes for rapid production of the end products. Industrial fermentation are carried out in fermentors. Initially the process of industrial fermentations were applied in the production of food and alcoholic beverages.Now these have been extended to a variety of other products.Antibiotics,enzymes,organic acids,Baker's yeast, ethanol, vitamins, steroid hormones are the important examples of industrial products of fermentation.

11.2.1 Antibiotics :

The secretions of microorganisms which are selectively toxic are called antibiotics. Certain fungi and bacteria produce antibiotic which kill pathogenic bacteria. First antibiotic to be extracted from the species of *Penicillium notatum*, is penicillin in the year 1928 by Sir Alexander Fleming. Since then a large number of antibiotics have been industrially obtained from microbes like fungi,bacteria and actinomycetes (Table 11.1).

Table - 11.1
Microbes in antibiotic production

Antibiotic	Microbe
Penicillin	*Penicillium* spp.
Streptomycin	*Streptomyces griseus*
Tetracyclins	*S. aureofaciens*
Chloramphenicol	*S. venezuelae*
Nystastin	*S. nouresi*

Using the strains of the microbes in fermentors containing carbohydrate source,mineral salts and corn steep liquor under vigorous aeration,there can be the maximal production of penicillin.This was the 'wonder drug' during the second world war to give relief to the wounded soldiers form pain and suffering.

The second antibiotic to be produced was from the bacterium, *Streptomyces griseus*. It was obtained from a fermentation medium containing glucose,soyameal and mineral salts. The p^H of the medium was maintained at 7.4 - 7.5 . The fermentation was carried out under submerged condition at 25-30^{0}c for 5 - 7 days.

Similarly hundreds of antibiotics have been produced, now-a-days, by the process of industrial fermentation. These are employed to cure many killer diseases like cholera, plague,diphtheria,tuberculosis,leprosy,typhoids etc.which have earlier caused havoc in the form of epidemics in the various parts of the world.

11.2.2 Alcoholic beverages :

The alcohol was produced by fermentation in the early days but for many years it was done by chemical means through catalytic hydration of ethylene. In modern era, attention has been paid to the production of ethanol for chemical and fuel purpose by microbial fermentation. Ethanol is produced by using sugar beet,potatoes,corn,cassava and sugar cane etc.

Both yeast like *Saccharomyces cerevisiae , S. uvarum , S. carlsbergensis , Candida brassicae C. utilis and* bacteria (*zymomonas mobilis*) have been employed for ethanol production in industries. Different types of alcoholic drinks are manufactured depending upon the raw material employed for the fermentation. For example, the alcoholic beverage, beer is obtained from the starch of barley grains. Likewise grapes are normally the raw materials for wine production.The process of alcohol production is called brewing and here, the byproduct carbon dioxide is used as bakery. Carbon dioxide (CO_2) is utilized to provide sponginess to the breads, cake and many other such products.

11.2.3 Production of organic acids:

Many organic acids such as acetic,citric,gluconic,fumaric etc. are produced by microbial fermentation. The list is given in the table below (Table - 11.2).

Table - 11.2

Microbes in organic acid production

Organic acid	Microbe involved
Lactic acid	*Lactobacillus* spp.
Acetic acid	Acetobacter spp.
Citric acid	*Aspergillus* spp.
	Penicillium spp.
Gluconic acid	*A . niger*
	P . chrysogenum
Fumaric acid	*Penicillium* spp.

The commercial production of vinegar (acetic acid) involves a preliminary fermentation of the juice to produce ethyl alchol and its secondary fermentation into acetic acid under aerobic conditions.

Lactic acid is produced from various types of carbohydrates such as corn starch,potato starch,molasses and whey.The starchy materials used are initially hydrolyzed to simple sugars. It is then fermented by the spp of *Lactobacillus* under suitable environmental condition to obtain the lactic acid.

Citric acid is the key intermediate of TCA cycle. Many fungi,bacteria and yeasts produce it. A variety of carbohydrate sources such as beet molasses,sucrose,commercial glucose, starch hydrates etc. are used as the raw material in the industrial citric acid production.

All these organic acids are used in our food items as preservatives,flavour enhancers,oxidation prevention,flavouring agent, prevention of turbidity etc.

11.2.3 Production of enzymes:

Microbes are known to excrete enzymes into their growth medium and these enzymes have many uses in pharmaceutical,food and textile industries.Some of the microbial enzymes and their uses are given in the table below (Table - 11.3)

Table - 11.3

Microbial enzymes and their uses

Enzymes	Organism	Substrate	Application
Amylase	*Aspergillus spp*	wheat bran	Digestive, preparation of glucose syrup
Protease	*A. niger* *Bacillus subtilis*	wheat bran	Digestive, meat tenderizer, clarifying beer
Pectinase	*Aspergillus spp*	wheat bran	Clarifying fruit juice
Lipase	*Rhizopus spp*	wheat bran	Digestive
Cellulase	*Trichoderma viridie*	cellulose	Digestive

The quality and quantity of enzymes produced depend upon the strain of the microbes and cultural conditions.

11.3 SEWAGE TREATMENT :

The human population is increasing at a very fast pace.. As a result of this and particularly after the industrial revolution, cities, towns, urban centers in the form of human settlements are coming up in large scale. From such habitations , huge amount of waste water is being generated every day. The waste water from such locations are called sewage. Conventional waste treatment practices like cess pits,septic tanks,sewage farms,gravel beds,percolating filters and activated sludge processes with anaerobic digestion have been in use in most countries from the time immemorial. These are less effective and somtimes nonproductive.

However, the waste water and domestic sewage contain good amount of degradable organic compounds. Bacteria, protists and many other microorganisms are capable of breaking down these organic matter.These microbes need to be supplied with adequate nutrients,oxygen and other essential elements for their growth which consequently enhance the rate of chemical degradation.

The treatment of such waste water is carried out in three steps: primary, secondary and tertiary.In the primary steps,unwanted coarse,nonbiodegradable particles are removed.

The secondary process consists of aerobic microbial degradation in open bioreactors. The organisms multiply and grow forming biomass known as sludge. The sludge is passed on to the anaerobic bioreactors for its anaerobic digestion to produce biogas and manure. The tertiary process is optional. It consists of chemical precipitation. The effectiveness of this process depends on the number of microbes coming in contact with the pollutant organic molecule. Therefore, the process is carried out in a constantly stirred open bioreactor, supplied with nutrients.

An alternative to the aforementioned bioreactor, is known as percolating or trickling filter bioreactor. The sewage is allowed to flow on the surface of stone gravel or plastic sheet, on which microbes have been immobilized. Another innovation in the waste water treatment is a deep shaft fermentation system. The deep shaft is a hole in the ground,divided to allow the cycling and mixing of waste water,air and microbes.

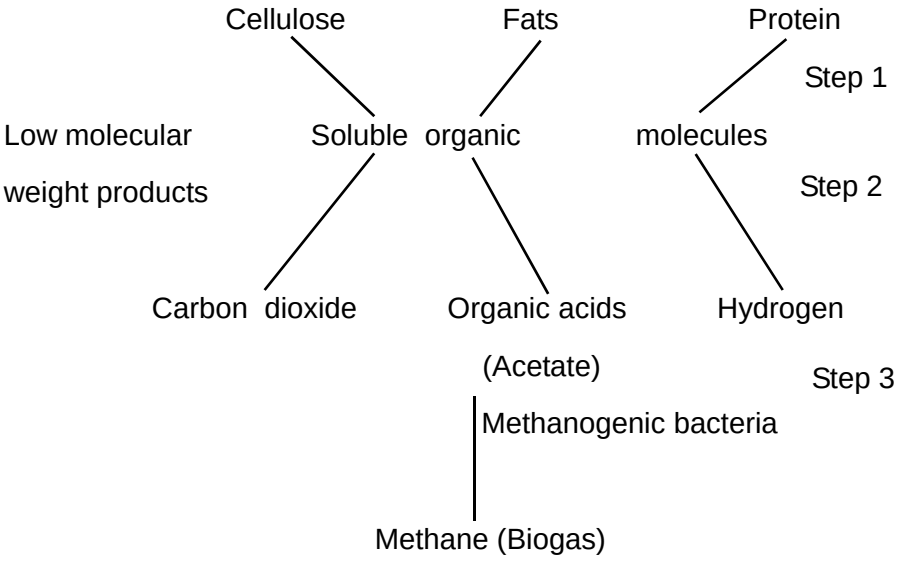
In countries,receiving high annual hours of sunlight,the algae-bacterial bioreactors have been developed. The biomass is used in biogas production or animal feed.

11.4 ENERGY GENERATION :

The modern life is completely energy dependant. Nonrenewable souces of energy are obtained from fossil fuels like coal and crude petrol. Another important source of energy is biogas. It is a complex mixture of gases such as methane, carbondioxide etc.Methane content is nearly 50-60 per cent. Other gases in biogas are relatively low; such as carbon dioxide nearly 25-30 per cent, hydrogen-1-5 per cent, nitrogen 2-7 per cent, oxygen percentage is 0.01. Biogas is inflammable gas produced by bacteria in the intestine of ruminant cattle and natural wetlands by anaerobic digestion of complex organic molecules. The methane producing bacteria are known as the methanogenic bacteria and process of methane production is called methanogenesis.

Man has been using biogas for over 200 years.In India,biogas is popularly known in India as Gobar gas. Since in our rural areas,the cattle population is the maximum,the biogas is produced from their excreta called cow dung or Gobar. Economically, viable biogas is produced in large vessels called bioreactors.

The anaerobic digestion of complex organic molecule is achieved in three steps. In the first step, cellulose, fat and proteins contained in the waste products are made soluble. In the second step, such products of low molecular weight are converted into organic acids,mainly acetate by microbial action and in the third step,acetate by methanogenic bacteria converted to methane and carbon dioxide.



Schematic presentation of anaerobic digestion of complex organic molecules to biogas (methane).

In recent years biogas is also obtained from municipal sewage,agricultural and urban wastes.

The biogas in Asia is mainly used in cooking and lighting purposes. There are many other uses of biogas, such as its use in internal combustion engines to power pumps and eletric generators.The sludge is used as biofertilizer. By this, the extent of enviromental pollution is reduced and demand for energy is met.

11.5 BIOCONTROL AGENTS :

During the golden era of green revolution in India, chemical pesticides have brought about dramatic and immediate effets in preventing the attack of plant pathogens and pests to our crop plants.Thereby, the crop production increased manifold.However, all these chemical pesticides are nonbiodegradable.They have a tendency to persist in the enviornment as such for more than 15-25 years and accumulate in increasing concentration in different trophic levels of the food chains. This phenomenon is known as bioaccumulation.It has significant adverse effets on a variety of life forms including the man.

Again the targeted pest population becomes increasingly resistant to the prescribed dosages of chemical pesticides. Consequently, higher dosages have to be used to increase crop productivity. The use of chemical pesticides,besides killing targeted, harmful pests destroy many beneficial insects,microbes etc.

Under the above backdrop of drawbacks of the use of chemical pesticides,alternative methods of controlling pests have been investigated over the past decade or so. Pesticides that are products of microbes and plants have been discovered. These natural pest killing agents biopesticides have become obvious choice because of the following reasons (1) high pest specificity (2) biodegradability.

Bacillus thuringiensis, a gram positive soil bactrium and baculovirus a virus, are typical examples. They encode for products having insecticidal properties. The encoding genes have been successfully cloned and introduced into the crop plants in culture by tissue culture techniques. Thus transgenic plants are formed. These transgenic plants possess inherent ability of producing specific biopesticide and protect them from the harmful pests. The pesticidal molecules are known as the biopesticides or microbial pesticides.

Baculoviruses are rod shaped double stranded DNA viruses that can infect and kill large number of invertebrate organisms. They infect mostly the larval stages of various insects of order Lepidoptera,Diptera, Hymenoptera,Coleoptera and Homoptera.Larvae of the insects damage the crop plants.

Therefore, baculoviruses are used as potential biopesticides for controlling such insect pests.An added advantage is that this virus does not harm non-target organism since viruses are obligate parasites and very much host specific. Insect larvae are infected when they ingest the plant material contaminated with baculoviruses. In the alimentary canal, the protein coat of the virus is digested by digestive enzyme of the larvae and eventually,the DNA is released. The viral DNA enters the nuclei of intestinal epithelial cells of the host and replicates into large number of viral particles. During the later phases, the virus causes lysis and death of the pest.

Now-a-days, a number of enzyme inhibitor such as plant protease inhibitor, cow pea trypsin inhibitor have been transferred by microorganisms to function as biocontrol agents.

11.6 BIOFERTILIZERS :

Plants require nutrients for their growth, development and other vital activities. Optimum supply of nutrients promotes healthy growth of the plants and their maximum productivity.Nitrogen and phosphorous constitute important elements in proteins, nucleic acids, co-enzymes and some lipids. Nitrogen occurs in gaseous state and approximately constitute 80 per cent in the atmosphere. Phosphorus occurs as insoluble phosphates in the soil sediments. Like many living beings, plants are not equipped with mechanism for utilizing atmospheric nitrogen and insoluble phosphates.

They depend upon microbes for such basic necessities. The soil microbes trap the atmospheric nitrogen and convert it into nitrates by a process known as the biological nitrogen fixation. Certain other soil microbes solubilize the insoluble phosphates present in the soil sediments and then make it avaliable to the plants. The biologically active products popularly known as the biofertilizers are formed due to the metabolism of microbes (bacteria, algae, fungi) so that nutrients can be provided to the plants. The nutrient requirements of the plants can be met by chemical fertilizers sold in the market with various trade names. The result of application of chemical fertilizers initially may be excellent. But the continuous use of the same in the crop field causes soil sickness,retardation in productivity, above all, enviromental pollution. Hence, scientific community all over the world are inclined to develop greater yield of biofertilizers like (1) nitrogen fixing (2) phosphate solubilizing.

Chemical fertilizers account for half of the nitrogen supply of the plants. The remainder is derived from nitrogen fixing organisms (diazotrophic) bacteria such as *Rhizobium*, *Frankia*, *Azotobacter*, *Azospirillum*, *Klebsiella*, *Rhodospirillum* and Cyanobacteria(blue green algae) . The process by which,the diazotrophioc microbes make the atmospheric nitrogen available to the plants in utilizable form is known as biological nitrogen fixation. Biofertilizer is the inoculum containing one or a few of the above mentioned nitrogen fixing or certain phosphate solubilizing microbes,packaged in carrier materials preferably sterile soil. This is the conventional method of producing the biofertilizer.

However, with the emergence of biotechnology, genetic engineers have tried to manipulate nitrogen fixing property into non-nitrogen fixing crop plants. The objectives are:

1. Modify either the microbes or the targeted crop plant so that each could benefit from an association with the other.
2. Modify non-nitrogen fixing bacteria occurring in close association with the crop plants which could fix nitrogen.
3. Genetically engineered crop plants by transferring nitrogen fixing (nif) genes from nitrogen fixing microbes,which could fix their own nitrogen from the atmosphere.

Several groups of microbes are selected for commercial use of biofertilizers. These include (1) *Rhizobium* (2) Azospirillum (3) *Azotobacter* (4) Blue green algae (5) Mycorrhiza(6)Acetobacteria (7) Phosphobacteria.

Rhizobium is a symbiotic bacterium which fixes atmospheric nitrogen in association with root system of legumes. *Azotobacter* is a free living nitrogen fixer. However, *Azospirillum* may be symbiotic or free living. Its inoculant is used for sugarcane, rice and cotton cultivation. Blue green algae called BGA or cyanobacteria are now-a-days used as an ideal biofertilizer. Mycorrhiza is a symbotic association where certain types of soil fungi live symbiotically with the root system of forest trees and fix atmospheric nitrogen. Phosphobacteria and Acetobacteria are used for providing essential nutrients to the crop plants.

———

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the blanks with correct answers from the choices given in the brackets of each bit.

(a) In curd making, _____ is useful in coagulation of milk protein (*Lactobacillus, Saccharomyces, Penicillium, Aspergillus*)

(b) Antibiotics Streptomycin is obtained from _____

(*S*. griseus, *S*. aureofaciens, *S*. nouresii, Saccharomyces cerecisiae)

(c) Citric acid is produced when fermentation caused by _____ . (*Lactobacillus, Aspergillus spp, Penicillium spp*, *Acetobacter spp)*.

(d) Lipase enzyme is produced by the activity of _____

(*Trichoderma viridie, Rhizopus spp, Aspergillus spp, Saccharomyces cerevsiae)*.

(e) In pest control of crop plants _____ has pesticidal properties. *(Baculo viruses, papilloma viruses, Pox viruses, Rhizobium)*

2. Answer in one word only :

(a) What is called to the process of heating and cooling of milk for inactivation of bacteria ?

(b) What is called to the secretions microorganisms which are toxic to pathogenic bacteria ?

(c) What is the commercial name of acetic acid ?

(d) What is called to the accumulated microorganisms and organic matter in the treatment of sewage ?

(e) What is the major component of biogas ?

(f) What can be called to the natural pest killing agent other than artificial chemical?

(g) What is called to the association between *Rhizobium* in the root system of legumes?

3. Correct the sentences in each bit without changing the underlined word/words :

(a) Antibiotic <u>tetracyclin</u> is obtained from *Penicillium notatum.*

(b) In biogas methane is produced due to the activities of <u>nitrogen fixing</u> bacteria.

(c) The first antibiotic extracted from bacterial culture is <u>*nystatin*</u>.

(d) Industrial production of organic acids through microbial cultures is due to the <u>oxidation</u> process by bacteria.

(e) <u>Acetic acid</u> is produced by *Lactobacillus spp*.

4. Fill in the blanks :

(a) In biogas production ——— bacteria are used.

(b) BGA used in biological nitrogen fixation are called ——— bacteria.

(c) Ethanol obtained by ——— fermentation is used in industry.

(d) Acetobacter converts ——— to vinegar by aerobic frementation legumes ?

GROUP - B
(Short Answer-type Questions)

1. Write notes on the following with atleast 3 valid points :

(a) Biogas

(b) Biopesticides,

(c) Biofertilizers

(d) Microbes in industry

(e) Microbes in antibiotics production

(f) Microbes in sewage treatment

2. Differentiate with atleast 2 valid points :

(a) Chemical fertilizers and biofertilizers.

(b) synthetic pesticides and biopesticides.

(c) Bakery and brewery.

(d) Symbiotic nitrogen fixation and mycorrhizal nitrogen fixation.

GROUP - C
(Long Answer-type Questions)

1. Give a delailed account of industrial application of microbes.
2. Explain how microbes are useful in pollution control and also in production of alternative source of energy.

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UNIT - IV : BIOTECHNOLOGY AND ITS APPLICATIONS

CHAPTER 12

PRINCIPLES AND PROCESS OF BIOTECHNOLOGY

Over the years, the population of our planet has gone up to a new dimension and it is ever increasing. New human settlements have come up at the cost of agricultural land and forests. This has resulted in a decrease in the arable land area, which consequently has led to a decrease in the food grain production. People have used fertilizer and pesticides to increase food grain production by conventional means. Forests have been cleared to accommodate new human settlements. The industrial base has gone up to meet the growing demand of the population. All these practices have polluted our environment to an extent that it has become unsuitable for living. The mankind has suffered from mysterious diseases, for which long term cure have not been discovered. There has been a shortage in the conventional medicines. Life scientists have worked hard in their search for a lasting solution to all the aforementioned problems. At last, biotechnology emerged as a possible answer to these problems.

Biotechnology, as it means, is not a pure branch of science, rather an integrated approach involving the knowledge, skill and techniques of many branches of science. It is very difficult to define the word in its totality, since it involves so many disciplines, together in the fold of its study. However, we have made a formal attempt to define biotechnology as underlined.

"Biotechnology is an application of knowledge and techniques of biochemistry, microbiology, genetics, immunology, tissue and cell culture, molecular biology, chemical engineering and computer science to living systems or parts thereof, for harvesting beneficial products and / or services for mankind".

Molecular biology and **Genetic engineering** are considered as two mainstays of biotechnology. Molecular biology refers to the study of structure and function of genes at fhe molecular level. Genetic engineering, on the other hand, means **manipulation or engineering of genes (DNA) towards a desired end.** Several synonyms such as **gene cloning, molecular cloning, gene manipulation** and **recombinant DNA technology** may substitute for genetic engineering. However, the word cloning has become so familiar with us that most have at least heard about the word than understood.

Although this branch of science seems to be relatively new, its application dates back to very early times of human civilization. Sumerians and Babylonians knew to prepare beer. Ancient people also knew cheese production and mushroom cultivation. All these processes involve biotechnological applications. **Antony van Leeuwenhoek observed microorganisms with his microscope** in the seventeenth century. **Louis Pasteur (between 1857 and 1876)**

established the fermentation ability of microorganisms. He is considered as the **father of biotechnology. The term 'biotechnology'was coined by Karl Ereky (1917),** a Hungarian engineer, to describe the large scale production of pigs by using sugar beets as the source of food. For around the next 45 years, the word biotechnology, as a scientific discipline, remained as an ambiguous connotation. This ambiguity ended in **1967** when, a Swedish microbiologist, **Carl Gören Heden suggested** the **changing of the title of a journal from the "Journal of Microbiological and Biochemical Engineering" to "Biotechnology and Bioengineering".** From this time on, biotechnology has unequivocally been associated with the study of "industrial production of goods and services by using organisms, systems and processes". It underwent a slow period of growth up to the third quarter of the last century. A **biotechnological revolution** started in 1970 after which, it underwent a period of exponential growth.

12.1 PRACTICE OF GENETIC ENGINEERING :

Biotechnology has been referred to as a branch of science that delivers products and services to the human society. Therefore, **it is considered as a trade.** Genes are the targets of this trade practice, since genes direct the synthesis of beneficial products. Therefore, the primary objective of this practice is to have as many identical copies of a beneficial gene. A beneficial gene is isolated and made to synthesize a useful product. One copy of a gene forms one molecule of the product at a point of time. More copies of the gene will form more product molecules. Therefore, the primary objective is to make a large number of copies of a target beneficial gene in a relatively simpler living system. This process of making a number of identical copies of a beneficial gene is known as **gene cloning.** Microorganisms, especially bacteria, are chosen as host cells for this work, which provide a suitable environment for replication (amplification) of genes. The gene is introduced into a bacterium by a process, known as **transformation.** For transformation, a gene is delivered into a bacterial host conjointly with a carrier, also DNA, known as a **vector.** The beneficial gene - vector combine is a heterogeneous combination of two'different DNAs. This combine is known as a **recombinant DNA** or **chimeric DNA** and the technology as **recombinant DNA technology.**

There are two methods of gene cloning: **host cell based cloning** and **Polymerase Chain Reaction (PCR) based cloning.**

12.1.1 Host cell-based cloning :

In this process, a host, a prokaryotic or a eukaryotic cell is used as a suitable environment for cloning a gene. Two important tools: **(1) enzymes and** (2) **nucleic acids** execute this process. The process consists of the following ten steps :

1. Isolation of the donor DNA.
2. Cutting of the donor DNA into fragments by a suitable restriction endonuclease (restriction enzyme).

3. Separation of the DNA fragments by agarose gel electrophoresis.
4. Transfer of the separated fragments onto a nylon membrane by blotting and identification of the donor fragment by molecular hybridization and autoradiography.
5. Isolation and cleavage of the vector (carrier) DNA.
6. Joining (ligation) of the donor DNA fragment to a vector DNA resulting in a recombinant or chimeric DNA
7. Delivery of the recombinant DNA into a host cell for amplification by transformation.
8. Plating and culture of the transformed host cells.
9. Screening and selection of the host cell clones containing the recombinant DNA.
10. Expression of the desired DNA fragment for a polypeptide product.

The fundamental steps in gene cloning are depicted in the Fig.12.1.

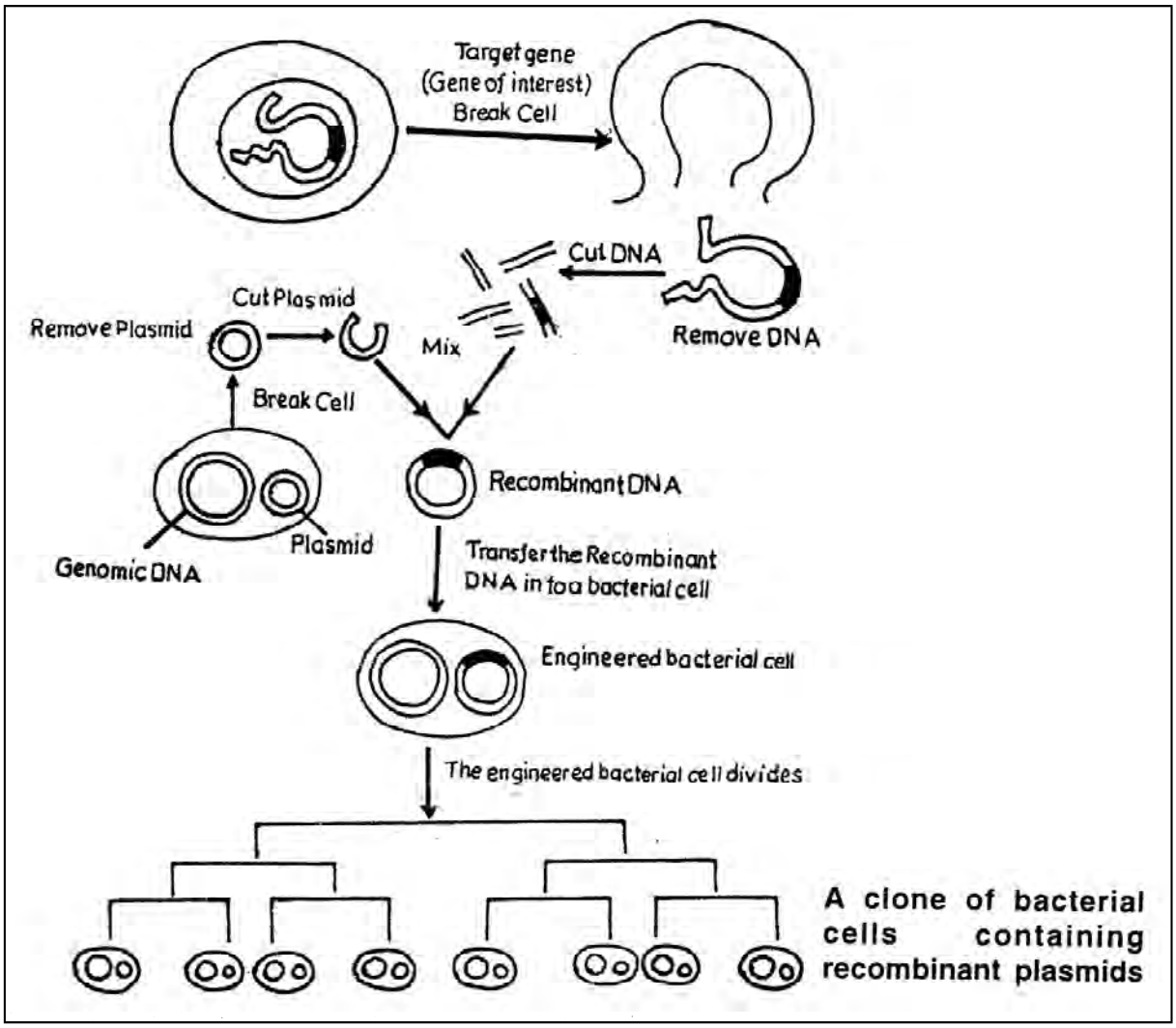

Fig.12.1 : Fundamental steps in gene cloning

12.1.1.1 Isolation of the donor DNA :

The DNA from the donor cell is isolated and purified following a standard procedure.

12.1.1.2 Cutting of the donor DNA :

The DNA is then cut into fragments by a specific group of enzymes called **restriction endonucleases** or **restriction enzymes (REs).** In addition to REs, other enzymes are also required in this practice. These enzymes, together with the vector DNA, constitute **important tools** of the trade.

Enzymes :

The enzymes fall under four general classes: **(a) Nucleases, (b) Polymerases, (c) Ligases and (d) DNA end modifying enzymes.**

(a) Nucleases : Nucleases are nucleic acid digesting or degrading enzymes. These break phosphodiester bonds joining the nucleotides in a polynucleotide chain. The nucleases are classified from different viewpoints. Some are nucleic acid specific (acting specifically on RNA or DNA) and some others are strand specific (acting on single or both strands). RNA nucleases are **ribonucleases** and DNA nucleases are **deoxyribonucleases.** Nucleases are classified as **exonucleases** and **endonucleases,** based on the site of action. An exonuclase degrades nucleic acids (DNA or RNA) from their free termini by breaking phosphodiester bonds and removing nucleotides one after another. Exonucleases are further classified as **exo (3'→ 5' or 5'→ 3'),** based on the direction of action on the strands. Conversely, endonucleases do not require free termini for action. They can act on linear as well as circular DNA molecules. They break internal phosphodiester bonds of single or double strands of DNA or RNA. Their action generates polynucleotide fragments of variable sizes. Most important among these are **restriction endonucleases or restriction enzymes** (REs). **Werner Arber**, **Daniel Nathans** and **Hamilton O. Smith** are credited with discovery and characterization of restriction endonucleases. For this discovery, they were awarded Nobel Prizes in Medicine or Physiology in 1978.

(i) **Restriction Endonuclease :** These are specific enzymes, which recognize specific sequences called, **recognition sequences** on DNA and make double stranded cuts either within the recognition sequence or at a variable distance from the recognition sequence. They act as scissors and therefore, often are referred to as **molecular scissors.** The point of cleavage is known as a **restriction site.** There will be as many restriction sites as the number of recognition sites. The REs that are used in the recombinant DNA technology fall under class II. These make double stranded symmetrical cuts within recognition sequences, generating cohesive or blunt ends.

(ii) **Nomenclature :** REs are named by a three-alphabet abbreviation after the name of the bacterial species, from which they are isolated. The first alphabet refers to the

genus, while the following two to the *species*. The three alphabets are followed by the strain of the bacterium. In some REs, Roman numerals like I, II, etc. follow to identify multiple REs isolated from the same species. The first enzyme is designated by the Roman numeral I and the subsequent enzymes by II and III and so on. For example, *Eco* **RI** is the first RE isolated from **RY 13 strain of** *Escherichia coli.* The subsequent enzyme from the same species and same strain is named as *Eco* **RII.** Around 500 REs have so far been isolated from bacteria. A few other REs, commonly used in the cutting of the DNA are Hin d III , Bam HI, Pst I and Hpa I.

Restriction Enzyme	**Source**
Hind III	*Haemophilus influenzae*
Bam HI	*Bacillus amyeloliquefaciens*
Pst I	*Providentia stuarti*
Hpa I	*Haemophilus parainfluenzae*

(iii) **Mechanism of Cutting :** All class II REs recognize specific short sequences in DNA called **palindromes** (Fig. 12.2) and cleave phosphodiester bonds on both the strands generating **3**'-OH and 5'-P ends. A palindrome is a sequence of base pairs, which reads the same on both the strands in the same direction. For example, the sentence **"Madam, I'm Adam"** reads the same in the reverse. This sentence is a palindrome.

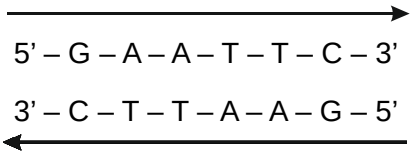

Fig. 12.2 : The recognition sequence of Eco RI depicting a palindrome

Some REs cleave both DNA strands **symmetrically around the line of symmetry** in the recognition sequence yielding fragments with **sticky** or **cohesive ends** [Fig.12**.**3 (b) & (c)]. Such type of cutting is known as **staggered cutting.** A cohesive end has an overhanging single stranded fragment, which is complementary to the cohesive end on the other strand. A fragment having two cohesive ends circularizes spontaneously by complementary base pairing between two overhanging cohesive ends. Such circles again become linear by heating. A few other REs cleave both the strands **on the line of symmetry** of the recognition sequence yielding **blunt** or **flush ended fragments** [Fig. 12**.**3 (a)]. REs, which recognize similar recognition sequences, are termed as **isoschizomers** *(Sau* **3A and** *Mbo* **I).** Although isoschizomers recognize the same recognition sequence, they break the phosphodiester bonds at different positions *(e.g.; Sma* I and *Xma* I)**.**

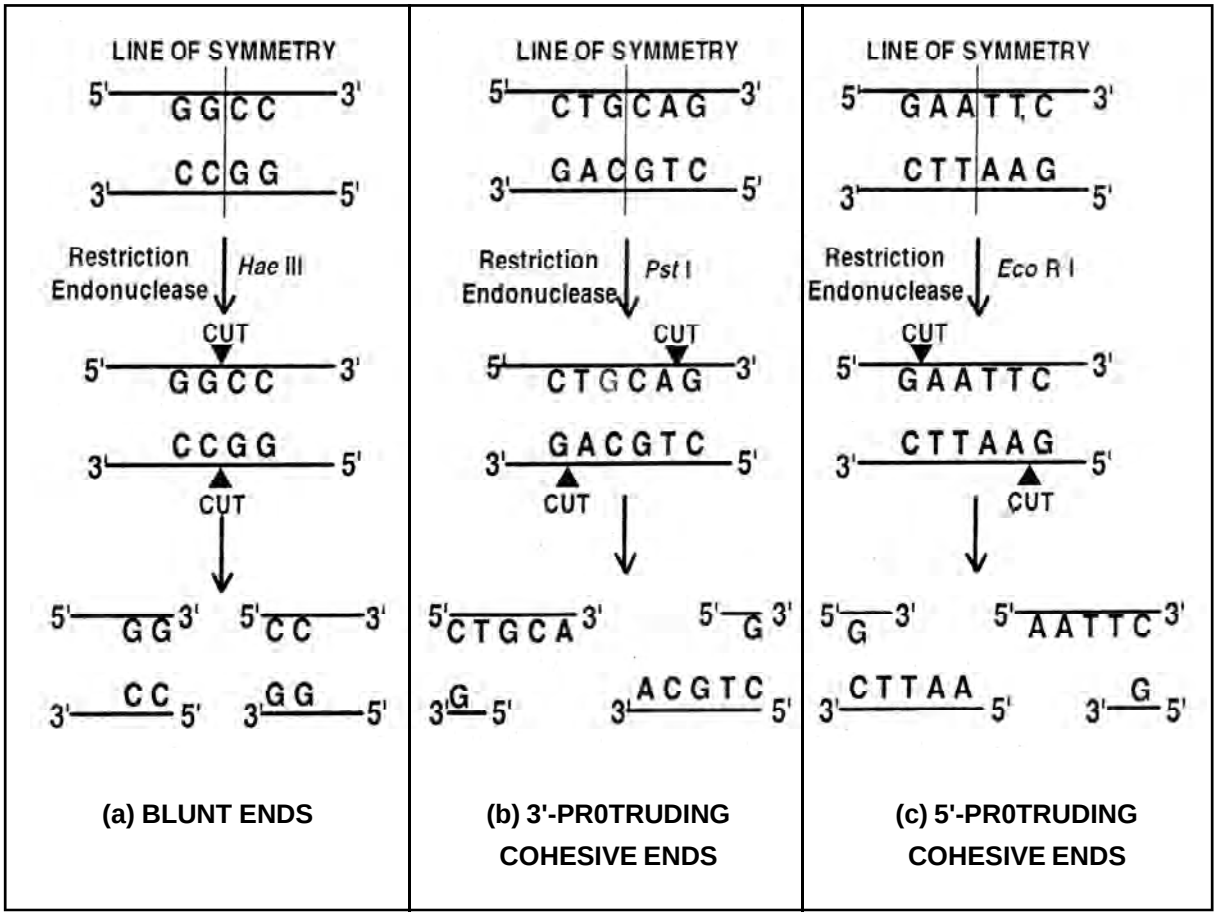

Fig. 12.3: Patterns of cleavage by restriction endonucleases. (a) Blunt (flush) ends; (b) 3'-Protruding cohesive ends; and (c) 5'- Protruding cohesive ends.

(b) Polymerases : Polymerases are enzymes that copy nucleic acid molecules. They are primarily classified as (1) RNA polymerase (DNA dependent RNA polymerase) and (2) DNA polymerase (DNA dependent DNA polymerase) based on the substrate. There is a third type of polymerase, the reverse transcriptase (RNA dependent DNA polymerase) in retroviruses. RNA polymerases catalyze the copying of a strand of DNA into RNA through a process known as **transcription.**

DNA polymerases are enzymes that catalyze the copying of a DNA strand into another complementary DNA strand through a process known as **replication.** A reverse transcriptase catalyzes the synthesis of a complementary DNA (cDNA) strand on an RNA template. This phenomenon is known as **reverse transcription.**

(c) DNA Ligases : A ligase is an enzyme that catalyzes the sealing or joining of a **3'**-OH end of a nucleic acid fragment with the 5'-P end of another fragment by forming a phosphodiester bond. It can therefore, be thought of as a **molecular glue,** which sticks DNA fragments together.

The enzyme used in gene manipulation is the **T4 DNA ligase,** which is purified from E *coli* cells, infected byT4 bacteriophages.

(d) End Modifying Enzymes : These enzymes modify the termini of DNA molecules in a variety of ways, which are used in gene manipulation. Important ones among these are **alkaline phosphatase, polynucleotide kinase** and **terminal transferase.**

12.1.1.3 Separation of the DNA fragments by electrophoresis :

Following the fragmentation of the DNA, the fragments are separated from each other by a technique known as **electrophoresis.**

Electrophoresis:

It is a technique of separating electrically charged molecules of different molecular weights in an electric field. DNA, RNA and protein molecules of differing molecular sizes are separated from their respective mixtures by this technique. This separation technique is known as **gel electrophoresis.** Here, a jeliy like porous and semisolid medium (gel) is used as the medium for separation of the molecules in an electric field. The semisolid gel is constituted by closely adhering gel particles, among which are very constricted passages. Following the application of a potential difference, the molecules migrate through these passages at differential rates. Therefore, the gel is often called **molecular sieve.** Two types of gels are used for molecular separations: **agarose** and **polyacryiamide. Agarose is used for molecular separation of nucleic acids** (both RNA and DNA), while **polyacryiamide for proteins.**

The rate of migration is inversely proportional to the molecular weight of the sepatating molecules and directly proportional to the strength of the electric current. Larger molecules migrate relatively slower, while smaller molecules migrate faster. The molecules are made negatively charged and hence, the migration is towards the anode (positively charged electrode). Nucleic acids can be conferred uniform negative charges in an alkaline buffer solution.This type of electrophoresis is known as **agarose gel electrophoresis** (Fig12.4).The separated DNA fragments are not visible as such. Therefore, the fragments are mixed with a suitable dye before loading the mixture of DNA fragments on to the gel. Two types of dyes are used: a visible dye **(methylene blue)** and an invisible dye that absorbs UV radiation and fluoresces orange **(ethidium bromide).** The molecular weights of the fragments are known from the distance, the fragments have travelled and then comparing with those of a set of standard fragments, also run parallel to the unknown.

However, a protein molecule contains many amino acids and all the amino acids do not have similar acid-base properties. Hence, a protein molecule cannot be conferred with uniform negative charges in a buffer solution. It is therefore, treated with **sodium dodecyl sulfate (SDS),** an **anionic detergent** that coats a protein molecule and confers negative charges

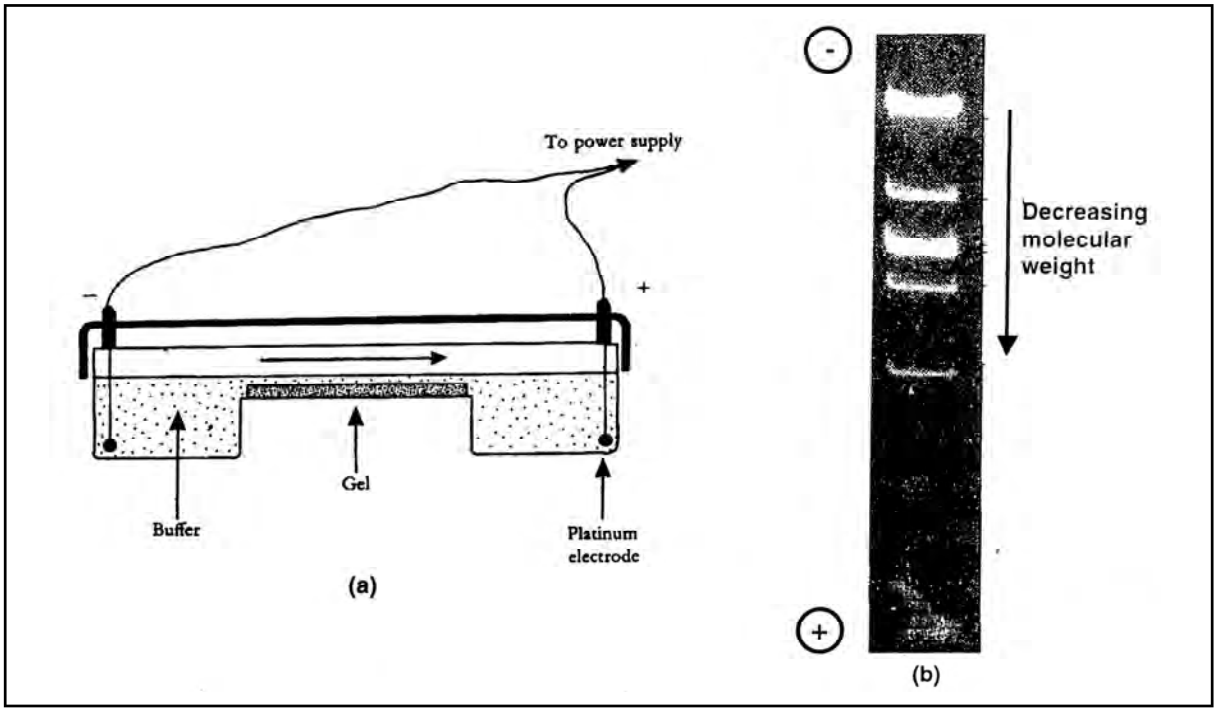

Fig. 12.4 : (a) Agarose gel electrophoresis apparatus, (b) Fluorescent bands on the agarose gel slab containing resolved DNA fragments with differing molecular weights. [Note that the migration is from cathode (-) to anode (+) and the rate of migration is inversely proportional to the molecular weight of the fragments.]

uniformly. Secondly, being a detergent, it breaks the bonds stabilizing the tertiary structure of proteins and thus facilitates their migration. A different gel known as polyacrylamide is used for proteins. The electrophoresis technique for proteins, therefore, is known as **Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS - PAGE).** The gel, containing the separated proteins, is stained with **Coomasie Brilliant Blue** or **Amido Black** stain solution. The stain resolves a series of bands along the vertical axis of the gel slab. The band, nearest to the start point, contains protein molecules with maximum molecular weight and hence, has least migration. Conversely, the band, farthest from the start point, contains protein molecules with least molecular weight and hence, has maximum migration.

