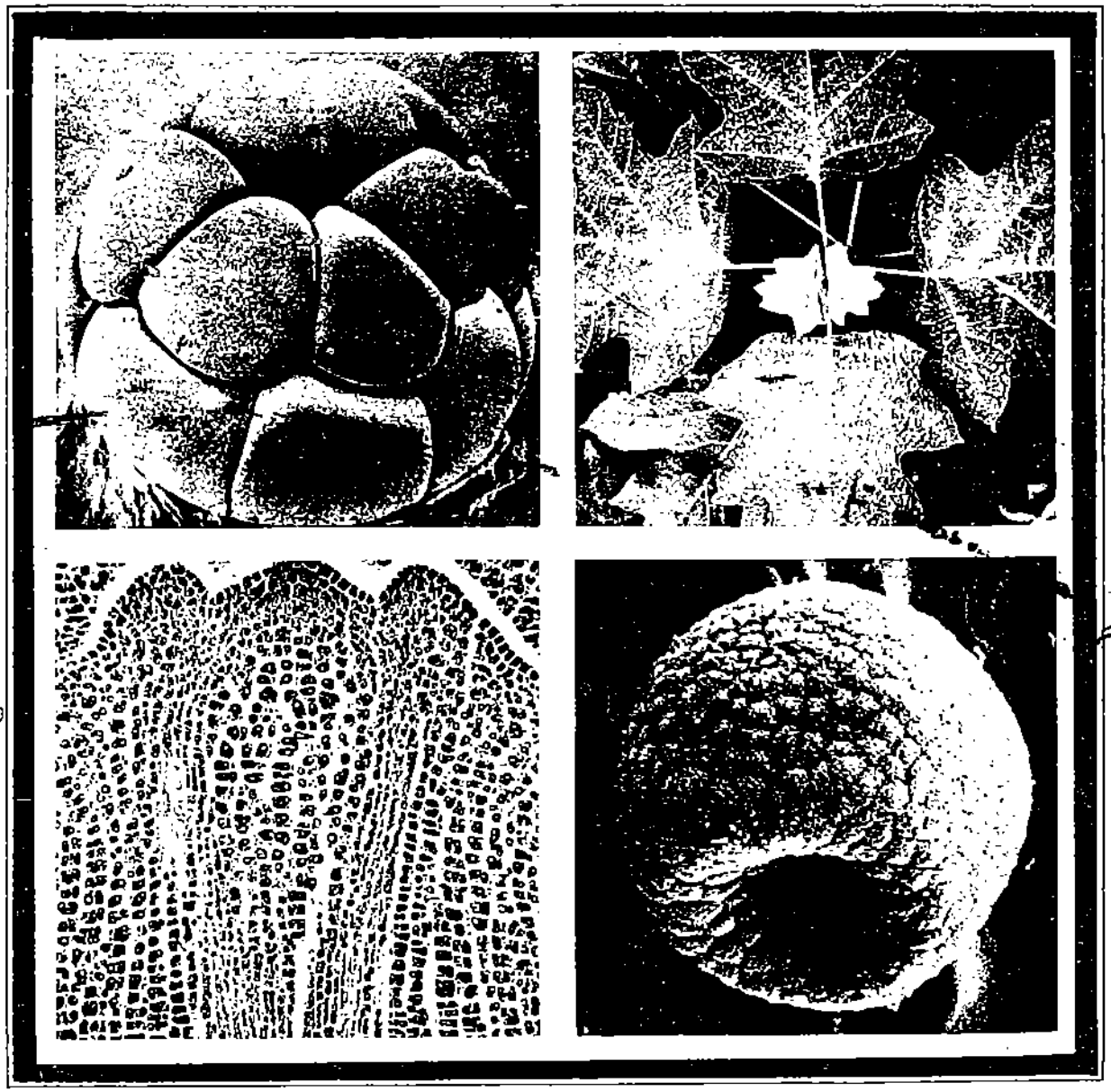
UG24/BY-09

Indira Gandhi National Open University
School of Sciences

LSE-06
DEVELOPMENTAL BIOLOGY



Plant Development-I

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UTTAR PRADESH
RAJARSHI TANDON OPEN UNIVERSITY

Developmental Biology

Block

1

PLANT DEVELOPMENT-I

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DEVELOPMENTAL BIOLOGY

Developmental biology is a vast, fast growing multidisciplinary science concerned with the study of developmental phenomena in animals, plants and microorganisms. A butterfly egg hatches into a caterpillar that grows and changes into pupa from which another butterfly emerges or a gram seed germinates, forming a plant, that in turn reproduces to form seeds all over again. All this is development. An adult lizard loses its tail and grows a new tail. This is also development.

Organisms are not produced fully formed as if by a miracle of creation. Their genesis proceeds and is completed through a series of orderly and progressive changes. In all sexually reproducing organisms, be they plants or animals, development begins from a single cell, the fertilized egg or zygote. Through a process of precisely coordinated step by step changes, the cells derived from zygote give rise to a new individual having the form distinctive of the parents characteristic of the species concerned. The main concern of developmental biology is to discover what processes are involved and how they are controlled particularly in complex multicellular organisms that originate from a single reproductive cell (zygote) or from a small group of somatic cells in case of asexual reproduction and regeneration.

In the early history of developmental biology, emphasis was laid mainly on the descriptive and comparative studies of development of embryo and various tissues and organs. There was an equal emphasis on the experimental approach and methods. Important scientific breakthroughs and technical advances since the middle of present century, particularly in biochemistry, genetics, cell biology, and molecular biology, have transformed developmental biology into a multidisciplinary science. In the present times the basic thrust in developmental biology is to understand the processes occurring at each level, from molecular to cellular to anatomical, at every stage of development from egg onwards and to discover how all of them together give rise to increasingly complex structures at successive stages. As we pointed out earlier, developmental biology now has become a broad-based multidisciplinary science, linked with almost all biological and also physical sciences benefiting immensely from new technologies originating in all these disciplines.

Organisation

This is a four credit course which is organised into four blocks. In this course the development in plants and animals have been dealt with independently, with two blocks each devoted to plant and animal systems. Such an arrangement became a necessity in view of the exclusive developmental processes and mechanisms characteristic of each group. Such processes and mechanisms are in fact to be viewed as adaptations to the mode of life which each group has evolved over millions of years. The diversity in developmental processes between plants and animals appears to be unique considering the fact that both the groups of sexually reproducing organisms begin their life cycle from a single cell viz. zygote.

Block-1 Plant Development-I: This block covers mainly the developmental aspects that are related to sexual reproduction in flowering plants.

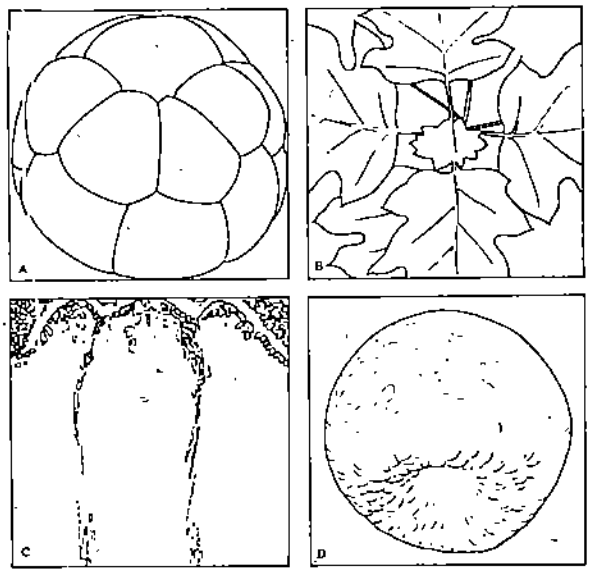
Block-2 Plant Development-II: The various developmental processes other than reproduction form the subject matter of this block. Phenomena such as root and shoot morphogenesis, apical dominance and secondary growth are discussed in this block. Further, this block also briefly deals with the current trends in developmental studies in plants.

Block-3 Animal Development-I: This block deals with development during embryonic period, more specifically the mechanisms that transform a single-celled zygote to a three layered structure the gastrula. We also discuss morphogenesis of specific organs and organ systems from the germ layers.

Block-4 Animal Development-II: This block comprises phenomena of metamorphosis, regeneration, ageing and cancer that occur during post-embryonic life, and further there is an unit on human development.

What you should already know

We assume that you are familiar with the our Cell Biology Course (LSE-01) as it is a prerequisite for this course. It will be useful to refresh your memory of what you have learnt in your Genetics course (LSE-03) particularly the units 14, 15 and 17; units 8 and 10 of Physiology-II (LSE-05); and unit 10 of Evolution-1 (LSE-07), before you start a study of this course.



A) Scanning electron micrograph of 16 cell stage of frog embryo (after L.M. Beidler).

B) Top view of plant showing opposite, decussate arrangement of leaves (Courtesy: Professor H.Y. Mohan Ram).

C) Longitudinal section of shoot tip showing apical meristem (Courtesy: Professor M.R. Vijaya Raghavan).

D) Early gastrula of sea urchin showing invagination in the vegetal plate (after Morril and Santos).

Dedicated to
Late Professor S.C. Goel

BLOCK 1 PLANT DEVELOPMENT - I

In flowering plants, like all sexually reproducing organisms, development commences from a fertilized egg or zygote. It develops into an embryo which lies protected in the seed within the fruit. On getting the conditions favourable for growth, the embryo develops into a plantlet. In the early stages of embryogeny two distinctive regions are set apart, approximately at apposite poles, that subsequently retain the capacity for continued growth. One of these, the shoot apical meristem, functions to form the shoot system. The other one, the root apical meristem similarly forms the expanding root system. The activities of apical meristems result in the production of the primary body of the plant. In many plants, there is an additional component of development — the secondary growth, which leads to an increase in girth of the axis. When the plants reach sexual maturity, they bear flowers that harbour the male and female reproductive organs. The gametes that are formed in these two organs contribute to form the zygote all over again, and thus begins the story of development of another plant.