12.1.1.4 Transfer of the separated fragments onto a nylon membrane (blotting) and identification of the fragment of interest by molecular hybridization and autoradiography :

The gel containing the separated fragments is then treated with an alkali to render the double stranded fragments single stranded by a phenomenon known as **denaturation.** These single stranded fragments are transferred onto a nitrocellulose filter paper or **nylon membrane** by a process known as **blotting.** There are three types of blotting procedures: **(1) Southern blotting, (2) Northern blotting and (3) Western blotting.**

(a) Southern Blotting (Fig.12.5) :

The blotting applied to DNA is known as Southern blotting. It has been named so after its discoverer **E. M. Southern (1975).** Southern blotting is followed by hybridization with a complementary DNA or RNA sequence **(probe)** that is labeled with ^{32}P (radioactive phosphorus). The probe finds its complementary target DNA sequence, binds to it by forming hydrogen bonds and forms a duplex. This operation is known as **molecular hybridization.** The procedure that combines Southern blotting and molecular hybridization is known as **Southern blot hybridization.** Then **autoradiography** is performed to identify, the target DNA sequence.

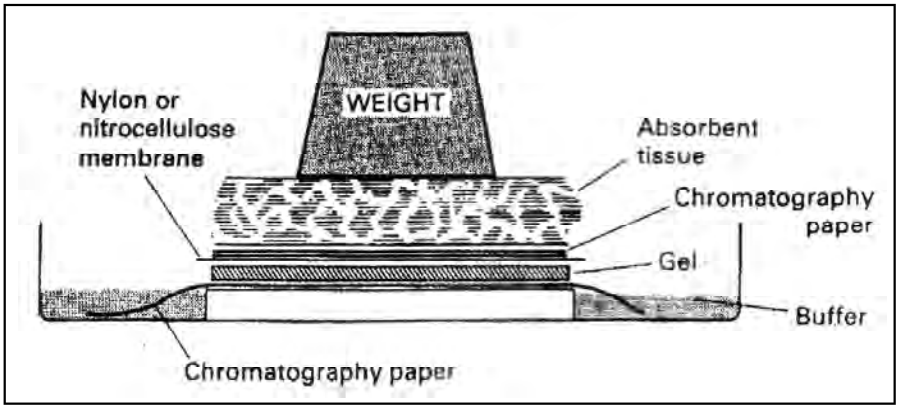

Fig.12.5 : A Southern blotting (capillary blotting) apparatus

(b) Northern Blotting :

The blotting of RNA is known as Northern blotting. The RNA molecules are separated by agarose gel electrophoresis and then transferred to a nylon membrane. The specific sequence can be identified by hybridizing the sequence with its complementary radiolabeled single stranded DNA probe followed by autoradiograpty.

(c) Western Blotting :

The transfer of protein molecules separated by SDS-PAGE onto a membrane is known as western blotting. Unlike that of Southern blotting, the separated protein molecules are transferred to the membrane by creating a potential difference across the polyacrylamide gel slab, a process, known as **electroblotting.**

Northern and Western terminologies are whimsically used, unlike that of Southern. Nobody has yet come up with Eastern blotting.

12.1.1.5 Host - Vector systems :

The identified target DNA fragment by Southern blot hybridization is, next, transferred to a **host cell** for cloning or amplification. Since, the fragment does not have a replication

property; it is joined to another DNA fragment having an autonomous replication property. This DNA is known as the **carrier** or **vector DNA.** The target DNA and the vector DNA are from two different sources. This heterogeneous combine is known as a **recombinant DNA** or **chimeric DNA.**

Both prakaryotjc and eukaryotic host cells are used for cloning of genes. The target DNA and the vector DNA fragments must be compatible with each other, on the one hand, and the recombinant DNA must replicate freely in the host cell, on the other. Therefore, each of the host cell, discussed above, has its characteristic compatible vector. The vector and the host cell are to be carefully chosen for the cloning of a gene. The host cell and the vector, thus, constitute another class of tools of the trade. The vector is also known as a **cloning vehicle** and a suitable vector has the following properties: (1) It is a DNA molecule; (2) It has an origin of replication; (3) It has one or more marker genes for screening; and (4) It has a cloning site at which the target gene is inserted.

Several types of cloning vectors are used in the cloning of a gene, based on the size of the target DNA fragment: **(i) Plasmid, (ii) DNA and RNA bacteriophages (iii) Hybrid plasmid -bacteriophage vector (Cosmid) (iv) Bacterial artificial chromosome (BAC) and (v) Yeast artificial chromosome (YAC).** The choice of the vector is very important in gene cloning. For example, genes in the range of 5 -10 Kb (Kb is Kilobase; 1 Kb = 1000 base pairs) can be stably cloned in a bacterial plasmid. When the size of the target gene is more than 5 - 10 Kb, it is cloned in bacteriophage vectors and cosmids. Human genes, having thousands of base pairs are cloned in special cloning vectors, such as BACs and YACs. In the underlying section, we have discussed about a plasmid, due to its simple and easy to use structure.

(a) Prokaryotic host cell :

Although, a recombinant DNA has an origin of replication, it can't replicate alone. It requires a system, containing all the bare minimums, like a replication enzyme (DNA polymerase), the building blocks (nucleotides) and other essential things. All these are available in the simplest prokaryotic cell, a bacterium. In essence, a bacterial cell functions as a factory for cloning of a gene and also for its expression for the formation of a useful product. **The most suitable host cell is *E. coil* K12 (K12 being the strain).** This cell functions as a cloning host. There are other cloning hosts. However, *E.coli is* the host of choice due to its simple structure.

(b) Prokaryotic cloning vector :

Many prokaryotic cell compatible vectors are in use for cloning of genes. More common among these are (i) plasmids and (ii) plasmid-bacteriophage hybrid (cosmids).

(i) Plasmids : Plasmids are small and double-stranded extrachromosomal DNA molecules having an origin of replication.They occur naturally in bacteria. Naturally occurring plasmids are not suitable for the cloning of genes. They are genetically engineered and

made suitable for cloning. Such plasmids are called **cloning plasmids.** A suitable cloning plasmid should preferably have the following properties: (i) it should be **non-conjugative;** (ii) it should have a **relaxed replication** and a **high copy number.** (iii) it should have an origin of replication. (iv) it should have unique restriction sites, and (v) it should have antibiotic marker gene for selection.

The most common cloning plasmid is designated as **pBR 322,** where p stands for the word plasmid and B and R signify the names of its engineers **(F.** Boliver and R. Rodriguez). **322** is a numerical designation that has a relevance to these workers, who worked out the plasmid.

The following are the features of pBR322 (Fig.12.6):

1. It is a small double stranded circular DNA molecule.
2. It has an origin of replication, ***ori C.***
3. It has two antibiotic resistance marker genes: (i) **ampicillin resistance gene** (A_p^r) and (ii) **tetracycline resistance gene (T_c^r)**
4. A unique ***Eco* RI** restriction site is engineered into a region between the A_p^r and T_c^r marker genes.
5. A unique site, each for ***Pvu* I** and ***Pst* I** restriction enzymes is engineered into the A_p^r gene.
6. A unique site, each for ***Bam* HI** and ***Sal* I** is engineered into the T_c^r gene.

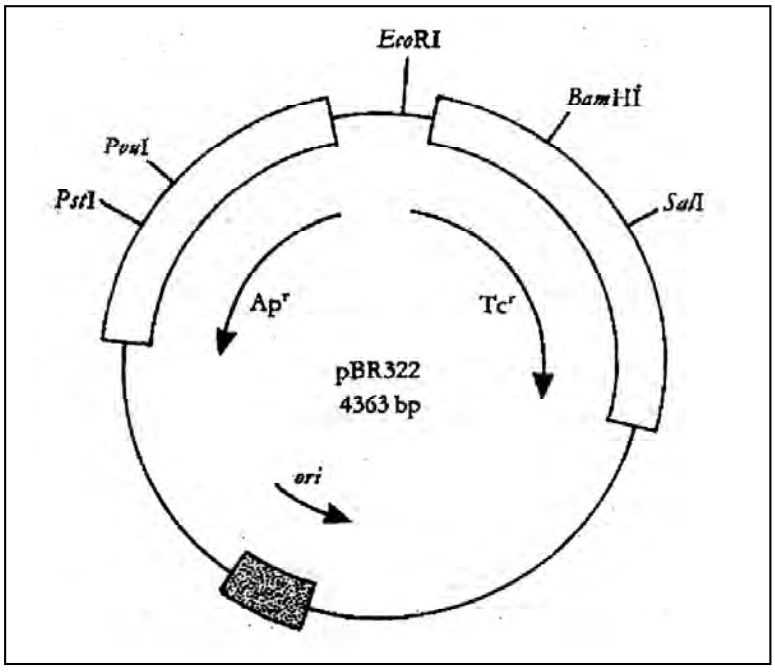

Fig. 12.6 : A map of pBR322 with an origin of replication ***(Ori),*** antibiotic resistance genes: ampicillin resistance gene ***(Ap^r)*** and tetracycline resistance gene (Tc^r) and a few unique restriction sites.

(ii) Plasmid - Phage hybrid cloning vector (Cosmid) : A cosmid is a **hybrid cloning vector** of a typical cloning plasmid and the **cos site** of a bacterophage DNA. The cos site refers to cohesive site, formed by complementary base pairing between two cohesive ends of the λ bacterophage DNA. This cos site is engineered into a bacterial plasmid to result in a cosmid. The cos site is significant from the viewpoint that it can accept a gene in the size range of 30–45 kb. It is a suitable cloning vector for cloning many mammalian genes.

12.1.1.6 Isolation and cleavage of the vector (plasmid) :

All plasmids used for gene cloning are genetically engineered and are available commercially, packaged into bacteria. There is a standard procedure for the isolation of plasmids from these bacterial cells. Following isolation, the cloning plasmid is cleaved by using the same RE that is used to cleave the target gene from the donor DNA. This generates complementary cohesive ends in the gene and the cleaved plasmid. This facilitates the joining of the gene onto the plasmid by complementary base pairing. The restriction site is carefully engineered into a suitable place of the plasmid so that the screening of the transformed host cells containing recombinant plasmids becomes relatively easier.

12.1.1.7 Joining of the target DNA fragment on to the vector DNA (plasmid) - Recombinant DNA :

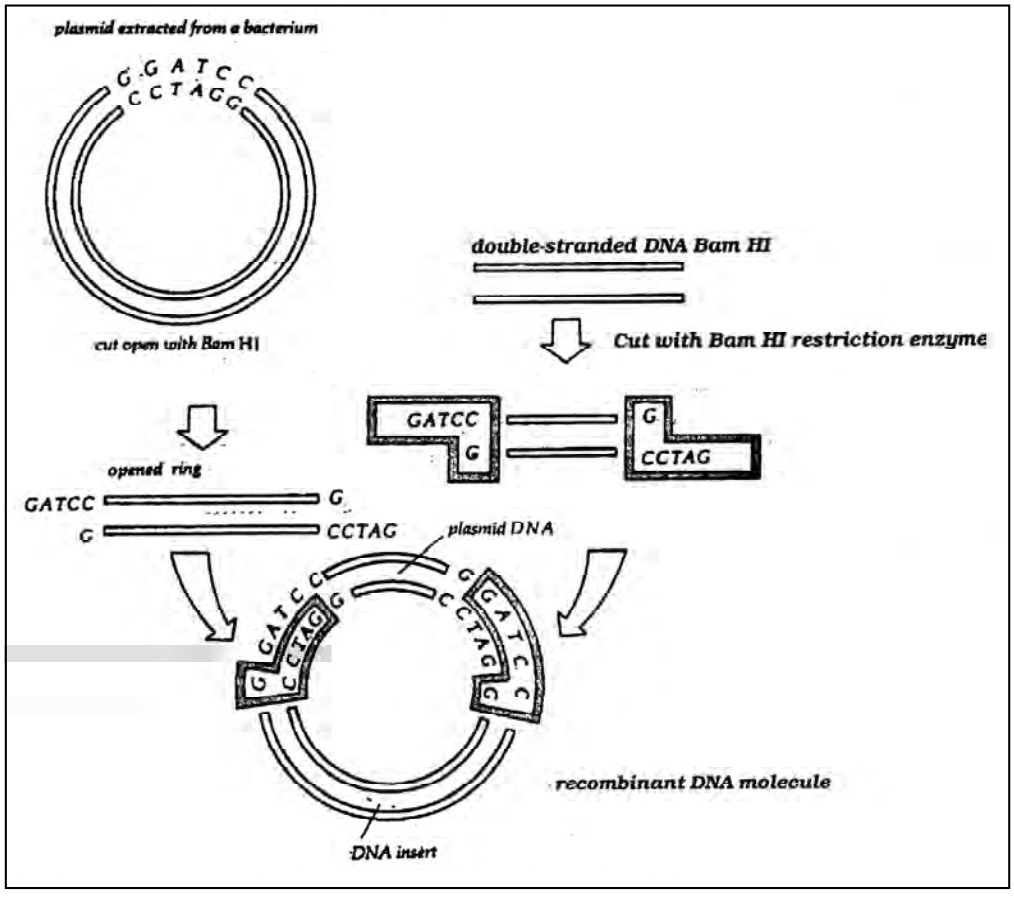

Fig.12.7 : Construction of a recombinant DNA molecule.

When the DNA inserts are mixed with the cleaved plasmids, the overhanging cohesive ends of both undergo complementary base pairing and form a double stranded circular DNA. The sites of joining of the two fragments, still have no phosphodiester bonds. The enzyme **DNA ligase** is used to form phosphodiester bonds between the donor DNA fragment and the plasmid, thus, forming a completely closed DNA circle. The new double stranded DNA circle, containing the target gene and the plasmid, is called a **recombinant DNA** or a **chimeric DNA** (Fig. 12.7). The process of formation of the recombinant DNA is called **recombinant DNA technology**. Recombinant DNA is constructed in the laboratory under controlled conditions. Paul Berg, Stanley Cohen and Herbert Boyer made significant contributions in the construction of a recombinant DNA. However, Paul Berg was awarded a Nobel Prize in Chemistry in 1980 for this significant work.

12.1.1.8 Delivery of the recombinant DNA into the host cell for amplication (Transformation):

(a) Conventional Method (Cold Calcium Chloride Method) : The recombinant DNA, formed in the preceding step, is delivered into a host cell for its amplification. The widely used prokaryotic host cell is ***E. coli.*** **The process of uptake of plasmid DNA is known as transformation.** Prior to transformation, the host cell is made competent, in which the plasma membrane is temporarily made permeable to the recombinant plasmids. This is achieved by soaking the host cells in an ice-cold solution of calcium chloride.

(b) Alternate Methods : The pant and animal cells can be transformed by one of a few direct physical methods outlined below.

(i) Microinjection (Fig.12.8) : It is a direct method of DNA delivery into plant and animal cells. In animal cells, especially in mammals, microinjection is the choice to deliver a piece of foreign DNA (transgene) into the fertilized egg. The microinjected DNA is in a linear form and is injected alone i.e. without the assistance of a vector DNA. The fertilized egg has two pronuclei, the male and the female before karyogamy. The male pronucleus is larger and can be traced by using a dissecting microscope. The egg is held on the tip of a glass micropipette by mild suction and the foreign DNA is injected into the male pronucleus with a glass micropipette (0.5 μ in diameter). The injected DNA recombines with the DNA of the male pronucleus and the male pronucleus in turn fuses with the female pronucleus to result in a zygote nucleus. All this is carried out *in vitro.* The microinjected eggs are implanted into the uterus of a female. The same practice is applicable to plant cells. However, the cellulose cell wall is removed by enzymatic digestion before microinjection. The procedure, apparently, seems simple but, indeed involves a lot of care and precision. The percentage of success is exceedingly low. For example, out of 1000 microinjected eggs, 30-50 only are successful.

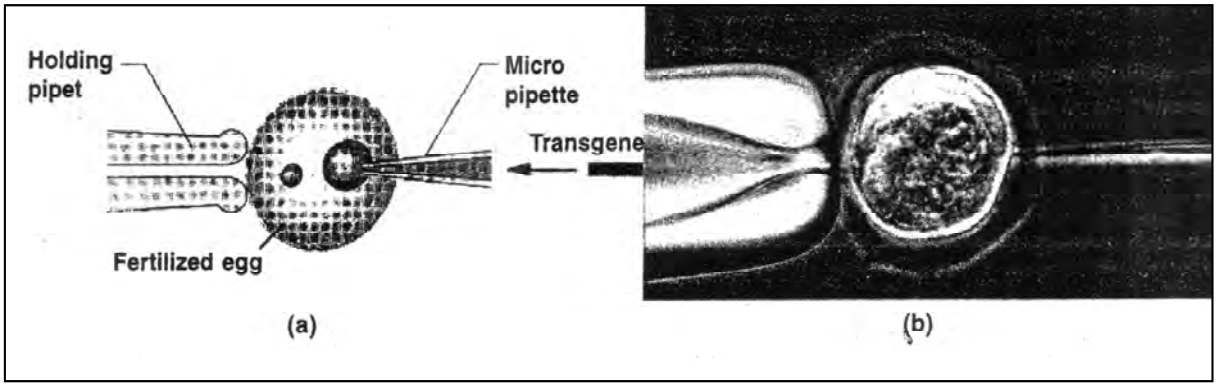

Fig.12.8: (a) An outline of ***in vitro*** microinjection of foreign DNA fragment into a mammalian fertilized egg. (b) Photomicrograph of the same.

(ii) Electroporation : Plant protoplasts can be transformed by foreign DNA by this method. The plant protoplast is a plant cell, whose cell wall is removed. The protoplasts are placed in a suitable medium containing foreign DNA. Brief pulses of high voltage electric current are passed through the medium. It creates temporary openings in the plasma membrane, through which the naked foreign DNA or recombinant plasmids can enter into protoplasts. The cell wall is regenerated following the entry of the DNA.

(iii) Microprojectile Bombardment: Variously named as **particle gun bombardment** or **biolistcs** or **shot gun,** the technique consists of bombarding spherical gold or tungsten particles coated with precipitated target DNA. The particles (1 - 4 mm in diameter) are coated with DNA, precipitated with calcium chloride or spermidine or polyethylene glycol. An apparatus called a **particle gun** is used to accelerate the coated particles to a high speed of 300 - 600 m/sec.The coated particles are called **microprojectiles,** which when bombarded, penetrate cell walls and membranes of plant cells.

12.1.1.9 Plating and culture of the transformed host cells:

The transformed cells are then plated on a selective nutrient media for propagation and colony formation. Streaking of the inoculated transformed host cells is done on the media to ensure pure colony formation. The culture plate with the inoculated transformed *E. coli* cells are incubated at 37° C in an incubator for growth and formation of single colony clones.

12.1.1.10 Screening and selection of the host cell clone containing the recombinant plasmids (Table : 12.1 & Fig. 12.9) :

The construction of the recombinant DNA and the transformation of *E. coli* cells by the recombinant DNA are very delicate and sensitive. The percentage of success in both is extremely low. Secondly, the success of these processes cannot be monitored under a microscope. Therefore, an analytical screening procedure is adopted to select the correct transformed clones,

before proceeding to the next step. There may be three possible outcomes from these manipulating procedures:

1. Some *E. coli* cells may not undergo transformation by the cloning plasmids.
2. Some *E. coli* cells may be transformed by unrecombined cloning plasmids.
3. Some *E. coli* cells may be transformed by recombinant plasmids.

In the event of the above-mentioned circumstances, it becomes imperative to locate the clones of *E. coli* having the incorporated recombinant plasmid. This process is known as **screening.** The right clones are selected and others are discarded. Screening is done by examining the antibiotic resistance properties of the *E. coli* clones. This screening procedure has been termed as screening by **insertional inactivation**. The target DNA, if is inserted into the T^r_c, the resistance property of the host cell to tetracycline is lost and the cell becomes sensitive to the antibiotic, but still is resistant to ampicillin. Alternately speaking the T^r_c is inactivated by the insertion of the doner DNA fragment.

Table-12.1 : Screening and selection of the right *E. coli* clones

CELLS OFTHE SINGLE COLONY CLONE	ANTIBIOTIC RESISTANCE	
	TETRACYCLINE	AMPICILLIN
1. Cells containing no plasmid	-	-
2. Cells containing unrecombined plasmid	+	+
3. Cells containing recombinant plasmid	-	+

(-), sensitive; (+), resistant

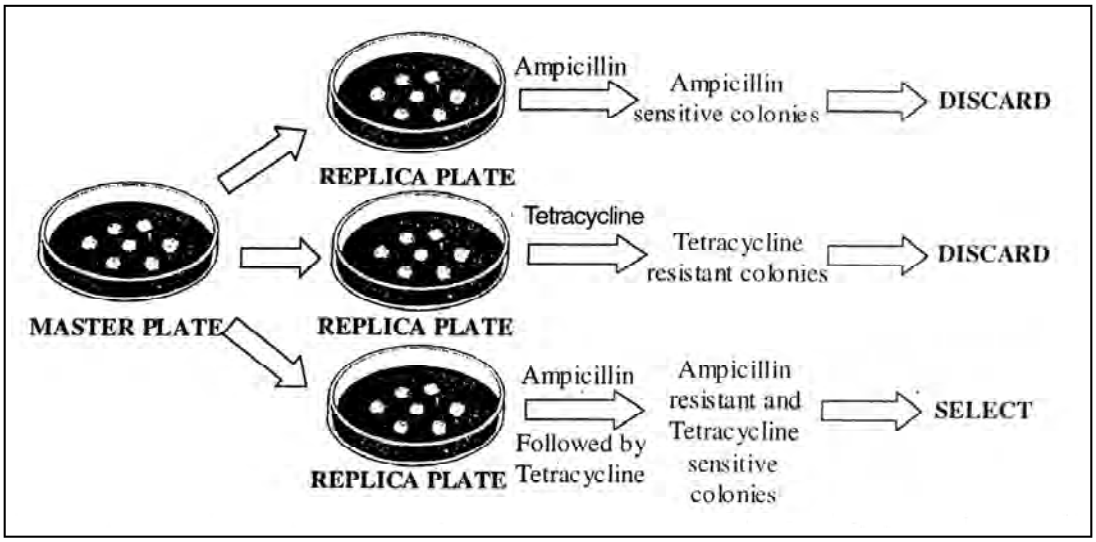

Fig. 12.9 : Screening of the recombinant colonies by insertional inactivation of tetracycline resistance marker gene (T^r_c)

12.2 POLYMERASE CHAIN REACTION (PCR) BASED GENE CLONING :

In the preceding sections, we discussed about the cloning of a gene in a suitable host cell. However, a gene can also be cloned or amplified without the assistance of a host cell by a specific reaction, known as **polymerase chain reaction (PCR).** PCR was discovered by **Kary B. Mullis** in 1983. The reaction is carried out in a **thermocycler** having a **thermal cycling (heating and cooling) programme.** The target gene is put along with other substrates in the thermocycler. The DNA fragment doubles at the end of the first cycle and quadruples after the second cycle and so on. In this amplification process, no host cell is used as the supporting system.

12.2.1 The reaction (Fig.12.10) :

The amplification of a segment of DNA takes place in the following three steps : **(1)** denaturation; **(2)** primer annealing; and **(3)** extension. All the constituents of the PCR are put in to a thermocycler in a PCR tube, programmed to an appropriate reaction condition. The input consists of the target DNA, primer (17-30 nucleotides in length), nucleotide precursors and a DNA polymerase for extension.

(a) **Denaturation :** The target DNA is heated to 94°C for 1.0 min to render it single-stranded.

(b) **Primer Annealing :** The reaction mixture is cooled to 56° C for 1.5 min. Oigonucleotide primers having complementarity are annealed to the 3' ends of both the denatured single strands. Primer selection is the most important task in the PCR amplification process.

(c) **Extension :** The reaction mixture is the heated to 72° C for 1.0 - 1.5 min.The DNA polymerase elongates the new strand by adding nucleotides in a 5'$\rightarrow$ 3' direction. Mullis used the **Klenow fragment** of the DNA polymerase I in their original work. DNA polymerase I has three functions: a polymerase (elongation) function and both 5'$\rightarrow$3' and 3' $\rightarrow$ 5' exonuclease functions. The 5' $\rightarrow$ 3' exonuclease function is removed by cleaving the enzyme. The remaining fragment retains the polymerase and 3' $\rightarrow$ 5' exonuclease functions. The latter fragment has been referred to as **Klenow fragment**. It is used to copy single stranded DNA fragments. A fresh enzyme source has to be used in each cycle of reaction because of the the denaturation step. **Lawyer *et al.,*** 1990 discovered a thermostable DNA polymerase from the **sulfur spring bacterium, *Thermophilus aquaticus.*** This polymerase was resistant to higher temperatures such as that of the denaturation temperature (94°C).This polymerase is designated as **Taq polymerase.** As a consequence of employing a heat-resistant enzyme, the PCR could be automated by putting the reaction mixture in a thermocycler with a suitable thermal cycling programme.

Taq DNA polymerase lacks a 3'—»*5* proofreading exonuclease activity. It cannot detect if a wrong base is incorporated during elongation Other thermostable DNA polymerases have been used to partly overcome this problem. However *Taq* polymerase remains the DNA polymerase of choice in PCR amplification reactions. It is important to sequence and compare the amplified DNA molecules following the extension reaction.

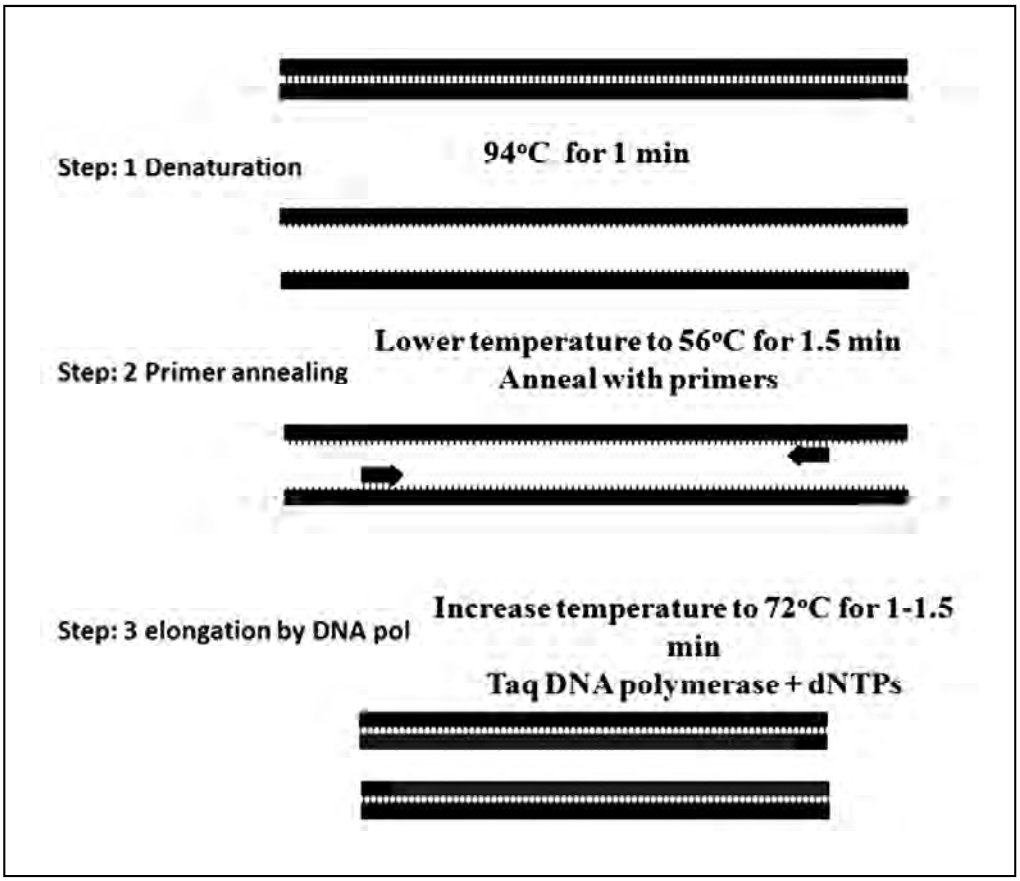

Fig.12.10 : Polymerase chain reaction (PCR).

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer:

(i) The double helical structure of DNA was proposed by:

(a) Jacob and Monod (c) Watson and Crick

(b) Sanger and Gilbert (d) Beadle and Tatum

(ii) Polymerase Chain Reaction was discovered by:

(a) H. Khorana (c) R. Holley

(b) K. Mullis (d) M. Nirenberg

(iii) Exonuclease is an enzyme that:

(a) Makes internal cuts in polynucleotide

(b) Polymerizes nucleotides

(c) Joins two polynucleotide fragments

(d) Removes nucleotides from the termini one after another

(iv) DNA ligase is commonly known as:

(a) Molecular scissors (c) Molecular glue

(b) Molecular marker (d) Molecular probe

(v) During electrophoresis, DNA fragments move from:

(a) Anode to cathode (c) Move randomly

(b) Remain static (d) Cathode to anode

(vi) The blotting of protein molecules to a nylon membrane is known as:

(a) Southern blotting (c) Northorn blotting

(b) Western blotting (d) Eastern blotting

(vii) Detection of a desired DNA fragment by using radioactive emission is known as:

(a) Hybridization (c) Autoradiography

(b) Denaturation (d) Electrophoresis

(viii) Choose the incorrect answer:

(a) A plasmid is small, double stranded circular DNA

(b) A plasmid contains an origin of replication

(c) A plasmid has several restriction sites

(d) A plasmid has telomeres

(ix) A cosmid is a:

(a) Plasmid Phage hybrid vector (c) Expression vector

(b) DNA bacteriophase vector (d) Viral vector

(xi) The example of a plant cell compatible vector is:

(a) Fertility plasmid (c) Tumor inducing plasmid

(b) Colicinogenic plasmid (d) Resistance plasmid

(xii) Amplification of DNA by PCR uses a DNA polymerase called:

(a) *Taq* DNA polymerase (c) DNA polymerase III

(b) RNA polymerase (d) Reverse transcriptase

2. Fill in the blanks with appropriate words:

(i) The phenomenon of fermentation was demonstrated by ____.

(ii) The word'Biotechnology' was coined by ____.

(iii) Class II restriction endonucleases (enzymes) recognize specific nucleotide sequences in DNA called ____.

(iv) Cohesive ends in the DNA fragments are generated by ____ cutting.

(v) The anionic detergent, used in polyacrylamide gel electrophoresis is known as _____.

(vi) The transfer of separated protein molecules from the gel into a nylon membrane is known as ____.

(vii) Detection of desired DNA fragment by the emission of ionizing radiation is known as ____.

(viii) The conjoint structure formed by the joining of the vector DNA and the target DNA fragment is known as ____.

(ix) The uptake of the recombinant DNA by the bacterial host cell is known as ____.

(x) The delivery of a foreign DNA fragment into the fertilized egg with a micropipette is known as ____.

3. Answer each of the following in one word:

(i) The technique of separation of DNA fragments based on their molecular weight and electrical charge.

(ii) The restriction endonuclease isolated from *Escherichia coli*.

(iii) The DNA digesting enzyme that removes nucleotides from the termini.

(iv) The enzyme that catalyzes the synthesis of RNA on a DNA template.

(v) The enzyme that catalyzes the replication of DNA.

(vi) The enzyme that catalyzes the synthesis of a complementary DNA strand on an RNA template.

(vii) The fluorescent dye used in agarose gel electrophoresis.

(viii) Transfer of DNA fragments from the agarose gel to a nylon membrane.

(ix) Breaking of hydrogen bonds in a duplex so as to make it single stranded.

(x) The DNA that helps carry the target DNA fragment to the host cell for cloning.

(xi) The plasmid phage hybrid cloning vector.

(xii) A plant cell, whose cellulose cell wall is digested.

(xiii) The plasmid present in *Agrobacterium tumefaciens*.

(xiv) Transfer of a DNA fragment into a host cell in a medium by passing brief pulses of electric current through the medium.

(xv) The instrument used in PCR amplification.

4. Match the words of Group 'A' with those of group 'B' to make meaningful pairs:

I.

Group A	**Group 'B'**
Resrricyion endonuclease	End modifying enzyme
DNA ligase	RNA dependent DNA polymerase
Exonuclease	Molecular scissors
Reverse transcriptase	Removes nucleotides from both ends
RNA polymerase	DNA dependent DNA synthesis
DNA polymerase	Molecular glue
Alkaline phosphatase	DNA dependent RNA synthesis

II

Group 'A'	**Group 'B'**
Polyacrylamide	*Agrobacterium tumefaciens*
Southern blotting	Thermocycler
Plasmid	Protein electrophoresis
Agarose gel	Denaturation
Cos	Taq polymerase
Tumor inducing plasmid	Cloning vehicle
Recombinant DNA	Molecular marker
DNA coated tungsten particles	Nucleic acid electrophoresis
Insertional inactivation	Chimeric DNA
Breaking of interchain hydrogen bonds	Particle gun
Equipment for PCR amplification	Screening of clones
Ampicillin resistance gene	Blotting of DNA
Therophilus aquaticus	Cohesive site

GROUP - B
(Short Answer-type Questions)

1. Answer each of the following in 50 words:

(i) Define biotechnology
(ii) Define gene cloning
(iii) What is a restriction endonuclease (restriction enzyme)? Why is the word restriction used to designate these?
(iv) Describe two types of cutting of DNA, executed by restriction endonucleases.
(v) What is electrophoresis? How many types of electrophoresis you have studied?
(vi) What is a palindrome? Give an example.
(vii) What is a polymerase? How many types of polymerases you have studied?
(viii) Why is DNA ligase called molecular glue?
(ix) What is Southern blotting?
(x) Why is SDS used in polyacrylamide gel electrophoresis?
(xi) What is autoradiography?
(xii) Enumerate the features of a suitable cloning plasmid.
(xiii) What is a recombinant DNA?
(xiv) What is microinjection?
(xv) Describe briefly electroporation.
(xvi) What is polymerase chain reaction (PCR)?

2. Write brief notes on the following:

(a) Restriction endonuclease
(b) DNA ligase
(c) DNA polymerase
(d) Southern blotting
(e) Agarose gel electrophoresis
(f) Cloning plasmid
(g) Cosmid
(h) Recombinant DNA
(i) Polymerase chain reaction
(j) Microinjection

GROUP - C
(Long Answer-type Questions)

1. Decribe briefly about the making of a recombinant DNA.

❑ ❑ ❑

CHAPTER 13

APPLICATIONS OF BIOTECHNOLOGY

Biotechnology is an integrated applied branch of science, which mainly deals with the use of microorganisms, plant and animal cells in culture systems or entire plants or animals for the production of **beneficial products (molecules)** and / or **services** through **gene manipulation** technique. Its applications range from agriculture to health care management and from industry to environment. It has revolutionalized human life and promises to bring much more in the future.

Some of the fore front applications in agriculture and forestry and medicine and healthcare sectors are discussed briefly in the underlying sections. However, some specific applications are discussed a little more elaborately as per the syllabus.

13.1 APPLICATIONS IN AGRICULTURE AND FORESTRY :

13.1.1 Plant cell and tissue culture :

Plant cell and tissue culture has been amalgamated with plant genetic engineering giving rise to a new branch known as **plant biotechnology.** Plant genetic engineering involves the manipulation of the plant genome by transferring a beneficial gene from one plant cell to another and then growing the transformed plant cell in an artificially enriched media. The totipotent cell divides. and re-divides and forms a mass of undifferentiated cells that constitute a **callus** (Fig. 13.1). On appropriate hormonal stimulation, rooting and shooting takes place and the

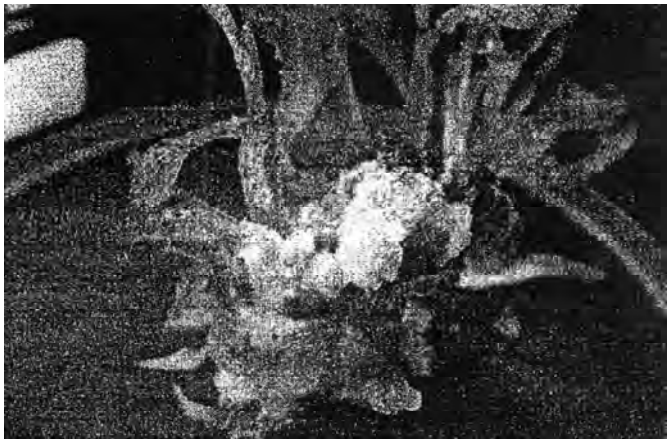

Fig.13.1 : A mass of undifferentiated plant cells (callus) regenerated from an explant cultured on a growth media.

callus turns into a **plantlet,** ready to be transferred to the soil. Novel and economically important plants combining beneficial characters of two plants have been generated by applying plant cell and tissue culture technique. Plantlets of large forest trees have been raised by plant tissue culture technique and transferred to barren and unutilized land to regenerate new forests.

13.1.2 Drought resistant plants :

Genetic engineers have identified genes, whose products may help the plant retain more water and withstand prolonged drought condition. These genes may be introduced into isolated plant cells in culture and novel plants generated by tissue culture technique.

13.1.3 Fungi and bacteria resistant plants :

Pathogenic bacteria and fungi infect a wide variety of agriculturally important plants, thereby decreasing harvest. However, some plants do possess the inherents properties of protecting themselves against these pathogenic organisms. They do so by encoding enzymes and other proteins. Their encoding genes are introduced into isolated plant cells in culture and fungi and bacteria resistant plants are generated by tissue culture technique.

13.1.4 Virus resistant plants :

Transgenic plants have been generated, which express the coat protein genes of infectious plant viruses. The coat protein, thus expressed, turns on the plant version of the immune system. Thus, the expressed coat proteins function as **plant vaccines.**

13.1.5 Biopesticide (*Bt* Protein) :

For an elaborate discussion refer to section 13.7.

13.1.6 Herbicide resistant plants :

Herbicide resistant transgenic plants have been generated by transferring bacterial herbicide resistant genes into plant cells grown in culture. **Glyphosate** is the most widely used broad-spectrum herbicide world over. A glyphosate resistant gene from ***Petunia*** plant is transferred into isolated plant cells in culture and glyphosate resistant plants generated.

13.1.7 Ice (Frost) protection :

Crops grown at sub-zero temperature are subjected to frost damage due to formation of ice crystals in their tissues. A gene in a soil bacterium, ***Pseudomonas syringae,*** is known to be involved in promoting ice nucleation.This gene is deleted by gene technology method and a mutant *P. syringae* engineered. This strain is known as **INA- or ice minus strain,** which when sprayed on crops prevents frost formation.

13.1.8 Bioplastic :

Natural plastic is a polymer of organic compounds. It is non-bio-degradable and hence, causes serious pollution problems through dumping. An alternative to this has been discovered in biotechnology. A group of microorganisms synthesizes biopolymers, similar to natural plastic. The gene encoding the enzyme in the synthesis of this biopolymer is isolated and transferred to corn plant cells in culture and transgenic corn plants are generated, which expresse the transgene and synthesize the biopolymer. This biopolymer is used as **bioplastic.** The advantage of bioplastic is that it is biodegradable and hence, does not cause any environmental pollution problem.

13.1.9 Novel transgenic plants :

Novel flower colours, not naturally found, are also generated by gene manipulation. A gene, encoding an enzyme, involved in the flower pigment biosynthetic pathway, is introduced into ***Petunia*** protoplasts in culture and transgenic plants generated, which bore pink flowers. Similarly, purple and brick red colours have also been engineered to appear. **Florigene,** a biotech company in 1996 released the first-ever genetically manipulated flower into the market. Experiments are underway to induce blue pigment formation in rose plant so as to produce blue roses.

A luminescent tobacco plant is another example of a novel plant generated through gene manipulation. The **luciferase gene of bacteria or firefly** expresses luciferase. It acts on the substrate **luciferin** and turns it into a product, which glows in dark. This phenomenon is known as **bioluminiscens**.The tobacco plant cells in culture are transformed by a recombinant *Ti* plasmid containing a luciferase gene. Plants, generated, glow in dark in the presence of the substrate **luciferin** (Fig. 13.2).

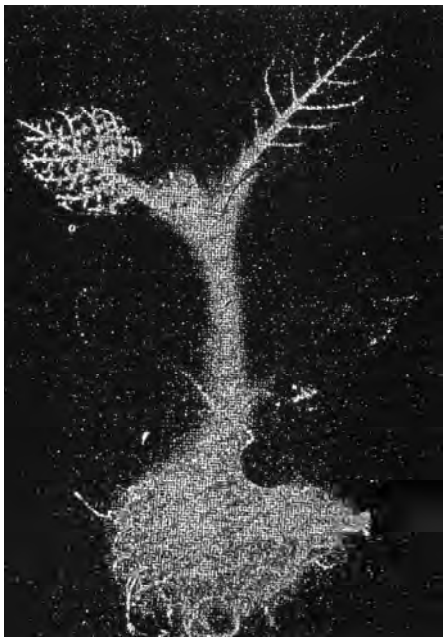

Fig.13.2 : A bioluminescent plant containing the luciferase gene of firefly.

13.1.10 Protein producing plants :

Now, transgenic plants are projected as **bioreactors** for the synthesis of many **therapeutic proteins.** Many **mammalian proteins,** such as **enkephalin (a neuro-peptide)** and **human serum albumin** have been successfully expressed in plants.

13.1.11 Golden rice:

Golden Rice is a transgenic variety of rice with an elevated level of **β-carotene (provitamin A), a precursor of vitamin A.** The genes encoding the enzymes of the β-carotene biosynthetic pathway are introduced into rice plant cells in culture. Transgenic rice plants are generated, which produced rice with β-carotene.

13.1.12 Delayed fruit ripening (Flavr-Savr tomato) :

Fruit ripening in tomato and other fruits and vegetables are delayed by manipulating a gene, involved in softening and ripening. A variety of tomato plant has been successfully engineeed, which bears tomatoes, known as **Flavr-Savr tomatoes.** This variety exhibits a delayed ripening.

13.1.13 Nitrogen fixation :

Majority of plants depend upon soil microorganisms for their nitrogen requirement. The atmospheric nitrogen is trapped by soil microorganisms and converted into soluble nitrate by a process, called **biological nitrogen fixation.** This process is catalyzed by an enzyme complex, **nitrogenase,** encoded by many **nitrogen fixing *(nif)* genes.** Non-nitrogen fixing plants are genetically modified by introducing nif genes from *Rhizobium* for nitrogen fixation.