In this course, we have discussed the details of development in plants, not exactly in the same sequence as described above but we have picked up the threads somewhere in between. In the first unit, Anther and Ovule — we begin by taking up the structural organisation in relation to the functional aspects of the male and female reproductive organs. These are the seats for the production of the pollen and embryo sac — the male and female gametophytes of the flowering plants.

The microspore nucleus undergoes mitotic division forming a large vegetative cell and a small generative cell. The latter further divides forming two sperm cells — the male gametes. On the other hand, the functional megaspore organises into an embryo sac. Present in the embryo sac is the egg cell which is the female gamete. In Unit 2 — Gametogenesis. you would study about the events that lead to the formation of male and female gametes.

Unit 3 — Pollination and Fertilization. After the formation of the male and female gametes, the two crucial steps of sexual reproduction follow, i.e., pollination and fertilization. The result is the formation of zygote that eventually develops as the embryo which remains protected in the seed. In nature, the successful accomplishment of these processes is dependent on a number of factors, that too, you would study in this unit.

Unit 4 — Endosperm. One of the peculiarities of angiosperms is double fertilization, resulting in the formation of zygote and a triploid tissue — the endosperm. The tissue is interesting not only from the point of view of its origin, but also of its structure. This in fact is the nutrient store house of the embryo locked up in the seed. In this unit you would study the salient morphological, structural and cellular details and functions of endosperm.

Unit 5 — Embryogenesis. After the fertilization of the egg cell, the zygote develops into an embryo. A study of this unit will familiarise you with the various events during the development of embryo. You would also learn how to differentiate the dicotyledonous embryo from the monocotyledonous one. The role of special structures such as suspensor and endosperm in the proper development of embr also dealt with in this unit. Usually one may expect one embryo per seed, but there are instances when more than one embryo also develops. We shall also explore these multiple embryos from the point of view of their origin, development and their utility.

Unit 6 — Seed and Fruit. The post-fertilization development of the ovule and the ovary results in the formation of seed and fruit respectively. In this unit you would study about the development of seed and fruit, the various kinds of appendages present on the seed surface, the nature of food reserves and the common seed dispersal mechanisms. Two interesting aspects, that is, parthenocarpic development of fruit, and the phenomenon of vivipary are also taken up in this unit.

Objectives

After studying this block you should be able to:

- describe the structural organisation and the function of various tissues composing the androecium and gynoecium;
- discuss and compare the events that lead to the formation of male and female gametes;
- elaborate the process of pollination and fertilization, highlighting the important factors that influence/affect these processes;
- explain the origin, structure and importance of endosperm;
- elucidate embryogenesis in dicotyledonous and monocotyledonous plants, highlighting the role played by special structures such as suspensor and endosperm
- describe the post-fertilization events that lead to the development of seed and fruit

UNIT 1 ANTHER AND OVULE

Structure

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1.1 INTRODUCTION

The first unit of developmental biology in plants describes the male and female reproductive structures, i.e., the androecium and the gynoecium, of the flowering plants. These two structures are responsible for producing the pollen and the embryo sac, respectively which represent the gametophytic generation. During the course of evolution, these structures, particularly in the angiosperms, have become highly specialised, i.e., they have developed various kinds of cells and tissues, each responsible for performing specific function. In this unit, you would study the development and structure of the androecium and gynoecium in relation to their functions.

Objectives

After studying this unit, you should be able to:

- describe the early developmental stages of an anther;
- depict the structural organisation of an anther;
- relate the origin, structure and functional interrelationships among the tissues that compose microsporangium;
- differentiate between amoeboid and secretory type of tapetum;
- explain the sequential events associated with microsporogenesis;
- depict the structural organisation of a typical ovule;
- trace the events in the development of ovule;
- discern the various types of ovules;
- recognise and explain the specialisation in various parts of the ovule;
- follow megasporogenesis.

Study Guide

Before you begin to study this unit, you should have a clear picture of the concept of androecium and gynoecium. *Androecium* represents the collection of *stamens* in a flower. And each stamen in turn consists of an *anther* and a *filament*. *Pollen grains* are produced in *pollen sacs* located inside the anther lobes. When pollen grains become mature, the anthers split open, releasing the pollen. The *gynoecium* represents a collection of *carpels*. You may recall that a carpel consists of a *stigma*, *style* and *ovary*. The ovary encloses the *ovule* or the *megasporangium*, in which the *megaspores* and the *female gametophyte* develop. The latter, after fertilisation produces the *embryo* and *endosperm*, while the entire microsporangium with its enclosed structures, becomes the seed — the unit that ensures link with the next generation.

In this unit you would see many diagrams. Do not skip them, but use them to clear your concepts and make your study of the unit meaningful. Therefore, spend some time on each diagram, as and when it is referred to. You will also come across several botanical names as examples of different situations. You need not memorise all of them, but try to remember at least a few.

Prior Reading

A revision of the following two units, would be helpful in a better understanding of some of the concepts dealt in this unit, particularly the Subsection 1.2.3.

Unit-17 of LSE-01, Cell Biology course;

Unit-03 of LSE-03, Genetics course.

1.2 ANTHER

A typical anther has four microsporangia, (tetrasporangiate) two in each lobe (see Fig. 1.1). One of the easiest methods of studying the structural details of the anther is by cutting a transverse section of the anther.

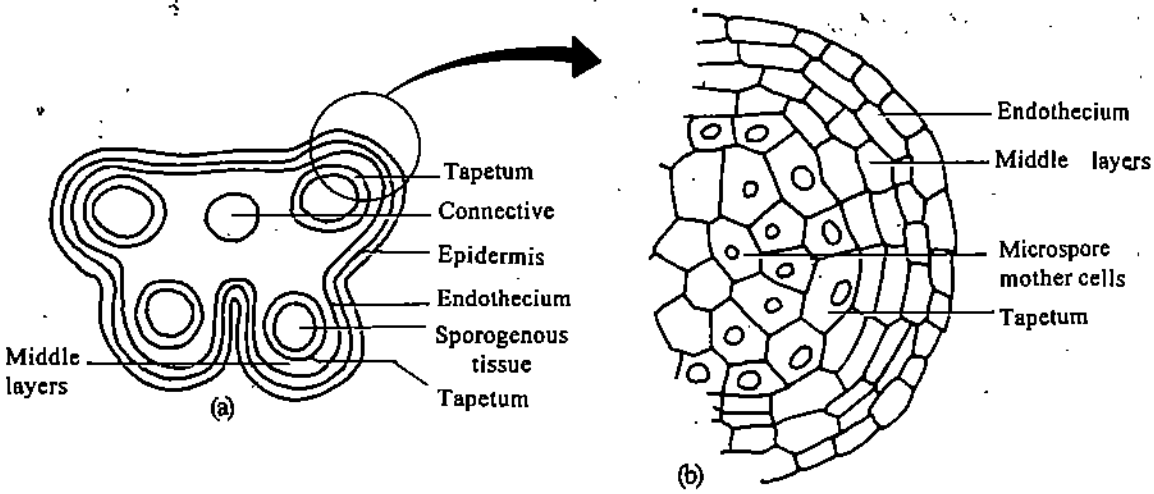


Fig. 1.1: (a) Diagrammatic representation of a typical anther, cut in transverse section. (b) A portion enlarged to show the basic cell layers/tissues.

1.2.1 Development

A young anther consists of a homogeneous mass of meristematic cells that are surrounded by an epidermis (see Fig. 1.2a).

As the anther develops further, it begins to assume a four-lobed appearance, and one or a group of two or more hypodermal archesporial cells differentiate in each lobe. These cells appear conspicuously different from the rest as they are larger, densely cytoplasmic and have prominent nuclei (Fig. 1.2b). These are the *archesporial cells* (see Fig. 1.2b, arrow). The archesporium may be either single-layered or made up of several vertical rows of cells. The latter in a transverse section of anther appears as a plate of cells (see Fig. 1.2c).

The archesporial cells divide in a plane parallel to the outer wall of the anther lobe. Such divisions are known as *periclinal divisions*. After one such division, one **primary parietal cell** (towards the epidermis, abbreviated as PPC), and another **primary sporogenous cell** (towards the interior of the anther sac, abbreviated as PSC) are formed (see Fig. 1.2d). The PPCs by repeated periclinal and anticlinal divisions (at right angles to the surface) give rise to 2 to 5 layers of the anther wall. And the cells of the primary sporogenous layer, either directly or after a few mitotic divisions form the **microspore mother cells** (abbreviated as MMC). The schematic representation of the ontogeny of the anther wall layers and the microspore mother cells is presented in Figure 1.3.