13.2 APPLICATION IN MEDICINE AND HEALTHCARE MANAGEMENT :

13.2.1 Recombinant therapeutic molecules:

Insulin and **human growth hormones** are among the first few therapeutic protein molecules, produced commercially by the application of recombinant DNA technology. Following this, a score of very important therapeutic proteins have been synthesized in genetically engineered organisms. Many have become commercial. Many peptide hormones, like **insulin, growth hormone, somatostatin;** blood products, like **coagulation factors VIII** and **IX;** anticoagulants, like **tissue plasminogen activator (tPA)** and **hirudin; haematopoietic growth factor, erythropoietin; interferons** and **interleukins; monoclonal antibodies** and **recombinant vaccines** are a few among many recombinant therapeutic molecules. The procedure for the synthesis of recombinant insulin is outlined in the section 13.3.

13.2.2 Tansgenic animals as bioreactors :

For an elaborate discussion refer to section 13.7.

13.2.3 Recombinant vaccines :

For an elaborate discussion refer to section 13.4.

13.2.4 Monoclonal antibodies :

Monoclonal antibodies are, produced by B-lymphocytes of a single clone. The B-lymphocytes are sensitized by an appropriate antigenic determinant and then fused to a myeloma cell (a cell having an unregulated division), resulting in a somatic hybrid cell, called a **hybridoma.** The hybridoma is cultured in an appropriate animal cell culture medium. Under a congenial environment, the hybridoma synthesizes antibodies of a single specificity. These are used for the treatment of some cancers and are also used in clinical investigations.

13.2.5 Clinical investigations of genetic disorders :

The principles of molecular biology, immunology, biochemistry and genetic engineering are used in clinical investigations of human genetic disorders. All genetic disorders are caused by chromosomal aberrations and / or gene (point) mutations. The abnormal chromosome number and structure, caused due to aberration and the location of the mutant gene on a chromosome, is first identified. Then a possible remedial measure is thought out. The remedial measures, involving the treatment of genetic disorders, are discussed under gene therapy.

13.2.6 Gene therapy :

For an elaborate discussion refer to section 13.5.

13.2.7 Medical diagnostics :

Gene and enzyme technologies are applied in several medical diagnostic procedures. **DNA fingerprinting** has become an indispensable part in forensic diagnosis. The technique helps establish the identity of a person, who has perpetrated a crime. It also helps in establishing close kinship. **Enzyme Linked Immunoabsorbent Assay (ELISA)** is another analytical technique, which detects the presence of a specific protein. The technique is used to detect the presence of HIV antigens in the blood of a person, suspected to have contracted AIDS.

13.2.8 Antibiotics :

Recombinant DNA technology has been applied to the production of novel broad-spectrum antibiotics with increased the activity and without side effects. Secondly and more importantly, the quantity yield lowers the cost of production and hence the price in the market. Consequently, it is within the reach of the common man.

13.2.9 Microbial synthesis of amino acids :

The biosynthesis of several amino acids is augmented by suppressing several steps in the complex bao-synthetic pathways. Suppression of a biosynthetic step requires a knowledge of the catalyzing enzyme and its encoding gene. This will facilitate the augmentation process *in vitro.* This type of manipulation has been termed as **metabolic engineering.**

13.2.10 Vincristine and vinblastine from plants :

Vincristine and **vinblastine,** isolated from Madagascar periwinkle, *Catharanthus roseus, are* used as anti-cancer or chemo therapy drugs. However, these are produced in such low amounts in *C. roseus* that the extraction is not economically viable. Therefore, large-scale plant cell and tissue culture systems (bioreactors) are set up for the synthesis of these products.

13.2.11 Microbial synthesis of vitamin C :

The conventional synthesis of vitamin C consists of one microbiological and four chemical steps. This five-step process was reduced to a two-step process by microbial gene cloning. This reduces the cost of production.

13.2.12 Vitamin E from plants :

Vitamine E is a group of hydrophobic compounds, such as γ and α tokopherols.The natural source of vitamin E is γ tokopherol, which is ten times less potent than α-tokopherol. Gene and enzyme technology and metabolic engineering are applied to augment the synthesis of α-tokopherol.

13.2.13 Microbial synthesis of indigo and melanin :

The microbial synthesis of the blue dye, **indigo** and the black pigment, **melanin** are two more examples of metabolic engineering. These two products are synthesized from tryptophan and tyrosine, respectively.

13.3 HUMAN INSULIN :

Insulin is a protein hormone, synthesized and secreted by the pancreas. It is hypoglycemic, i.e. it decreases any excess of glucose that is present in the blood and stabilizes its concentration at equilibrium. Under some pathological conditions, the pancreas is unable to secrete the required amount of insulin. This results in an increase in the blood glucose concentration, leading to a pathological condition known as **diabetes mellitus**. In this situation, exogenous insulin injection is necessary to maintain the blood glucose concentration at equilibrium.

Prior to the emergence of recombinant DNA technology, human insulin requirement was fulfilled by insulin recovered and purified from the pancreas of slaughtered animals. However, two problems were encountered in securing this insulin. Firstly, this insulin was a little different from human insulin in its aminoacid sequence and hence, generated an immune response in the recipient's body. Secondly, the production cost was high due to its complex extraction and purification processes. Additionally, the purified insulin was contaminated by many pathogenic viruses. These problems have been overcome by the use of recombinant DNA technology. Insulin that is produced by recombinant DNA technology is known as **recombinant human insulin**.

13.3.1 Strategy for insulin synthesis :

Insulin is a dimeric protein, i.e. it consists of two polypeptides, A and B, joined by two disulfide bonds. Primarily, it is synthesized as a single polypeptide, which is then cleaved to yield two polypeptides, A and B. The two polypeptides are then joined by two disulfide bonds to

form mature insulin in a natural environment. Keeping an eye on this, two approaches have been developed.

In the first approach, the insulin gene is isolated from beta cells of the pancreatic islets and purified. It is then cloned in an *Escherichia coli* cloning host cell. The cloned genes are then expressed in an expression host cell of the same species. It is synthesized as a single polypeptide. Following its synthesis, it is extracted, purified and enzymatically cleaved into A and B polypeptides. The two polypeptides are next joined by two disulfide bonds to yield mature human insulin. This insulin is identified as **recombinant human insulin**.

A second approach is to use a yeast host cell for the expression of the cloned insulin genes. This approach has an advantage over *E. coli* host cell in that, yeast, being an eukaryotic cell, expresses, cleaves and joins two polypeptides correctly. Despite this advantage, most of the human insulin manufacturing biotech companies rely on the use of *E. coli* as the cloning and expression host.

13.3.2 Human insulin manufacturing companies :

Genentech is the first biotech company to manufacture recombinant human insulin in 1978 using bacteriophage vector and E. coli as the cloning and expression host cell. Later, this technology was licenced to **Eli Lilly Corporation** of USA. The recombinant human insulin was termed as **HUMULIN**. It was approved as the first recombinant drug by the **Food and Drug Administration (FDA)**, USA for human use. Since then, several companies, world over, have been manufacturing recombinant human insulin on a commercial basis. Some noteworthy companies are: **Novo Nordisk of Denmark; Hoechst and Aventis of Germany and Pfizer of USA. Wokhardt Limited,** a pharmaceutical company has been manufacturing human insulin in India under the trade name of **WOSULIN**.

13.3.3 Types of recombinant insulin :

Insulin is marketed in two forms: **injectable** and **inhalable**. Injectable insulin is available in two forms: **pen** and **vial**. All the companies except Wokhardt Limited, manufacture the pen form, while Wokhardt markets it in a vial form. **Pfizer Inhale Corporation of USA** is the only enlisted company manufacturing inhalable insulin.

13.4 RECOMBINANT VACCINES :

A vaccine is defined as an antigenic agent, which, when administered into the of an animal, generates an active acquired immune response. The antigenic agent varies from vaccine to vaccine. It is generally of three classes: **attenuated (inactivated) whole organisms, isolated antigenic proteins,** such as **coat proteins of viruses** and **inactivated toxins**. The latter two fall under the **subunit vaccine** class, where a part of the organism, possessing an antigenic property is used in the vaccine.

Ever since the discovery of small pox vaccine, research and investigation picked up for the development of effective vaccines and in the process, infallible vaccines for human and domestic animals have been developed. This endeavour has culminated in the search for more effective vaccines by applying recombinant DNA technology. Success has been achieved in developing some such vaccines. These vaccines have been termed as **recombinant vaccines**. However, identifying the antigens and the antigen coding genes in the pathogenic microorganisms is of paramount importance.

13.4.1 Strategies for recombinant vaccine synthesis :

Several strategies have been employed in developing such recombinant vaccines. In the first strategy, the surface coat proteins of pathogenic viruses, such as those of hepatitis A and B have been used successfully. However, there was a problem in their commercial production to meet the growing demand. It was solved by using the gene technology procedure.

The gene expressing the surface coat protein of hepatitis B virus (HBV) was identified. It was isolated and purified. It was then cloned in a prokaryotic host cell to a high copy number and then expressed in a yeast host cell to produce surface antigen proteins (HBsAg). This protein is used in the hepatitis B vaccine. Hepatitis B vaccine enjoys the distinction of being the first recombinant vaccine for human use.

In another approach, naked DNA of the pathogenic microorganism, encoding the antigen is used as a vaccine. The naked DNA is inserted into a suitable DNA vector to result in a recombinant DNA. This recombinant DNA is directly introduced into the host. In the host, the inserted DNA directs the synthesis of antigens, which evokes an active acquired immune response. Several DNA vaccines are available for veterinary use. Currently, no DNA vaccine has been approved for human use. A DNA vaccine for Zika virus began testing at National Institute of Health (NIH) in 2016. It has not yet been approved.

13.4.2 Recombinant vaccine manufacturing companies :

Following this success, several multinational biotech companies started commercial production of recombinant vaccines. The common brands presently available for human use are: Recombivax (Merck); Energix B (Glaxo Smithkline); Elovac (Human Biologicals Institute, a division of Indian Immunological Limited); Genevac B (Serum Institute) and Shanvac B. All these are hepatitis B vaccines. Twinrix, manufactured by Glaxo Smithkline is the only combination vaccine used against hepatitis A and B.

13.5 GENE THERAPY :

Gene therapy is a therapy or treatment of a gene, which has been mutated. It is a therapy to set it in a right order. A genetic disorder is expressed, when a gene is mutated. The mutant gene, as it is known, encodes a different polypeptide other than a normal. This polypeptide

is the root cause of the expression of symptoms of a genetic disorder. The wrong polypeptide may block a normal biochemical pathway, which results in a failure of the formation of an appropriate product. Many such disorders have been thoroughly studied and documented. Some of these are biochemical disorders, like alkaptonuria, phenylketonuria and albinism, while others are chromosomal and gene disorders, like Down syndrome, Turner syndrome, Klinefelter syndrome, fragile X syndrome, cri du chat, Huntington's disease, Tay Sach's syndrome and many more.

There is no effective treatment for such disorders. However, with the establishment of Genetic Engineering as a discipline, a small breakthrough has been made in the search for the treatment and cure of these disorders. Attempts have been made to rectify or replace mutant genes, so that they express normally. This replacement process is known as gene therapy or gene replacement therapy. It may be defined as a therapy and treatment of a genetic disorder by replacing a mutant gene with a normal gene in the cells of an affected person in order to restore normal cellular functions.

13.5.1 Types of gene therapy :

There are two types of gene therapy: **somatic cell gene therapy** and **germ cell gene therapy**.

13.5.1.1 Somatic cell gene therapy :

In this therapy, a mutant gene in the affected somatic cells of a person is replaced by a normal one, so that the normal cellular functions are restored. This kind of a change is confined to a generation only. This therapy is successful for genes, which follow simple mendelian inheritance. The first ever somatic cell gene therapy was conducted at the National Institute of Health (NIH), USA in 1990. A four year girl, named Asanthi DeSilva was suffering from an inherited immunodeficiency disorder, **Severe Combined Immunodeficiency (SCID)**. It is caused due to a failure of synthesis of an enzyme, **adenosine deaminase**. Its absence was attributed to a mutant *ADA* gene.

Her bone marrow stem cells were transformed *in vitro* by a correct *ADA* gene. The transformed cells were reimplanted into the bone marrow. In the succeeding years, the transformation yielded encouraging result. Following this treatment, several such SCID cases were treated in the same manner. Another disorder, **familial hypercholesterolemia (FH)** was also treated in a similar manner to that of SCID in 1992. This disorder is caused due to an absence of **low density lipoprotein receptors (LDL receptors)** on the hepatocyte surface.

13.5.1.2 Germ cell gene therapy :

It refers to the replacement of a mutant gene with a normal gene in the gametes. The genetic changes conferred on the gametes pass on to the next generation and thus, perpetuate

through generations. However, bioethics forbids the practice of germ cell gene therapy in all countries of the world.

13.5.2 Gene therapy methods :

There are two methods of gene therapy: ***ex vivo*** and ***in vivo***.

13.5.2.1 *ex vivo* gene therapy :

The affected cells are removed from the body and transformed by the remedial gene *in vitro*. The transformed cells are grown in a cell culture medium to a sufficient number and then returned to the body by transfusion or transplantation.

13.5.2.2 *in vivo* gene therapy :

This practice is still in an experimental stage. Here, the cloned genes are directly introduced into the affected cells of the person. This is an option, where the individual's cells can't be cultured to a sufficient number.

13.5.3 Ethical concerns :

Bioethics forbids germ cell gene therapy in its totality. In respect of somatic cell gene therapy, the process has to be tested on animal models and humans for its safety and efficacy. Before it is dedicated for human use, it is mandatory that it is approved by an appropriate regulatory authority for a legal status.

13.6 GENETICALLY MODIFIED ORGANISMS (GMOs) :

A Genetically Modified Organism (GMO) is one, whose genetic material has been altered by genetic engineering. The first GMO was created by **Herbert Boyer** and **Stanley Cohen in 1973**. The kanamycin antibiotic resistance gene of a bacterium was transferred into another bacterium thast was sensitive to the antibiotic. The recipient bacterium consequently acquired the kanamycin resistance property and became resistant to kanamycin. Following this success, they transferred genes from a toad to a bacterium and were able to express these genes successfully in the new environment.

Ever since these discoveries, many GMOs have been created for the benefit of mankind. Thus, transgenesis emerged as an offshoot from gene technology, which means successful transfer and expression of genes across species barriers. The genes, transferred across, were termed as **transgenes**. Novel and beneficial plants and animals could be generated by using this technology. Such genetically modified plants and animals were termed as **transgenic plants** and **animals**.

Although the process spells very simple, it is a very expensive, time consuming and difficult process to execute. It involves many analytical molecular biological techniques like, identification of a beneficial gene in a donor organism, isolation and purification of the said

gene, transfer of the gene to a recipient cell and generation of a transgenic organism from the transformed cell. All these essential processes have been described in Chapter 12. Of these steps, the transfer of a gene across is most difficult. Many gene transfer techniques have been developed looking at the structures of the recipient cells. Among these techniques, **microinjection** is the technique of choice for animal cells, while **electroporation** for plant cells. The transgenes are either transferred alone or in conjunction with a vector or carrier DNA. Several species of animal viruses have been used as vectors for animal cells, while the *Ti* plasmid (Tumor inducing plasmid of *Agrobacterium tumefaciens*) is the vector of choice for plant cells.

GMOs have wide ranging applications in agriculture and animal husbandry; medicine and healthcare management and environmental monitoring and management. A few of these applications are briefly described in the preceding sections of the present chapter. Transgenic plants and animals have proved to boost productivity. This and much more is discussed a little more elaborately in the succeeding section.

13.7 *Bt* CROPs :

The major objective of the agricultural scientists and animal breeders is to increase productivity to feed the millions. This is partially achieved by the use of modern agricultural practices and improved animal breeding techniques. Agricultural scientists have been using improved varieties of seeds, inorganic fertilizers and pesticides. Pesticides are used to kill a variety of organisms, which damage crop plants through infections and destroy stored food grains following harvest. These organisms are collectively called **pests**. Pesticides, which are used to kill these pest organisms are bionondegradable complex organic compounds. These are highly toxic too. There have been large-scale uses of pesticides, which accumulate in the environment, causing serious environmental pollution problems. Secondly, these enter into food chains and consequently, by the process of eating and being eaten, enter into the body of organisms. These are not metabolized and hence, accumulate in the living tissues dose by dose in a phenomenon called **bioaccumulation**. Over a threshold level, these produce adverse effects, which are expressed as some pathogenic symptoms. This process is known as **biological magnification**.

Biological scientists have searched for alternate and equally potent methods of containing the pests without inflicting a damage on the environment. At last, they have succeeded in their endeavor in discovering a species of bacterium having an insecticidal property. It is a **Gram positive soil bacterium** belonging to the species, ***Bacillus thuringiensis***.

13.7.1 Insecticidal toxin of *Bacillus thuringiensis* :

The bacterium forms endospores within a sporangium during adverse environmental conditions. Sporulating cells produce parasporal crystalline inclusions of proteins. This protein

is identified in several synonyms like, δ **endotoxin** or **insecticidal crystalline protein** or **cry protein** or ***Bt* protein**. *Bt* protein is hydrolyzed by an alkali into 250 kD (kilodalton) units, known as **protoxins**. Each protoxin consists of two 130 kD polypeptides. The 130 kD polypeptide is digested into a 68 kD toxin polypeptide in an alkaline pH. When caterpillars eat the leaves of crop plants, on which the bacterial spores are deposited, they ingest the spores. The spores germinate in the alimentary canal, the bacteria grow in size and produce *Bt* protein. This protein is digested into 68 kD toxin polypeptides in the intestine of the larva. The action of the polypeptide, eventually kills the larva. The alimentary canal of mammals, including human, produces an acid, which degrades the *Bt* protein. Thus, it is apparently harmless to human and other mammals. Since, this pesticide is produced from an organism, it has been identified as **biopesticide**.

13.7.2 Strategy for protection by *Bt* protein :

Two strategies have been employed to develop insect resistance in crop plants by using *Bt* protein. In the first strategy, *B. thuringiensis* spores are sprayed with water on the crop plants. However, the insecticidal effects are temporary. Therefore, repeated spraying becomes necessary for a long term effect. This problem has been circumvented by the application of genetic engineering. The *Bt* protein is encoded by a gene, ***cry*** present on a plasmid. There are two lines of actions with the *cry* gene. Firstly, the gene is isolated and then introduced into other species of bacteria like, *E. coli* or *Pseudomonas fluorescens*, which are better suited for survival in the field. These transformed bacteria may be sprayed on the crop plants. In the other approach, the *Bt* protein gene is isolated and then inserted into an expression *Ti* plasmid (Tumor inducing plasmid) of *Agrobacterium tumefaciens*. Isolated plant protoplasts are transformed by the recombinant *Ti* plasmid and then cultured *in vitro* to generate insect resistant plants.

13.7.3 *Bt* Crops, A ground reality :

Many crop plants have been genetically modified by the *Bt* protein gene (*cry*). **Monsanto of USA** genetically engineered and marketed *Bt* cotton seeds bearing the trade name of **Bollgard**. **Plant Genetic Systems** (later became **Aventis Crop Science**) is the creator of a variety of corn seeds, carrying *cry* gene. It was marketed in the trade name of **Star Link corn**. Later, the seeds of this brand of corn were withdrawn from the market because it was not suitable for human consumption. Several other *Bt* plants like, tobacco, coffee, cocoa, walnut, soybean, etc. have been successfully generated by using *cry* gene.

13.7.4 Potential risks:

One major risk of widespread use of *Bt* technology is an increasing fear of resistance of the pests to the *Bt* protein and appearance of resistant varieties of pests in the future.

13.8 TRANSGENIC ANIMALS :

In the last quarter of the last century, there was brisk research and investigation in the field of agriculture and animal husbandry to meet the ever growing demand of food for the millions. The farmers and the animal breeders were under an increased pressure for discovering scientific methods for increasing productivity. The animal breeders were looking for animals, which would grow faster, yield more milk, lay bigger eggs and so on. They were successful in combining traditional breeding with gene technology, which yielded encouraging results. It involved selecting, isolating, purifying and transferring beneficial genes of one species to another to harvest a beneficial effect. The animal that is created by the transfer of the beneficial gene (genes) is a **genetically modified animal** or a **transgenic animal**. The gene that is transferred is known as a **transgene**. The transgene is transferred by using one of the several methods of gene transfer in practice. Microinjection is found to be most suitable for animal cells.

13.8.1 Transgenic mouse :

R. L. Brinster and **R. Palmiter** (**1982**) successfully created the first transgenic mouse by transferring the rat growth hormone gene into the fertilized mouse egg by microinjection. This act was carried out *in vitro*. Following the transfer, the fertilized egg was implanted into the uterus of a pseudopregnant mouse. The mouse gave birth to mice that were relatively larger in size, possibly due to an increased synthesis of growth hormone directed by the rat growth hormone transgene. This mouse was termed as a **supermouse** because of its abnormal growth.

This success story inspired the scientists to generate many transgenic fishes, birds and mammals like, cows, pigs, goats and sheep. Some such transgenic animals increased productivity, while others served as decorative pet animals. For example, a Pacific transgenic salmon was generated by a growth hormone transgene. This fish was commercially important in view of its growth compared to the wild fishes. Similarly, brightly coloured transgenic glo fishes have been engineered by fluorescent colour producing transgenes. These are put in an aquarium as a part of aesthetic sense.

13.8.2 Pharming :

Pharming is a word, used in biotechnology to describe the commercial use of transgenic animals as sources of important pharmaceutical products. Recall the supermouse that was created by the transfer of the rat growth hormone transgene into the mouse fertilized egg. The super mouse was genetically altered to produce **tissue plasminogen activator** (**tPA**), an agent that dissolves blood clot. Several other pharmaceutical products like urokinase, a1 antitrypsin, insulin, growth hormone, blood coagulation proteins (factors VIII and IX), fibrinogen and lactoferrin (an infant nutrition formula) have been successfully harvested by using transgenic animals as bioreactors.

13.8.3 Animal cloning and making of Dolly :

Ian Wilmut of Roslin Institute in Scotland came up with a cloned sheep, named Dolly in February, 1997. He used nuclear transplantation technique to create Dolly, a clone of a sheep. This was the first ever clone of an animal. Following this, two other sheep, named, Polly and Molly were also created in the same manner.

13.8.4 Ethical concerns :

Following the generation of transgenic animals and cloned sheep, there was a growing debate among the scientists, social activists, lawyers and general public at large about the safety concerns of the gene manipulation experiments of the kind. Social, moral and legal issues were deliberated upon and in the process, there was a unanimous agreement as to exercising extreme care and caution in generating transgenic animals and animal clones.

13.9 BIOSAFETY ISSUES :

In the last quarter of the last century, research and investigation in the area of biotechnology have undergone an exponential growth. New innovations are made and applied in the manufacture of beneficial products and services for the mankind. Noteworthy among the products are many pharmaceutical products, which have revolutionized healthcare services. Among the processes are the creations of many transgenic plants and animals for increasing productivity, creation of many genetically modified organisms, especially for treating environmental pollution and gene therapy procedures for treating genetic disorders. Amid these developments, biotechnology was confronted with an important issue, i.e. the safety in manufacture and / or designing and applications of these products and processes. Moreover, the safety concerning the legal rights of ownership of the inventor was also threatened and there was a threat of theft and transfer of useful biological resources from one country to the other. All these issues are classed together as biosafety issues since the harvested products and services involve living organisms.

Biosafety, in a broad sense, refers to the prevention of loss of biological integrity of biological processes and products, harvested by using living organisms. For example, recombinant insulin was manufactured in a complex biological process putting in thought, knowledge, skill and execution method of the inventor. Secondly, a lot of energy and money was spent in the successful execution of the process. Therefore, the right of the inventor needs to be protected by law considering it as a property. On the other hand, insulin that is manufactured a prescribed trial process to prove that it is suitable for human use. Another potential hazard was the release of genetically modified organisms ((GMOs) into the wild. There was a threat that it might sexually reproduce with organisms of its own species and exchange genes, consequently changing the structure of the gene pool. This might have produced an adverse effect on organic evolution. Thus, normal biological diversity might be destabilized.

All these afore mentioned problems are addressed by national and international regulations, formulated by regulatory authorities. Among these, United Nation Convention on Biological Diversity (CBD) and Cartagena Protocol on Bio safety are the first of the kind.

13.9.1 Convention on Biological Diversity (CBD) :

The convention was established in 1992 under aegis of United Nations Organization with a focus on conserving biological diversity. It raised a concern on the potential hazards on biotechnological applications on plants and animals that may have an adverse impact on biological diversity. It emphasized on the safe handling of biotechnological products. Its regulations and guidelines have been the base for the establishment of Cartagena Protocol on Biosafety.

13.9.2 Cartagena Protocol on Biosafety :

CBD established a working group to develop a draft protocol on biosafety in 1995. After several rounds of negotiations, a draft protocol was adopted in 2000 as an international legal binding agreement, addressing potential risks of GMOs. The protocol outlines regulations for safe transfer, use and handling of GMOs and their transboundary movement to prevent the adverse impacts on biological diversity and risks to human.

13.9.3 Patent :

The capability of the brain to think something novel or innovative is called **intellect**. When someone invents something by using his intellect, it becomes his property, especially intellectual property. He possesses all rights to use it the way he likes. There are laws at the national as well as international levels, which forbid the misuse of this property and confer the ownership on its creator. Misuse in any form is legally enforceable like those in movable and immovable properties. There are several forms of intellectual properties like **patent, design, trade mark, trade secret, geographical indications** and **copyright**. In the following section, patent and laws governing the award and use of patent is discussed.

Patent is an open letter. It is a set of legal right, privilege and authority granted by a sovereign state to a person or an institution for a limited period of time for an invention using scientific and technical knowledge. All sovereign countries have enacted their own Patent Acts to regulate the use of such properties. India enacted the Patent Act in 1970. The Act has undergone amendments in 1999, 2002, 2005 and 2006. The head quarters is in Kolkata, West Bengal. The nodal centre for Indian biosafety network is the Department of Biotechnology, Government of India. **Patents are granted for inventions** and not for discoveries. An invention involves new knowledge, while a discovery is an application of the knowledge. For example, the double helical model proposed by Watson and Crick was a discovery and hence, doesn't qualify to be patented, while new forms of DNA, such as recombinant DNAs have been patented.

13.9.3.1 Prerequisites for a patent :

An invention qualifies to be patented following the fulfillment of the following conditions as per the Indian Patent Act of 1970:

1. The invention must be novel, i.e. it must be new or innovative. Alternately speaking, it should not be available to the public earlier.
2. The invention must have an inventive step.
3. The invention must have an industrial application.
4. The invention must be described in sufficient details before filing for a patent, so that an average skilled person in the relative field can rework the invention without further experimentation.

13.9.3.2 Patent related case studies :

(a) Diamond vs Chakraborty case : A genetic engineer, named Anand Mohan Chakraborty was working for General Electric in USA. He developed a bacterium species of **Pseudomonas** genus that could eat the oil and consequently clear oil spill. He applied for a patent for the bacterium, but was denied by the patenting authorities on the plea that patent couldn't be granted because the bacterium was a living organism. He then moved to the Supreme Court and argued in his favour. After several hearings, the apex court in 1980 ruled in favour of Chakraborty.

(b) Neem patent case : The multinational agribusiness company, W. R. Grace of New York and United States department of Agriculture, Washington D.C filed for an European patent in the European Patent Office (EPO) for grant of patent in favour of W.R. Grace. It was stated that neem oil controlled fungal growth on plants. The plea was accepted and a patent was granted. Following the publication, Dr. Vandana Shiva of Research Foundation for Science and Technology & Natural Resource Policy, New Delhi and others filed a legal opposition to the grant of patent in the EPO. The case was admitted and after several rounds of hearing a verdict was awarded in favour of Dr. Shiva and others. The patent granted to W. R. Grace was withdrawn.

(c) Turmeric patent case : A US patent on turmeric was awarded to two US based Indians in 1995, specifically for the use of turmeric powder in wound healing. Two years later, a complaint was filed by the Council of Scientific and Industrial Research (CSIR) challenging the novelty of the invention. The validity of the patent was examined and finally in 1997, the patent was revoked.

13.9.4 Biopiracy :

The patent law is enforceable for inventions, which are made on microorganisms, plants and animals as source materials. The source material itself in its natural state can't be patented.

The varies source materials in the natural environment is diverse and and hence constitute a biological diversity. In order to expand the knowledge and applications in biotechnology, the source materials need to be surveyed thoroughly. THe survey with this particular intention is known as bioprospecting. Bioprospecting can be done by scientists, who are trained in this area.

There is a fear that during the course of bioprospecting, scientists may transfer any biological resource, which they may consider as novel. However, CBD has recognized the sovereign rights of a country over its natural resources. Illegal transfer of biological resources has been termed as **biopiracy**. It describes a practice, in which indigenous knowledge and practice used by indigenous people of a region is used by others for profit without permission from and with little or no compensation or recognition to the indigenous people themselves. This is an illegal practice and enforceable in the court of law. Three following case studies will suffice the context.

13.9.4.1 Neem patent case : Refer to section 13.9.3.2(b).

13.9.4.2 Turmeric patent case : Refer to section 13.9.3.2(c).

13.9.4.3 Basamati rice patent case :

Basamati rice is an aromatic variety of long grain rice, indigenous to the Indian subcontinent. In 1997, the US Patent and Trademark Office (USPTO) granted a patent to a Texas based American company, Rice Tec Inc for basamati rice line and grains. The patent application was based on claims of Rice Tec of having invented the said rice. However, due to people's movement against Rice Tec, USPTO partially rejected the patent.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer :

(i) Golden rice is produced by rice plant having a transgene encoding an enzyme in the biosynthetic pathway of

(a) b Carotene (c) Glyphosate

(b) Luciferin (d) *Bt* protein

(ii) Fruit ripening is delayed by preventing the expression of the enzyme

(a) Luciferase (c) Nitrogenase

(b) Polygalacturonase (d) Adenosine deaminase

(iii) Humulin is manufactured by

(a) Pfizer (c) Eli Lilly

(b) Hoechst (d) Aventis

(iv) Genetic correction of inflicted cells is made *in vitro* and then reimplanted into its natural environment. This therapy is known as

(a) *ex vivo* gene therapy (c) *in vitro* therapy

(b) *in vivo* therapy (d) *in toto* therapy

(v) The first genetic disorder treated by gene replacement therapy is

(a) Familial hypercholesterolemia (FH)

(b) Cystic fibrosis (CF)

(c) Duchenne muscular dystrophy (DMD)

(d) Severe combined immunodeficiency (SCID)

(vi) Patent is not granted for

(a) A novel invention

(b) An invention having an industrial application

(c) A discovery made by previously existing knowledge

(d) An invention having an inventive step

(vii) Which of the following is not related to biosafety ?

(a) Convention on Biological Diversity

(b) Cartagena Protocol

(c) World Trade Organization

(d) UNICEF

(viii) Which of the following patent cases, India is not directly or indirectly connected with ?

(a) Soyabean patent case (c) Turmeric patent case

(b) Neem patent case (d) Chakraborty patent case

(ix) The supermouse is a genetically modified animal with

(a) Insulin transgene (c) Growth hormone transgene

(b) Lipid biosynthesis transgene (d) Steroid hormone transgene

(x) Which is the nodal center for Indian biosafety network ?

(a) Department of Biotechnology

(b) Department of Science and Technology

(c) Indian Agricultural Research Institute

(d) Department of Forest and Environment

2. Fill in the blanks with appropriate words:

(i) The mass of undifferentiated plant cells in a plant tissue culture media is known as _____.

(ii) Herbicide resistant plants are generated by plant tissue culture technique by transferring _____ gene of a bacterium into a plant protoplast.

(iii) A bacterium species of _____ genus is genetically engineered to prevent frost formation in plants.

(iv) A bioluminescent plant is generated by transferring _____ gene of a firefly into plant protoplasts.

(v) Golden rice producing plant is a transgenic plant , whose cells have a transgene encoding _____.

(vi) Delayed ripening in tomato is due to the inhibition of expression of an enzyme _____.

(vii) The fist recombinant human vaccine produced and marketed is _____ vaccine.

(viii) Recombinant insulin in the trade name of HUMULIN is manufactured by _____.

(ix) Monoclonal antibody is synthesized and secreted by a cell known as _____.

(x) Severe combined immunodeficiency (SCID) is expressed due to the absence of an enzyme, _____.

(xi) A forensic analysis of DNA for establishing the identity of a person is known as _____.

(xii) An immunological technique, applied to detect the presence os very minute quantity of antigen in the serum is known as _____.

(xiii) A biopesticide, known as *Bt* protein is expressed by a bacterial species, _____.

(xiv) A legal right, privilege and authority granted to a person for a limited period for an invention is known as _____.

(xv) The use of novel biological resource of a sovereign country without its due permission is known as _____.

3. Answer the following in one word each:

(i) The tomato plant variety that bears tomatoes exhibiting delayed ripening.

(ii) The somatic hybrid cell, which produces monoclonal antibodies.

(iii) Genetically engineered rice, rich in vitamin A.

(iv) An insecticidal protein, produced by *Bacillus thuringiensis*.

(v) A broad spectrum herbicide that is used world over.

(vi) The biotech company, which commercially manufactured the first recombinant human insulin.

(vii) The first genetic disorder that was treated by gene therapy.

(viii) The gene transfer into the mammalian fertilized egg with a micropipette.

(ix) The gene transfer method practiced by passing intermittent pulses of electric current through the medium containing plant protoplasts.

(x) The corn was genetically engineered by transferring *Bt* protein gene into plant protoplasts. The brand was marketed and later was withdrawn due to safety reasons.

(xi) The biosafety protocol that was drafted in 1995 and adopted in 2000.

GROUP - B
(Short Answer-type Questions)

1. Answer each of the following within 50 words:

(i) What is golden rice?

(ii) What is Flavr Savr tomato?

(iii) What does the recombinant hepatitis B vaccine contain?

(iv) What do you understand by *ex vivo* gene therapy?

(v) What do you mean by a biopesticide? Give an example.

(vi) What is a super mouse?

(vii) Explain biopiracy.

(viii) Ennumerate and explain in brief two biosafety issues, biotechnology is confronted with.

(ix) Describe the evolution of Indian Patent Act.

(x) Describe the neem patent case.

2. Write brief notes on the following:

(i) Herbicide resistant plants

(ii) Humulin

(iii) Recombinant vaccine

(iv) Gene therapy

(v) Biopesticide

(vii) Transgenic animal

(viii) Patent

(ix) Biopiracy

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UNIT - V : ECOLOGY AND ENVIRONMENT

CHAPTER 14

ORGANISMS AND ENVIRONMENT

14.1. ORGANISMS AND ENVIRONMENT :

Ecology is a branch of biology which deals with the study of the inter-relationships between living organisms and their non living environment. The basic idea behind study of ecology is to understand how different organisms interact with their counterparts and physical environment as a group, forms different units such as population, community, ecosystem or even the biosphere.

All living beings are made of protoplasm; protoplasm is organized into cells; cells into tissues; tissues into organs and organs into **organisms**. Organisms of one kind often live together to form a small or large assemblage called **population**. In any habitable area, several populations belonging to different plant and animal species interacting amongst themselves to form an integrated whole called biotic community or **community**. A community interacting with its physical environment constitutes an **ecosystem**. The environment provides energy and nutrients such as water, carbon, nitrogen, oxygen, phosphorus, calcium, potassium, iron and several other essential minerals without which no organism can sustain. The environment is thus an essential part of the ecosystem. The largest ecosystem is the entire habitable part of the earth and its environment, called **biosphere** or **ecosphere** where living and non living parts of the communities interact to maintain a stable and steady system.**(Fig. 14.1)**

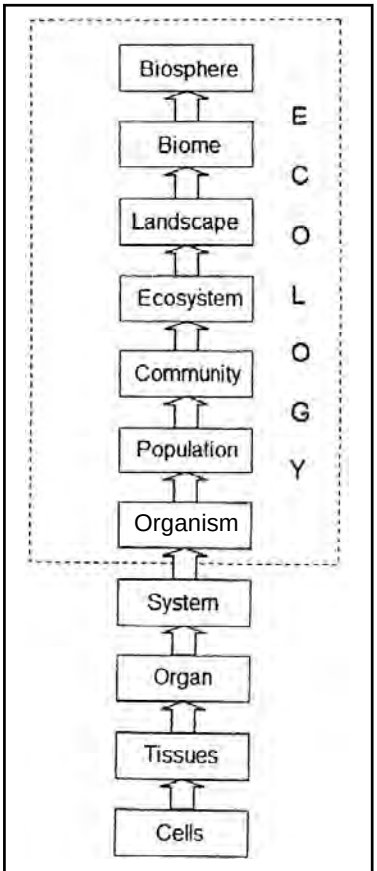

Fig. 14.1: Showing the levels of organization in biology and the position of ecology.

14.1.1. Habitat and Niche:

A habitat is a natural abode or locality where a plant/ animal grow. Basing on the environment, the differences in the vegetation and species of different places are observed. Environment means everything outside the specific organism which influences in anyway the life of that organism.

Each organism plays a particular role in its surrounding. A niche is the role a speices plays in its ecosystem. In other words, niche is how an organism makes a living. A niche includes the role of the organism in the flow of energy through its ecosystem. An organism's niche also includes how the organism interacts with other organisms and its role in cycling of nutrients.

The external factors which influence the organism can be light, temperature, water, soil, etc. For instance, water is used as habitat by aquatic organisms and it comprises three major categories: marine, brackish and freshwater habitats. Similarly, the land is used as a habitat for numerous terrestrial organisms. It includes many major categories of land masses which are called **biomes**. Biomes are distinct large areas of earth with relatively homogeneous climatic factors, flora and fauna e.g., deserts, tropical forests, tundra, etc.

Thus, habitat is the physical area where a species lives. The many factors are used to describe a habitat. These include- climatic, edaphic and topographic. Climatic factors are light, temperature, precipitation, humidity, wind. Edaphic factors are factors related to soil whereas topographic factors are physical factors related to slope, altitude and others concerned with earth surface. Of these, some of the major abiotic factors are discussed below:

(a) Light: Light can be said as the visible part of the spectrum of solar radiant energy i.e., 390-760nm. The visible spectrum consists of seven different colours which is available in specific bands of wavelength. Radiations below the visible spectrum include cosmic, gamma, x-rays and above are infra-red, radio waves. **(Fig. 14.2)**

Sunlight is the primary source of light. It plays an important role in almost all ecosystems. The entire food chain starts with the organisms that are photosynthetic (producers). So without sunlight, all life excluding some microbes would perish, not just the plants. The total amount of light that falls on the earth varies according to the season, latitude, altitude and conditions of the atmosphere. The quality of light is modified by the clouds and fog. Red and blue light are most effective in photosynthesis by green plants. UV rays have injurious effect on the cells.

As we all know the photosynthesis is an important process carried out by the plants where chlorophyll takes a major role. The synthesis of chlorophyll is dependent on light. Light has a major role in the transpiration process which help in the gaseous exchange and also maintaining the temperature of the plants. The photoperiodic movement of the plants is directly regulated by the plants and the flowering. Other processes which are affected by the light include growth, development, reproduction, etc. Depending on the requirement of light intensity, there are two groups of plants: (i). Heliophytes - which require high light intensity also called shade intolerant species and (ii). Sciophytes - requiring low intensity of light also called shade tolerant species.

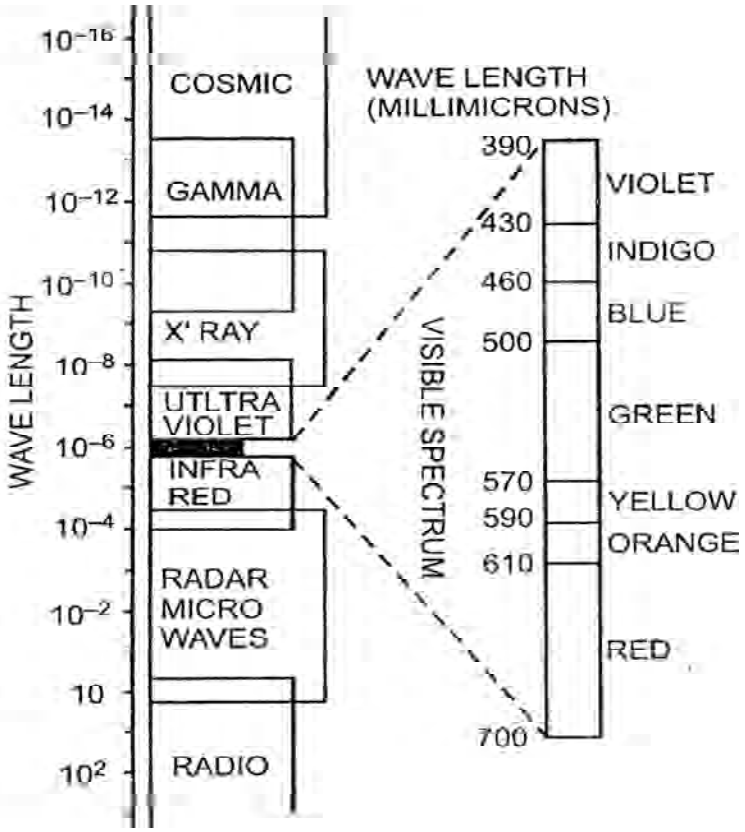

Fig.14.2 : The spectrum of radiant energy and visible light

Light has a role in the growth, colouration of plumage or body, migration, reproduction and various activities of insects, birds, fishes, reptiles and mammals.

Basing on the availability of light and oxygen on the water bodies, there have been some zones created: (i). Littoral Zone: It is exposed to wave action and is highly productive, (ii). Limnetic Zone: This includes all the waters beyond the littoral zone and down to the light compensation level. The limnetic zone derives its oxygen content from the photosynthetic activity of phytoplankton and from the atmosphere immediately over the lake's surface. The region which receives the maximum light above the light compensation point is Euphotic Zone where as the area receive diffused light around the light compensation point is Disphotic Zone(or twilight zone), (iii). Profundal Zone: Region with absolutely no light and (iv) Benthic Zone: Bottom zone or the lowest surface including the sediment surface of a water body.(**Fig. 14.3.**)

(b) Temperature : Temperature regulates all the chemical processes of plant metabolism. The metabolic process begin at a certain minimum and increase with rise of temperature until they reach the maximum at a temperature called optimum. Each species has its minimum and maximum beyond which its life activity ceases. The plants have the capacity to act according to

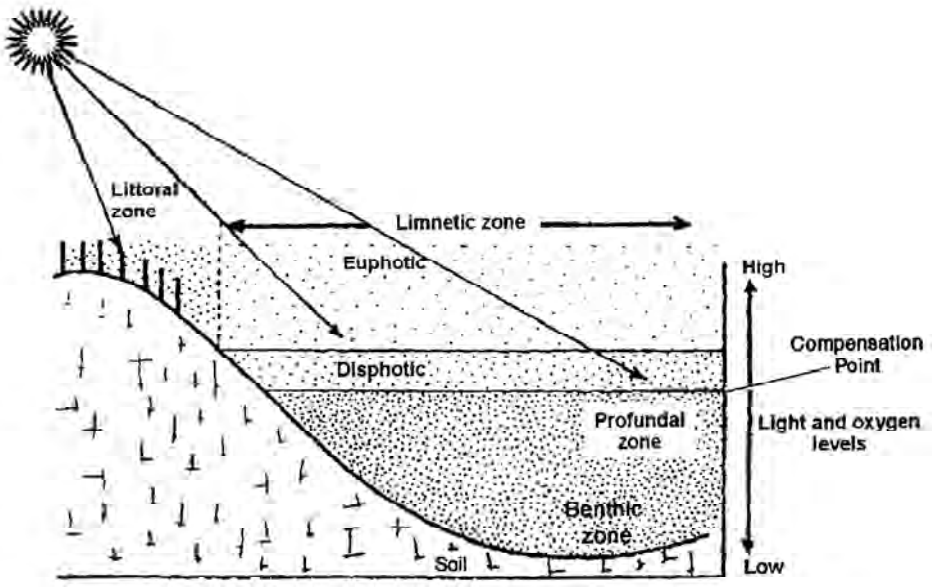

Fig. 14.3 : Zonation in deep lake showing gradient of light and oxygen

the environmental temperature. Although plants can survive in a wide range of temperature conditions, still they become accustomed to the temperature of a particular habitat by constant association with the result that the habitat of a species more or less become restricted to a particular temperature range. The maximum temperature the plants can endure also vary with different species. It ranges from 40 - 70° C depending on the habitats.

(c) Precipitation : The major forms of precipitation are rainfall, hailstorm etc. Rainfall is very important as most of the plants absorb water from the soil. Precipitation is an indirect factor which affects plant life through atmospheric humidity and water content of the soil. However, it may be stated that the vegetation of any area is determined primarily by the amount of rainfall in that area. The organisms which are found in water are called aquatic organisms.

(d) Soil : It is more associated with the plants having roots. The plants derive minerals and water out of the soil as soil has the holding capacity. Soil contains organic, inorganic colloids, organic matters and soil organisms. Microorganisms found to grow in the soil containing moisture. Water is a solvent found in the soil which is the basis of life of the living organisms. The soil-air is important edaphic factor that determines the types of microorganisms, soil animals and vegetation that will grow on the soil. The organisms such as bacteria, fungi, lichens, earthworms, nematodes help in modifying the soil structure and to increase the soil fertility and humus as well. The soil – air interface has numerous communities of different kinds of micro- and macro-organisms**. (Fig. 14.4)**

Besides these prominent abiotic factors, the biotic factors are also forming a part of the habitat which will be discussed in the subsequent chapters.

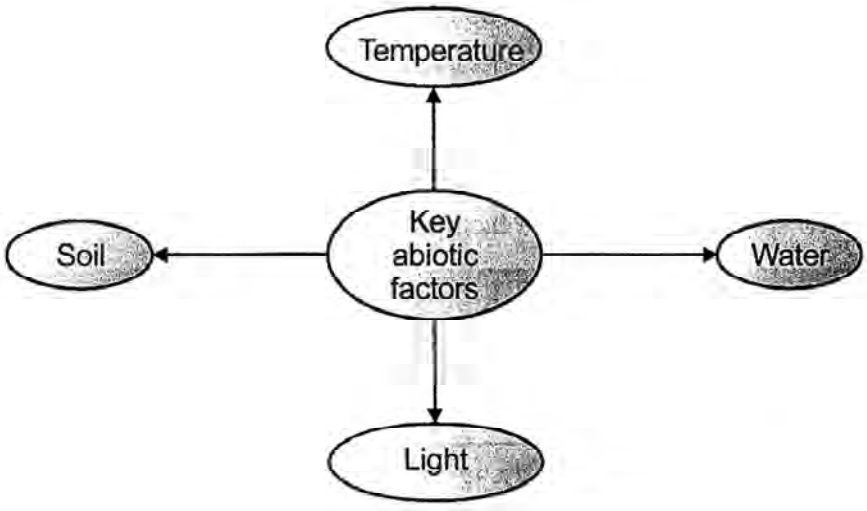

Fig. 14.4 : Key abiotic factors

14.2. POPULATION AND ECOLOGICAL ADAPTATIONS :

Individuals does not live in isolation rather individuals of same species assemble to occupy a particular space in a given time is called population. It is a part of the ecosystem and its study is called population ecology. Individuals of the populations require different necessary conditions for the easy survival and they undergo some morphological, anatomical or physiological changes which are called adaptations.

Depending upon the water requirement or more specifically upon the quantity of water available in their habitats, we find three ecologically distinct groups of plants populations. These are :

(a) Mesophytes: Plants growing in habitats that are neither very dry nor very wet.

(b) Hydrophytes: Plants growing in or near water,

(c) Xerophytes: Plants growing in habitats where there is poor availability of water (dry habitat).

Ecological adaptations in the two important ecological groups- hydrophytes and xerophytes are described below.

14.2.1 Mesophytes :

Mesophytes are terrestrial or land plants. These land plants are capable of growing in moist habitats, and well aerated soil. The plants can grow luxuriantly in the soil and air of moderate humidity. The plants generally lack structural, anatomical and physiological adaptations of xerophyte and hydrophytes. These plants form extensive vegetation in the land masses, called forests. The features of mesophytes are :

(1) Root system is well developed and extensive.

(2) Stems are generally aerial, solid and freely leranched.

(3) Leaves are generally large, broad, thin and have variable shapes with green colour.

(4) Cuticle in aerial parts are moderately developed.

(5) Epidermis is well developed without any hair or waxy coatings and chloroplasts are present in epidermal cells.

(6) Stomata may be dorsiventral or isobilateral respectively in dicots and monocots.

(7) Mesophyll is differentiated into palisade and spongy parenchyma.

(8) Vascular and mechanical tissue are well developed.

14.2.2 Hydrophytes :

Hydrophytes are plants that live in abundance of water or in wet places. They are either partially or wholly sub- merged in water, when they are found in abundance of water. In wet places, their roots or rhizomes are exposed to sufficient water.

Types of hydrophytes :

An aquatic environment, in general, provides the following conditions for the plant:

(i) A matrix for plant growth.

(ii) Availability of nutrients in dissolved state.

(iii) Minimum fluctuations in temperature.

(iv) Decreased availability of light and oxygen with the increase in depth.

(v) Water movements, weak or strong.

Hydrophytes may grow in ponds, pools or rivers which are called fresh water. But when they grow in salty water of seas, salty lakes or oceans, they are known as marine plants.

Not all the conditions described above are encountered by every hydrophyte because they show different types of distribution. Basing on their relation to water and air, hydrophytes are grouped into the following categories.

(i) Submerged hydrophytes

(ii) Floating hydrophytes

(iii) Amphibious hydrophytes

14.2.2.1 Submerged hydrophytes :

These are plants that grow in water, totally submerged and are not in contact with atmosphere. These plants are either free floating *(Ceratophyllum, Myriophyllum, Utricularia* etc.) or rooted *(Hydrilla, Vallisneria, Potamogeton, Chara, Nitella* etc.) (Fig. 14.5 & 14.6). They use dissolved oxygen in their respiration.

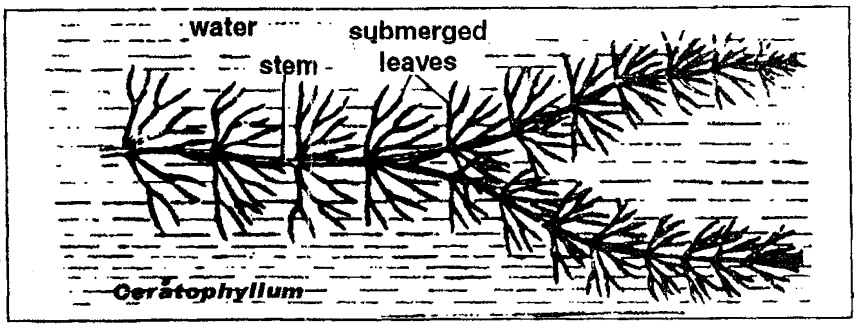

Fig.14.5 : Submerged floating hydrophyte

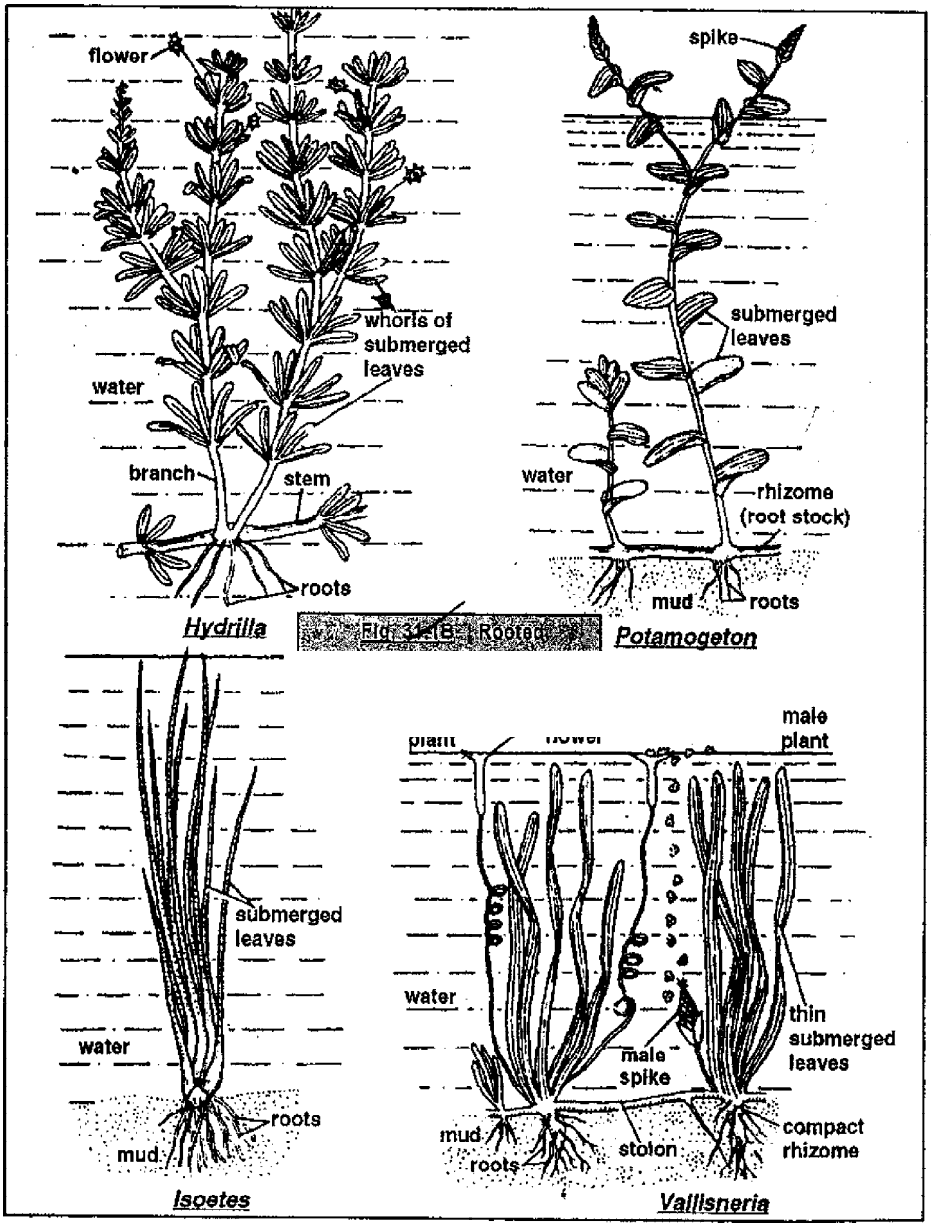

Fig.14.6 : Rooted submerged hydrophytes

14.2.2.2 Floating hydrophytes :

Plants that float on the surface or slightly below the surface of water but are in contact with air are called floating hydrophytes. These plants are either free floating or rooted to substratum.

(i) Free floating hydrophytes: These plants float on or just below the surface of water but are not rooted to the soil. Duck weeds *(Lemna and Wolffia)*, water hyacinth *(Eichhornia crassipes)* and water ferns *(Azolla and Salvinia)* are examples of this category (14.7).

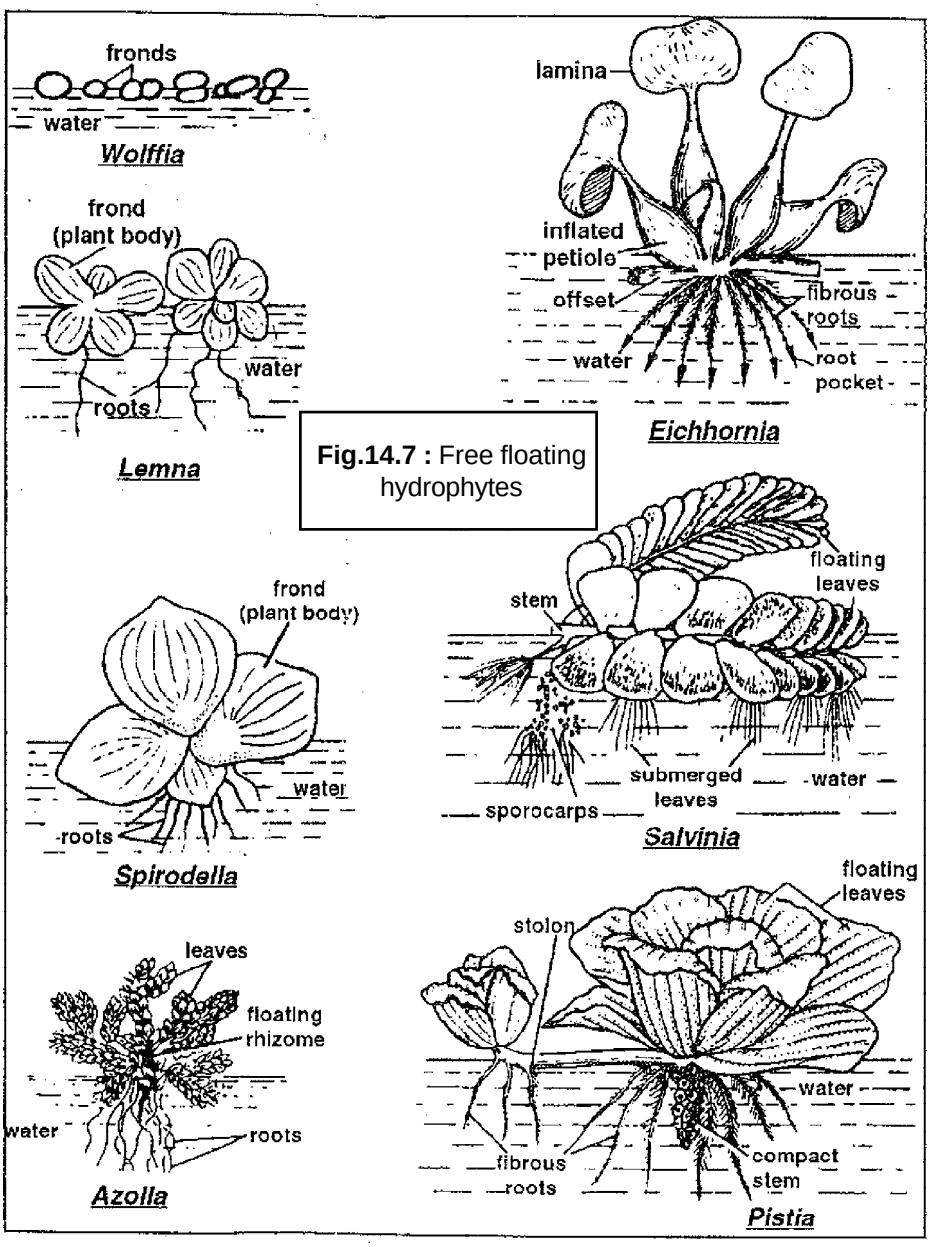

Fig.14.7 : Free floating hydrophytes

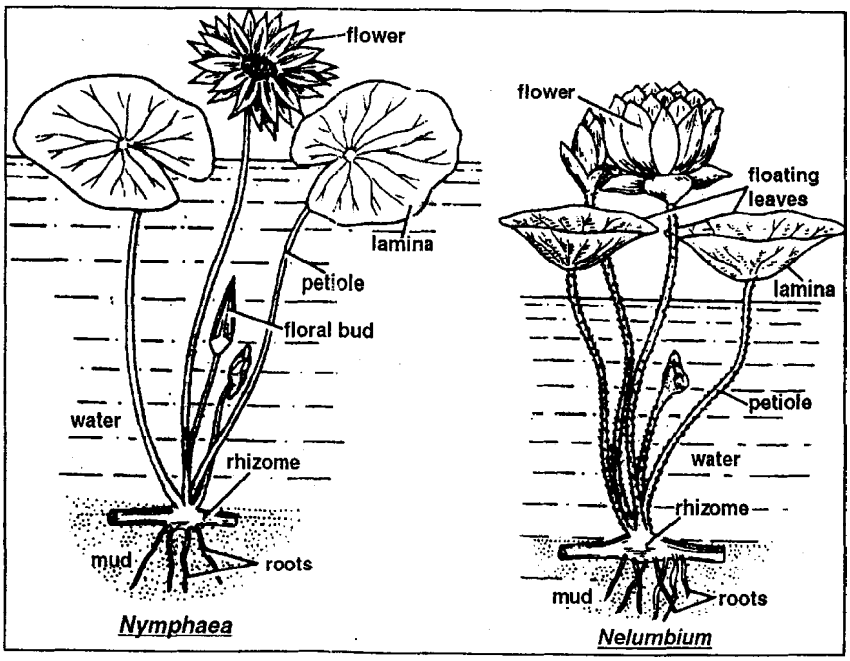

Fig.14.8 : Rooted hydrophytes with floating leaves

(ii) Floating but rooted hydro- phytes: This category includes plants that are rooted to the sub- stratum of their habitat (pond, lake or river) but their leaves and flowering shoots either float on the surface of water or just emerge out of water. Lotus *(Nelumbium)* and water lily *(Nymphaea)* are examples of this category (Fig.14.8).

14.2.2.3 Amphibious hydrophytes :

These are plants that are partly in water and partly in air. The aquatic part may be in shallow water or muddy sub-stratum. Morphological and anatomical features of these plants are different in parts that are in direct contact with water and the parts exposed to air. In some plants of this category like *Ranunculus* and *Sagittaria* (Fig.14.9), the roots and parts of shoot are in water; whereas in others like *Scirpus* and *Cyperus* (Fig.14.10), have their roots in water, but their shoots are completely exposed to air. The second category of plants are also called marshy plants. There is also a category of plants which are partly submerged only when there is inundation of sea water. Such plants are called halophytes.

14.2.2.4 Adaptations in hydrophytes :

Adaptations in hydrophytes can be discussed under three headings: morphological, anatomical and physiological.

(i) Morphological adaptations :

Hydrophytes show various kinds of morphological adaptations in their roots, stems and leaves.

Roots

Roots of hydrophytes are not of much importance, because most hydrophytes are partly or wholly immersed in water.

(i) Roots are totally absent in plants like *Utricularia, Ceratophyllum*, *Myriophyllum, Salvinia*.

(ii) Poorly developed roots are found in submerged plants like *Hydrilla, Vallisneria* .

(iii) Root pockets in place of root caps are found in floating hydrophytes like *Pistia* and *Eichhornea* that project the root tip.

(iv) Root hairs are poorly developed in most hydrophytes.

(v) Some plants like *Jussiaea* have two types of roots; one type being normal but the other spongy type and negatively geotropic.

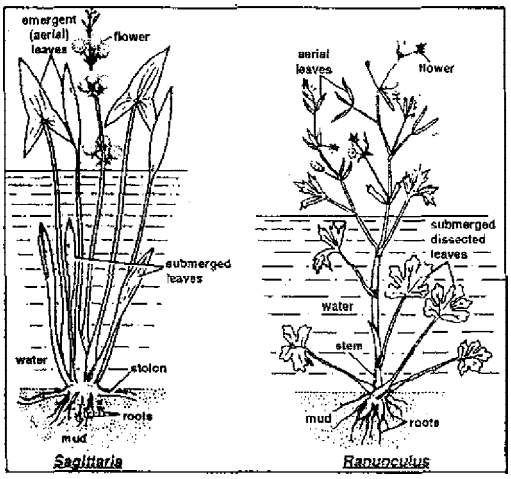

Fig.14.9 : Amphibious hydrophytes

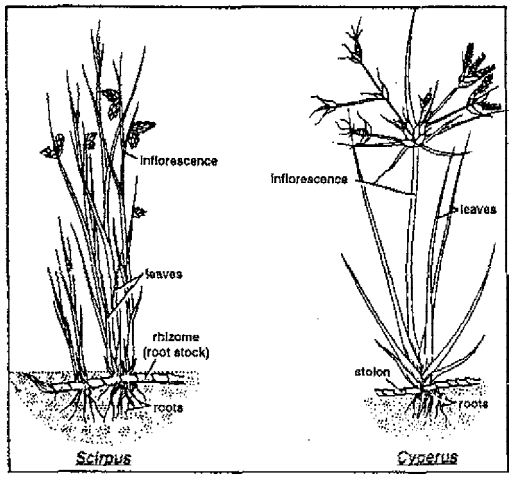

Fig.14.10 : Marshy plants.

Stem

(i) In submerged hydrophytes the stem is slender, spongy, flexible and long as in *Hydrilla, Potamogeton*.

(ii) In some floating hydrophytes like *Azolla, Pistia* or *Eichhornia,* it is horizontal, spongy and floating.

(iii) In rooted hydrophytes like *Sagittaria, Cyperus, Scirpus, Potamogeton,* the stem is a rhizome or stolon .

Petioles

Some hydrophytes show special features in the petioles.

(i) Petioles in submerged plants, with free floating leaves like *Nymphaea* and *Nelumbium,* are long, slender and spongy.

(ii) In the free floating hydrophyte *Eichhornia,* the petiole is swollen and helps in floating.

Leaves

Hydrophytes show a number of variations in the structure of their leaf lamina.

(i) In submerged hydrophytes like *Utricularia, Myriophyllum* and *Ceratophyllum*, the leaves are finely dissected and in plants like *Vallisneria,* they are long and narrow. In both types of adaptations, the intention is to offer little resistance to water currents.

(ii) In free floating hydrophytes, the leaves are smooth, shining and coated with wax. Presence of wax not only prevents water clogging, but also protects from physical and chemical injuries.

(iii) In floating but rooted hydrophytes like *Nelumbium* and *Nymphaea,* the petioles are long and the lamina are peltate with their lower surfaces in direct contact with water and the upper surfaces exposed to air.

(iv) One important feature that is usually shown by amphibious hydrophytes is heterophylly (leaf dimorphism), i.e., the presence of two types of leaves. In plants like *Sagittaria, Ranunculus* and *Limnophyla heterophylla,* the submerged leaves are ribbon shaped or dissected and the leaves above the surface of water are broad.

(ii) Anatomical adaptations (Fig. 14.11 - 14.16) :

In general, hydrophytes show the following trends in anatomical features:

(i) Reduction in protecting structures.

(ii) Reduction in mechanical tissue.

(iii) Reduction in conducting tissue.

(iv) Increase in aeration.

As the above features are seen in most organs of a plant, the anatomical adaptations are discussed on the basis of such features rather than on the basis of organs.

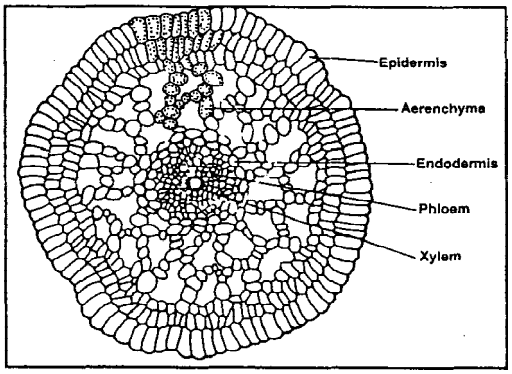

Fig.14.11 : T.S. of *Hydrilla* stem

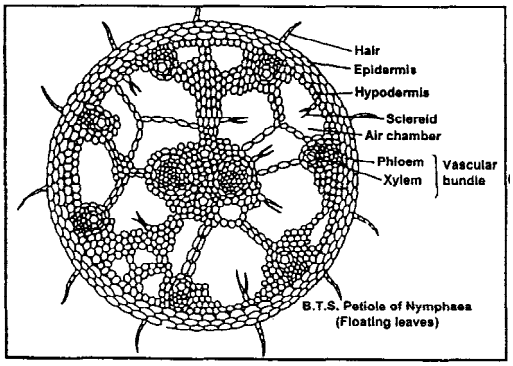

Fig.14.12 : T.S. of petiole of *Nymphaea*

Reduction in protecting structures

(i) Absence of cuticle in the submerged portions.

(ii) Use of epidermis as an absorbing or photosynthesizing (when epidermis has chloroplasts) organ rather than a protecting organ.

(iii) Poorly developed hypodermis.

Reduction of mechanical tissue

(i) Total absence or poor development of sclerenchyma in the submerged portions.

(ii) Presence of special type of sclereids called asterosclereids in some hydrophytes that provide mechanical support in the absence of sclerenchyma.

(iii) Presence of sclerenchyma in little or moderate quantities in the aerial portions.

Reduction of conducting (Vascular) tissue

(i) Vascular bundles are reduced to few or even one (*Hydrilla*) and located at the centre.

(ii) Xylem cells are very few as there is hardly any need of conduction.

(iii) Phloem is usually ill developed but in some cases it is well developed.

(iv) Secondary vascular tissue is never developed.

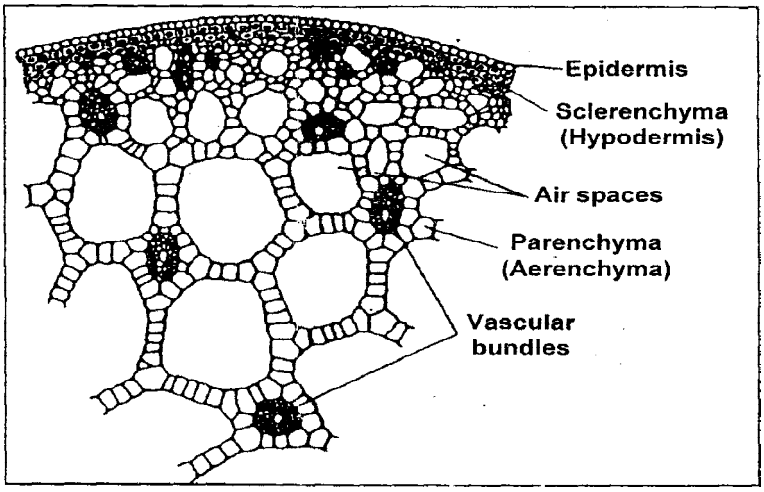

Fig.14.13 : T.S. of petiole of *Eichhornia.*

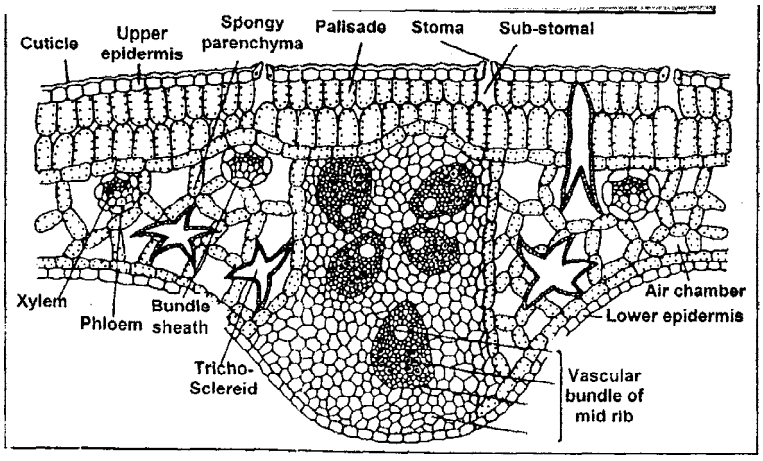

Fig.14.14 : T.S. of leaf of *Nymphaea*

Increase In aeration

(i) Stomata are totally absent or vestigeal in submerged parts.

(ii) Stomata are confined to upper surface leaves of rooted but floating hydrophytes.

(iii) In amphibious plants, stomata are scattered on the aerial portions.

(iv) Roots, stems and leaves of most hydrophytes have parenchymatous tissue with air chambers. These chambers store gases like CO_2 and O_2 and help in respiration and photosynthesis. These are hence, called arenchyma. Besides, the air chambers help in buoyancy and provide mechanical support.

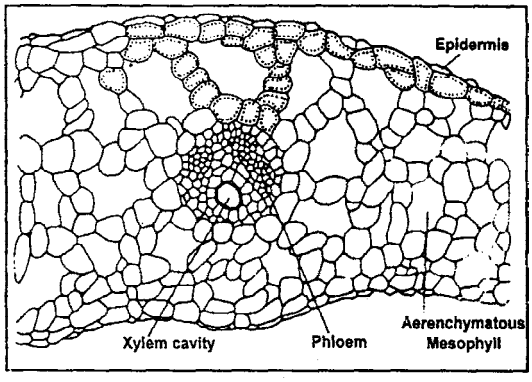

Fig.14.15 : T.S. of submerged leaf of *Vallisneria.*

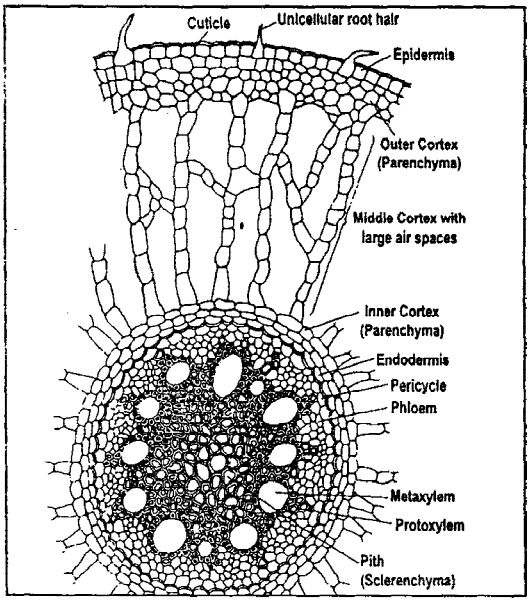

Fig.14.16 : T.S. of root of Typha.

(iii) Physiological adaptations :

Besides their adaptations in the morphological and anatomical characters, hydrophytes also show physiological adaptations.

(i) Osmotic concentrations of cell sap is low.

(ii) No transpiration from submerged plants.

(iii) Photosynthetic and respiratory gases are retained in air chambers for future use.

(iv) As far as reproductive physiology is concerned, hydrophytes mostly prefer vegetative reproduction.

14.2.3 Xerophytes :

Xerophytes are plants that live in conditions of water scarcity. Places where there is scarcity of water are called xeric habitats. Xeric habitats are of two types:

(i) Physically dry habitats are those in which water cannot be retained (deserts, rock surfaces).

(ii) Physiologically dry habitats have plenty of water, but the water is not available to the plant.

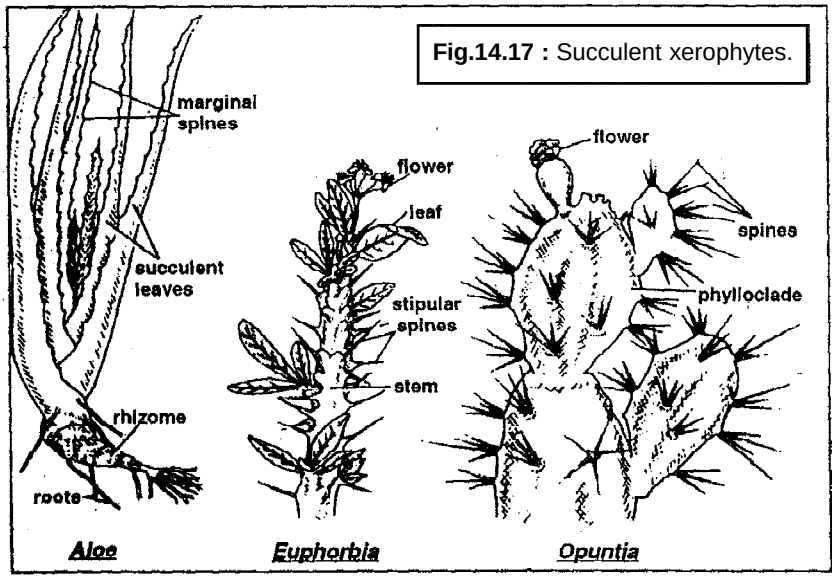

Fig.14.17 : Succulent xerophytes.

Types of xerophytes

Xerophytes are plants that can withstand conditions of water scarcity and the adaptations in such plants aim at the following.

(i) Absorb more water from the surroundings

(ii) Retain water in their organs

(iii) Reduce transpiration

(iv) Reduce utilisation of water

Basing on their adaptation to water scarcity or drought conditions, xerophytes are classified into the following.

(i) Drought resistant plants

(ii) Drought enduring plants

(iii) Drought escaping plants (ephemerals)

Drought resistant plants develop features (adaptations) that enable them to survive in extreme conditions; drought enduring plants can tolerate drought though they may not have distinct adaptation; drought escaping plants are short-lived that complete their life cycles before dry conditions are reached (ex. *Artemisia, Astragalus).*

Basing on their capacity to store water, xerophytes are classified as succulents (Fig. 14.17) and nonsucculents (Fig. 14.18). Succulents like *Opuntia,* have their organs swollen due to accumulation of water, whereas non-succulents are considered as true xerophytes.

14.2.3.1 Adaptations in xerophytes (Fig. 14.19 - 14.22) :

Adaptations in xerophytes are of two types:

(i) Xeromorphic adaptations are those which are inherited whether the xerophyte grows in xeric conditions or not. For example, a Cactus has the same feature, whether it is in a desert or in a normal land.

(ii) Xeroplastic adaptation are the ones that are induced temporarily but disappear when the conditions are favourable.

Xerophytic adaptations may be morphological, anatomical or physiological.

(i) Morphological adaptations :

Xerophytes exhibit a number of special features in their morphological organs.

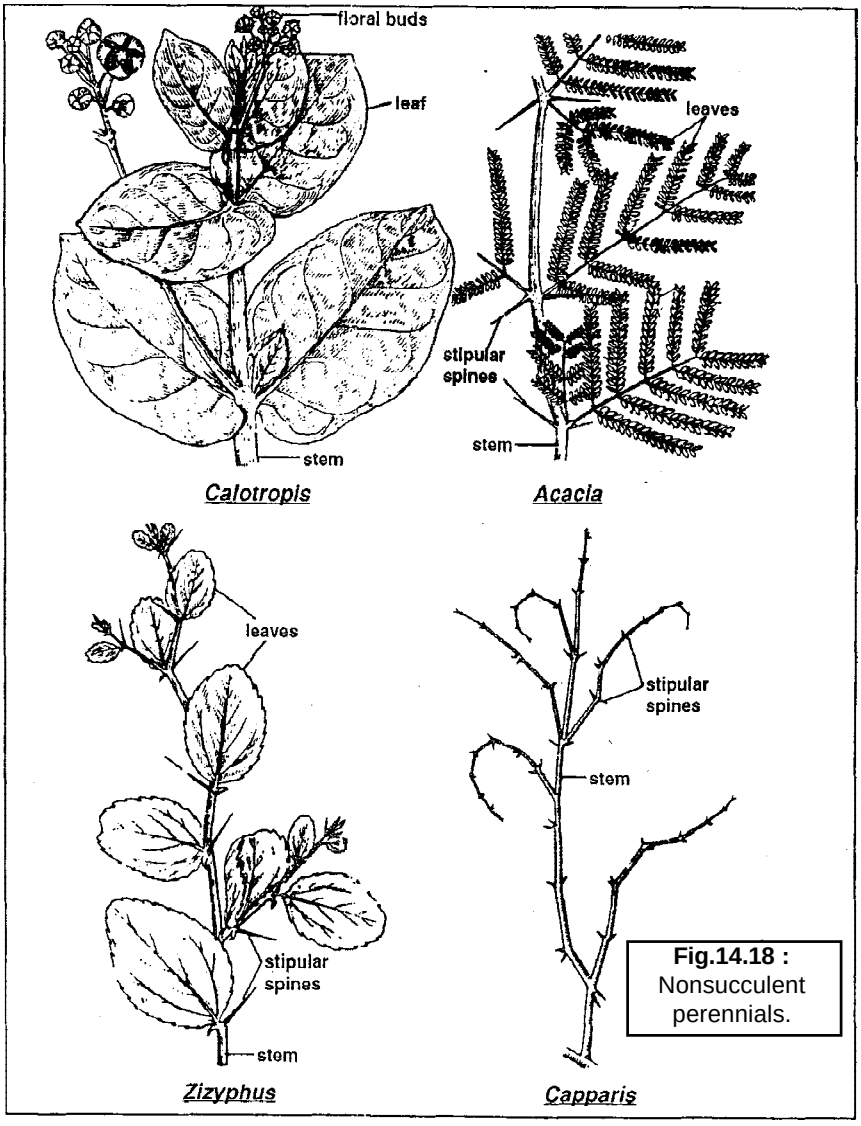

Fig.14.18 : Nonsucculent perennials.

Roots :

(i) The root system is well developed, extensive and much branched.

(ii) Roots of perennial xerophytes reach greater depth to absorb water but some xerophytes have shallow root system especially when water is available in the surface layers.

(iii) Root hairs are profuse.

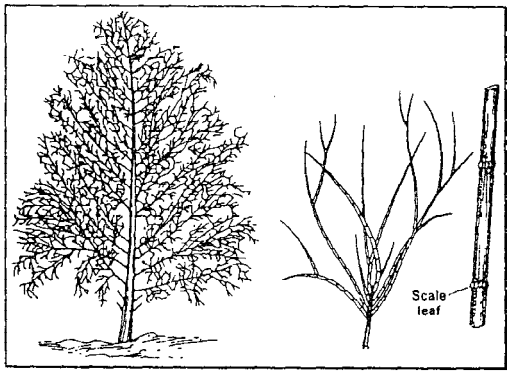

Fig.14.19 : Casuarina plant (left), a branch (middle) and a portion of branch showing microphyllous (scale) leaf (right)

Stems :

(i) Stems are hard and woody.

(ii) Some stems are covered with dense hairs *(Calotropis),* coated with wax *(Opuntia)* or silica *(Equisetum).*

(iii) Stems in some xerophytes are modified to thorns *(Duranta).*

(iv) Succulents have their stems modified into structures like phylloclades *(Opuntia*.); **cladodes** *(Asparagus)* or leaf like structure *(Ruscus).* All such structures are usually meant for water storage.

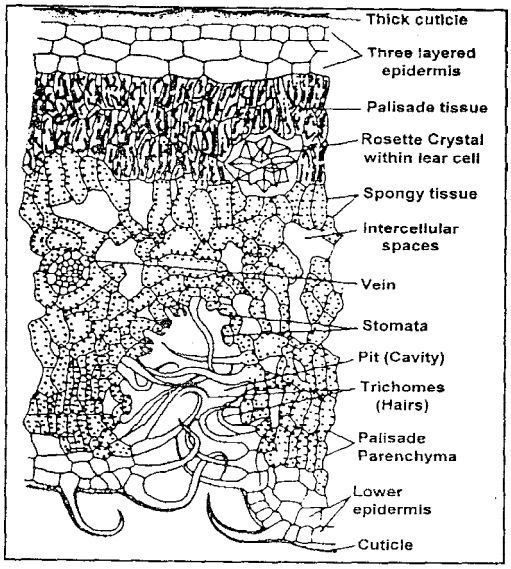

Fig.14.20 : T.S. of *Nerium* leaf.

Leaves :

Usually leaves of xerophytes are reduced or modified to various kinds of structures to minimise transpiration. The following types of condition are seen:

(i) Microphyllous when the leaves are small scaly *(Casuarina* : *Asparagus)* or needle like *(Pinus)*

(ii) Trichophyllous when the leaves are covered with hairs *(Nerium, Calotropis*) (iii) Macrophyllous when the leaves are soft and fleshy *(Begonia)*

(iv) Sclerophyllous when the leaves are stiff and hard. *(Banksia)*

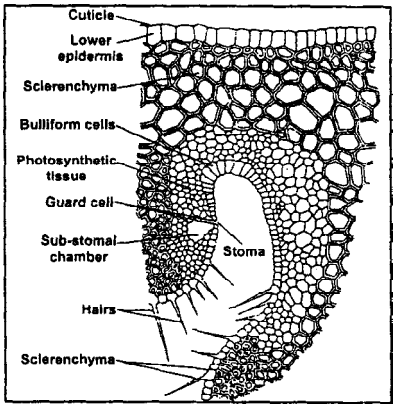

Fig. 14.21 : T.S. of leaf of Ammophil showing bultin form cells.

(v) Many xerophytes have no leaves *(Capparis*) or they fall very early (caducous) as in *Euphorbia*.

(vi) Rolling of leaves is observed in some xerophytes like *Ammophila* where the stomata are directed inwards.

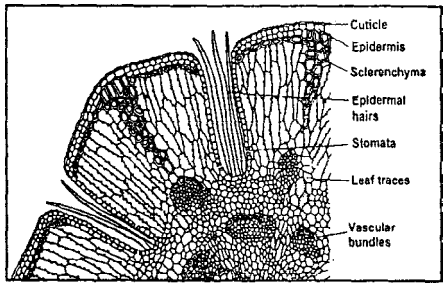

Fig.14.22 : T.S. of *casuarina* stem.

(ii) Anatomical adaptation :

Anatomical adaptations can be conveniently discussed under the headings- *epidermis, hypodermis, ground tissue* and *vascular tissue.*

Epidermis :

(i) Some xerophytes have multiple epidermis *(Nerium)*.

(ii) Epidermis is with thick cuticle and deposition of waxes, resins etc.

(iii) There are epidermal hairs especially in grooves (furrows) that protect the sunken stomata.

(iv) Mostly stomata are sunken and are in pits.

(v) Stomatal frequency is low.

(vi) Leaves that have the capacity to roll have specialised cells called bulliform cells that help in rolling.

Hypodermis :

Hypodermal layers of xerophytes are thick and well developed.

Ground tissue :

(i) In stems, there is abundant mechanical tissue in the form of sclerenchyma as in *Casuarina* stem.

(ii) Since leaves are reduced, the stems usually have chlorenchyma.

(iii) In succulent plants, cortex is filled with water, mucilage, latex etc.

(iv) In plants that have leaves, palisade parenchyma is well developed.

(v) In *Pinus,* mesophyll cells are modified.