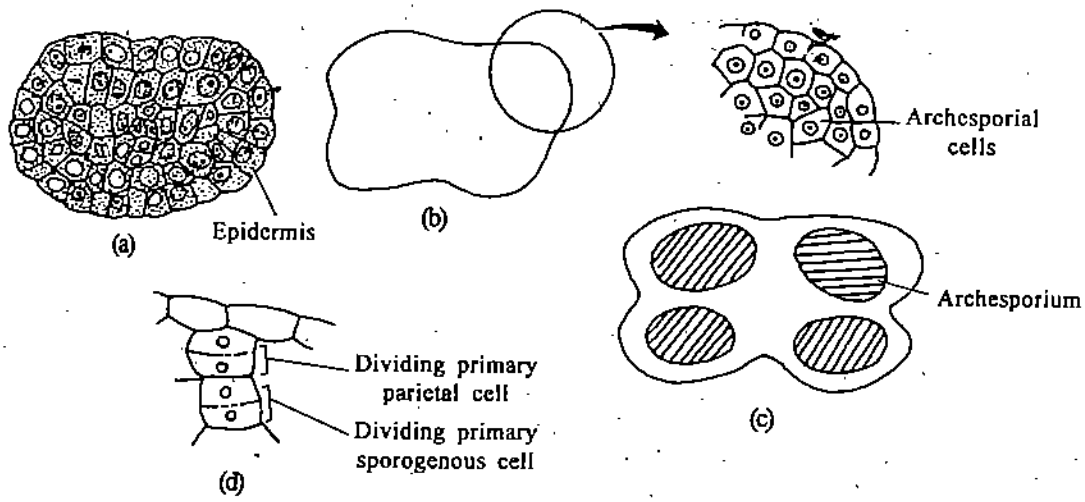


Fig. 1.2: Diagrammatic representation of different stages of development of microsporangium: a) An undifferentiated anther showing homogeneous mass of cells surrounded by epidermal layer; b) anther that has begun to assume four lobed outline, a portion of the same enlarged to show the prominent archesporial cells; c) an outline diagram of a four-lobed anther. The shaded areas in each lobe represent archesporial cells, d) a portion of an anther lobe enlarged to show the two products of the archesporial cell. The one towards the epidermis is the primary parietal cell and the inner one is the primary sporogenous cell.

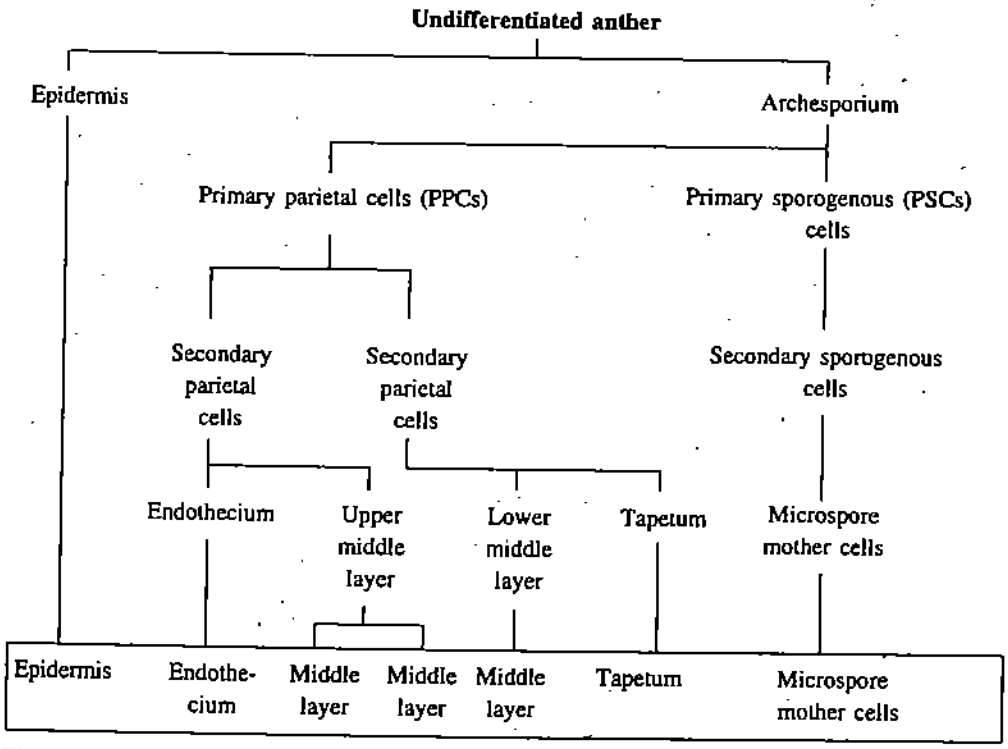


Fig. 1.3: Schematic representation of the ontogeny of anther wall layers and microspore mother cells in young anther of *Alectra thomsoni* (After Vijyaraghavan and Ratnaparkhi, 1973).

1.2.2 Anther Wall Layers

The wall of a mature anther consists of the following layers, from the epidermis inwards: epidermis, endothecium, middle layers and tapetum.

Epidermis

The epidermis is the outermost layer of the anther wall. It undergoes only anticlinal divisions. In this manner it is able to cope up with the rapidly enlarging internal tissue. In a mature anther, the epidermal cells appear greatly stretched and flattened. The function of the epidermal cells is mainly to provide a protective covering to the internal tissues.

Endothecium

The layer of cells lying immediately next to the epidermis is the endothecium, which is responsible for the dehiscence of the anther. It is usually single-layered, but sometimes multilayered as in *Nicotiana tabacum*. You may recall that the endothecium originates from the PPCs (Fig. 1.3). The endothecium mostly differentiates in the four protuberant or bulging parts of the anther (see Fig. 1.4a, thin arrows). Sometimes, it also develops near the connective region of the anther (see the shaded regions in Fig. 1.4). In some plants like *Triticale* an entire ring of endothecium is present.