(vi) Intercellular spaces are greatly reduced.

Conducting tissue :

Vascular tissue (xylem and phloem) is very well developed in xerophytes.

(iii) Physiological adaptations :

Xerophytes show a number of physiological features:

(i) Transpiration is well regulated.

(ii) Osmotic concentration of the cell sap is high.

(iii) Succulents have high pentosans (derived from polysaccharides) resulting in accumulation of water.

14.3 POPULATION INTERACTIONS :

Under a natural condition, no organism can remain separated from all other living forms. An individual of a population is influenced directly or indirectly by a number of different species. Even the autotrophic organism like plant species cannot survive alone. This has to depend on soil microbes (for breakdown of organic matter in soil) to fulfill their inorganic nutrient requirements. The plants also depend on some animals or insects for pollination activities. Some vital processes like growth, nutrition and reproduction depend upon the coactions or interactions of other members within the species or between individuals of different species (population). The first one is called intraspecific interaction and the second is interspecific interactions. Such interactions can be beneficial (positive interaction) or detrimental (negative interaction) or neutral (neither beneficial nor harmful) to one or both the individual species. Basing on these interactions some relations have been developed:

14.3.1 Mutualism :

It is an interaction between two or more species in which all are benefited out of the relationship. It is a type of obligate association of two organisms where both live together and cannot live separately.

Some of the best **plant** examples include- (a) Lichens: Here, the photosynthesizing phycobiont (algae) who prepares food through photosynthesis creates a mutual relation with mycobiont (fungi) which absorb nutrients and provides support. (b) Mycorrhizae: It is an association between the roots and fungi. This mutual relationship help fungi to provide essential nutrients from soil whereas the roots of the plant provide energy in the form of carbohydrates.

There are some **plant-animal** examples observed which fit into this relationship- (a) the plants often require the insects for facilitating pollination and for the seed dispersal where as the insects collect nectars and honey from the plants. (b) The relationship of wasp as the pollinator and species of fig plants is an example of this. For each fig species there is a requirement of wasp, without which the pollination will fail to occur. The wasp pollinates the fig flowers while searching for a suitable egg laying place. On the other hand, the female wasp lays egg in the fig fruit or developing seeds for deriving nutrition for its larvae.

14.3.2 Competition :

The name suggests that this is a kind of interaction where two individuals or species compete for a limited resource. This can happen between the members of the same species (intraspecific) or between members of different species of a community (interspecific).

The intraspecific competition occur for the resources which are in short supply such as food, space or mate. These are of two types – (a) Contest competition: where each organism claims a part of the resource but due to competition some are successful to get but failures denied access to the resources. (b) Scramble Competition: In this case the resource gets divided to many smaller parts to which all have access. Individual organisms scramble for resources. Each individual obtain a small amount of resource that makes it unable to survive.

The interspecific competition are of two types – (a) Competitive exclusion: Two closely related species competing for the similar resources cannot survive together indefinitely. One species eliminates the other completely from a region, perhaps leading to extinction. (b) Competitive coexistence: In this case, species may live in the same habitat but use different foods or exploit them at different times, which avoid competition. Or, the species can remain together despite being strong competitors. The environment first favours one species, then in the later stage, environment favours to another species.

14.3.3 Predation :

In predation one animal kills another animal or plant for food. So to say one species called predator is benefitted and the interaction is detrimental for the other species i.e., prey. Examples of predators are Tiger, Lion, Larger Fish, Animal, etc. and prey include Deer, Smaller fish, Plants, etc.

Predators have definite roles in the management of ecosystem.

(i) By the predator-prey interrelation the energy transfer across one trophic level to the other takes place.

(ii) The predators help to keep the prey population under control. For example, the growth of grass population is kept under control by the herbivores such as goat, cow, etc. Basing on this principle the biological pest control has been successful in agriculture.

The prey has also adapted various defence majors to escape or fight the predators. Some of these are as follows:

For animals : 1. Camouflage- There are some animal species who can change their colour to escape from the predators. 2. Chemical emission- Some animal species emit poisonous chemicals from their body to evade from the predators. 3. Mimicry- It refers to the resemblance

of one organism to another or to the natural objects among which it lives, that secures its concealment, protection or some other advantage.

For Plants : The plant systems have adapted more effective morphological and chemical defence measures against their predators. Presence of spines in *Cactus*, *Argemone*, poisonous chemicals like glycosides in *Calotropis* are some of the examples. Chemicals like caffeine, quinine, strychnine, opine, nicotine, etc. are produced in the plants which have been the defences against the grazers.

14.3.4 Parasitism :

This is a relationship between two individuals wherein one individual called parasite receives benefit at the expense of the other individual called host. It is a harmful interaction between two individual species. Parasitism is mainly a food coaction but the parasite derives shelter and protection from the host, too. The parasite ordinarily does not kill the host until this completes its reproduction. It may so happen during the course of these coactions the host may die due to some other secondary infections. Human is a better example who is beset with many parasitic organisms like tape worms, flukes, round worms etc. in the digestive system. There are many plant examples like rusts, smuts which are parasites on crop plants, *Cuscuta* twines on the host plant and derives nutrition through haustoria, being a good parasite.

Many parasites are host specific i.e., they can act upon a single species of host.

Parasitic Adaptation :

The ectoparasites and endoparasites have following parasitic adaptations-

(i) Reduction of unnecessary sense organs and locomotory organs.

(ii) Developed some clinging organs like hooks, suckers, etc. to cling on to the host. Also some sucking organs to suck the blood in animals or sap in plants.

(iii) Loss of digestive system.

(iv) High reproductive capacity.

Effects of Parasites on host :

(i) Parasites always do not kill the host immediately but they make the host to suffer the damage to the structures and in excess may cause death.

(ii) The parasites can cause the reduction of growth and reproduction of host.

(iii) Parasites also help in reduction of the host population.

There are two types of parasites basing on the occurrence in the host body: (a) Ectoparasite- these type of organisms live outside and derives nutrition from the host, example; lice on human, *Cuscuta* on plants. (b) Endoparasite-These live within the host to cause harm, example; worms in human.

14.4 POPULATION ATTRIBUTES :

Population is a unit of ecosystem through which the energy flows and nutrients get cycled which helps in maintaining its stability. A population has some features such as birth rate, death rate, growth form, age structure, density, etc. which are under discussion.

(a) Growth : Populations show growth which characteristically increase in size in a sigmoid, 'S' shaped or logistic fashion. When a few organisms are introduced to a particular unoccupied area, the growth of the population is at first slow (positive acceleration phase) then becomes very rapid (logarithmic phase) and finally slows down because of the increased environmental resistance (negative acceleration phase). The population size never increases beyond a saturation limit called the carrying capacity.

Another kind of population growth curve in 'J' shaped form is observed where the density of the organisms increases rapidly (i.e. exponential or compound interest fashion) but stops suddenly due to environmental resistance or other causes. (**Fig.14.23)**

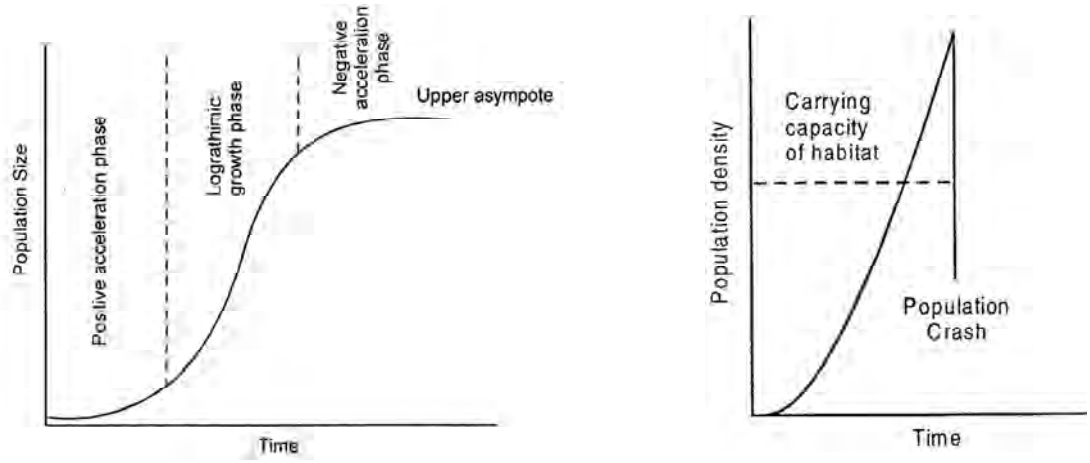

Fig. 14.23 : The sigmoid and exponential growth curves

The growth rate of a population can also be determined:

$$\text{Growth rate (g)} = \frac{\text{Number of births in the area (b)} - \text{Number of deaths in the area (d)}}{\text{Average population in particular time}}$$

The population size for a given species is not a static parameter but it is ever changing basing on different factors including food availability, predation pressure and weather adversity. The population thickness in a definite habitat fluctuates due to these four changes.

(i) **Natality :** This is the number of births during a given period of time in a population which adds to the initial density.

(ii) **Immigration :** This is one way inward movement which allows to increase the population level causing overpopulation. The immigration may also lead to increase beyond the carrying capacity which can result in increased mortality among the immigrants or decreased reproductive capacity of the individuals. Both emigration and immigration are initiated by weather and other abiotic and biotic environmental factors.

(iii) **Mortality :** Number of deaths taking place in a particular population in the unit time.

(iv) **Emigration :** This is one way outward movement due to overcrowding or population pressure. By dispersing into new localities, there is an opportunity for interbreeding with other populations which may lead to create better variability and adaptability. However, continuous emigrations are rare in occurrence but when these occur, leads to depopulation.

Migration is a periodic departure and return of organisms from or to the population. Most two way migratory movements are rhythmic processes of population and regular periodicity is a common feature. Normally environmental periodicities such as day and night rhythms, lunar periods, tides, changing seasons, etc. control these migratory movements.

(b) Birth and Death rates : The rate of increase or decrease of population can be attributed through birth and death. Natality is equivalent to birth rate which expresses the rate of new born individuals (reproduction) in the population. Here, two aspects of reproduction such as fertility and fecundity should be distinguished. Fertility is the actual level of performance based on numbers born and fecundity is the potential level of performance (or physical capacity) of the population. Example: the fertility rate for human population may be one birth per eight years per female in its proper reproductive age but fecundity rate reflects one birth for human female in each interval of 9 to 11 months in the proper reproductive age.

Thus, natality or birth rate of a population can be expressed as :

$$\text{Birth rate } (b) = \frac{\text{Number of new borns per unit time } (dNn)}{\text{Average population } (Ndt)}$$

where, b = natality per unit time, d = changing value of the entity, N = initial number of individuals in population, Nn = number of new individuals added, and t = unit time.

Similarly, the death rate is the death or loss of individuals from the population in unit time and can be expressed as:

$$\text{Death rate } (d) = \frac{\text{Number of deaths per unit time}}{\text{Average population}}$$

The death rate has a relation with natality because of the overcrowding, predation and spread of diseases. The mortality rate in many species varies from one age level to another, thus a mean death rate has only general significance.

(c) Sex Ratio : Another attribute characteristic of a population is sex ratio. It represents the ratio of organisms of different sexes of the population. In plants, most of them are bisexual but some are either male or female. But in animals, the sex is either male or female which creates a difference in the population.

(d) Age Distribution : A population at a given time is composed of individuals of different age groups such as pre-reproductive, reproductive and post-reproductive. The population age distribution is related to the growth rate of the population and this can be used to calculate whether the population is expanding or contracting. Ordinarily a rapidly expanding population would have a large proportion of young individuals, a stationary population with even distribution of age groups and a declining population contain a large proportion of old individuals.

These age groups of the population can be portrayed through the graphical age pyramid representations. In human populations, the age pyramids generally express age distribution of males and females in a combined diagram. **(Fig. 14.24)** The shapes of the pyramids reflect the growth status of the population. The pyramids can be of three different types as follows:

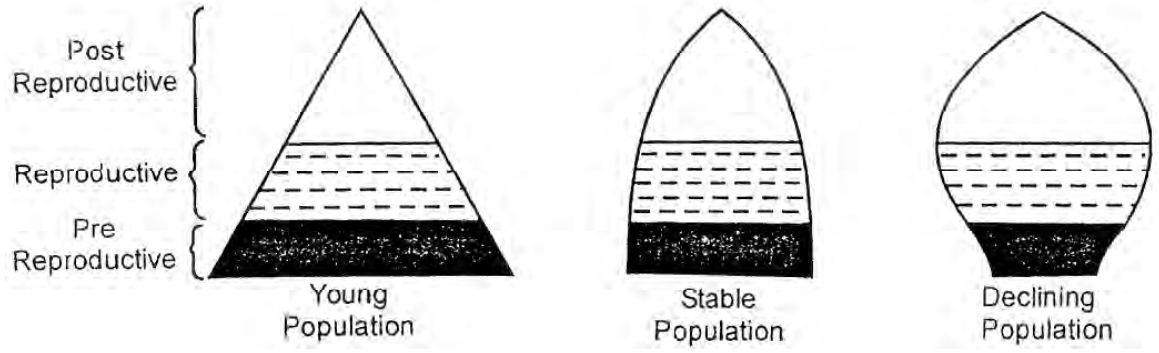

Fig. 14.24 : Age pyramids

(i) **Triangular :** This is a type of growing population which can be graphical shown like a triangle. The population carries a high proportion of pre-reproductive individuals followed by reproductive individuals and post-reproductive individuals. Because of the very large number of pre-reproductive individuals, more and more of them enter reproductive phase and rapidly increases the size of the population.

(ii) **Bell shaped :** This type of pyramid will denote a stationary or stable population having an equal number of young and middle aged class of individuals.

(iii) **Urn-shaped :** This group has a small number of pre-reproductive individuals followed by a large number of reproductive individuals. As there is less number of

individuals in pre-reproductive groups, the population size will decline with time and the growth rate will be zero. Such a population will show a negative growth or declined growth.

(e) Population Density : The population density means the size in relation to unit space at a particular time. The size of a population can be measured in a several ways. The parameters include abundance (absolute number in population), numerical density (number of individuals per unit area) and biomass density (biomass per unit area). The density of a species population can be expressed either with reference to the total area (i.e. crude density) or with reference to the actual area of habitat available to the species (ecological density). For example: there is a crude density of 200 tigers per square kilometer but if only half of the area had suitable area for tigers, the ecological density would be 400 per square kilometer of tiger habitat. If the size of individuals in the population is relatively uniform, as mammals, birds, then this density is expressed in terms of number of individuals (or numerical density). On the other hand, when the size of individuals is variable such as fishes, plants called biomass density. The density of organisms in an area varies with season, weather, food supply, etc. The existence of a population in a particular area at a given time depends on its rate of reproduction (natality) and mortality besides ingress or egress.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Choose the correct answer from the choices given under each bit :

(i) A population is a group of

(a) Individuals in a species (c) Species in a community

(b) Individuals in a family (d) Communities in an cosystem

(ii) Exponential growth occurs when there is

(a) A great environmental resistance (c) A fixed carrying capacity

(b) No biotic potential (d) No environmental resistance

(iii) In a population, unrestricted reproductive capacity is called as

(a) Carrying capacity (c) Birth rate

(b) Biotic potential (d) Fertility rate

(iv) Two opposite forces operate in the growth and development of a population. One of them is related to the ability to reproduce at a given rate. The force opposite to it is called

(a) Environmental resistance (c) Mortality

(b) Fecundity (d) Biotic control

(v) The carrying capacity of a population is determined by its

(a) Population growth rate (c) Mortality

(b) Limiting resources (d) Natality

(vi) Which of the following is a conduit for energy transfer across trophic levels?

(a) Mutualism (c) Photocooperation

(b) Parasitism (d) Predation

(vii) Phenomenon of inhibition of growth of one species by other species through secretion of some chemicals is termed as

(a) Commensalism (c) Allelopathy

(b) Mutualism (d) Predation

(viii) Predation performs all, except

(a) Transfer of energy (c) Loss of sense organs

(b) Keeps prey population under control (d) Maintains species diversity

(ix) Two important factors that influence the life of organisms are

(a) Soil, temperature (c) Soil, light

(b) Light, water (d) Water, temperature

(x) Ecology describes

(a) Interactions between living organisms only

(b) Intraspecific competitions only

(c) Interactions between members of a single species

(d) Interactions of organisms and abiotic components around

2. Answer in one word only :

(i) Study of interrelationship between the environment and a plant species

(ii) Amount of water vapours actually present in the air at any given time

(iii) The total amount of water in the soil, except the gravitational water

(iv) Association of fungi and algae

(v) The study of soil

(vi) Vegetation where the annual rainfall is more than 50 inches

3. Correct the sentences changing the underlined word only.

(i) Plants those grow in soil and mud are xerophytes.

(ii) Sunken stomata is a characteristic of hydrophytes.

(iii) Air pockets are found in mesophytes.

(iv) The pre reproductive mass is found more in urn shaped pyramid

(v) Population consists of different kinds of species.

4. Fill up the Blanks

(i) Shallow water region present on the edge of lakes is called ————————.

(ii) The most relevant ecological factor is ————————.

(iii) Mortality and ———————— contribute to a decrease in population density.

(iv) J-shaped curve represents ———————— growth

(v) Geometric representation of age structure is a characteristic of ————————.

GROUP - B
(Short Answer-type Questions)

1. Answer in three sentences :

(i) Camouflage

(ii) Edaphic factor

(iii) Habitat

(iv) Temperature

(v) Biomes
(vi) Population
(vii) Competition
(viii) Abiotic factors
(ix) Population density
(x) Necessity of Adaptations

2. Differentiate between :

(i) Habitat and Niche
(ii) Mutualism and Parasitism
(iii) Hydrophytes and Xerophytes
(iv) Birth rate and Death rate
(v) Fertility and fecundity
(vi) Logarithmic and exponential growth

GROUP - C
(Long Answer-type Questions)

1. Explain what is population? Describe the different characteristics of population.
2. What do you understand by population. Explain the different attributes of the population.
3. Explain how different organisms interact in a population emphasizing on the possibilities of various relationships.
4. What is a habitat. Describe the different types of abiotic factors present in the habitat.
5. What are the various adaptations different plants adapt for their survival in different habitats.

❑ ❑ ❑

CHAPTER 15

ECOSYSTEMS

Ecology includes the life habits of millions and millions of different kinds of organisms and considers all types of influences and interactions among them and their nonliving environments. In fact, the scope of ecology is very extensive. However, the concepts of ecology can be presented in some remarkable basic and simple principles. The logical starting place of it is the “Ecosystem”.

The term ecosystem comprises of structural and functional units of living organisms and non-living substances interacting to produce an exchange of materials between themselves. With the ecosystem, the terms like “environment” and “habitat” come into discussion. Environment, literally means “to surround” (derived from French verb environner) and therefore, includes all conditions, circumstances and influences, surrounding and affecting an organism or group of organisms.

Habitat is derived from the Latin word, habitare (to dwell). Thus it includes all features of environment in a given locality. Ecosystem, apart from including habitats and environments, specifically refers to the dynamic interaction of all parts of environments, focusing particularly on the exchange of materials between the living and non-living components. Some authors make the scope of environment all inclusive and define ecosystem as a ‘viable unit of environment’. The entire biosphere can be regarded as a “global ecosystem”. Since this system is too much big and complex, it is convenient to divide it into two basic categories, like terrestial or land ecosystem and the aquatic ecosystem. Thus forest, grassland and desert are some examples of terresrial ecosystems. Similarly, pond, lake, river and ocean are some examples of aquatic ecosystem. Crop fields and aquariums can be regarded as man-made ecosystems.

15.1 ECOSYSTEM COMPONENTS :

The ecosystem is divided into two main components viz. Biotic (living) and Abiotic (non-living). The living component is usually comprised of producers, consumers and decomposers, while the non-living component is divided broadly to two types viz. materials and energy. Let us start the ecosystem structure with biotic components as the abiotic components have been discussed in previous chapters.

15.1.1 Biotic component :

Living organisms are highly organized. In order to survive and maintain, this internal order organisms need supplies of relevant nutrients. From the nutritional point of view, an ecosystem shall have three types of organizations viz. Producer, Consumer and Decomposer organisms.

(i) Producer :

These are the green plants which synthesize organic compounds and are called autotrophic or self - productive. They take inorganic compounds from the surrounding and manufacture organic moleclues and living protoplasm from them. All green plants are photoautotrophs as they obtain their energy from the sunlight by the process called photosynthesis. There are many photosynthetic bacteria having various ways of obtaining their carbon compound. Besides the photosynthetic organisms, there are chemosynthetic organisms which utilize hydrogen sulphide (H_2S) and bond energy of such inorganic components. Obviously, all life depends upon the basic productive capacity of green plants and bacteria.

(ii) Consumer :

Consumers are animals which utilize the organic materials directly or indirectly manufactured by autotrophs. They are called heterotrophs. Primary consumers or herbivores directly consume the organic compounds of plants. Mammalian herbivores mostly are predators while most insect herbivores are parasites. Secondary consumers are carnivores which feed on herbivores and smaller carnivores. An omnivore is an organism that eats both plants and animals. Humans are omnivores par excellence.

(iii) Decomposer :

These organisms break down the organic waste products and dead remains of organisms into the inorganic substances needed by the producers. Decomposers are often microorganisms. They play a vital role in nature. Their nutrition is saprophytic, that is, associated with rotten and decaying organic materials. In a sense, they are the digestive organisms of an ecosystem - converting complex chemicals to simpler forms. They provide the final essential link in the cycle of life.

15.1.2 Abiotic Component :

The abiotic components of an acosystem can be divided into 3 parts. (a) inorganic nutrients like C, N, H etc. (b) organic compounds which constitute the living body (c) environmental factors. These may be respectively categorized under physical environment, nutrients and energy providers.

The interaction of various biotic and abiotic components result in a functional structure characteristic of each type of ecosystem. The plants and animals of any ecosystem form its species composition. To understand the basic components and their specific functioning the example of a pond ecosystem can be considered.

15.1.3 Pond ecosystem :

I. Biotic Components

(a) **Producers :**

These are the algae and other green plants growing in the pond. The plants are either rooted to the soil or are found floating in water (phytoplanktons). All these plants produce food through photosynthesis and are responsible for basic food supply to the pond.

Some of the common algal species found floating in pond water are *Zygnema, Ulothrix, Spirogyra, Oedogonium, Diatoms, Anabaena.*

The aquatic macrophytic populations are of *Typha, Saggitaria, Hydrilla, Vallisnaria, Nelumbium, Eichhornia, Pistia, Azolla, Wolffia, Lemna* etc.

(b) Consumers

These are largely herbivores feeding directly, the plants growing there These are primary consumers, which are divided into Benthos (insects, crustaceans, mites, fishes, molluscs etc.) and Zooplanktons (protozoans like *Euglena*, rotifers like *Brachionus* and crustaceans like *Cyclops* etc.)

The primary consumers are food for another set of organisms, together called as "**secondary consumers**" e.g. big fish feeding on small fishes, beetles feeding upon zooplanktons etc. There may be yet another group constituting "**tertiary consumers**" which feed on the secondary consumers.

(c) Decomposers

These are, of course, heterotrophs. Majority of them are saprophytes feeding on dead organic matter which they absorb in solution or ingest in very small pieces. The decomposers help release of basic materials for reuse. Important species functioning as decomposers are members of the group of bacteria, fungi or prokaryotes.

Generally speaking the upper layer of a pond is "Productive Zone" of plants and the bottom layer is a "Detritus Zone" of decomposers and these two zones together constitute a balanced ecosystem.

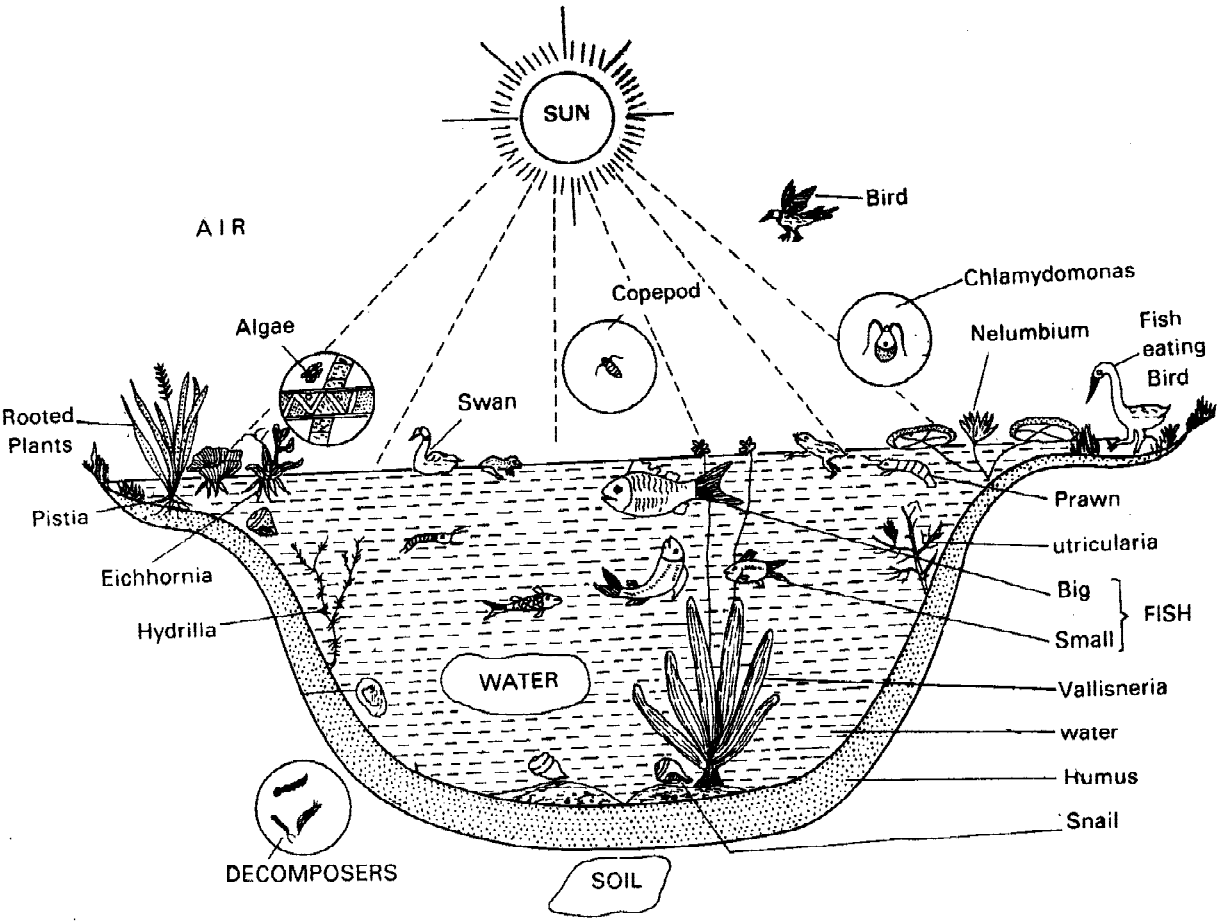

Fig. 15.1 : Pond as aquatic ecosystem

II. Abiotic Components :

It includes water, air, inorganic elements, organic compounds of various types. These are mostly in utilizable form. Some of the matters are there which are in available form while much of them are in the living organisms occurring there. This component also includes solar radiation falling on the surface of the pond. (Fig. 15.1)

Similarly forest ecosystem is an example of **terrestrial ecosystem**. Unlike a pond, the ecosystem, here, is complex one Since many small and big ecosystems operate, one within the other. Being large, there are different strata of layers of plant covers with different species of herbivores and predators, whose population is regulated by space, food, water, parasites and natural disasters. The same ecological principles like the ponds are applicable in this case. There are aoutotrophs, herbivores, carnivores and decomposers but with different species composition from the ones we have discussed in pond ecosystem. In most forest environments,

the rate at which litter (dead organic matter to the soil in the form of leaves, barks, stems etc.) is added equals the rate at which it is removed by decomposition. When dead materials decay, they form humus, which is functionally of great importance. The humus acts as a reservoir holding minerals and water until they are absorbed by plants, or in other words "recycled". Hence, the humus helps build a balanced ecosystem.

An ecosystem, thus, has following functional atributes (i) Productivity, (ii) Decomposition, (iii) Energy flow and (iv) Nutrient cycling.

15.2 PRODUCTIVITY :

Living organisms are highly organised. In order to survive and maintain its internal order, organisms need constant supply of energy. Organisms utilize energy when they make new cells, this condition is called growth. Respiration releases energy where relatively large molecules, such as glucose, are broken down to smaller less ordered molecules i.e. carbon dioxide and water. Organisms need to have nutrients (for example, glucose) in which they conserve their energy.

Primary production in the world is mostly due to photosynthesis, which the autotrophs do when they convert carbon dioxide and water into larger structural molecules. With this process, the plants grow in size. So primary production is defined as the amount of biomass or organic matter produced per unit area over a fixed time period by plants due to photosynthesis. It is expressed as weight in grams per square meter per day ($gm^{-2} d^{-1}$) or energy in kilocalories per square meter ($Kcal\ m^{-2}$).

15.2.1 Gross Primary Productivity - (GPP) & Net Primary Productivity (NPP) :

We have discussed the rate of biomass production is productivity. It can be of two types. GPP- Gross Primary Productivity and NPP- Net Primary Productivity. GPP is a measure of total amount of organic matter production due to photosynthesis by an ecosystem. All organism, including autotrophs respire . During respiration, some of the matter from the gross primary productivity is utilized and lost. After this loss, whatever remains is **Net Primary Productivity (NPP).** Hence, **NPP = GPP - Respiration.** Different plant communities have different net primary productivity. In a terrestrial community, GPP is approximately 2.7 times of NPP while the same is 1.5 times in oceans. Net primary productivity is, thus, available biomass for consumption by herbivores and decomposers. Secondary productivity is the rate of new organic matter production by consumers.

15.2.2 Capture of Light by Plants :

Green Plants have the capacity to combine carbon dioxide and water to produce sugar using the energy of sunlight. The process is called Photosynthesis. It is the mainspring of all life

and it represents the basic productive capability of any ecosystem. Ecologically, the most important aspect of this process photosynthesis requires (1) plants containing chlorophyll (2) light (3) carbon dioxide (4) water (5) some oxidant ions such as Fe or Mg and finally (6) phosphorus in the form of phosphates. Green plants are not just the producers of glucose and carbohydrates, they also synthesize highly ordered molecules like lipids, proteins and vitamins, all of which are fundamental chemical constituents of protoplasm.

Even in the most productive communities, plants trap only about 1-3% of the energy which they receive from sunlight. Generally speaking, the efficiency of phtotosynthesis which is also called “ecologic efficiency” averages less than 1%. In large bodies of water such as ocean, the ecologic efficiency is as low as 0.18%.

15.3 DECOMPOSITION :

As discussed earlier in this chapter, decomposers help release of basic materials by breaking down complex organic matter into inorganic substances. This process is known as decomposition. The dead remains of plants and animals and the fecal matters of animals constitute detritus, which are decomposed by fragmentation, leaching, catabolism and mineralization. Fragmentation is the process of breaking down the detritus into smaller particles. By the process of leaching water soluble smaller particles percolates down the soil and get precipitated. Microbes like bacteria and fungi degrade detritus into simpler inorganic substances by catabolism. Humification and mineralization occur during decomposition in soil. Humus formation results from humification which is highly resistant to microbial action. Humus undergoes very slow degradation by certain microbes to release inorganic nutrients through a process called mineralization.

15.4 ENERGY FLOW :

The transfer of energy from plants through a series of other organisms constitutes food chains. The term trophic level refers to the parts of a food chain in which a group of organisms secure food in the same general way. Thus, all animals which obtain their energy by directly eating grass, such as grass hoppers and cattle are part of the same trophic level. An assemblage of trophic levels within an ecosystem is known as “trophic structure”. An ecosystem may have 3 to 6 trophic levels through which energy and organic materials pass. A very common example of a short practical food chain would be grass - cow - man. Another example of food chain is grass - grasshopper - frog - snake - eagle.

In aquatic ecosystem, algal phytoplanktons occupy the same trophic level as the grass while the animals like crustaceans, insects and herbivorous fishes occupy the same trophic level as the cattle.

Trophic structures tend to be simple in the polar region as they become more complex on progressing through the temperate regions to the tropics. The concept of food chain, although simple to understand, practically becomes very complicated. This is because the organism are eating a variety of other organisms that may be at different trophic levels. This net - like trophic interrelationship is called a "food web" (Fig. 15.2). For example, a bird may feed on fruits / seeds and also fishes or insects depending on supply position. In fact, food chain is only of academic interest, but the rule of nature is food web. Food web provides ecologic strength and security in complex trophic structures usually seen in stable ecosystems world over.

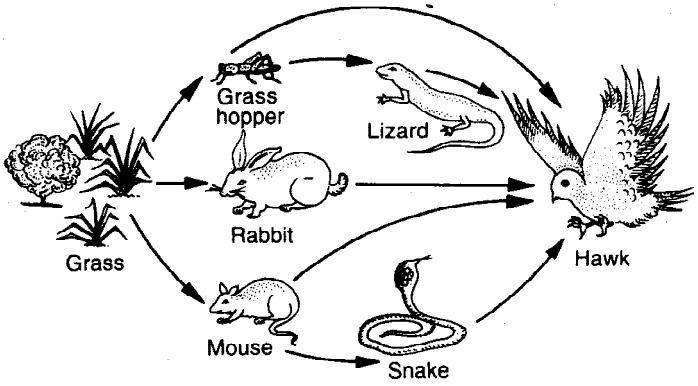

Fig.15.2 : Food web of a grass land ecosystem

Energy Flow efficiency in an ecosystem.

Transfer of energy from one trophic level to another i.e. from producers to primary consumers to secondary consumers to tertiary consumers is governed by "Lindemann's Law of Trophic Efficiency". Lindemann (1942) was an ecologist working on the ecology of a small lake in USA, produced the first measures of trophic efficiency. Lindemann's law of trophic efficiency states that the efficiency of energy transfer from one trophic level to the next is about 10%. This means that only around one-tenths of the net primary productivity of producers passed on to herbivores. Then the same pattern is followed when energy is transterrred to next trophic level the carnivores and one-tenth of the net productivity of first level carnivores goes to the second level carnivores and so on.

It, thus, demonstrates that light or solar energy is converted to carbohydrates, i.e. chemical energy by green plants. If corroborates first law of thermodynamics of conversion of a form energy into another form and it is neither created or nor destroyed. But when the fixed energy in green plants is transferred through different trophic levels is 90 percent lost. Similar to second law of thermodynomics, there is an increased trend towards disorderness which can be expressed by huge loss and only 10 per cent transfer of energy at every successive trophic level.

We should remember, Lindemann's law of trophic efficiency is more of a suggestion than being universal. Recent data have shown that the efficiency may vary from less than 0.1% to 20% depending on the behaviour and physiology of the organisms concerned.

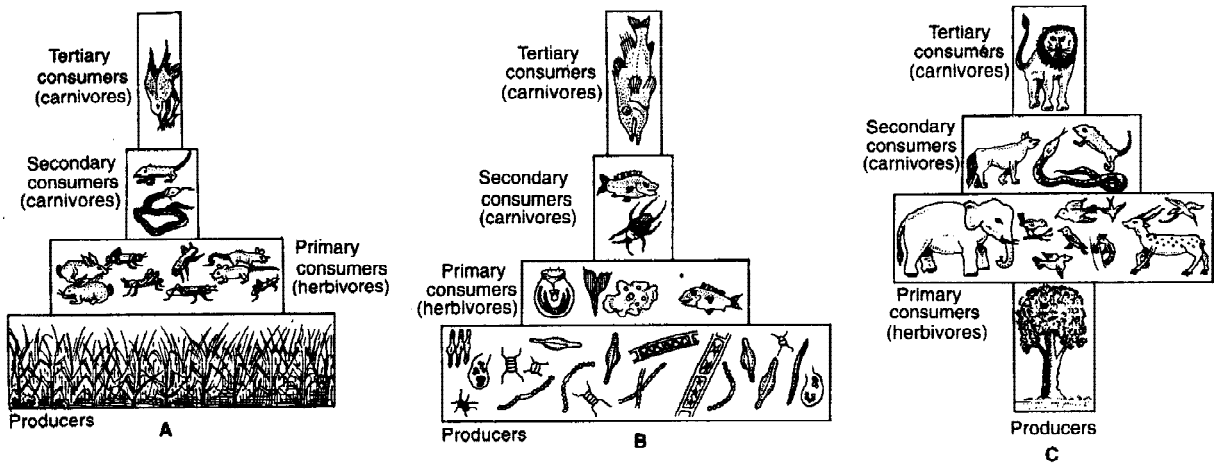

Fig.15.3 : Pyramids of Numbers : A. Grassland; B. Pond; C. Forests

15.5 ECOLOGICAL PYRAMIDS :

Ecologic pyramids are diagrams of data representing the standing crops at each trophic level. The first attempt to provide a quantitative law concerning the trophic level in a community was given by an English ecologist Elton (1927). He coined the term pyramid of numbers in the different stages of a food chain commonly observed in the field. He visualized that the food chain accompained by concommitant energy losses at each step was not only an elegant flowing model but also as a static pyramid. Pyramid of numbers helped portray the relative numbers of organisms at each of the 4 trophic levels - producers, herbivores and two levels of predators in a forest or a lake ecosystem. However, the existence of parasites frequently leads to inverted pyaramids of numbers. Again, inverted pyramids of numbers result when, for instance, a single tree support much smaller organisms (15.3).

Pyramids of biomass (Fig. 15.4) were thought to be more representative than mere numbers in successive trophic stages in a community. As the name suggests, pyramids of

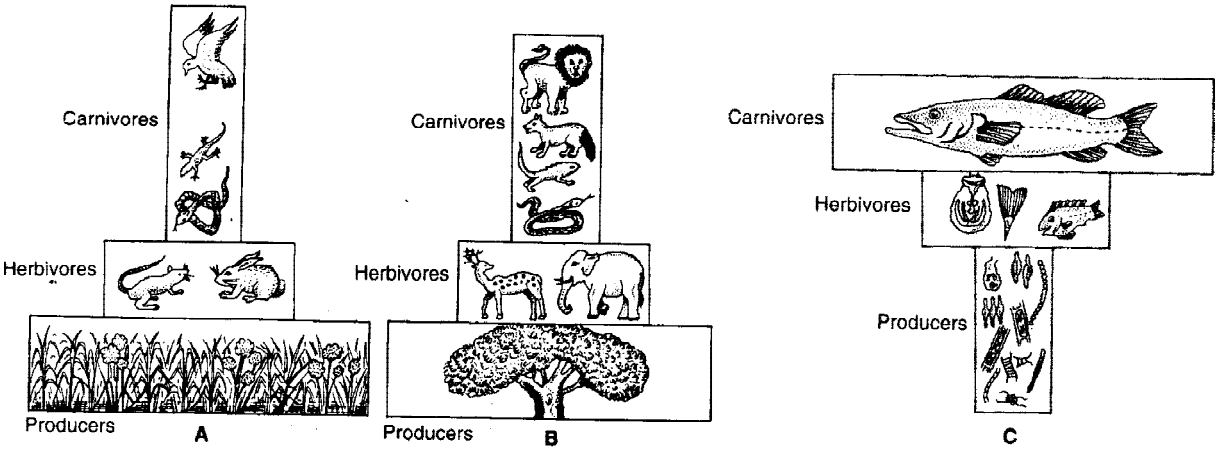

Fig.15.4 : Pyramids of Biomass : A. Grass land; B. Forest; C. Pond

biomass are calculated, by determining for a given unit area, the biomass of producers or the organic weight, the biomass of herbivores, the biomass of first level carnivore and so on.

It is a fact that very few pyramids of biomass have ever been determined, for this is quite a difficult and time-consuming job, although it is likely to remain pyramidal in shape and not inverted. However, in the oceans where the rate of reproduction of the phytoplankton is much greater due to their much smaller size, may support a larger mass of zooplankton at certain time in a year. Here again, the pyramid may appear inverted.

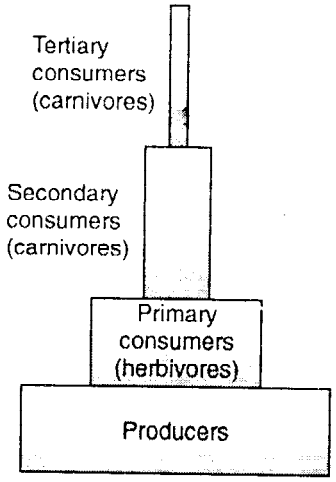

Fig. 15.5 : Pyramid of

There is a thrid sort of ecologic pyramid to indicate the trophic relationships within a community. This is the **pyramid of energy (Fig. 15.5)**. It shows the flow of energy from one trophic level of a community to the next. The units of pyramids of energy are energy / area / time i.e. kJ / ha / yr. It shows the rate at which energy flows up a food web, measured over a stated period of time.

Theoretically, the pyramid of energy can never by inverted. This flows directly as per the first law of thermodynamics or the law of conservation of energy which states that energy can neither be created nor destroyed but is always conserved.

It is worth emphasizing that the pyramid of energy gives complete information only on the flow of energy in an ecosystem. It should not be seen as "better" than pyramids of numbers or biomass, since an ecologist seeks different kinds of information in different ecosystms and therefore, applies different kinds of pyramidal studies depending on data of interest.

15.6 NUTRIENT CYCLING :

Any ecosystem requires a constant supply of nutrients like carbon, nitrogen, phosphorus calcium etc. The amount of nutrients present in the soil or atmosphere at any given point of time is called standing state. The standing state shows a seasonal and climate variation. It also varies in different ecosystems. These nutrients are never lost from the ecosystem. The nutrients move through different components of the ecosystem and are recycled back. This movement of nutrients through different components of ecosystem is called nutrient cycle or biogeochemical cycles. The reservoirs for the gaseous nutrients like Nitrogen and Carbon dioxide is the atmosphere and for sedimentary nutrients like sulphur and phosphorus is Earth's crust. Many environmental factors like soil, moisture, pit, temperature etc. regulate nutrient cycle.