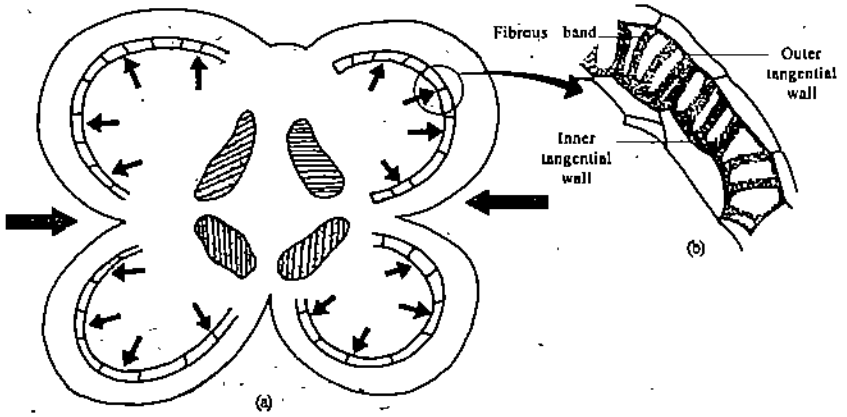


Fig. 1.4: (a) Diagrammatic sketch of the transverse section of an anther showing the position of the endothecium (small arrows). Large arrows point to the two dehiscing regions of the anthers showing longitudinal dehiscence.
(b) A few endothecial cells enlarged to show the characteristic endothecial thickenings.

The endothecial cells are radially elongated (see Fig.1.4.b). Their full development is attained by the time the anther is ready to dehisce to discharge the pollen grains. At maturity the endothecial cells can be easily recognised by the presence of *fibrous bands* (Fig.1.4b). They usually arise from the inner tangential walls. The fibrous bands radiate outwards and upwards ending near the outer wall of each cell. Hence, the inner tangential wall appears thick, whereas the outer tangential wall appears thin. These thickenings contain a high proportion of cellulose and a small amount of pectin.

As mentioned above, the endothecial cells, owing to their thickenings help in the dehiscence of the anthers. This is brought about due to the differential hygroscopic expansion of the outer and the inner tangential wall of the endothecial cell. You may recall, that the inner walls are thicker due to the presence of more α-cellulose than the outer ones. Therefore, the inner walls absorb more moisture and expand more than the outer ones. This results in the rupturing of the anther wall. This rupture or opening up of the anther occurs at the 'weak' points of the anther, that is, areas in the anther that lack such thickenings. In the longitudinally dehiscing anthers which are of most common occurrence, there are usually two such 'weak' areas (see Fig. 1.4a, solid arrows). There are also some variations in the endothecium structure and function. In the cleistogamous forms (whose flowers never open), the endothecial fibrous thickenings are absent. These cells may also not develop in plants whose anthers dehisce by apical pores as the dehiscence of anther takes place by dissolutions of certain cells at the apex of the anther.

Middle Layers

Inner to the endothecium lie one to three layers of cells, which are collectively termed middle layers. These originate from the PPCs (you may look at Fig.1.3 again) and have a nutritive function. The cells comprising these layers are generally rich in reserve food such as starch, during early stages of anther development. These cells are generally ephemeral. When the microspore mother cells undergo meiosis, the cells of the middle layers usually become flattened and crushed.

Tapetum

Tapetum is the innermost and the most important layer of the anther wall. It is generally

composed of a single layer of cells. These cells completely surround the sporogenous tissue (Fig.1.5), and they attain maximum development when the sporogenous tissue is at the tetrad stage. The tapetum is of dual origin in most of the angiosperms; the cells of the outer side originate from the derivatives of the PPCs, while those of the inner portion originate from the cells of the connective. Usually tapetal cells of different origin also appear different in size and structure. Tapetum of dual origin is also known as **dimorphic tapetum.** In *Alectra thomsoni*, the tapetal cells on the outer side are much smaller than those on the inner side (Fig.1.6).

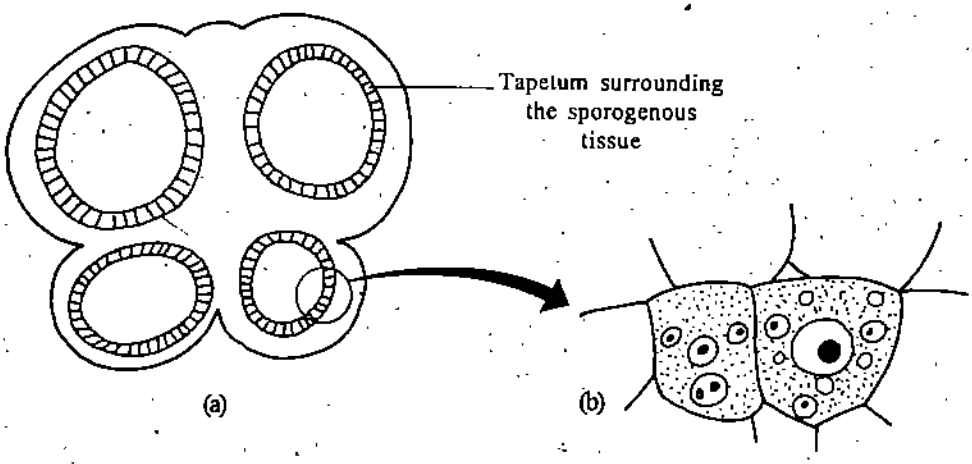


Fig. 1.5: (a) Diagrammatic sketch of transverse section of an anther showing the position of tapetum. (b) A few cells enlarged to show the dense cytoplasm and multinucleate condition of the tapetal cells.