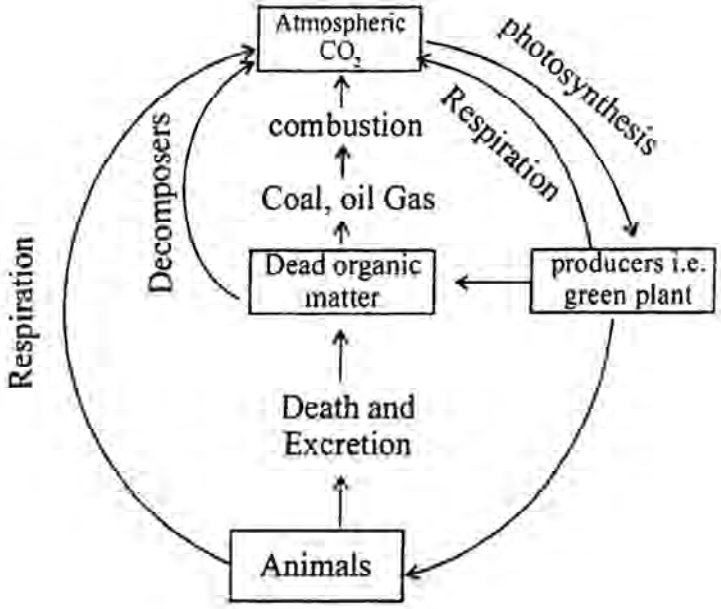

Fig. 15.6 : Carbon cycle

15.6.1 Carbon cycle (Fig. 15.6) :

All organic matters are composed of carbon. In fact about 45% of the dry weight or organisms is carbon. Ocean, atmosphere and fossil fuels are the reservoirs of carbon. In fact ocean contain about 71% of total carbon dissolved in it and also regulate the amount of carbon dioxide in the atmosphere. Carbon cycling occurs through atmosphere, ocean and organisms.

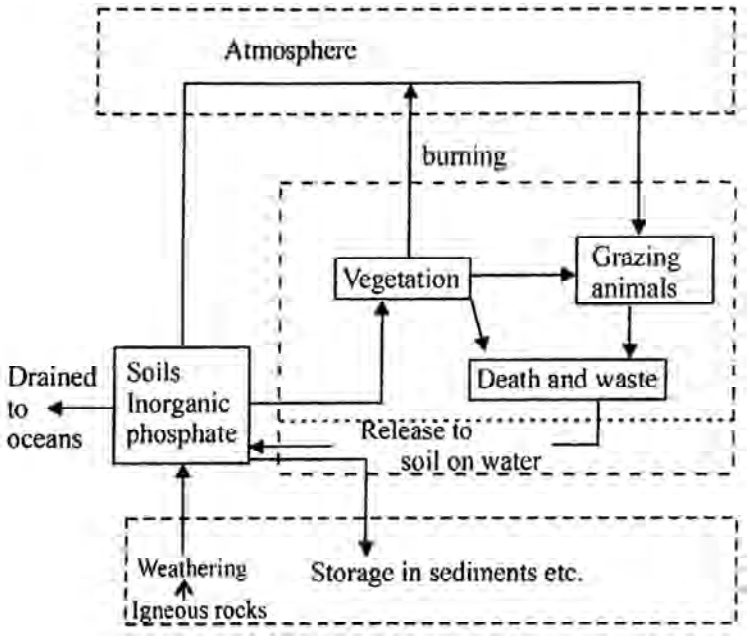

Fig. 15.7 : Model of phsophorus cycle

The atmospheric carbon dioxide is converted to organic matters through photosynthesis. As per estimate 4×10^{13} Kg of carbon is fixed annually through photosynthesis. Most of the CO_2 are returned back to the atmosphere through respiration. The decomposers also return a substantial amount of carbon dioxide to the atmosphere. The other processes through which CO_2 is released to the atmosphere include burning of wood, forest fire, volcanic activities, fossil fuel burning in automobils etc. A very small amount of CO_2 is lost to sediments and thus is kept out of cycling. Human activites like deforestation, urbanisation, fossil fuel burning have increased release of more CO_2 to the atmosphere thun is actually fixed. This causes an imbalance resulting in atmospheric pollution.

15.6.2 Phosphorus cycle (Fig. 15.7) :

The interchange of phosphorus in between different segments of environment is known a phosphorous cycle. Phosphorus is an essential component of an number of biomoleculaes like nucleic acids, rich phosphate compounds etc. it is also a constituent of shells, bones and teeth. Phosphorus occur as sediments in natural rock as calcium flurophosphate or fluorapatite, iron phosphate or aluminium phosphate. Dissolved phosphate is absorbed by plants and remain in the plants as organic phosphates. When herbivores eat plants, phosphorous enter into animal system, moving from one trophic level to another. The animal wastes and dead animal and plant bodies are decomposed by detritus to liberate phosphorous soluble organic phosphorous (phosphates) more from soil to streams, rivers, lakes and oceans where these are deposited in the form of segments. A fraction of the soluble phosphorus solution precolates into deeper soil layers to be deposited as semiments, sedimentary rocks enter the cycle by weathering process.

15.7 ECOLOGICAL SUCCESSION :

Succession is a dynamic characteristic of a community. A community goes on changing until a balance is established between community and environment. The equilibrium state is called climax. Continuous change (succession) and climax are characteristics of a community.

Besides the above characteristics, a community also shows certain features when considered along with other communities. Different communities under similar environmental conditions show ecological equivalents (organisms of different species but showing same type of adaptations due to their exposure to similar environment).

When one community merges into another, the transition zone is called the *ecotone* zone. An ecotone shows edge effect (presence of greater diversity along with members of both communities).

15.7.1 Changes in community :

Plant succession

A community is always dynamic. Environmental conditions influence the living organisms present in a community and the organisms, in turn, also influence their habitat. Thus, the habitat and the components of community keep on changing. *The process of change in the habitat accompanied by the change of vegetation, one after the other is called* plant succession.

The basic concept of plant succession is that the interaction between the habitat and the plants colonising the habitat, result in some changes in the climate. Change in the climate may not be suitable for the existing plant community. Thus, new types of plants that can survive in the changed environment, invade that habitat and replace the old ones. The process of replacement of one community by the other continues till climax or stable community is reached.

Clements (1916) defined succession as *a natural process by which "the same locality becomes successively colonised by different groups of plants"*. Knight (1965) defined succession as *orderly and progressive replacement of one community by another:*

From the above definitions, it is clear that a plant community is not static. It involves a regular, orderly, progressive and predictable change, ultimately, leading to the replacement of one community by another. Odum (1971) considers succession as development of a community.

15.7.2 Types of succession :

Ecological succession is classified on the basis of various *parameters of classification:*

Based on the time of succession :

(i) **Primary succession:** This type of succession starts on a substratum where there is no living being. The living organisms that establish themselves for the first time on such a substratum are called pioneers or primary colonisers. The community thus formed is called primary community.

(ii) **Secondary succession:** Here the succession starts on a substratum where there was living matter previously, but has disappeared due to some natural calamity.

Based on cause :

(i) Autogenic succession: When the organisms of a community modify the environment in such a way that the community itself is replaced by a new one, the succession is called autogenic.

(ii) Aerogenic succession: Here the causative factor behind succession is not the community itself but the physical surrounding.

Based on nutritional status:

Autotrophic succession: In this type, the dominant organisms are the autotrophs; and it starts in an inorganic environment.

Heterotrophic succession: Here the dominant organisms are heterotrophs; and it starts in an organic environment, where the energy and organic matter gradually decreases.

Based on the habitat :

Succession may start in aquatic, xeric, saline, sandy, rocky habitats and -accordingly successions can be classified as hydrosere, xerosere, halosere, psamosere and lithosere respectively.

15.7.3 Process of succession :

The process of succession involves the following steps:

(i) **Nudation:** This is a process by which a bare area is created. The reasons behind the creation of a bare area may be topographic (soil erosion, land slide, volcanic activity); climatic (glaciers, storms, frost, fire etc.), or biotic (anthropogenic activities like industrialization, agriculture, etc.).

(ii) **Invasion:** This is the process in which new species reach and establish themselves in the bare areas. There are three substages of invasion.

(a) Migration: This is the entry of propagules like seeds or spores by agents like air or water into the new area.

(b) Ecesis: Once the propagules reach the new area, they germinate as they are to survive in the new environment. Most plants fail to survive. The process of successfully establishing in the new environment is called ecesis. The first species that establishes itself is called the pioneer.

(c) Aggregation: Increase in the number of individuals after ecesis is called aggregation.

(iii) **Competition and coaction:** There is always a competition among or within the species for nutrition and space. Due to competition, there is coaction in which one influences the other leading to survival of the fittest.

(iv) **Reaction:** The organisms that have established themselves and are competing for nutrition and space influence the environment and bring in a change in the surroundings. Such a process is called reaction. Reaction causes a change or destruction of one community and establishes another. Each such stage that appears due to reaction is called seral stage.

(v) Stabilization or climax: It is the final stage in which the community that has developed is not replaced and is called the climax community.

A climax community, according to Clements (1916) shows three characteristics– unity, stability and phylogenetic relationship.

Climax is usually explained by two concepts:

(a) Monoclimax theory: According to this theory given by Clements (1916), only one community exists in a particular geographical area (climate), other communities present in the area are described by the terms *proclimax, subclimax* or *post-climax* and may collectively be called subor- dinate climaxes.

(b) Polyclimax theory: This concept given by Tansley (1935) states that a number of communities may coexist in a climate.

15.7.4 Hydrosere :

It is a type of plant succession that occurs in aquatic environment in which water disappears and is replaced by land. Hydrosere starts in a new and virgin (no life) area and terminates in a forest. The different stages in a hydrosere are as follows. (Fig. 15.8)

1. **Phytoplankton stage:** This is the first stage of hydrosere in which spores or algae or bacteria enter the body of water. These organisms multiply and flourish. Such organisms called the pioneers. They not only, add organic matter and nutrients due to their life activities, but also settle at the bottom after their death. Thus a layer of mud is formed at the bottom of the pond.

2. **Submerged** stage: The mud formed at the bottom of the pond allows submerged hydrophytes to grow there. Plants like *Utricularia, Ceratophyllum, Myriophyllum, Vallisneria* etc. grow in this stage, when water depth is about 10 feet. When these plants die, they get deposited at the bottom of the pond or lake. This, along with the eroded soil, raises the bottom of the pond or lake, making the water shallow. The shallow water habitat becomes less suitable for the submerged vegetation.

3. **Floating** stage: When the depth of water of the pond or lake becomes 4 to 8 feet, the submerged vegetation gradually disappears giving way to plants like *Nymphaea, Nelumbium, Typha, Pistia, Eichhornia.* Luxuriant growth of these plants prevents light penetration leading to total disappearance of submerged vegetation.

4. **Reed swamp stage:** When the water depth of a pond or lake becomes one to three feet, the habitat is not suitable for floating plants and gives way to amphibious plants. Amphibious plants can very well survive in such conditions as they can successfully

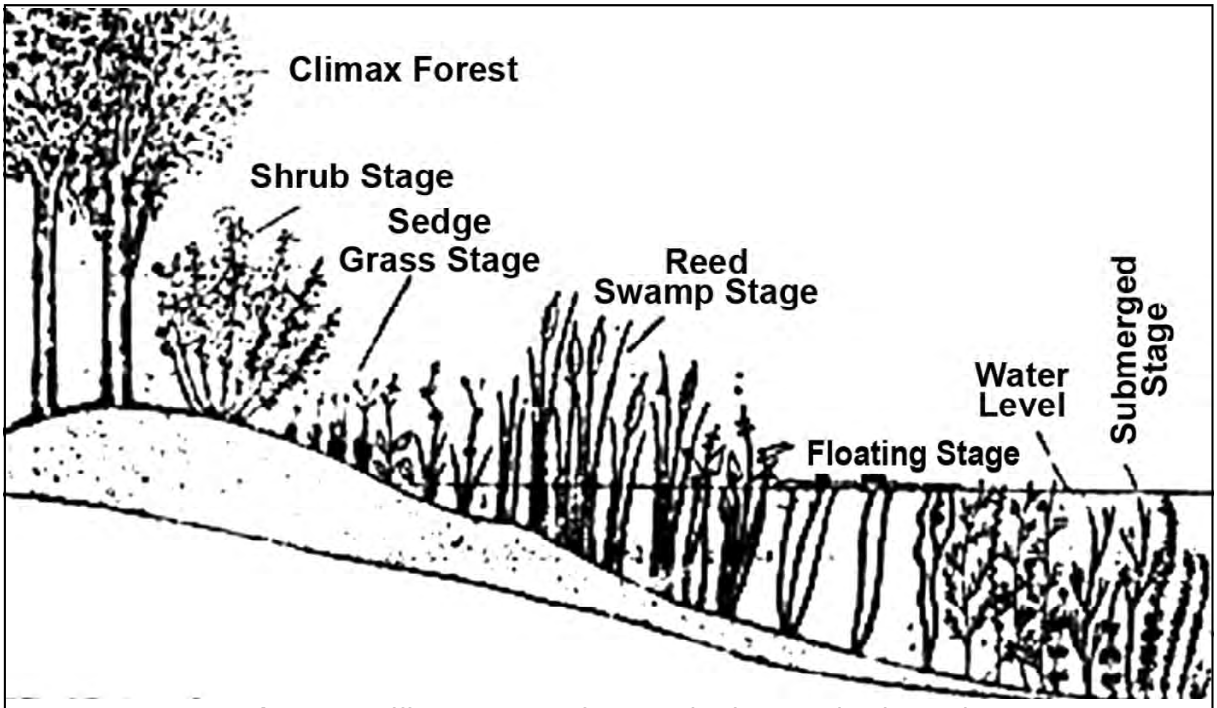

Fig. 15.8 : Different stages of succession in water (Hydrosere)

survive in aquatic and aerial environment. Examples of such plants are *Typha, Polygonum, Marsilea, Sagittaria* etc. Leaves of these plants form a cover over the submerged and floating plants. Such covering cuts off light from submerged and floating plants that are underneath. There is a gradual disappearance of submerged plants (if any) and the floating plants. Plant debris and deposit of soil reduces the depth of water and the habitat becomes less suitable for the reed-swamp plants.

5. **Sedge grass stage:** This stage which is also called sedge marsh or meadow stage. It is the result of the formation of marshy soil due to further decrease in water level after the death of preexisting community. In the beginning, plants belonging to the families Poaceae and Cyperaceae start growing in the habitat. Examples of plants that belong to this stage are *Carex, Juncus, Mentha, Iris* etc. Growth of these plants causes excessive absorption and transpiration. This, in turn, greatly modifies the habitat and makes it unsuitable for existing plants. Accumulation of plant debris and deposit of soil particles along with absorption-transpiration patterns of the plants create an environment totally unsuitable for the growth of hydrophytes. Gradually mesophytes appear and the sedge vegetation gets replaced.

6. **Shrub stage:** Creation of a mesophytic habitat allows shrubs and medium sized trees to grow. These plants not only produce more shade but also absorb and transpire large quantities of water. Shade loving herbs or shrubs may grow under the trees. Examples of plant species belonging to this stage are *Acacia, Cassia, Salix* etc.

7. **Climax stage:** With the continuous creation of humus due to deposit of plant debris and addition of more soil, a suitable habitat is created not only for the growth of microorganisms, but also huge plants. Creation of such a community is called the climax after which further succession is not possible.

The hydroseric succession takes a very long time (thousands of years) but is responsible for the creation of a forest from a pond.

15.7.5 XEROSERE :

Xerosere is defined as *a succession which begins in dry habitat and reaches a climax.* The habitat, besides being deficient of water, is devoid of organic matter.

The most typical xerosere is the lithosere that starts on a bare rock. Different stages involved in a xerosere (lithosere) are described below. (Fig. 15.9)

1. **Crustose lichen stage:** A bare rock is the substratum for the growth of the pioneers. The rock is exposed to high temperature and has very little moisture and organic matter. The only category of plants that can grow here are crustose lichen like *Rhizocarpon, Lecanora etc.* These lichens secrete some acids that cause weathering of the rock. Death of these provide dead organic matter. The soil formed by weathering of the organic matter make the substratum suitable for the growth of foliose lichens.

2. **Foliose lichen stage:** Foliose lichens like *Parmelia, Dermatocarpon* appear on the substratum which gradually flourish and replace the crustose types. Presence of foliose lichens not only adds organic matter but accumulates dust particles and thus helps in the creation of humus. Formation of thin layer of soil creates a new habitat where moss can grow.

3. **Moss stage:** Formation of soil layer makes the substratum suitable for the growth of xerophytic mosses like *Polytrichum, Grimmia* etc. There is a competition between the lichens and the mosses resulting in a further increase in soil layer.

4. **Herb stage:** Extensive growth of mosses cause accumulation of soil, minerals and organic matter. Shallow rooted grasses like *Aristida* and *Poa* appear in the habitat. These annual grasses are gradually

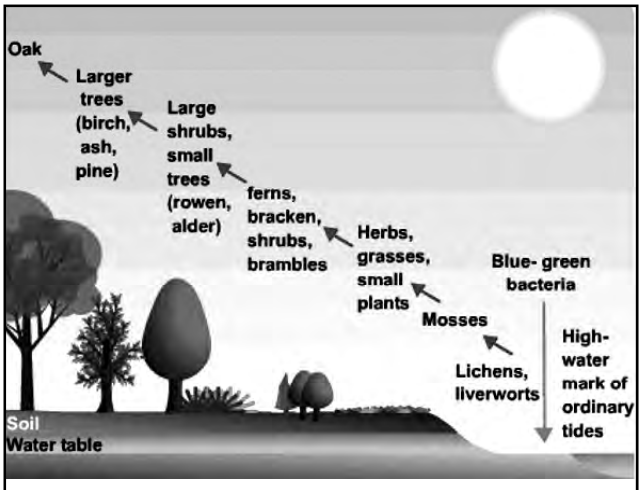

Fig.15.9 : Different stages of succession on bare rock (Xerosere)

replaced by the biennials and perennials. The thickness of soil layer increases and the xeric condition gradually become mesophytic. Such a change encourages the growth of shrubs.

5. **Shrub stage:** Accumulation of soil causes migration of shrub species like *Phytocarpus* and *Rhus* to the area. By overshadowing the herbs, the shrubs add more organic matter and thus add more humus and humidity to the soil. There is an increase in species diversity.

6. **Forest (climax) stage:** The climax vegetation of trees depends on the climate of the region. If the climate is dry, trees like *Acacia* grow. In relatively moist and wet climate mesophytic trees grow and a dense climax forest is formed.

Along with the changes in the plant life in a xerosere (as also in a hydrosere), there is also a change in the animal life. The colonization starts with ants and spiders and goes up to a variety of arthropods, birds and mammals by the time the climax community is formed.

15.8 ECOSYSTEM SERVICES :

Natural ecosystems provide multitude benefits to humankind which are collectively known as ecosystem services. Ecosystem services were popularised and their definitions were formalised by United Nation 2005 Millennium Ecosystem Assessment (MEA). MEA is a four year long study involving more than 1,300 scientists worldwide. This MEA report 2005 defines Ecosystem services as **benefit people obtain from ecosystem** and distinguished four categories of ecosystem services. The following list represents samples of each.

A. Provisioning services : The products obtained from ecosystem, including genetic, genetic resources, food and fibre and fresh water.

B. Regulating Services : The benefits obtained from regulation of ecosystem processes for example

- Carbon sequestration and climate regulation
- Waste decomposition and detoxification
- Purification of water and air.
- Crop pollination
- Pest and disease control

C. Supporting services : This includes, ecosystem services that are encessary for the production of all other ecosystem services like :

- Nutrient dispersal and cycling
- Seed dispersal
- Primary production

D. Cultural Services : This includes, non-material benefis people obtain from ecosystem through :

- Cultural, intellectual and spiritual inspiration
- Recreational experiences (including ecotourism)
- Scientific discovery.

Recently the term ‘‘Supporting services’’ has been replaced by ‘‘Habitat Services’’.

The study of ecosystem services include the following steps:

1. Identification of ecosystem Services providers (ESPs)
2. Determination of community structural aspects that influence house ESPs, function.
3. Assessment of key environmental (abiotic) factors influencing the provision of services.
4. Measurement of spatial and temporal scales ESPs and their services.

Some examples of ecosystem services are given here.

15.8.1 Carbon Fixation :

It is the process of long term storage of atmospheric carbon dioxide or other forms of carbon to either mitigate or defer global warming and avoid dangerous climate change. It is a way of slowing down atmospheric and marine accumulation of greenhouse gases. Carbon dioxide is naturally captured from the atmosphere through bioligical, chemical and physical processes. Artificial processes like use of saline aquifiers, reservoirs, ocean water, aging oil fields or other carbon sinks are also in use. Bioligically carbon fixation is done by reforestation, urban forestry, wetland restoration and agriculture. In ocean, carbon fixation is mostly done by sea weed cultivation.

15.8.2 Pollination :

Pollination of crop plants by insects such as bees is required for about 15-30% of crop plants. In USA, majority of large scale farmers import non-native honey bees to provide service. A study reveals that in California’s agricultural region, wild bees alone could provide partial or complete pollination services or enhance the services provided by honey bees through behavioural interactions. However, intensified agricultural practices can quickly erode such pollination process through less of species and those remaining fail to compensate such differences. Research also shows that wild insects or non-native insects within 1-2 Km radius of farm areas ensure proper pollination thus, a potential insurance policy for the farmers can be raised through this service.

13.8.3 Oxygen Release :

One of the ecosystem services provided by different types of vegetation is the net release of oxygen which is used by other life forms for respiration. Oxygen is produced by trees as well as planktons and weeds in ocean. In fact the marine ecosystem particularly planktons produce more than 50% of the available oxygen. The plants, absorb CO_2 for photosynthesis and release oxygen thus acting as a filter or purifier of air we breath. The net production by oxygen by healthy tree depends on the species, size, (biomass), health and location. On existing literature and survey report, it is concluded that two healthy trees of large size produce enough oxygen as is required by one individuals in one year.

_____

SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Fill in the blanks with correct answers from the choices given in the brackets of each bit.

(i) Forests represent _____ ecosystem.
(Aquatic, terrestrial, estuarian, grassland)

(ii) Decomposers are generally _____.
(green plants, microorganisma, phytoplanktons, insects)

(iii) Man is a _____.
(herbivore, carnivore, Omnivore, producer)

(iv) Ecologic efficiency is less than _____ percent.
(1, 10, 5, 0.5)

(v) The efficiency for energy transfer from one trophic level to another is nearly ____ per cent.
(1, 10, 5, 20)

(vi) Pyramid of _____ can not be inverted.
(Energy, biomass, number, ecosystems)

(vii) Succession that starts at _____ habit is called hydrosere.
(sandy, rocky, aquatic, xeric)

(viii) Succession that starts at sandy habitat is called _____.
(Halosere, lithosere, psamosere hydrocere)

2. Answer in one word only :

(i) What can be called primary consumers?

(ii) What is called to decomposers living on dead, decaying substratum?

(iii) Through which process is energy lost form living organisms?

(iv) Which type of ecological pyramid is never inverted?

(v) What is called to the process of creation of bare area ?

(vi) What is the form of climax forest called ?

3. Correct the sentences in each bit without changing the underlined word/words :

(i) <u>Pond</u> represents a forest ecosystem.

(ii) <u>Ecosystem</u> has structural and energy aspects.

(iii) <u>Biogeochemical</u> cycle may be otherwise called energy cycle.

(iv) All heterotrophs are capable of Photosynthesis.

(v) Animals are responsible for utilizing dead, decaying substances, thereby cycling of materials becomes feasible.

(v) Flow of nutrients is unidirectional.

(vii) Net primary productivity is calculated by taking into consideration gross primary productivity and photosynthesis.

(viii) Food chain shows a complicated net like interrelationship in trophic levels.

(ix) Pyramid of biomass takes into consideration the number of organisms in each trophic level.

(x) Pyramid of energy is always inverted.

(xi) A stable community shows high species dominance.

(xii) Primary succession starts where there was living matter previously.

4. Fill in the blanks :

(i) Green plants are called _____ as they fix Co_2.

(ii) In forest ecosystem, pyramid of number is _____ type.

(iii) Common decomposers form _____ ecosystem.

(iv) Secondary products are called _____ in a food chain.

(v) The second trophic level in pond is _____.

GROUP - B
(Short Answer-type Questions)

1. Write notes on the following with at least 2 valid points

(i) Ecosystem (ii) Phytoplankton

(iii) Ecesis (iv) Nudation

(v) Ecological pyramid (vi) Food chain

(vii) Food web (viii) Climax forest

(ix) Plant succssion (x) Species diversity

2. Differentiate the following with at least 3 valid points

(i) Parasite and saprophyte

(ii) Producer and consumer

(iii) Food chain and food web

(iv) Herbivore and predator

(v) Primary Succession and secondary succession

(vi) Primary productivity and Net productivity

(vii) Pyramid of biomass and pyramid of numbers

(viii) Abiotic component and Biotic components

(ix) Species diversity and species dominance

GROUP - C
(Long Answer-type Questions)

1. What is ecosystem ? Describe the different component of ecosystem.
2. Give an account of energy flow in an ecosystem.
3. Write a brief note about ecological services.

❑ ❑ ❑

BIODIVERSITY AND ITS CONSERVATION

CHAPTER 16

16.1 CONCEPT OF BIODIVERSITY :

Diversity in simplest term refers to variation. There is a proverbial saying that diversity (variation) adds spice to life. If there were no variation, life would be monotonous. Why just to our life only, the concept applies to each and every visible matter around us. It applies to physical matter and biological organisms as well. The diversity among biological organisms has been referred to as **biological diversity** or **biodiversity**. We need to study the minute details of this biodiversity since it affects all aspects of our as well as other organisms' lives. The diversity occurs at all possible levels, such as **species, populations** and **communities.** Biodiversity is affected by the physical environment in a bigger way and changes with time. This change leads to the appearance of better adapted and more diverse organisms. This change is positive and indefinite and has been referred to as **organic evolution**. However, the indiscriminate action of human has caused irreparable damage to the physical environment and the existing diverse groups of organisms. The physical environment changes adversely and this adverse environment is the main causative factor for the extinction of many species of organisms. We have studied many episodes of mass extinction in the history of biological organisms, out of which the mass extinction of dinosaurs is one.

The term biodiversity was first used by **Lovejoy (1980)** to refer to the number of species of a region. **Norse** and **Mc Manus** in the same year added two more, such as **genetic** and **ecological diversities** to the one described by Lovejoy. They in 1986 explained biodiversity at three levels, such as genetic (diversity within species), organismal (diversity among species) and ecological (diversity among communities).

Hence, biodiversity is natural variations at all levels, such as **genetic diversity** (diversity within species), **organismal diversity** (diversity among species or populations), and **ecological diversity** (diversity among communities). This categorisation is called the hierarchy of biodiversity

16.1.1 Genetic Diversity :

Genetic diversity fundamentally means variation in the genetic material among individuals of a species. The genetic variation may be in the nucleotides, genes, entire genome or chromosomes. This diversity arises through genetic recombination during sexual reproduction and mutation. It leads to natural selection and the origin of new species. Thus, genetic diversity increases adaptability and is essential for the evolution of new species.

16.1.2 Organismal (Species) Diversity :

It refers to the number of species in a population in a given space and time. It is measured by species richness (the number of species in a given area at a particular point of time), species composition (list of species) and relative abundance (relative number of individuals of different species). The International Union for Conservation of Nature and Natural Resaurces (IUCN) recognises three types of species diversity : (1) **alpha diversity**, (2) **beta diversity,** and (3) **gamma diversity**. Alpha diversity refers to the veriety of species within a community. It is also referred to as the **species richness**. Beta diversity is the diversity of species among communities, while gamma diversity is the diversity of species across a wide geographical range.

16.1.3 Ecological Diversity :

Ecological (ecosystem) diversity explains about the different types of ecosystems present in the biosphere. The community composition (assemblage of several interacting populations in a given space at a particular point of time) is affected directly by the environment. A change in the ambient environment induces a change in the ecosystem composition and its functioning. It is also, referred to as **community diversity**.

16.2 PATTERNS OF BIODIVERSITY :

Biodiversity is not evenly distributed throughout the world. Some regions are very rich in diversity and some are poor. Several geographic and climatic factors are responsible for this uneven distribution of diversity. Both latitude and altitude are two important geographic factors influencing both climatic conditions as well as species distribution.Scientists have observed that species diversity decreases as one moves from the Equator towards the pole, either north or south. There is no doubt that the tropics (latitudinal range of 23.5^0N to 23.5^0S) are very rich in species diversity.This pattern can be seen in terrestrial, marine and freshwater habitats. The gradient is steep in northern hemisphere and less strong in the sourthern hemisphere. Tropical rain forests are amazing in the sense that they contain 70% of species of the world only in 7% of the Earth's land surface.Many of the tropical rain forest areas also show high level of endemism..The tropical rain forests have drawn world-wide attention because of their high biodversity and rapid destruction of habitats. The factors making tropical rain forests rich in biodiversity are (i) tropical latitudes have remained undisturbed for million of years allowing speciation, (ii) tropical environments are relatively constant throuhout the year which promotes niche specialisation and greater diversity and (iii) high productivity leading to greater diversity. It is also found that species diversity increases with area (species-area curves), how it peaks in areas with intermediate productivity or intermediate rates of disturbance.The more variable the habitat, the greater the species diversity within it. This pattern was offered as one of the reasons why there are more species in a bigger area (more area covers a greater variety of habitat).

India is situated north of the equator between 8°4' to 37°6' north latitude and has 2.4 per cent of the total surface area of the world. As compared to the land area it is is bestowed

with an incredible species diversity. The country harbours about 7% of the world's species of animals and plants Around 40,000 species of plants and 90,000 species of animals are spread over an area of 329 million hectares. Around 140 genera of plants, 138 species of amphibians, 214 species of reptiles, 176 species of birds and 44 species of mammals are endemic to the country. It is due to this species diversity that India is crowned as one among the **seventeen mega biodiversity countries** of the world (As per the Conservation General Report 1998). Three large **biodiversity hotspots** are in India: one being the **Eastern Himalayas** and the other, the other two are **Western Ghats** and **Indo-Burma region**.

17.2 IMPORTANCE OF BIODIVERSITY :

Biodiversity has been playing an important role in maintaining the well-being of human society. Scientists have classified the values of diversity as: **(1) direct**, and **(2) indirect**. This classification may also be viewed as ecosystem goods and ecosystem services and values, respectively. Ecosystem goods refer to natural products harvested from ecosystems, directly used by human. These include food, clothing, shelter, medicines and biological resources directly used in industry. Ecosystem services refer to different ecosystem processes, which help sustain both the ecosystems and human life.

(a) Direct value:

(i) Food: The diverse groups of plants and animals meet all the food requirement of human in the form of cereals, pulses, vegetables, fruits, milk, meat, fish, egg and many others. Besides food additives, such as colours, flavouring substances; and spices and condiments are also harvested from plants and animals. Natural drinks and beverages are extracted directly from many plants or are prepared by using parts of plants. The best examples are tea and coffee. Drinks like wine and beer are prepared by fermentation using microorganisms. Some other fermented products used as food are soya sauce, cheese, yoghurt, sauerkraut. Cooking media, such as vegetable oils and vegetable ghee are extracted from plant seeds.

(ii) Clothing: People used natural fabric, made of cotton and natural silk before the invention of polymers. Cotton is a plant product, while silk is harvested from silk moth. This apart, many household articles are made out of jute, again a plant product.

(iii) Shelter: Before the scientific innovations relating to the construction of houses, people relied completely on plants or thir products for the building of shelters. However, presently, knowledge from building research has substituted the conventional building materials by industrially manufactured products. Nevertheless, many such materials are still products of plants as on today. Whatever the case may be, the conventional building materials have no substitute.

(iv) **Medicines:** Ancient people used several plant and animal products of medicinal importance for their requirements. One typical example is that of **Quinine**, the antimalarial drug, quinine obtained from the bark of *Cinchona* plant can cure the dangerous desease. This is used since time immemorial. Now more than 50% of the medicines are manufactured using microbial organisms, plant and animal extracts. Many conventional drugs and specialized drugs, such as **anti-cancer drugs** and drugs used to treat cardiac diseases and neural disorders have been formulated from plant and animal extracts. Two examples would explain this context more elaborately. **Anticoagulants**, extracted from the blood sucking animals are used as active principles in the manufacture of antihamorrhagic drugs. Secondly, **snake venom** and **toxins** produced by many animals are the source of many drugs for **neural** and **muscular disorders**. Antibiotics, such as **penicillin, tetracycline**, **streptomycin**, etc are extracted from microorganisms. Beetles, millipedes, snails and ants produce many **biocidal compounds**, which are used in manufacturing many antibiotics.

(v) **Industrial Products:** A wide variety of industrial products are directly made out of biological resources. These are **timber, fuel wood, fibers, dyes, resins, gums, adhesives, natural rubber, oils, waxes and perfumes**. Paper, a daily use item of the common man is manufactured from wood pulp. Plants provide raw materials for making wooden furniture, cane furniture, mats and baskets. Natural rubber is made from the alkaloid secretions of many species of plants. Animal skin is used in making leather. Everybody has an increasing fascination for products made out of leather.

(b) Indirect Value :

(i) **Biological Control:** The concept of parasitism and predation is used in the biological control of insect pests and weeds. The biological control programmes have become successful against around 300 species of insect pests and 200 species of weeds. Some microorganisms are genetically modified to provide a beneficial service to mankind. These microorganisms are used in the manufacture of **antibiotics, oral contraceptives** and **several medicines and management of pests, recovery of metals (bioleaching), increasing soil fertility, generating biofuel, monitoring environmental pollution, cleaning oil spill,** and **treatment of sewage** and **solid waste**.

(ii) **Environmental Modulation:** The flora and fauna of an area influence and modulate the environment directly or indirectly. These influence the hydrology and soil nutrients, prevent soil erosion, drought and flood and play many other beneficial roles. Some animals and plants are very good **ecosystem engineers**.

The beaver (a mammal) is considered as a very good ecosystem engineer. It creates dams by using logs in river channels, modifies nutrient cycling and influences decomposition dynamics. Consequently it influences the plant and animal communities and the entire biodiversity of the watershed area in question. These species are known as **key stone species**. Extinction of key stone species reduces the relative abundance of species in the community.

(iii) Ecosystem Functions and Services: All organisms in an ecosystem interact with each other and with the environment they live in a concerted manner and influence its functions. They recycle the life sustaining elements so that there is air to breathe, water to drink and nutrient-rich soil to grow crops. The consequence is that these elements are generated as much as these are used so that a **state of equilibrium** or **homeostasis** is maintained. Let us use the natural resource for our benefit and preserve an equal share for our posterity. This concept has been referred to as **sustainable development**. Overexploitation of the biological resource and other natural resource will derail the normal ecological functions, which will affect the human well being in a bigger way. For example, the decrease in flora of an area increases the carbon dioxide content in the atmosphere, which elevates the temperature of that area. The decline in the microflora population prevents the recycling between the complex organic matter and simple inorganic elements and compounds. Another simple example will explain the context. Carnivore and herbivore populations in an ecosystem ought to be maintained in equilibrium for its optimum functioning. An increase in the carnivore population will decrease the herbivore population by predation, which will increase the vegetation. Conversely, a decrease in the carnivore population will increase the herbivore population, which in turn, decreases the vegetation by overgrazing. The consequence is a destabilization of the balance of the ecosystem.

(iv) Ecotourism: The diverse biological resource of a country motivates people from around the world to undertake tours to enjoy the diverse wild life and charismatic landscape. The country earns quite a large sum of foreign currency as revenue. Tourists use such tours as **recreational activity.**

16.4 LOSS OF BIODIVERSITY :

Species extinction is the most common cause of loss of biodiversity. Like every other thing, the physical environment changes with time. Species extinction is due to loss of adaptability with the changing physical environment. Therefore, extinction is a natural phenomenon. **We have known five episodes of mass extinction of species in the history of biological organisms.** However, human intervention is the immediate cause of sixth extinction of many species, which would not have otherwise happened. IUCN estimates that 12259 species have

become extinct since the origin of life on earth. **The factors, which induce species extinction, are termed as drivers**. The drivers are of two classes: **direct** and **indirect**.

Direct drivers influence ecosystem processes, which bring about mass extinction of species. These include: (1) land use change (habitat destruction and fragmentation); (2) climate change; (3) overexploitation of natural resources; (4) environmental pollution; and (5) invasion of exotic species. Indirect drivers change one or more direct drivers. These include: (1) population growth; and (2) income and lifestyle of the people.

(a) **Habitat Destruction and Fragmentation:** The destruction of habitats is the primary reason for the loss of biodiversity in terrestrial and coastal ecosystems. Conversion of forest land for agriculture, developmental projects, mining operations, etc leads to the destruction of natural habitats of organisms. Natural habitat loss is further due to filling of wetlands,ploughing of grassland, etc. These changes kill or force out many species of plants, animals and microorganisms of their habitat. Birds, mammals and plants are most affected due to habitat loss.

Developmental projects, such as upcoming railway track or a highway passing across a natural habitat, fragments it into two or more smaller habitats. Smaller habitats are more susceptible to ecological succession. Over the years, a new ecosystem comes up in place of the old one. The consequence is the total extermination of the existing species. Secondly, when a large population fragments into smaller ones, there is more inbreeding and there is an inbreeding pressure on the gene pool. The frequenting of elephants into human habitats in recent years is the concequence of habital fragmentation resulting from developmental prejects.

(b) **Introduction of Alien Invasive Species:** A plant or animal species introduced to a country intentionally or unintentionally, may at times become invasive. This alien species overpower the native flora or fauna and become wild. For example, *Parthenium* entered India with imported food grains in the mid-1950s. Because of favourable environmental conditions in our country, this has become wide spread, and now it covers more than 25 million hectares of farm land. Similarly, *Lanata* and *Eicchornia* introduced as ornamental plants have now become wild in India because of invasiveness. It may grow at the cost of the native species and consequently may be that the native species may be eliminated.

(c) **Climate Change:** The climate of the globe changes erratically by anthropogenic activities. Global warming due to accumulation of greenhouse gases and gradual thinning of the stratospheric ozone are two major causes of erratic climate changes. The polar ice caps are slowly melting with a rise in the sea level. This may submerge some low lying coastal habitats in the near future. Secondly, the animals and plants fail to adapt themselves to the very fast changing climate. The consequence is their elimination.

(d) **Overexploitation of Natural Resources:** Human population growth has changed the land use pattern to meet the ever growing need. Natural habitats of native animals and plants are being used for construction of new housing complexes in urban areas, expanding agricultural practice and construction of industrial establishments. The consequence is habitat loss resulting in extinction of species. This situation is so menacing that wild animals are entering into urban areas in search of food and water. Moreover, there is an overexploitation of forest resources, which has squeezed the natural habitats of many wild animals. In addition, indiscriminate hunting of wild animals has put their status as either endangered or critically endangered.

(e) **Environmental Pollution:** Environmental pollution is a secondary but yet a powerful cause of loss of biodiversity. Pollution may reduce and eliminate populations of sensitive species. Nonbiodegradable pesticides cause serious threats to some species, DDT affected decline in fish-eating birds and falcons by interfering their reproduction. In South Asia, population of vultures in the genus *Gyps* declined by more than 95% due to the toxic effect of Diclofenac.In nature vultures eat up the dead animals This anti-inflammatory drug passed on from these dead bodies to vultures and cause renal failure and death of the vultures.

(f) **Population Growth:** Human population has grown by all leaps and bounds over the last century and still continues to grow. It is the solitary primary cause of the loss of biodiversity. All evil deeds, described have given rise to secondary causes of loss of biodiversity. If this trend continues, most of the species will face extinction in a few years now. It is therefore, imperative to have effective laws in all the countries to put a check on of human population growth. Lavishness of people without caring for the natural resources is most disatrous.

16.5 EXTINCTION OF SPECIES :

Extinction means a species which no longer exists and its individual remains are available only in fossil form. Extinction is the gravest aspect of the biodiversity crisis: it is irreversible. While extinction is a natural process, human impacts have elevated the rate of extinction by at least a thousand times the natural rate. Species have disappeared and new ones have evolved over million of years in the history of earth. Naturally extinction goes on with change in environmental conditions.This process is a slow one.

It is learnt from geological history of earth that large number of species became extinct due to catastrophes. In the history of our planet, five big **mass extinctions** occurred, the most serious extinction took place in end-Cretaceous in which dinosaurs and many marine species were wiped out. These extinctions are gone and still we are bestowed with biodiversity. The serious concern is the present **anthropogenic extinction**, the extinction caused due to human

activities. The previous mass extinction took million yeras where as the anthropogenic extinction is only 200 years phenomenon. This man-made extinction results in severe depletion of biodiversity and the rate of this **Sixth Extinction** (man-made) is several hundred fold higher than the previous ones. The IUCN (International Union for Conservation of Nature and Natural Resources) Red List 2004 has recorded a total loss of 784 species in the last 500 years. These include 733 animals (mostly vertebrates and molluscs), 110 plants and one red alga. In recent years extinction of dodo in Mauritius, quagga in Africa are notable.The species that became extinct in 2003 was the plant *Nesiota elliptica*, St. Helena Olive a small tree in Saint Helena Island, in the South Atlantic Ocean. The IUCN red list of threatened species founded in 1964, is the worlds most comprehensive inventory of global conservation status of biological species.

Red data book : Red data book is the source published by the IUCN which provides important information about endangered species. More than 1000 creatures are threatened with extincttion, some very soon, some within a decade or so.

Besides many species being extinct, more than 15,500 species are facing the threat of extinction. The **IUCN prepares the Red Lis**t of threatened organisms. The Red List is a catalogue of taxa that are facing extinction.It provides information about the taxa need conservation and seroius attention of public and policy makers. The World Conservation Union has recognised several categories in the Red List. Some of these are

Extinct : A taxon is Extinct when there is no reasonable doubt that the last individual has died. Example- Indian Cheetah.

Critically Endangered : A species is critically endangered when is facing an extremely high rate of extinction in the wild in the immediate future. Example- One horned Rhinoceros.

Endangered : A species is endangered but not critically but is facing a very high risk of extinction in the wild in the near future. Example- Giant Panda, Polar Bear.

Vulnerable : A taxon is vulnerable when it is facing a high risk of extinction in the wild in the medium-term future. Example- Sparrow.

16.6 HOT SPOTS OF BIODIVERSITY :

The British biologist Norman Myers coined the term “biodiversity hotspot” in 1988. A biodiversity hotspot is a biogeographic region with a significant reservoir of biodiversity that is under threat from humans due to habitat loss. According to Myers, to qualify as a biodiversity hotspot, a region must meet two strict criteria:

(a) it must contain at least 0.5% or 1,500 of the world’s 3,00,000 species of vascular plants as endemics, and

(b) should have lost at least 70% of its primary vegetation.

Around the world, at least 34 areas qualify under this definition. These sites support nearly 75% of the world's most threatened mammals, birds and amphibians, and about 50% of

all plant and 42% land vertebrates (Conservation International, 2005). As many as 16 hotspots are in the tropics and about 20% of the human population live in the hot spots. Tropical Andes hot spot has 20,000 endemic plants and 1567 vertebrates and it is at the top of the list. Four regions that satisfy the criteria of hot spots comes under India and these are:The Western Ghats and Sri Lanka, The Eastern Himalayas, Indo-Burma (North-Eastern India south of Brahmaputra river)and Sundaland (Nicobar Islands).

The Western Ghats are a chain of hills that run along the western edge of peninsular India. These regions have moist deciduous forest and rain forest. The region shows high species diversity as well as high levels of endemism. Nearly 77% of the amphibians and 62% of the reptile species found here are found nowhere else. There are over 6000 vascular plants belonging to over 2500 genera in this hotspot, of which over 3000 are endemic. Much of the world's spices such as black pepper and cardamom have their origins in the Western Ghats. The region also harbors over 450 bird species, about 140 mammalian species, 260 reptiles and 175 amphibians. Over 60% of the reptiles and amphibians are completely endemic to the hotspot.

The Eastern Himalayan hotspot has nearly 163 globally threatened species including the One-horned Rhinoceros, the Wild Asian Water buffalo and in all 45 mammals, 50 birds, 17 reptiles, 12 amphibians, 3 invertebrate and 36 plant species. Hot spots should be very much protected for biodiversity conservation.

16.7 BIODIVERSITY CONSERVATION :

Biodiversity is everything to us and it is our responsibility to conserve for future generations. The **International Union for Conservation of Nature and Natural Resources**, also identified as **World Conservation Union (IUCN), World Wide Fund for Nature (WWF), Food and Agricultural Organization (FAO)** and **United Nations Educational Scientific and Cultural Organization (UNESCO)** evolved the **world conservation strategy** in 1980 for the conservation and sustainable use of biological resource. Two major types of conservation strategy of biological diversity were framed: ***in situ*** (**on site**) and ***ex situ*** (**off site**). *In situ* conservation refers to protecting the plants, animals and microorganisms in their natural habitat including its lithosphere, hydrosphere and atmosphere. On the other hand, *ex situ* conservation refers to conservation of biodiversity outside the boundaries of their natural habitats. The *in situ* practice is, by far, the most effective method of protecting and propagating the species and improving the quality of their habitats. IUCN has designated six different categories of land as protected areas for the conservation of all populations of plants and animals and all the ecosystems operating there. The institution has developed guidelines for their management. **A protected area, as defined by the IUCN, is an area of land or sea, especially dedicated for the protection and maintenance of biological diversity and associated cultural resources and managed through legal and other effective means.** Some protected areas discussed here are **National Parks**, **Wildlife Sanctuaries** and **Biosphere Reserves**.

(a) National Park :

A national park comes under **category II** of the protected areas, specified by IUCN. It is an area dedicated to conserve the environment, natural and historical objects and the wildlife and to provide enjoyment in such a manner and by such a means that will leave them unimpaired for the enjoyment of the future generations. A national park is established by a special statute by the central government and therefore, it has a permanent status. The legal implication comes into force from the date of its notification. In a national park all privileges and rights become null and void and all forestry operations and other uses, such as grazing of domestic animals are prohibited. However, the general public may enter into it with a due legal permission from an appropriate authority for the purpose of observation and study. IUCN (1975) has adopted the following definition for a national park:

A national park is relatively a large area

1. Where the operating ecosystems are not materially altered by human exploitation and occupation, where plant and animal species, their habitats, and geomorphological sites are of scientific, educational and recreative interests or which contains natural landscapes of great beauty and aesthetic value.
2. Where the highest competent authority has taken steps to prevent or eliminate exploitation or occupation in the entire area and to enforce conservation measures effectively in respect of ecological, geomorphological and aesthetic features.
3. Where visitors are allowed to enter under special conditions for inspirational, cultural and recreative purposes.

Hailey's National Park in Uttarakhand was the first national park in India established in 1935. It is renamed as Corbett National Park. There are 103 national parks in India (as on 2015) out of which, in the state of Odisha, Bhitarkanika in Kendrapara district is a National Park, the other one Similipal in Mayurbhanj district, is proposed to be a National Park.

(b) Wildlife Sanctuary :

A wildlife sanctuary comes under the **Category IV** of the protected areas, specified by IUCN. It is created by a gazette notification by the State Forest Department, where vulnerable, endangered and critically endangered wild animals are protected. It can, therefore, be abolished in a similar manner. Human intervention, poaching and grazing are strictly prohibited by law. There are **537 wildlife sanctuaries in India** (as on 2015), covering an area of 1,22,867.34 Km^2, which is 3.74% of the total geographical area of the country. **The state of Odisha has a network of 19 wildlife sanctuaries**.

WILDLIFE SANCTUARIES OF ODISHA :

Sl.No.	Name	District	Protected wildlife
1.	Sunabeda	Nuapada	Tiger, Chital, Gaur, Hyena, Barking deer, Leopard, and Sambhar
2.	Chilika (Nalabana)	Khurda, Puri and Ganjam	White-bellied sea eagle, Jacana, Purple moorhen, Greylag geese, Herons and Flemingos
3.	Nandankanan	Cuttack	Zoological park harbouring a variety of animals in enclosures
4.	Satkosia Gorge, Tikarpada	Angul, Cuttack and Boudh	Gharial, Mugger and Fresh water turtle
5.	Bhitarkanika	Kendrapada	Estuarine crocodile, King cobra, Indian rock python and Water monitor lizard
6.	Simlipal	Mayurbhanj	Tiger, Elephant, Sloth bear, Leopard, Deer, Gaur Bison, Langur and Mugger.
7.	Karlapat	Kalahandi	Tiger, Sambhar Leopard, Gaur and Chital
8.	Lakhari Valley	Gajapati	Tiger, Hyena, Elephant, Deer, Sambhar and a variety of birds and reptiles.
9.	Kuldiha	Balasore	Tiger, Leopard, Elephant, Gaur, Sambar, Giant squirrel and a number of bird species
10.	Khalasuni	Sambalpur	Tiger, Elephant, Bison, Deer, Sambhar, Spotted deer, Mouse deer, Barking deer and Wild dogs
11.	Kotagarh	Kandhamal	Tiger, Elephant, Gaur, Sambhar, Spotted deer, Peafowl, Red jungle fowl
12.	Hadagarh	Keonjhar and Mayurbhanj	Tiger, Leopard, Fishing cat, Jungle cat, Hyena, Elephant, Langur, Pangolin
13.	Debrigarh	Sambalpur	Chousingha, Tiger, Leopard, Gaur, Sambar, Spotted deer, Sloth bear, Resident and migratory birds
14.	Balukhand - Konark	Puri	Blackbuck, Spotted deer, Monkey, Squirrel, Jungle cat, Hyena, Jackal and Mongoose
15.	Gahirmatha (Marine)	Kendrapada	Olive Ridley turtle
16.	Chandaka - Dampara	Khurda and Cuttack	Elephant, Chital, Barking deer, Leopard, Mouse deer, Wild pig, Common langur, Small Indian civet, Rhesus monkey and Common Indian mongoose,
17.	Ushakothi (Badarama)	Sambalpur	Elephant, Leopards, Tiger, Sambhar, Gaur, Spotted deer, Wild bear, Bison and Black panther
18.	Baisipalii	Nayagarh	Fauna is same as that of Satkosia
19.	Kapilash	Dhenkanal	Elephant, Sloth Bear, Spotted Deer, Barking Deer, Hayena and Python

(C) Biosphere Reserve :

A biosphere reserve comes under **category V** of the protected areas, specified by IUCN. It is an undisturbed natural area, where *in situ* conservation of all forms of life is enforced along with its support system, so as to serve as a referral system for monitoring and evaluating the changes in natural ecosystems. It was initiated under the **Man and Biosphere (MAB) programme** by the United Nations Scientific Educational and Cultural Organization (UNESCO) in 1971. The first biosphere reserve was established in 1979 and since then, the network has gone up to 669 in 120 countries which includes 16 trans-boundary sites (as on 2016). There are **18 biosphere reserves in India** and **Odisha has a share of one in Similipal**. 8 out of 18 biosphere reserves constitute a part of **World Network of Biosphere Reserves** based on UNESCO's MAB programme.

The objectives of this programme are to:

1. Conserve representative samples of ecosystems.
2. Provide long term *in situ* conservation of genetic diversity.
3. Provide natural areas for basic and applied research in ecology and environmental biology.
4. Impart opportunities for environmental education and training.
5. Promote appropriate and sustainable management of living resources.
6. Disseminate the experience so as to promote sustainable development elsewhere.
7. Promote international cooperation.

A protected area, declared as a biosphere reserve should satisfy the following essential features.

1. It should contain abundant genetic diversity.
2. It should be unique in itself.
3. It should have adequate long term legal protection.
4. It should be of an appropriate size for effective maintenance of natural populations so that there is no genetic drift.
5. It should have sufficient natural resource available for ecological research, education and training.
6. It should be a natural home for the endangered species of plants and animals.

Outline of a Biosphore Reserve : The Biosphore Reserve integrates human activities with conservation of biodiversity. It consists of four zones, namely (i) core zone, (ii) buffer zone, (iii) transition zone and (iv) zone of human encroachment.

BIOSPHERE RESERVES OF INDIA :

Sl.No.	Name	Year	Location
1.	Achanakamar-Amarkantak	2005	Parts of Anupur and Dindori districts of Madhya Pradesh and parts of Bilaspur district of Chhattishgarh.
2.	Agasthyamalai	2001	Neyyar, Peppara and Shendurney wildlife sanctuaries and their adjoining areas in Kerala.
3.	Cold Desert	2009	Himachal Pradesh
4.	Dehang-Dibang	1998	Part of Siang and Dibang Valley in Arunachal Pradesh.
5.	Dibru-Saikhowa	1997	Part of Dibrugarh and Tinsukia Districts (Assam)
6.	Great Nicobar	1989	Southern-most islands of Andaman And Nicobar Islands.
7.	Gulf of Mannar	1989	Indian part of Gulf of Mannar between India and Sri Lanka.
8.	Great Rann of Kutch	2008	Gujarat
9.	Khangchendzonga	2000	Parts of Khangchendzonga hills and Sikkim.
10.	Manas	1989	Part of Kokrajhar, Bongaigaon, Barpeta, Nalbari, Kamprup and Darang districts of Assam.
11.	Nanda Devi	1988	Part of Chamoli, Pithoragarh, and Bageshwar districts of Uttarakhand.
12.	Nilgiri	1986	Part of Wayanad, Nagarhole, Bandipur, Madumalai, Nilambur, Silent Valley and Siruvani hills of Tamil Nadu, Kerala and Karnataka.
13.	Nokrek	1988	Part of Garo hills of Meghalaya.
14.	Pachmarhi	1999	Parts of Betul, Hoshangabad and Chindwara districts of Madhya Pradesh.
15.	Panna	2011	Madhya Pradesh
16.	Simlipal	1994	Parts of Mayurbhanj district of Odisha.
17.	Sunderbans	1989	Part of delta of Ganges and Brahamaputra river systems of West Bengal.
18.	Seshachalam Hills	2010	Andhra Pradesh

The core zone is strictly protected to maintain the ecological diversity and integrity. The buffer zone concentrically surrounds the core zone. Recreational activities and sustainable utilization of natural resources are allowed in this zone. The transitional zone is ecologically least sensitive and hence anthropogenic activities, research and sustainable development are permitted. The zone of human encroachment is the outermost part, where normal anthropogenic activities are allowed.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Multiple Choice Questions :

(i) Genetic diversity refers to
(a) Variation in the genetic material (c) Variation in the number of species
(b) Variation in the populations (d) Variation in the animal distribution

(ii) Species diversity means
(a) Number of species (c) Species composition
(b) Relative abundance of species (d) Genetic diversities

(iii) The Forest Conservation Act was enacted in
(a) 1972 (c) 1980
(b) 1952 (d) 1991

(iv) Conservation of wild animals and plants in sanctuaries and national parks is
(a) *ex situ* conservation (c) *in vitro* conservation
(b) *in vivo* conservation (d) *in situ* conservation

(v) Corbett National Park is situated in
(a) Uttarakhand (c) Uttar Pradesh
(b) Jharkhand (d) Himachal Pradesh

(vi) Following mass extinctions, recovery to the same level of biodiversity has taken
(a) hundreds of years (c) thousand of years
(b) millions of years (d) billions of years

2. One word substitution :

(i) A species originated in one place and found nowhere else.
(ii) Organism whose no living representative is seen.
(iii) Biogeographic region with high endemism and habitat destruction.
(iv) Conservation of biodiversity in its natural site
(v) Diversity of all life forms in the Earth

3. Correct the statement by changing the underlined words only :

(i) Hybrid plants of a species are the source of disease resisatant genes
(ii) Bhitarkanika is a hotspot.
(iii) Hotspots are characterised by low endemism and habitat destruction
(iv) Botanical gardens are meant for *in situ* conservation of biodiversity
(v) WWF has enlisted endangered species in red data book.

4. Fill in the blanks with suitable words:

(i) The term "Biodiversity'' was coined by _____.

(ii) The three levels of biodiversity are _____ diversity, species diversity, and _____ diversity.

(iii) There are _____ numbers of megabiodiversity countries in the world.

(iv) India has _____ numbers of biodiversity hotspots.

(v) The Wildlife Protection Act was enacted in _____.

(vi) The UN Conference on Human Environment was held in _____ in 1972.

(vii) The expanded form of IUCN is _____.

(viii) The first national park of India is _____ national park.

(ix) Odisha has _____ numbers of national park.

(x) There are _____ numbers of wildlife sanctuaries in Odisha.

(xi) India has _____ numbers of biosphere reserves.

(xii) The concept of biosphere reserve made a beginning under _____ programme instituted by a UN body, namely _____.

GROUP - B
(Short Answer-type Questions)

1. Write notes on each of the following:

(i) Ecological diversity

(ii) Wildlife Protection Act (1972)

(iii) *in situ* conservation

(iv) *ex situ* conservation

(v) Biosphere Reserve

2. Differentiate between :

(i) *in situ* and *ex situ* conservation.

(ii) Genetic diversity and species diversity

(iii) National park and Sanctuary

(iv) Extinct and endangered species

GROUP - C
(Long Answer-type Questions)

1. What is meant by biodiversity ? Write the causes of loss of biodiversity?
2. How can the biodiversity be conserved ? Add a note on importance of biodiversity.
3. Give an account of biodiversity and its conservation measures.

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CHAPTER 17

ENVIRONMENTAL ISSUES

17.1 ENVIRONMENTAL POLLUTION :

The living organisms and the environment, both exist, interact and affect each other in a reciprocal manner so that an equilibrium is established. With time the environment changes, which in turn, induces adaptability in organisms better for their survival. By acquiring adaptive characters, new species evolve. This is a slow but continuous routine process. We have had the evidences in its favour from the history of life on earth. However, besides some stray natural calamities, anthropogenic activities have influenced the environment in an adverse manner so that it has become unsuitable for living. The quality of the environment has changed by the dumping of unnecessary toxic materials into it. This has caused serious health hazards to human beings and other living organisms. Human being applies his wisdom to escape from the problem to some extent. However, animals and plants, which do not have any means to counter this problem, face extinction. **This change in the quality of the environment has been referred to as environmental pollution** and is considered as a major problem among others in the present century. Population explosion is attributed as one single primary cause for environmental pollution. There are many secondary causes arising out of the primary cause. **Environmental pollution may, therefore, be defined as any qualitative change in the natural environment that threatens the existence of humans and other living organisms.** The study of environmental pollution is classed under: **air pollution; water pollution; soil pollution; thermal pollution; radioactive pollution; and noise pollution etc.**

17.2 AIR POLLUTION :

Air is a mixture of several components like oxygen, carbon dioxide, nitrogen, water vapour, dust particles and many others, each present more or less in a definite percentage. Animals and plants respire by taking oxygen and releasing carbon dioxide. Plants and other photosynthetic organisms take carbon dioxide and release oxygen during photosynthesis. Many microorganisms assimilate nitrogen from the atmosphere for nitrogen fixation. There is a complex mechanism of recycling of these gases such that the percentage of each gas is maintained at equilibrium. This is a continuous phenomenon. However, some natural catastrophic phenomena and human activities release many harmful gases into the atmosphere in a continuous manner so that the quality of the air changes beyond managable limits. This has been termed as air pollution. Air pollution occurs from two sources: (1) Natural Source and (2) Human Source.

17.2.1 Natural Source :

Natural disasters like earthquake, volcanic eruption, dust storm and soil erosion release a large volume of particulate matter, dust particles and noxious gases into the atmosphere. These elements cause severe health hazards and loss of vegetation. Radon is a naturally occurring colourless and odourless radioactive noble gas formed from the decay of radioactive elements. It releases radium into the atmosphere, which causes severe health hazards. Fur of animals, pollen grains, fungal spores, bacteria and viruses are also considered as naturally occurring pollutants that contribute towards illness.

17.2.2 Human Source :

This is the major source of air pollution. This source includes fumes released from the burning of fossil fuels like coal, petroleum products, and natural gas in the industrial establishments. Mining operations release huge amount of grit and dust into the atmosphere. Emission from the exhaust pipes of vehicles contains noxious gases like carbon monoxide, carbon dioxide, oxides of nitrogen, sulphur dioxide, hydrocarbons and suspended particles. Air pollution occurs due to accidental leakage of gases from industries, nuclear weapon testing, armed conflicts between countries and festival celebration e.g. extensive fireworks during Diwali. Have you ever read about the **Bhopal gas tragedy of 1984?** It is considered as a major industrial disaster of the world. Nearly 42 tons of toxic methyl isocyanate gas leaked from the Union Carbide's pesticide factory in Bhopal on 2nd December, 1984, when the city was fast asleep. More than 8,000 people died and 500,000 people over an area of 100 km^2 were affected. Many more still continue to suffer.

Pollutants are classified as either **primary** or **secondary** based on their formation. Primary pollutants are directly released into the atmosphere from their sources, while secondary pollutants arise by chemical reactions among the primary pollutants.

17.2.3 Primary Pollutants :

Primary air pollutants include **particulate matter, carbon monoxide** (CO), **carbon dioxide** (CO_2), **oxides of nitrogen** (NO_x), **sulfur dioxide** (SO_2), **hydrocarbons, toxic heavy metals, chlorofluorocarbons** (CFC_s) **or freons, ammonia**, and **radioactive elements**.

(i) Particulate Matter : These include both solid particles and liquid suspensions. Solid particles are **soil particles**, **soot**, **asbestos** and **fly ash**. Dry land without vegetation is subjected to soil erosion, which releases excess minute soil particles into the atmosphere. These together constitute dust. Natural calamities like dust storm, land slide, earthquake and volcanic eruption release a huge amount of dust into the atmosphere. Mining operations also release considerable amount of dust. Thermal power stations produce minute suspended particles, constituting fly ash. It contains silica, alumina, oxides of iron and toxic heavy metals like lead,

arsenic cobalt and copper. Excess fly ash deposition on the ground reduces soil fertility and hence vegetation growth. Thick black soot arising from the chimneys of industries contains minute suspended particles. Vehicular emission contains particulate matter that contributes further towards air pollution. **Aerosols** are particulate matter that are formed by the combination of gas and liquid particles together. These contain particles of less than 2.5µm in diameter. Aerosols permanently keep floating by the circulation of air. Particulate matter causes severe respiratory problems in animals (irritation of the respiratory tract leading to asthma) and loss of vegetation.

(iii) Carbon monoxide (CO): It is a colourless, odourless and highly toxic gas generated from the incomplete combustion of carbon. Burning of fossil fuel, natural gas, wood, vehicular emission, industrial exhaust release this gas into the atmosphere. Natural sources are volcanic eruption, natural gas emission from pits and electric discharge during thunderbolt.

The gas binds to haemoglobin forming **carboxyhaemoglobin**, which reduces the oxygen binding capacity of the blood and hence leads to **hypoxia** and death.

(iv) Carbon dioxide (CO_2): It is a naturally occurring greenhouse gas emitted from the incomplete combustion of carbon. Although, it is not as harmful as CO, it still acts as a primary pollutant.

Oxides of Nitrogen (NO_x): Nitrogen combines with oxygen forming several oxides of nitrogen, such as nitrous oxide (N_2O), nitric oxide (NO) and nitrogen dioxide (NO_2), of which NO_2 is dominant. These gases arise from the burning of biomass and fossil fuel. Thus, automobile exhaust, incinerators, furnace stacks and thermal power plants are the principal sources of these gases. Nitrogen based fertilizers also add NO_x to the atmosphere. Microbial nitrogen fixation also contributes to the emission of these gases to some extent.

NO_2 is a reddish brown toxic gas soluble in water. In the presence of water vapour, it forms nitric acid (HNO_3) in the atmosphere, which falls on the ground causing **acid rain**. NO_x, principally NO_2 is responsible for forming tropospheric ozone (O_3) through a series of photochemical reactions. Tropospheric O_3 acts as a secondary pollutant. NO_2 reacts with stratospheric O_3 and depletes it stratospheric O_3 absorbs ultraviolet (UV) radiation from the sun and thus acts as a protective shield. The depletion of O_3 layer allows UV radiation to reach the earth surface and cause severe health hazards. Photochemical reactions involving NO_2 and hydrocarbons induced by sunlight produce **photochemical smog**.

(v) Sulphur dioxide (SO_2): SO_2 is released into the atmosphere by the incomplete combustion of fossil fuel like coal and petroleum products. Thus, vehicular emissions and industrial exhausts are the primary sources of SO_2. Volcanic eruption also releases a huge quantity of SO_2 into the atmosphere. It is the source of sulphur trioxide (SO_3), a secondary pollutant.

It is soluble in water forming sulphuric acid (H_2SO_4). When present in the atmosphere, it reacts with water vapor forming H_2SO_4, which falls on the surface of the earth causing extensive damage, primarily to vegetation. This phenomenon is known as **acid rain**. SO_2 induces **bronchitis** and **asthma**.

(vi) Hydrocarbons: Hydrocarbons are volatile organic compounds (VOC), emitted into the atmosphere by natural biological activities as well as human activity. Human activity accounts for 15% of the total hydrocarbon emission into the atmosphere. Methane (CH_4) is the principal form of hydrocarbon emitted from natural sources like landfill deposits, oil wells and coal mines. Paddy cultivation and digestion of cellulose by ruminants also account for release of CH_4 from natural sources. Human source includes vehicular emission. Around 20 hydrocarbons have been identified in the vehicular emission.

Although nontoxic, CH_4 is a potential greenhouse gas that stays in the atmosphere for a pretty longer period of time and forms an explosive mixture with other gases. Hydrocarbons have a definite role in the formation of photochemical smog. Non-methane hydrocarbons induce the formation of tropospheric O_3, which acts as a potential greenhouse gas.

(vii) Ammonia (NH_3) : This is a highly toxic gas with a pungent smell. It is mainly emitted from agricultural processes. Food, fertilizer and some drug industries use NH_3 as a precursor. The industrial emissions and slow decomposition of garbage and sewage are the other sources of this toxic gas.

(viii) Toxic Heavy Metals : The toxic heavy metals such as lead, chromium, zinc, mercury, cadmium and copper are persistent elements in air, emitted from various sources. These elements bioaccumulate in the food chains, enter into the body of humans and other animals through the food chains and induce a wide range of health hazards. Lead damages liver, central nervous system, kidney and induces anemia and infertility. Cadmium causes bone deformity, kidney damage, testicular dysfunctioning and hypertension. Copper induces hypertension and zinc induces vomiting and renal damage. Chromium causes ulcer and nephritis, while mercury causes mental disorientation and deafness. Among these, lead is noteworthy. A major part of this element is released from automobile exhaust. Tetraethyl lead was used as an anti-knocking agent in the automobile fuel. However, it has been phased out from automobile fuel with effect from February, 2000.

(ix) Chlorofluorocarbons (CFCs) or Freons : These are potential greenhouse gases emitted from refrigerators, air conditioners and other cooling systems. CFCs move up to the stratosphere and under the influence of high energy UV radiation disintegrate releasing chlorine atoms. These chlorine atoms speed up the breakdown of O_3 into molecular oxygen (O_2) and nascent oxygen (O). The gradual depletion of stratospheric O_3 causes thinning out of the ozone layer through which

UV radiation reaches the surface of the earth. There are reports of ozone layer at arctic and antarctic regions upto alarming proportion.

(x) Radioactive Pollution: The natural sources of radioactivity are the cosmic radiation coming from the sun and even beyond. These extraterrestrial radiations collide with nuclei of atoms releasing subatomic particles, gamma radiation and ions. Another natural source of radioactivity is the occurrence of radioactive elements in the lithosphere. Man made sources are making and testing of nuclear weapons, use of nuclear fuel in nuclear power plants and use of several radioisotopes in scientific research and investigation. The most hazardous aspect of it is the disposal of the radioactive waste following their use. The ionizing radiations have been proved to be mutagenic and carcinogenic acting directly on the genetic material (DNA). Chernobyl and Fukuslima nuclear power plant disasters and their consequences are fresh in the memory.

(xi) Persistent Organic Pollutants (POPs): POPs is a group of organic chemicals, which remain in the environment for long, carried to distant places and accumulate in the body in undetectable doses through food chains. These include polychlorinated biphenyls (PCBs), DDT, dioxins and endosulfan. Most of the POPs are either produced intentionally or as byproducts. The **Stockholm Convention** on POPs was adopted in 2001, which seeks an elimination or production and use of all intentionally used POPs.

17.2.4 Secondary Pollutants :

Secondary pollutants are produced by chemical reactions among the primary pollutants. Some of the examples discussed below are tropospheric ozone, photochemical smog and sulphuric and nitric acids.

(i) Tropospheric ozone: Nitrogen dioxide (NO_2) released from primary sources dissociates into nitric oxide (NO) and nascent oxygen (O) under the influence of UV radiation. The nascent oxygen reacts with molecular oxygen forming ozone in the troposphere. Ozone is a toxic and corrosive greenhouse gas. It traps heat and adds to the greenhouse effect. It also participates in the formation of photochemical smog.

$$2NO + O_2 \rightarrow 2NO_2$$

Nitric Oxide Nitrogen Dioxide

$$NO_2 \xrightarrow[\text{radiation}]{\text{UV}} 2NO + [O]$$

Fig. 17.1 : Chemical Reactions in the troposphere generating Ozone.

(ii) **Peroxyacetylnitrate (PAN) :** When sunlight reacts with nonmethane hydrocarbons and nitrogen oxides, PAN is formed. Acetaldehyde, methyl glyoxyl and various bypoducts of oxidation of aromatic compounds are all precursors of PAN. The vehicular emission contains several primary pollutants. These primary pollutants react among themselves giving rise to several secondary pollutants. Peroxyacetyl nitrate (PAN) is one among these. It is an important constituent of photochemical smog.

(iii) **Photochemical Smog:** Smog is a conventional term used to denote the combination of smoke and fog formed during winter. Water vapour surround smoke, dust or soot particles forming secondary particles, which remain suspended in the air. This smog is reducing in nature and should be distinguished from photochemical smog, which is oxidizing. During warmer sunny days, oxides of nitrogen and sulphur and hydrocarbons from vehicular exhaust undergo a series of photochemical reactions producing many photochemical oxidants. These combine with tropospheric ozone forming a brownish hazy fume called **photochemical smog**. It irritates the eyes and lungs and causes extensive damage to vegetation and rubber goods.

(iv) **Acid Rain:** Acid rain is another consequence of the generation of primary air pollutants into the atmosphere. Burning of fossil fuel like coal, petroleum products and natural gas; forest fire; vehicular exhaust and other human activities release a number of oxides of nitrogen, sulphur dioxide and chlorine into the atmosphere. All these primary pollutants react with atmospheric water vapour and form constituent acids, such as nitric acid, sulphuric acid and hydrochloric acid. These fall on the ground in the form of rain. This phenomenon has been referred to as acid rain. These acids have a range of adverse effects on living organisms and inanimate objects. Historical objects like old buildings and statues are the targets of these acids. Taj Mahal in Agra is threatened by the acid rain caused by the industries in its vicinity.

17.2.5 Control of Air Pollution :

Many laws both at the national and international levels have been framed and many agencies at both levels have been set up to monitor environmental pollution. Several national and international conventions have been held to assess the quantum of environmental damage and suggest remedial measures for minimizing the damage. But it is not relenting. Pollution is on the rise steadily and irreversible damages are done to the environment. Bulk of the pollution is anthropogenic i.e. of human origin. The exponential growth in human population and the changes in the lifestyle are two important causes of this problem. There are very stringent laws for the protection of the environment. However, a change in the mindset of the citizens is

primary. This may be done by exposing them to the causes of occurrence and the consequences of major environmental crises through environmental education. A few conventional control measures of air pollution are presented below. We have learnt from the preceding sections that the industries and the automobiles are the main culprits of air pollution. These contribute particulate matter and toxic gases to the atmosphere.

(i) **Control of Particulate Matter Emission:** Particulate matter emission is reduced by fitting **smoke stags, electrostatic precipitators** and **wet scrubbers** in industries. The state and central Pollution Control Boards routinely measure the ambient air quality in major industrialized cities of India by estimating the quantity of suspended particulate matter, nitrogen dioxide and sulfur dioxide in the air.

(ii) **Control of Industrial Emission:** There are two fundamental practices for controlling emission from industries: (1) the gaseous pollutants are controlled or confined at source and (2) the pollutants are diluted in the atmosphere before they reach their targets. The first practice is executed by two methods: (a) modifying the processes so that the pollutants do not form beyond a permissible concentration and (b) reducing the concentration of the pollutants to a tolerable limit before they are released into the environment. The latter is the option of choice. This is practiced by **combustion, absorption** and **adsorption**.

Combustion: This is the method of choice, when the pollutants are organic in nature. **Flame combustion and catalytic combustion** are two combustion methods, which convert the pollutants into water vapour and relatively less harmful carbon dioxide. Flame combustion uses **incinerator**, while catalytic combustion uses **catalytic converters**.

Absorption: The emitted gas is passed through a scrubber containing a liquid absorbent to remove or modify it.

Adsorption: The gas is passed through a porous solid adsorbent, like silica gel, lime stone, activated carbon, etc. The toxic constituents are held at the interface of the adsorbent.

(iii) **Control of Vehicular Emission:** The vehicular emission is the major contributor in bigger cities. The use of tetraethyl lead as an antiknocking agent has been phased out from automobile fuel with effect from February, 2000. The Pollution Control Boards have set up pollution monitoring centres for periodic checkups of vehicular exhausts. The government encourages for the use of cleaner and fuel efficient cars. One of the options for reducing pollution by vehicular emissions is the use of electric cars running on power from rechargeable batteries. There should be an immediate shift from diesel to natural gas for trucks and buses. The Hon'ble Supreme Court of India has issued a series of orders from 1985 in an effort to reduce the level of environmental pollution. There will be mandatory use of compressed natural gas in all buses and commercial vehicles and shifting of all

polluting industries out of Delhi. Indian government passed a regulation for automobile emission in 1991. **The Bharat emission norm** modelled on Euro norm of European Union was introduced in 2000. Bharat III norm for new passenger cars came into force from April, 2010. Bharat IV norm was introduced in 12 Indian cities. A policy for mass transit system was passed to discourage the use of personal cars.

17.3 WATER POLLUTION :

Water is essential for all forms of life. Water constitutes about 70% of the total body weight of human. About 80% of the earth's surface is covered by water. A large part of it is held as salt water of the oceans, seas, lagoons, polar ice caps and glaciers and underground water. Only a small part of it is available for domestic and agricultural uses and industrial consumption. Due to an exponential growth in the population and industry, a large part of it is contaminated and becomes unworthy for use. The ever growing population and the expanding industrial network have an increasing pressure on whatever little water remains uncontaminated and pure. This is the story about the surface fresh water. The sea water and the ground water reserves are no exceptions too.

More than 90% of the surface water is unfit for use due to water pollution. Water pollution may be defined as contamination of water by filthy and toxic materials, which make it unsuitable for consumption by organisms including man. Pollution comes from two sources: **point source** and **non-point source**. Point sources, such as sewers and industries discharge the pollutants directly into the water bodies. When pollutants enter into a water body from all around, the source is non-point. For example, rain water runoff flushes all pollutants it comes across, such as sediments, human faecal matter, pesticides and fertilizers into a water body. In this case, there is no fixed point of discharge. Such a source is known as a non-point source. Water pollution is discussed under three sections: **surface water pollution; ground water pollution; and marine pollution**.

17.3.1 Surface water pollution :

(i) Suspended Solids and Sediments: Excess amount of soil particles arising from soil erosion and other suspended particles of the industrial effluents make water bodies cloudy. Some settle as sediment at the bottom. The rest is carried by the flowing water of the rivers, canals and springs to the sea, where sediments are formed at the bottom over the years. Continuous drainage makes the water turbid. The flowing water bodies are clogged. This range of events affects the aquatic ecosystems and the organisms in an adverse way.

(ii) Domestic Sewage: Sewage is waste water that is laden with human and animal faecal matter, soaps and detergents, nutrients like nitrates and phosphates and

millions of bacteria. The sewage in urban areas is drained either by underground sewers or in open drains. Whatever is the case, the sewage is directly flushed into water bodies like rivers, canals and coastal water of the sea. Due to the presence of organic matter, nitrates and phosphates, the water is enriched with nutrients, which promotes the growth of microbes, aquatic plants and algae. Their overgrowth blocks the penetration of the sunlight into the deeper layers of water. As they decompose the organic matter, they use more dissolved oxygen. Many aquatic organisms suffer from a deprivation of oxygen and consequently die.

(iii) Toxic Chemicals:

Metals: Metals like lead, copper, zinc, cadmium, mercury and arsenic are released into flowing or stagnant water bodies by industries. Copper, iron, zinc and magnesium are beneficial for physiological activities at lower concentrations but tend to produce toxic manifestations at higher concentrations. The consequences of mercury poisoning is discussed below as a reference.

Mercury poisoning has been recorded as one of the global industrial disasters. **Chisso Corporation in Japan** had been dumping mercury into the **Minamata Bay** since its inception. Hundreds of inhabitants died by eating methyl mercury contaminated fishes between 1950 and 1983. Many more suffered from blindness, convulsions and brain damage. Investigations found that the disease was due to mercury poisoning. The disease, as identified as **Minamata**, created a furor all around the world.

Detergents: Domestic use of detergents as cleaning agents is very common in every household. The domestic sewage run off containing detergents contaminate water bodies into which it is discharged. Detergents are non-degradable organic compounds such as **alkyl benzene sulfonyls** and **alkyl sulfonates**, which are highly toxic to all forms of aquatic life forms including fishes.

(iv) Radioactive Waste: Radioactive waste from nuclear power plants, uranium refining and nuclear weapon production factories and radioactive element mining are not safely disposed off. Traces may leak and be released into water bodies. What to talk of using this contaminated water, anybody close to the source may receive ionizing radiation and get affected. The radiation causes chromosomal aberrations, mutation, cancer, congenital deformities and miscarriages. Besides the conventional leakages, disasters occur in nuclear facilities and uncontrolled ionizing radiation is emitted. The civilization can never forget about the horrific consequences in the aftermath of two such major disasters in Chernobyl in Ukraine and Fukusima in Japan.

(v) Infectious Organisms: The water bodies into which the sewage is discharged are rich in biodegradable organic chemicals. These are the breeding grounds of many infectious organisms, such as bacteria, viruses and worms. The sewage contaminated water is responsible for many waterborne diseases and kill thousands of people in developing countries.

(vi) Hot water emission :

Hot water from industries, such as thermal power plants, when discharged into natural water bodies elevates the temperature. Dissolved oxygen depletes and the organisms are deprived of oxygen and die of hypoxia. Secondly, by a sudden elevation of the temperature, the organisms living there, get a thermal shock and may consequently die. Apart from the above chemical fertilizers and pesticides, extensively used in our agricultural practices for enhancing crop productivity fow through the run out water and finally pollute the water bodies. These aspects are discussed under 17.4.

17.3.2 Ground water pollution :

About one third of world's population depend on ground water for their daily requirement. It is a commonly held idea that surface water that sips through the soil is filtered and thus the ground water is free from contaminants. However, the story of arsenic poisoning tells us about the ground water contamination by seepage of arsenic contaminated surface water through the soil sediments.

Arsenic poisoning was reported in the 24-Praganas district of West Bengal in 1983. Under a mysterious circumstance the inhabitants developed spotted skin and nodules in the hand, which turned into gangrenes and then cancer. Investigations proved arsenic poisoning was the cause of such developments. It spread like an epidemic in Bangladesh also. People in these areas depended heavily on deep bore wells for their daily water requirement. Arsenic deposits are present in the aquifers. The element may have been released into the underground water. This is one theory. Another theory spells that there is arsenic in the headwater deposits of the Ganga and Brahmaputra rivers in the Himalayas, which may have percolated into the ground water through the soil.

Another way of groundwater contamination occurs through the leaching of contaminants from the sediments into aquifers. This is exemplified by the fluoride poisoning of the groundwater. All the 1200 inhabitants of the village, Jharana Khurd near Jaipur in Rajasthan look old irrespective of their age. The same is the story in many villages of Uttar Pradesh, Bihar, Jharkhand, and Andhra Pradesh. In the Angul district of Odisha too an identical situation has been discovered. All this is due to drinking of fluoride contaminated ground water that leads to a bone and tooth deformity disease known as **fluorosis**. Fluorosis leads to blackened or cracked teeth, joint deformities, gastro-intestinal and neurological problems. Fluoride contamination occurs in the deep aquifers by the fluoride leaching from the sediments. Fluorosis is now endemic in 19

Indian states with 65 million people including 5 million children affected. As on now, there is no full scale initiative for combating the menace. UNICEF, however, has introduced low cost domestic defluorination filter units, which can be used in homes.

Pollutants from septic tanks, waste dumps, landfills and underground tanks containing petroleum products and chemicals may sip into the underground water. Such contaminations last longer since the water is not flushed nor it is decomposed by microorganisms. Contaminants like lead, arsenic and fluorides remain in the groundwater for many years.

17.3.3 Marine pollution :

The sea water too is not exempted from pollution. Industrial effluents and agricultural runoff water containing many biodegradable organic pollutants, pesticides, fertilizers and a variety of toxic chemicals are directly discharged into the sea. The flushing of nitrogen and phosphorous from the agricultural runoff into the coastal water brings about eutrophication. This induces the multiplication of many toxic algae causing **algal blooms**. One such bloom is known as **red tide**. The toxin released into the water kills marine organisms *en mass*.

Oil spill is the major contributor of marine pollution. We have had a record of several major incidents of oil spills and the magnitude of damage done to the marine life. In 1989, an oil tanker, Exxon Valdez hit a coral reef off the coast of Alaska and spilled 38000 tons of crude petroleum into the sea. The spill spread along 4000 km of the coastline affecting all marine flora and fauna. Similarly, in 2010, an explosion in an offshore oil drilling facility in the Gulf of Mexico leaked 5 million barrels of oil and inflicted damages of the same magnitude in the gulf. The marine fauna was very badly affected.

Many human activities require radioisotopes. Following their use, radioactive wastes are generated, which should be safely disposed. However, the wastes are dumped in the soil, or dumped into rivers and seas. The ionizing radiations emitted from these elements inflict a lot of damage to the marine flora and fauna.

17.3.4 Control of Water Pollution :

The major contributors to water pollution are the domestic sewage, industrial effluents, agricultural runoff water, toxic deposits in the underground aquifers, oil tankers ferrying through seas and other minor practices like commercial fish catching and pisciculture. The control of water pollution mainly revolves around the treatment of waste water of the sewage, industrial effluents and agricultural runoff.

(i) Purification of water for domestic use: Water for domestic use is collected from rivers, springs, ponds, pools, reservoirs, wells and bore wells. In urban areas, the municipalities supply the domestic consumption from a reservoir through a network of pipelines following its treatment. Most of the water whether it is surface or underground is contaminated. The usual practice of bulk cleaning relies on

chemical treatment to settle the suspended particulate matter and then filter and disinfect. This is water for gross use in homes. However, drinking water is subjected to several treatments like **boiling, reverse osmosis** (RO) and **UV treatment**. Now many compact water purifying devices are available in the market for purifying drinking water.

(ii) Waste Water Treatment: The waste water of the sewage, industrial effluents and agricultural runoff is subjected to the same type of treatment for removing the contaminants. First, the gross solids, oil and grease, present if any, are removed and then the fundamental treatments begin. These fall under three categories: **primary, secondary** and **tertiary.**

In the **primary treatment**, the suspended solids are removed by **sedimentation** in large **sedimentation tanks**. Acidic or alkaline water of the industrial effluents is neutralized by appropriate chemical treatment. In the secondary treatment, the dissolved and colloidal organic matter is removed by biological processes using microorganisms. This is either aerobic (occurring with oxygen) or anaerobic (without oxygen).

In the aerobic process, the dissolve and colloidal organic matter is: **(1) coagulated; (2) oxidized to carbon dioxide;** and (3) **nitrogenous organic matter degraded to ammonia** and then to nitrate through nitrite. The secondary treatment is carried out in large **aerated oxidation ponds or ditches.** The most common biological oxidation is known as **activated sludge process**. The microorganisms are suspended in the waste water in a stirred aeration tank. The oxidation takes place and the organic matter is converted into **sludge**. The microorganisms multiply and form a flock known as **activated sludge**. This is removed for recharging of another cycle of the process. The highly toxic sludge is sedimented and removed for further treatment to remove contaminants. The anaerobic process is mainly used for the digestion of the sludge by fermentation. The tertiary treatment is carried out to disinfect the sludge to a maximum extent. The sludge arising from the domestic sewage is used as organic manure, while the one from industrial effluents and agricultural runoff is highly toxic and is disposed off safely. The water released from these processes is used for irrigation or pisciculture or is recycled for use in the industries.

(iii) Oil Spill Remediation: Spilling of crude petroleum from the tankers into the sea has been mentioned in the preceding section with some past episodes. It has been a problem for clearing the spill, which occurs in a sprawling area in the sea. Mechanical and chemical treatments were only partly successful. Even more than 20 years later, Alaska's coastline has the foot print of the spill in the form of a glaze. However, innovative biotechnology has genetically engineered hydrocarbon eating bacteria that are used successfully in clearing oil spills faster and more effectively. The use of organisms in clearing oil spills has been referred to as **bioremediation**.

Water is a precious resource. Therefore, it is to be judiciously used for any purpose. The industries are the bulk users of water. Therefore, a strict regulation should be imposed to rely on the recycling of water of the effluents. Secondly, the use of water should be appropriately levied by the government and there should be a periodic monitoring of the use from the natural sources. The government should have a mandatory policy for rainwater harvesting in all homes. In doing so the underground water table is regularly recharged. The recharged rain water in the wells can be used by the local people affected by drought and fluorosis. The Balisana village story in the state of Gujarat will suffice the context. The village was hit both by continuous drought and fluorosis. The NGO, Utthan taught the people to collect the runoff rain water and recharge it into dried up wells and tanks. The water recharged underground aquifers. Consequently, the dried up wells now have potable water.