The tapetum is a nourishing tissue. In a young anther, the tapetal cells remain in contact with the microspore mother cells through plasmodesmata till the formation of **meiocytes.** As soon as meiosis is completed these connections become plugged. Tapetum also plays an important role in the formation of exine and deposition of tryphine and pollenkitt. You will know more details about these two terms in the next unit. We shall now study the structure of the tapetal cells.

The sporogenous cells that have entered the meiotic phase are termed as meiocytes.

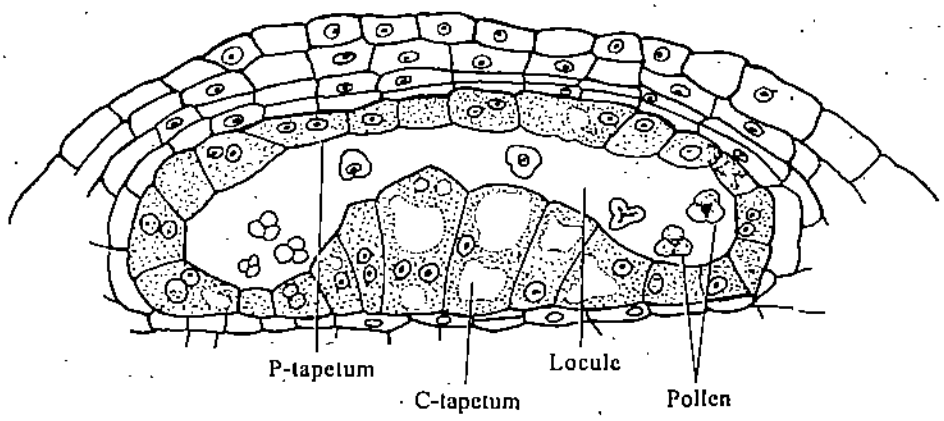


Fig. 1.6: A part of microsporangium of *Alectra thomsoni* showing dimorphic tapetum.Note the larger cells on the inner side (C-tapetum). These are derived from the connective region. And those from the outer side (P-tapetum) are smaller and are the products of the PPCs. (After Vijyaraghavan and Ratnaparkhi, 1973).

To begin with the tapetal cells are densely cytoplasmic and uninucleate. They often become from two to four and at times even up to 16 nucleate. The multinucleate condition results from nuclear divisions not accompanied by cytokinesis. Tapetal cells also become polyploid, either due to *endomitosis, polyteny* or by the formation of *restitution nuclei.*

Endomitosis — Mitotic division in which chromosome duplication and chromatid separation take place within the intact nuclear membrane and without spindle formation.

Formation of restitution nuclei — The mitosis goes on normally up to the early anaphase stage but the two sets of chromosomes formed, become enclosed within a common nuclear membrane. Such a nucleus is known as the restitution nucleus.

Polyteny — This refers to an increase in the number of chromonemata per chromosome.

Thus, there is an increase in DNA content, but there is no change in the number of chromosomes in the nucleus.

For the normal development of pollen grains, development of tapetum is very important. If the tapetum fails to develop or function abnormally, viable pollen grains are not formed; *pollen sterility* results.

In angiosperms there are two principal types of tapetum: the *amoeboid* and the *secretory*. In the secretory type of tapetum, the constituent cells maintain their individuality and position, whereas in the amoeboid type, the cells change their position and shape during ontogeny. We shall now study a few more details about these two types of tapetum.

Amoeboid Tapetum: It is also known as *invasive* or *periplasmodial* tapetum. This type of tapetum is more prevalent in the monocotyledons (*Arum*) than in the dicotyledons (*Helianthus*). In the amoeboid tapetum, there is a break-down of the inner tangential cell wall followed by the enlargement and movement of protoplasts into the anther sac, where they fuse and form *periplasmodium* (Fig. 1.7). The formation of periplasmodium occurs at different developmental stages ranging from meiotic prophase to tetrad stage of pollen development, in different species. In the anther sac, such fused protoplasts or the periplasmodia closely surround the developing microspores and play an important role in pollen development. At the tetrad stage the tapetal cytoplasm produces the enzyme callase which breaks down the callose wall around the microspore tetrads, releasing the microspores into the periplasmodium. Towards the end of pollen development, the periplasmodium degenerates, dries up and becomes coated on the surface of the pollen wall as pollen kitt material.