In the concluding remark, we would like to mention about a landmark episode on water pollution and how judicial activism can set things right. It is the story of polluted Ganga, cherished by all Indians as the sacred river. M.C.Mehta, an environment lawyer filed a public interest litigation case in the Supreme Court of India in 1985 against two polluting factories. Justice Kuldip Singh heard the case every Friday for over two years and passed a landmark judgment that the polluting industries be shut down if they did not install effluent treatment plants. The consequence was that hundreds of factories installed effluent treatment plants and many small factories, which could not, were shut down.

17.4 AGROCHEMICALS :

Chemicals that are used for maximizing productivity of crop plants by providing essential nutrients or by protecting the crop plants from pests and pathogens are called as **agrochemicals.** They are the **fertilizers** and **pesticides.**

17.4.1 Fertilizers :

Modern agriculture involves the application of chemical fertilizers as well as bio-fertilizers into the fields. These provide valuable nutrients to crop plants and maximize productivity. Phosphorus and nitrogen are the principal nutrients limiting plant growth. Farmers frequently apply more fertilizers (nutrients) than are actually assimilated by crops. The excess nutrients including phosphorus and nitrogen accumulate in the soil and finally flow in the runoff water (due to rain or irrigation) to the nearby water bodies. The water bodies are consequently enriched with nutrients which support the excessive growth of aquatic plants and algae. The excessive growth of algae in water bodies making the water look green is called as **algal bloom.** The excessive growth of unwanted algae and other phytoplanktons is called **eutrophication**. Algal bloom blocks sunlight to reach the bottom. As a result plants below the algal bloom cannot get sunlight to carry out photosynthesis. They eventually die and sink to the bottom where bacteria decompose the remains and use up oxygen for respiration. The water becomes depleted of oxygen that causes larger life forms such as fishes to suffocate and die. The algal bloom often also secretes several toxins which kills other organisms.

17.4.2 Pesticides :

Pesticides are chemicals that are used to kill a variety of harmful organisms. These include **insecticides, fungicides, herbicides** and other chemicals. These have been increasingly used in modern agricultural practice to kill the pests so as to have good harvest. However, the excess pesticides are drained to water bodies as runoff water, which inflict a great deal of damage to the aquatic organisms. The immediate target is its user i.e. human. **Dichloro Diphenyl Trichloroethane** (**DDT**) is the most widely used organochlorine pesticide against crop pests and disease carrying insects, particularly mosquitoes. It has already been banned in USA and many industrialized countries. However, in developing countries, it is still used to combat malaria. In India, its use is banned in agriculture, but still used against malaria. **Benzene Hexachloride (BHC)** and **endrine** are two more organochlorine pesticides. These are persistent pollutants that are stored in the liver and fat bodies of animals. These compounds inhibit, the synthesis of an important respiratory enzyme cytochrome oxidase. These affect the nervous system and inhibit sex hormone synthesis in vertebrates. Most of the water that we use on a daily routine basis are contaminated too by pestcides. The drinking water is contaminated by pesticides. Packaged drinking water that is sold in the market may be cited as an example.

The packaged drinking water that is thought to be contamination free is also contaminated with pesticides. Deadly pesticides like, **lindane, DDT** and **malathion** were present well above the European norm. The Union Ministry of Health and Family Welfare in 2003 notified new standards for pesticide residues in bottled water. The display of Bureau of Indian Standards certification on packaged drinking water bottles has become mandatory.

Endosulfan is another deadly pesticide that inflicts a huge damage on all life forms. In Kerala, this pesticide was aerially sprayed on cashew nut plantations to control the invasion of pests. In Kasaragod district of the state, there was a high incidence of cancer, psychiatric problems, and mental retardation among the youth below the age of 25 years. All these problems were later traced to endosulfan poisoning. In 2004, the use of endosulfan was banned on the basis of an order passed by the Hon'ble Kerala High Court. The Stockholm Convention on Persistent Organic Pollutants recommended a global ban of endosulfan in 2010. The story of **Agent Orange** (code name for a defoliant herbicide), used during US-Vietnam War reminds us of its damaging potentiality.

Many deadly pesticides are banned in industrialized countries. However, they continue to manufacture and sell the said pesticides to developing countries. Looking at the potential threats, some developed countries either sell or shift the manufacturing units to the developing countries. This reminds us of the Union Carbide's pesticide factory disaster in Bhopal in 1984.

Polychlorinated Biphenyls (PCBs): PCBs are stable organic compounds having insulation properties, fire resistance and low electrical conductivity. These persist for long in the

environment and are very toxic. When burnt, it turns into a toxic ash and when buried, it leaches into the ground water. It is washed away into rivers and seas. It then enters into the food chain and then into the bodies of aquatic animals. It affects the reproductive system in a big way. When the reproduction is hampered, it may lead to the extinction of the species. It is classed among persistent organic pollutants (POPs), whose manufacture and use has been regulated by Stockholm Convention on POPs, 2001. All the presticides are never metabolized by the organisms who assimilate it. It gets, in contrast, accumulated and passed on to next trophic level of the food chain. Hence, when it reaches the top consumers like the human beings, the effects becomes fatal. This is called biomagnification or bioaccumulation.

17.5 SOLID WASTE MANAGEMENT :

Anything that is no longer in use and hence, thrown away is called waste. Living organisms use so many essential things, utilize and generate waste from many biological processes. These wastes are generally organic and biodegradable. These are decomposed into their elements by natural processes and are returned to the nutrient pool for reuse. This called recycling and is an important process occurring in nature. Recycling is essential for the perpetuation of life. These natural wastes stay only for a short period and pose some pollution problems. However, these problems can be overcome by public health and hygiene education. It can be said that where there is consumption, there is waste generation. Nature handles these two processes so elegantly that there is equilibrium and there is no stockpiling of waste. In this case the input and output are held in balance. However, anthropogenic activities have generated so much of both biodegradable and non-biodegradable wastes that these remain stockpiled in the environment for long and create serious health and other hazards. Appropriate management is required to nullify the toxic effects of these wastes. When we talk about solid waste management, it reminds us of the story of Alang, the ship breaking yard on the coast of Gujarat. Here, heavy ships are dismantled making big mountains of scraps. This action generates a huge amount of dust and toxic fumes. Not to mention here that most ships that come here for dismantling carry toxic wastes. The toxic fumes have a direct impact on the health of the workers and people living in nearby villages.

17.5.1 Classes of Solid Wastes :

(i) Domestic Waste : These include the human and animal faecal matter, sewage, household garbage and waste generated from home and office appliances and motor cars.

(ii) Industrial Waste: Solid wastes and effluents from the factories and industries include blood and viscera from slaughter houses and effluents from breweries, tanneries, paper mills, steel plants, power plants, many chemical industries producing hazardous chemicals and oil refining industry.

(iii) **Construction Waste:** The real estate industry is increasing in urban areas by leaps and bounds. Huge amount of debris in different forms is generated either by the demolition or construction of buildings.

(iv) **Extraction and Processing Industry Waste :** Solid waste from mining and quarrying operations and slurries from the processing of ores are generated. Food processing industries generate huge amount of organic waste.

(v) **Agricultural Waste:** Solid organic waste from agricultural practice during the growing and harvesting seasons are dumped on the soil, which decompose and are washed away into nearby water bodies. These bring about eutrophication of water bodies, which affects the local biotic potential.

(vi) **Biomedical Waste:** Solid wastes from hospitals include infected syringes, needles, gauze, blood pouches, normal saline and dextrose pet bottles, parts of the body following surgery, date expired medicines, etc. These are either stockpiled in a place close to human settlements or are land filled in low lying areas. These are the sources of many disease causing organisms.

(vii) **Radioactive Waste:** Radioactive waste is generated from nuclear power plants, nuclear weapon manufacturing facilities, cancer treating hospitals and research laboratories using radioisotopes in investigations. This waste is to be disposed off safely by observing the standard guideline because it remains for a very long period and continues to emit ionizing radiation. The ionizing radiation is extremely hazardous to health of all forms of life.

(viii) **Electronic Waste:** This is the most recent form of solid waste generated from discarded electronic gadgets after use. The technology input into electronic gadget manufacturing industry changes very quickly. The consumers also like to buy such things with the most recent technology base. The consequence is the stockpiling of the old ones like televisions, computers, refrigerators, washing machines, microwave ovens, music systems, cell phones, note books, etc. The net result is that there is a rapid increase in the electronic waste. Such waste contains hazardous materials like lead, copper, zinc, aluminium, plastic casing and insulated cables. Industrialized countries export the e-waste to the developing countries. China and India import such waste and recycle and manufacture electronic items forgetting about their potential hazards. The only option left is that these sell relatively at a lower price.

(ix) **Plastic:** Carry bags and pet bottles, once thought to be useful have now become a nuisance. These are non-biodegradable and secondly increase the volume of municipal waste. Animals and birds often eat the plastic carry bags and die. The plastic carry bags play even a more nuisance because these are displaced to

distant places with the wind. Dumping of municipal waste is a common sight in the outskirts of metropolitan cities in India. The options for their safe disposal are not within the affordable limit of the local bodies monitoring their management. In some places, the disposal is practiced by burning these discarded articles. But this is a dangerous option, since burning generates very toxic fumes, which make the air even more polluted.

(x) Waste from Natural Disasters: Natural disasters like earthquake, volcanic eruption, flood and cyclone generate a lot of waste in the form of dust, smoke, ash, slag and organic silt.

17.5.2 Management :

1. The household waste is separated into different categories like organic waste, paper, glass and other containers. These are dumped in differently coloured bins marked for each category. These dumpings are collected by the local body in vans for final disposal or treatment. In doing so, it becomes easier to identify the category of waste from the bulk and decide on the method of disposal.

2. Municipal sewage is semisolid to liquid. Both forms are chemically treated in big tanks to precipitate out the solid things. The precipitate is separated and treated in a separate tank to turn into non-toxic compost. The water is chemically treated to make it contamination free. This water is either used in irrigation or returned to natural water bodies. This method of cleaning is called remediation. In an advanced technology, microorganisms are used to facilitate the bioremediation, i.e. decomposition and detoxification of wastes.

3. **Sanitary land filling** is a common method of solid waste management in bigger cities, where the hazardous waste is isolated from the environment until it is found safe. The waste is deposited in thin layers and compressed mechanically. Several such layers are formed and covered by a layer of compact soil. The waste inside the landfill breaks down and generates methane. Methane is often collected through a vent created on top of it and used as a fuel.

4. When adequate land is not available for land filling, incineration is executed. In this process, the solid organic waste is subjected to controlled combustion to produce nontoxic residues and gases.

5. Many toxic products after their use are recycled for making a variety of products. For example, plastic carry bags and pet bottles are thrown as garbage following their use. Such products create serious environmental pollution problems. These are recycled to produce a variety of products made out of plastic. In India, rag pickers, mostly women and girls collect reusable and recyclable materials from

the garbage dump and sell these to scrap traders. These materials are then sent to recycling units. The rag pickers work under an extremely unhygienic conditions, but yet render a great ecological service to the society.

Industrialized countries are faced with a serious waste management problem. The people have a continuous consumption and hence produce more waste. Due to lack of appropriate land filling sites and dumping yards and enforcement of strict environmental laws, these countries prefer to export the waste to developing countries, where the disposal is cheaper and environmental regulations are not that rigid. The developing countries expose their citizens to the damaging effects of the waste for some money in return.

In 1986, a cargo ship, Khian Sea, carrying 14000 tons of toxic fly ash generated from the incinerator in Philadelphia, travelled 16 years in the sea in search of a suitable place for unloading the ash. Thanks to the good efforts of the environmental organization, Green Peace that the ship was not given an entry into any harbour. Finally, in a secret effort, the crew succeeded and hardly 4000 tons were unloaded on the Haiti shore. Then the ship disappeared and was finally seen off the coast of Singapore, but this time without the load. The public protest against the act of unloading of the ash mounted in Haiti and the US government was forced to take back the ash. In 2000, the US government agreed to the demand and returned the ash to Florida. This is just a single wrong deed concerning the disposal of solid waste. There are many more such incidents.

17.6 RADIOACTIVE WASTE :

The conventional sources of energy such as oil and coal are slowly but steadily drying up and we all are faced with an energy crisis. Scientists searched for an alternate source and finally struck. This was nuclear energy. But looking at its massive destructive power, there was a rethinking. However, scientists and administrators assured the people that nuclear power was safe. Yet accidents have occurred all around the globe. The accidents in Chernobyl (1986) in Ukraine and Fukusima (2012) in Japan have forced all of us to rethink about the continuation of this practice. Accidents of minor scale have also occurred in the Sellafield Nuclear Complex in UK (1957), Hanford Nuclear Complex, USA (1973) and Three Miles Island Power Plant, USA (1979). Developed countries are now planning to switch over from nuclear power to another less hazardous source. There are two sources of radioactive waste : (1) Natural and (2) Anthropogenic or Human.

17.6.1 Natural Sources :

The cosmic radiation from the space and radioisotopes present in the soil sediments constitute the natural sources. The cosmic radiation is of extraterrestrial origin and consists of high energy photons and some heavier nuclei. These particles collide with the gas molecules of the atmosphere and bring about intense ionization accompanied with the release of neutrons,

mesons and gamma rays. All these particles together reach the surface of the earth as cosmic rays. Another natural source is the radioisotopes of carbon, potassium, lead, strontium, uranium and thorium occurring in the lithosphere as reserves. These also add to the ionizing radiation.

17.6.2 Anthropogenic Sources :

Man made sources are the major causes of ionizing radiation. These include: nuclear power plants; nuclear fuel processing plants; nuclear weapon manufacturing facilities; nuclear warfare; and research and investigation practice requiring radioisotopes. **There are three types of radioactive waste: low level**; **intermediate level**; and **high level**. Hospitals and laboratories emit low level of radiation. This level is not considered as a health hazard. Substances from nuclear power plants, like cleaning agents and sludge emit intermediate level radiation. These substances are encased in bitumen or concrete before being stored for disposal. Until 1980, this waste was dumped into the sea. However, an international convention prohibited sea dumping and recommended deep land filling. Spent nuclear fuel emits high level of radiation. This waste must be safely isolated for thousands of years.

1. Of all these sources, the nuclear power plants contribute most towards this misdeed. Firstly the safety of the plants is a major concern. We are very skeptical about this aspect because we have seen several episodes of nuclear disasters. Secondly, the safe disposal has been another major problem. It is estimated that by 2035, there will be 100,000 tons of this deadly residue. This is the story of spent nuclear fuel only in USA.
2. Besides the dropping of two nuclear bombs on Hiroshima and Nagasaki in 1945, there has not been any nuclear warfare so far. Who knows, man will not repeat it. The conduct of nuclear tests either on land or underground or underwater releases ionizing radiations and the fallout has serious consequences.
3. The mining operations of radioisotopes also pose a threat of radioactive fall out. The uranium mining facility in Jadugoda in the state of Jharkhand spells the whole story. The biodiversity in the area has depleted beyond recovery. The rare earth in the sandy beach of Chhatrapur in Odisha is another case in point.
4. Research and investigation activities also use radioisotopes of hydrogen, carbon, iodine, etc. Following the investigation, the spent isotopes must be disposed off safely so that there is no fallout. Bhaba Atomic Research Centre (BARC), Mumbai has fixed a standard for the safe disposal of this waste.

17.6.3 Radioactive waste management :

At each stage of radioactive fuel cycle, there are proven technologies to dispose of the radioactive wastes safely. Unlike other industrial wastes, the level of hazard of all nuclear wastes, its radioactivity diminishes with time. Each radioactive nucleides contained in the wastes has a

half life time taken for half of its atoms to decay, thus for it to lose half of its radioactivity. The most effective methods adopted for radioactive waste management or diluting the waste so as to make it harmless. This is done by deep and permanent burial.

17.7 GREEN HOUSE EFFECT AND GLOBAL WARMING :

People are in the habit of saying that the summer is really hot, hotter than yester years. They do not just bear it in mind. They experience it. Year after year the temperature is rising slowly but steadily. It is not a local, but a global phenomenon as evident from the Tuvalu story. Tuvalu is a chain of nine coral islands in the South Pacific Ocean with an area of 25 Km^2 and 11,000 inhabitants. The people of Tuvalu are now feeling the heat of global warming in a true sense. The main roads are washed by the high rising sea waves and the palm trees half submerged. Who knows, Tuvalu will not be eliminated from the political map of the world in 25-30 years now if the present trend continues. Tuvalu is not alone. Island nations such as Kiribati, Vanuatu, Marshall Islands, Cook Islands, Fiji and Solomon Islands may have the same consequence in the future. Many big cities of the world like Tokyo, New York, Mumbai, Shanghai, and Dhaka and parts of Florida, Netherlands, Belgium and Bangladesh may be submerged. It is said that all this is due to global warming, which is due to a steady rise in the temperature. The latter is known as **greenhouse effect.**

17.7.1 Greenhouse Effect :

We get heat from the solar radiation. The solar radiation is transmitted through the atmosphere and reaches the surface of the earth. However, a part of the heat energy is reflected back, which is absorbed by the cloud, water vapour and a number of gases. These gases have a property to trap the heat in much the same way as a green glass house. The concept of a greenhouse has relevance in countries, where the temperature is very low. At this temperature, the plants will not grow. Therefore, the plants are grown in a green house. The heat energy is trapped in the green house and the temperature is elevated thus facilitating the growth of plants. In the absence of green house gases in the atmosphere, the heat would radiate out and the surface would be far colder, largely covered by ice. Thus, the greenhouse effect is beneficial within its limit, while it becomes a concern, when the gases exceed the limit.

17.7.2 Greenhouse Gases:

Among the greenhouse gases, carbon dioxide is primary one. Others include methane, nitrous oxide, nitrogen dioxide, chlorofluorocarbons or freons, halons used in fire extinguishers, and tropospheric ozone.

(i) Carbon dioxide: Carbon dioxide is the most common and abundant greenhouse gas. It is generated from industrial and vehicular exhausts and by burning fossil fuel, solid waste and wood. Burning of fossil fuel (petrol and diesel) contributes

36%, coal contributes 35% and natural gas 20% of carbon dioxide. The rest is from the industries. The carbon dioxide concentration in the atmosphere has been rising steadily as evident from the recorded data. It has increased from 315 ppm (parts per million) in 1958 to 355 ppm in 1992 and then 389 ppm in 2010. If this rate of emission persists, a temperature rise of 1.5-4.5°C is likely by 2030. The major contributor of carbon dioxide is USA followed by Russia and European countries. China is no exception. It produced most amount in 2006. It is not a matter of serious concern for India at present. Better late than never. It is better to monitor the level of emitted carbon dioxide right from now.

(ii) **Methane:** Methane or marsh gas contributes only 4-9% to the global temperature rise. It is emitted from bacterial decay of organic matter, sewage treatment plant, landfill, paddy stubble and oil well.

(iii) **Nitrous Oxide:** It is emitted from the combustion of fossil fuel, decomposition of solid waste, industrial exhaust and agricultural practice.

(iv) **Nitrogen Dioxide:** It is emitted by burning fossil fuel and from vehicular exhaust. A part of it is also released by microbial action.

(v) **Chlorofluorocarbons ($CFCl_3$):** Chlorofluorocarbon is used as a common refrigerant and bromine containing compounds are used in fire extinguishers. These two are potent greenhouse gases. $CFCl_3$ remains inert in the troposphere. It moves up and under the influence of UV radiation, it dissociates into chlorine atoms. The highly reactive chlorine atoms react with stratospheric ozone releasing molecular oxygen.

$$CFCl_3 \xrightarrow[\text{radiation}]{UV} + CFCl_2 + [Cl]$$

Chlorofluoro carbon — Nascent Chlorine

$$O_3 + [Cl] \longrightarrow ClO + O_2$$

$$O_2 \xrightarrow[\text{radiation}]{UV} 2[O]$$

Nascent Oxygen

$$ClO + 2[O] \rightarrow O_2 + [Cl]$$

Fig. 17.2 : Chemical reactions leading to the depletion of stratespheric Ozone

(vi) Fluorinated Gases: Perfluorocarbons and sulphur hexafluoride are two potential greenhouse gases emitted mostly as industrial byproducts.These are often used as substitutes for CFCs and halons.

(vii) Tropospheric Ozone: Nitrogen dioxide (NO_2) released from primary sources dissociates into nitric oxide (NO) and nascent oxygen (O) under the influence of UV radiation. The nascent oxygen reacts with molecular oxygen forming ozone in the troposphere. Ozone is a toxic and corrosive greenhouse gas. It traps heat and adds to the greenhouse effect.

(viii) Water Vapour: This is a potential constituent contributing to greenhouse effect. It has enormous capacity to trap heat radiating from the surface of the earth. Following a rainy day, if there is sunshine, the temperature rises and often the climate is referred to as hot and humid.

17.7.4 Effects of Global Warming :

It is very difficult to predict the exact degree of effects of global warming. However, computer application studies on the climate indicate that there may be an average rise of 3°C by 2100. The effects may be very wide ranging such as on : (1) climate; (2) oceans and coasts; (3) glaciers and ice caps; (4) water and agriculture; and (5) plants and animals.

(i) Climate: The conventional climate pattern will change. There will be longer summer and shorter winter. We have started experiencing this change already. There will be unusual extreme weather conditions like heavy rainfall and flood and long stretching drought etc. There will be high intensity cyclonic storms hitting the coastline. We have had a recent experience of a super cyclone hitting the coastal Odisha in 1999.

(ii) Oceans and Coasts: A rise in the global temperature will speed up the melting of polar ice caps and glaciers. Consequently, the ocean water level will rise submerging many island nations and many coastal cities. The warmer water will accelerate the vanishing coral reefs.

(iii) Glaciers and Ice caps: Scientific data shows that the ice cover of the Arctic Ocean has declined over the past 30 years. This will have serious consequences like rise in the ocean water level and extinction of indigenous animals such as polar bears, seals and some sea birds. The ecology of the region will completely change. The mountain ice caps and glaciers will melt. This will induce untimely land slides and flood in the rivers originating from these glaciers. If this process continues, the rivers will gradually dry up. This apprehension is taking root in respect of Ganga, Yamuna and Brahmaputra originating from Himalayan glaciers. The recent menacing effects of cloud burst, flash flood and land slide in Uttarakhand is an eye opener. There was a heavy loss of property and human lives in the disaster.

(iv) **Water and Agriculture:** Accompanied by the rise in global temperature melting of glaciers and rising sea level, there will be loss of arable land and whatever agricultural practice done will yield decreased productivity.

(v) **Human, Animals and Plants:** Due to the changing climate, thousands of species of plants and animals will become extinct within a short period of time. Extreme climate will force the people living in the coastal areas to migrate.

17.7.4 Reducing Greenhouse :

When it was felt that the global warming was a fact, the World Meterological Organization and United Nations Environment Programme (UNEP) jointly set up the Intergovernmental Panel on Climate Change (IPCC) in 1988, a task force of climate scientists from nearly 100 countries. The group issued the first assessment report in 1990. The report aroused the participating nations at the Earth Summit in Rio de Janeiro (1992) to create the United Nations Framework Convention on Climate Change (UNFCC), which came into force in 1994. The objective was to establish national inventories of greenhouse gas emissions and their removal. This was a benchmark for the stakeholders in **Kyoto Protocol in 1997**. The convention was held in Kyoto, Japan in 1997, which was signed by 160 countries. The objective was to reduce the emissions of carbon dioxide, nitrous oxide and methane by 5%, besides reducing hydrofluorocarbon, perfluorocarbon and sulfur hexafluoride. With the Kyoto Protocol due to expire in 2012, the **Copenhagen Conference** was held in 2009 under the aegis of UNFCC. There was no unanimity in agreement among the participating countries and hence it failed.

17.8 OZONE DEPLETION IN THE STRATOSPHERE :

Ozone (O_3) is a poisonous gas made up of three atoms of oxygen. It is an extremely rare gas in the atmosphere. 90% of it occurs in the uppermost part of the atmosphere i.e. stratosphere between 10 – 50 km above sea level. It forms a blanket of the atmosphere. The rest is formed as a secondary pollutant in the troposphere. The ozone layer of the stratosphere absorbs the UV-B radiation of the sun. It also absorbs more dangerous UV-C radiation completely. More UV-B radiation will reach the surface of the earth by the depletion of the ozone layer. The consequence will be an increasing susceptibility to skin cancer, cataract, weaker immunity and decreased plant productivity.

CFCs, used as a common refrigerant and aerosol propellant, release nascent chlorine (chlorine atom), when activated by UV radiation. The chlorine atom reacts with stratospheric ozone and dissociates it into molecular oxygen and atomic oxygen. Bromine atom from halon (used in fire extinguisher) has a similar effect.(see Fig. 17.2) These are called ozone depleting substances (ODS). Scientific investigations reveal that the ozone layer is depleting alarmingly over Arctic and Antarctic regions. The ozone layer has become thinner by 30% over the Arctic and over Europe and many other places by over 5-30%. If this trend continues, the stratospheric

ozone will be confined to books only and the earth will receive sun's most of the UV radiation The quantum of catastrophic changes is unthinkable.

17.8.1 Reducing Ozone Depletion :

The depletion of the ozone layer became evident only in the 1980s. This initiated the negotiations among many countries in 1981 for an international agreement to phase out the ozone depleting substances. This effort led to the adoption of the **Vienna Convention** for the protection of ozone layer in 1985. However, there was no legal binding on the issue. This fact boosted the governments to adopt stronger measures for reducing the production and use of CFCs and many halons. As a result, the **Montreal Protocol** on Substances that Deplete the Ozone Layer was adopted in 1987. Ninety-six ozone depleting chemicals were listed for phasing out. The protocol became the first ever UN treaty to be ratified by all the member countries in 2009. This indicates the world community's commitment towards creating a healthy environmental cover for all.

18.8.2 Impact of Montreal Protocol :

The Montreal Protocol started paying dividends. The global ozone depleting substance concentration was decreasing and it decreased by over 98% since the enforcement of the Montreal Protocol. It is assumed that with this rate of decrease in the ozone depleting substances, the global ozone layer will recover by 2050. The Antarctic region, however, is expected to recover by the 2060-2075.

17.9 DEFORESTATION :

Like other valuable resources, the forest cover is also depleting very fast. From 6 billion hectares, 8000 years ago, it has declined to 3.6 billion hectares in 1999. 56 countries have lost 90-100% of their forest resources. This was a report by the World Commission on Forests and Sustainable Development (WCFSD). A similar finding was presented by the Food and Agricultural Organization (FAO) independently in 2010.

Forests provide an appropriate habitat to diverse groups of plants and animals. It provides timber, fuel wood, and many valuable products like honey, lac and medicinal plants. It absorbs carbon dioxide, prevents soil erosion, holds excess water and recycles nutrients.

Forests are cleared for timber, fire wood, making paper, new human settlements, setting up industries and mining operations. These are all anthropogenic sources of depletion. Natural calamities like earthquake, volcanic eruption, landslide, and forest fire are some natural sources of depletion. Deforestation leads to the destruction of natural habitat of many species of wild plants and animals. These either migrate to human settlements in search of shelter and food or face extinction. The topsoil is eroded and this accelerates silt deposition in dams and reservoirs.

Trees absorb a considerable amount of carbon dioxide, released by anthropogenic activities. Therefore, deforestation increases carbon dioxide concentration and adds to global warming. Local and global climate changes may also occur. By clearing forests, the local communities lose their sources of food, fuel wood, construction materials and much more.

The importance of forests and the circumstantial loss due to deforestation is noteworthy. The Andaman and Nicobar Islands have one of the world's finest evergreen forests. Another uniqueness of the forest is the local communities like the Great Andamanese, the Onge, the Jarawas, and the Sentinelese. They have lived there for at least 20,000 years. The population of these four tribal communities was 5,000 around 150 years ago. But today their population is left at a mere 500. If appropriate measures are not adopted by the government, they could become extinct soon. The forest resource has been plundered from time immemorial for the finest ever timber. This act still continues unabated. A 340 km long Andaman Trunk Road from Port Blair in South Andaman to Diglipur in North Andaman cutting across the evergreen forest was constructed. The consequence was: (1) clearing of a considerable part of the virgin forest and (2) more importantly, the original way of life of the native tribal communities, particularly of the Jarawas was eroded. Therefore, It is to be noted that deforestation does not merely result in a loss of biodiversity, it also ends ancient cultures.

(i) Forest Conservation: It is now or never situation. Whatever is gone is gone forever. Whatever little is left should be conserved for the benefit of the present and future generations. This concept applies to all the natural resources so to forests.

1. 21st March is observed as World Forestry Day as a token for making people conscious about the importance of forests in the sustenance of their lives.
2. The government should pass strong legislations concerning the conservation of forests with provisions for penalties in appropriate form for any violation and indeed the Government of India has passed a Forest Conservation Act in 1980 and it is in force.
3. The management of forests should be practiced by the government and the local community jointly. This is known as **joint forest management** . This concept came into force in 1980s. The local communities are involved in the planning and conservation of the forests. They are allowed conditional access into the forest for collecting the resources in a sustainable manner. In return, they safeguard the forests. This practice has started paying dividends.
4. Local community should be encouraged to plant trees in unused and degraded government land. The trees should be fast growing which would meet the fuel wood need of the communities. This concept, known as **social forestry**, was introduced in 1976 in India.

5. The involvement of the local communities in the conservation of forests and its success is exemplified by the **Chipko movement** in Tehri Garhwal of Uttarakhand. Inspired by Sundarlal Bahuguna and Chandi Prasad Bhat, women from villages put strong resistance to forest contractors in a bid to prevent them from cutting trees. This movement was grandly successful and was highly acclaimed globally.
6. The management should be sustainable such that the local communities harvest the forest products and conserve the resource for harvest by the next generation in the same token.

17.10 SUCCESS STORIES OF ENVIRONMENTAL CONSERVATION :

17.10.1 Community Participation for Conservation of Forest - a Case Study (I):

Degradation of the environment is a major challenge for mankind. Its effects are clearly visible on land, water and atmosphere. The problem is becoming more and more serious. The primary cause of this is the loss of forest cover. Deforestation occurs due to increase in human population, industrialization, overutilization of natural resources, and our failure to act in a responsible manner at all levels. There are lots of discussions on forest conservation to tackle deforestation. However, these are generally not translated into action. Generating public awareness and continuously involving them in the process can make a lot of difference. This has been demonstrated by the conservation of the **Andhari forest** of Jharsuguda district of Odisha.

The Andhari forest is located in the Laikera and Kirmira Blocks of Jharsuguda district. It has an area of more than 1000 hectares and is one of the biggest ‘Sacred Groove’ in the country. The highest peak of Andhari, called Chounabandh(1400 ft) is believed to be the abode of Goddess Andhari. People have been worshipping Goddess Andhari from time immemorial. This bond of faith with the Goddess became instrumental for its Conservation.

The dense Andhari forest was badly denuded by 1980. In 1988 Government introduced the Joint Forest Management scheme with people’s participation. A local social activist, Er. Subrat Kumar Naik, persuaded people of 15 villages around the Andhari forest to form Van Samrakshyan Samiti - Forest Protection Society (VSS) in their respective villages. The VSS are registered under the Forest Department and local Forest Officials supervise their activities. Uniting all the 15 VSS a Federation, the ‘Maa Andhari Bana Surakshya Samiti Mahasangha’, was constituted. Representatives of all 15 VSS constitute the Mahasangha. Members of each VSS are responsible for guarding and protecting the forest area assigned to them. They are allowed to collect dry fire wood for their use free of cost. For timber needed for construction of house and for agricultural tool (plough etc.), they pay a subsidized price to the VSS. Meeting of the Mahasangha is held in every village by turn at least once a month. Disputes, if any, within

the VSS or between neighbouring VSS is sorted out by the Mahasangha. Volunteers of the Mahasangha go round the forest from time to time, to check unauthorised cutting of trees. Occasionally volunteers drawn from all VSS make surprise checks of every household in all villages under the Mahasangha at the same time in the presence of Forest Department Officials. Fines are imposed, whenever necessary, which goes to the Mahasangha fund.

The key to success of conservation of the Andhari forest was the unity of all 15 VSS around it through the Mahasangha. The uniting factor is the faith of people on Goddess Andhari. Subrat explains that the real Puja of 'Maa Andhari' would be to conserve the forest. With this aim he started the Maa Andhari -Banadurga Puja in 1994 with the cooperation of all 15 VSS. It is held on the Eighth Day of the bright lunar fortnight of the month of Kartik. An idol of Maa Andhari - Banadurga is taken to the hill top (Chounabandha) for worship. People assemble in large numbers and Durga Puja is performed without animal sacrifice. The next day (Anla Navami) the idol is brought to the weekly market place at Pakelkhol-Kuanrmal and kept for 'Darshan'. In the evening a meeting is held for generating environmental awarewness and children enact conservation related drama. Such sustained efforts from 1994 to 2008 resulted in the regeneration of the Andhari forest which is visible in satellite imagery.

In 2008 Subrat died of a road accident. His father, Prof. D. R. Naik, Former Vice-Chancellor of Sambalpur University (1998-2001) is continuing his son's work. He has added some new dimension to it. A souvenir ANDHARI is published to motivate school children. A cycle rally by school children and villagers is held during the Dusshera festivals. Prof. Naik leads the rally himself. The rally goes around the Andhari forest touching all 15 villages and covers a distance of about 25 kms to spread the message of forest conservation.

The conservation of Andhari forest should serve as an example for others. Tiny rain drops make big oceans. Similarly we should conserve the small patches of forest in our neighbourhood to make a Green Earth. Let us think globally and act locally.

Community Participation for Conservation of Forest - a Case Study (II)

It is worth mentioning here that, in 1970s, an organized resistance to the destruction of forests spread throughout India and came to be known as the **Chipko movement**. The name of the movement comes from the word 'embrace', as the villagers hugged the trees, and prevented the contractors from felling them. The first Chipko action took place spontaneously in April 1973 in the village of Mandal in the upper Alakananda valley and over the next five years spread to many districts of the undivided Uttar Pradesh. It was sparked off by the government's decision to allot a plot of forest area in the Alaknanda valley to a sports goods company. This angered the villagers. With encouragement from a local NGO, Dasoli Gram Swarajya Sangh (DGSS), the women of the area, under the leadership of an activist, Chandi Prasad Bhatt, went into the forest and formed a circle around the trees preventing the men from cutting them down.

The success of the Chipko movement in the hills saved thousands of trees from being felled. Mr Sunderlal Bahuguna, a Gandhian activist, was later involved in this movement. His appeal to Mrs Indira Gandhi, the then Prime Minister of India, resulted in the green-felling ban. Mr Bahuguna coined the Chipko slogan: 'ecology is permanent economy'. The Chipko protests in undivided Uttar Pradesh achieved a major victory in 1980 with a 15-year ban on green felling in the Himalayan forests of that state by the order of the Prime Minister of India. Since then, the movement has spread to other states in the country.

17.10.2 Controlling Air Pollution in Delhi – a Case Study:

Air pollution is the single most critical problem faced by the inhabitants of Delhi. World Health Organisation (WHO) reports Delhi as the fourth most polluted city in the world in terms of suspended particulate matter (SPM). Delhi's air pollution problems include heavy smog and very high concentrations of harmful SPM arising out of a mixture of dust, smoke, soot, liquid droplets and pollens which become dangerous and very often hazardous for life. Harmful pollutants reach level more than 16-times of safe limit of 60. Air quality of Delhi is the worst for the last several years. The sources of pollution load of Delhi are principally due to road dust, automobile exhaust, industrial emissions and open fires including cooking by burning wood. Deforestation adds to the deteriorating air quality. Airborne pollutants rarely stay within safe limits even during summer when wind speed is stronger that can disperse particulate matters easily. Air quality worsens heavily during winter. Due to bad air quality, the people of Delhi suffer from serious health problems. They suffer from respiratory disorders; feel suffocated with the burning of eye anytime during the day.

Government of India as well as the Government of Delhi NCR have taken several measures to keep the air pollution level of Delhi within limits. The Hon'ble Supreme Court of India and the National Green Tribunal have also taken the deteriorating air quality of Delhi very seriously. The Central Pollution Control Board is monitoring the air quality rigorously. The Government initially encouraged and subsequently mandated Delhi's fleet of local buses to run on compressed natural gas (CNG). Taxis, auto rickshaws and domestic vehicles mostly run with CNG. CNG is not only less polluting, it is also cheaper than diesel. The polluting industries have been shifted to the outskirts of the city. They have been asked to take appropriate control measures to keep their emissions free of pollutants as far as possible. To decrease the pollution level, the government had launched an experimental scheme for a short period to reduce the number of motor vehicles to half on roads of Delhi by allowing the vehicles on odd and even days depending on the last digit of the registration number. As the burning of huge quantity of agricultural wastes cause serious pollution problem, the power plants have been asked to buy paddy and wheat stubbles to use as fuel for power generation. A number of awareness programmes have been launched for the purpose of keeping air pollution free.

Recently the Hon'ble Supreme Court of India has banned the sale vehicles not compliant with Bharat Stage IV (BS IV) emission standards after 31st March 2017 through out the country for curbing vehicular pollution. Honest attempts are being made to keep the air quality of Delhi within tolerable limits.

17.10.3 Community Participation for Rain Water Harvest to Fight Drought – a Case Study of Lapodiya Village of Rajasthan :

Lapodiya, a village of 200 households about 80 km from the state capital Jaipur in Rajasthan, is a shining example of how the residents of this village have managed to fight drought and save the village from pollution and natural disasters. Here, people have adopted innovative water conservation practices which have since been improvised and perfected over three decades. The villagers plan out an innovative practice of creating 'Chauka'. These are the square dykes that the villagers have dug in the fields that trap just enough water for soil productivity and allow excess water to flow through. Chaukas form a series of interconnected water dyke with a gap left on one side, so that there is an unhindered flow of water from one Chauka to another. Rows of Chaukas have been dug five feet apart. Utilizing each drop of rainwater, Chaukas replenish aquifers and also serve as drinking troughs for the village livestock. *A series of interconnected Chaukas have been dug in Lapodiya village that help conserve enough water for soil productivity and check water logging during rains.* With adequate water, different varieties of grasses have been sown along Chaukas, to provide fodder for village livestock. One can find about 30 types of grass here.

The residents have seen for themselves the results of water conservation. Adjoining villages suffered seriously from drought in 2003 and again in 2007. In both years, the 100-odd wells in Lapodiya remained full. Residents of Lapodiya have also cleaned three ponds that had been dug some two decade ago but had never been maintained. The cleaning out has helped improve the water table and there are more grasses growing on the banks. The renovated ponds have now been reserved for specific purposes. Phool Sagar (flower pond) is used only to water plants. Dev Sagar (pond for the gods) is used only for religious rituals. Anna Sagar (food pond) is used for irrigation. In an annual celebration, residents pay homage to these ponds. For the last several years, the residents have also been tracking climate change. They have obtained a small weather station, and keep regular measurements of rainfall, humidity and wind velocity. They also track the water table, biodiversity and other environmental parameters.

Entering into Lapodiya, the first sign board one notice thanks residents for their voluntary labour that has helped the village common pasture to flourish. The board requests every one not to pester the wild animals that share the pasture and notifies a complete ban on cutting trees or bushes or on any encroachment in the pasture. It also warns that anybody breaking these rules will be punished. The mindset of the villagers has changed for the better. Conservation is a part of religion in the village. At an open temple, there are clear instructions that water

bodies belong to Indra Devta (the rain god). If anyone spoils the ponds or spills garbage, Indra would get angry and the entire village would suffer from famine.

An NGO, Gram Vikas Navyuvak Mandal (GVNML), works on the conservation in Lapodiya and a cluster of 50 villages around it. The villagers have been inspired by slogans like “Shradha Karm” (efforts with humility). This adds a sense of pride to the villagers to serve their habitats in their own capacities. This is active volunteering by the local people. They spend their time and sweat for the sake of village development work and take collective decisions for all community initiatives. Whether it is digging pits for water harvesting, cleaning drinking water bodies routinely, planting trees in appropriate season or toiling for maintenance of protected areas, they participate for each work. In this drought prone region, villagers of Lapodiya clearly understand the nature of the environmental problems and its implications. And they are successfully taking steps to tackle the climatic change and conserve nature.

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SAMPLE QUESTIONS

GROUP - A
(Objective-type Questions)

1. Answer from the choices given under each bit:

(i) Which gas leaked from Union Carbide's Pesticide Plant in December 1984 is responsible for Bhopal Gas Tragedy?

(a) Methyl salicylate (b) Methyl isocyanate
(c) Ammonia (d) Hydrogen sulphide

(ii) Minamata disease is caused by the consumption of fish contaminated with:

(a) Lead (b) Copper
(c) Zinc (d) Mercury

(iii) The toxic metal used as an anti-knocking agent in petrol for automobiles is:

(a) Chelated copper (b) Tetraethyllead
(c) Iron sulphide (d) Leadchloride

(iv) Bharat Stage emission standards came into force from the year:

(a) 1998 (b) 2000
(c) 2006 (d) 2010

(v) Bone and tooth decay disease is caused by drinking water contaminated with:

(a) Fluoride (b) Borate
(c) Silicate (d) Aluminium

2. Express in one word:

(i) Removal of toxic substances from water by using living organisms.

(ii) Toxic compound formed by the reaction of carbon monoxide with haemoglobin in blood.

(iii) Enrichment of water bodies with excess amount of nutrients as a result of run off from surrounding land leading to overgrowth of plants and algae.

(iv) A kind of air pollutant named for the mixture of smoke and fog in the air.

3. Correct statement, if necessary, by changing underlined word(s) only:

(i) Fifth June of each year is usually observed as World <u>Food Security</u> Day.

(ii) The process of nutrient enrichment in water bodies is called as <u>biomagnification</u>.

(iii) Particulate matter formed by the combination of gas and water vapour is called as <u>smog</u>.

(iv) Chipko movement was organised for the protection of <u>water bodies</u>.

4. Fill in the blanks :

(i) The environment Protection Act was enacted in the year _____.

(ii) The common refrigerant responsible for the depletion of ozone layer of the atmosphere is _____ .

(iii) Carbon monoxide binds with haemoglobin forming _____ .

(iv) Depletion of ozone layer is speeded up by the _____ atom present in CFC.

GROUP – B
(Short Answer-Type questions)

1. Write within three valid points :

(i) Aerosol

(ii) Greenhouse effect.

(iii) Eutrophication.

(iv) Acid rain

(v) Photochemical smog

(vi) Global worming

2. Differentiate between the pairs:

(i) Aerosol and Smog

(ii) Renewable resources and non-renewable resources

(iii) Bioremediation and Eutrophication

(iv) Primary pollutant and Secondary pollutant

GROUP – C
(Long Answer-Type Questions)

1. Give an account of secondary air pollutants.
2. How can the industrial and vehicular emissions be controlled, describe.
3. Write the causes of ground water pollution and state how this can be controlled.
4. Write about the different classes of solid waste.
5. What are green house gases? Write about their effect on the environment.
6. Write the causes and consequences of global warming.

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