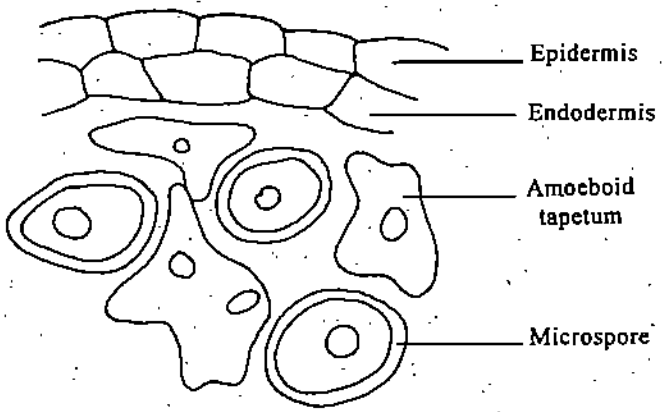


Fig. 1.7: A part of t.s. of anther showing amoeboid tapetum.

Secretory Tapetum

Secretory tapetum has other names: *parietal, cellular* or *glandular* tapetum. Secretory tapetum is more commonly observed in the dicotyledons. Unlike the amoeboid tapetum, the cells of the tapetum remain intact in their original position, till the maturation of the pollen grains. The tapetal cells secrete and liberate substances to the anther sac from their inner walls (Fig. 1.8).

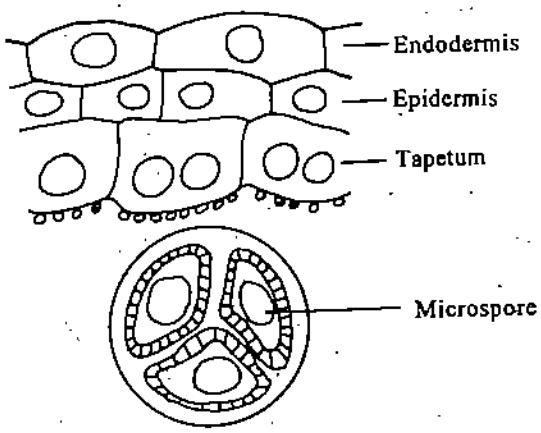


Fig. 1.8: A part of t.s. of anther showing secretory tapetum. Note the orbicules on the outer wall of the tapetum facing the microspore tetrad.

In several taxa that are characterised by secretory tapetum, there is deposition of sporopollenin granules on the inner faces of the tapetal cells. These granules are referred to as the orbicules or as 'Ubisch bodies', and these can be easily seen through the light microscope. The orbicules are generally absent in plants that show plasmodial type of tapetum. The orbicules originate in the cytoplasm as lipoidal pro-orbicular bodies (pro-Ubisch bodies) with a limiting membrane. These pro-orbicular bodies accumulate below the plasmalemma, and are extruded to the cell surface (facing the locule) where they acquire a sporopollenin coating. It has been believed that the orbicules have a role in pollen exine formation and pollen dispersal.

1.2.3 Sporogenous Tissue

You may recall that the primary sporogenous cells (PSCs) result after the periclinal division of the archesporial cells. The PSCs may undergo mitotic divisions and increase in number before functioning as MMCs or the PSCs may function directly as MMCs. The MMCs undergo meiosis and give rise to haploid microspores. The details of meiosis have been extensively studied.

Anthers are ideal systems to study meiosis. This is mainly due to the fact that, they are readily available and accessible for experimentation; and each anther contains a large number of mejocytes, mostly exhibiting synchrony. Most of the information on meiosis has been obtained from investigations on anthers.

Box 1.1: Meiosis in anthers.

Many interesting studies have been carried out on meiosis in anthers by using tissue and organ culture techniques. It is possible to remove anthers at different stages of development, culture them on a nutrient medium and investigate microsporogenesis. When the anthers are excised before the initiation of meiosis and cultured on a medium, meiosis is not initiated. However, if anthers are cultured after initiation of meiosis the division is continued in cultured anthers and results in the formation of microspores even on a simple nutrient medium. These results show that anthers require some special factor(s), apart from basic nutrients for the initiation of meiosis.

What is the exact nature of the stimulus for inducing meiosis in the sporogenous cells is not clear as yet although it appears that meiosis is tissue specific. It is believed that the stimulus originates elsewhere in the plant, and is transmitted to the anther. This stimulus is highly specific and functions only in the sporogenous cells, and not even in the nearby tapetal cells, through which it must pass to reach the sporogeneous tissue.

Meiosis is a significant event in sexual reproduction. You may recall from your study of Unit 3, Section 3.2 of LSE-03 course that, apart from reducing the diploid chromosome number to half, meiosis provides genetic variability due to recombination and crossing-over. These events lead to new genotypic combinations. This is the essence of sexual reproduction.

As you have studied, the meiotic cycle can be divided into two phases. Meiosis-I consists of a reduction division, resulting in two haploid cells or nuclei and meiosis-II is more or less like normal mitosis. In this unit, we shall not discuss the details of the process of meiosis. But in case you wish to revise them, you may refer to Unit-17, of LSE-01 course.

Syncytium Formation

In a young anther, before the onset of meiosis, the different cell layers; i.e., the anther wall layers, tapetum and sporogenous tissue (Fig. 1.9a) exhibit plasmodesmatal connections between the cells of adjacent layers. This means that there is a flow of nutrients between different cell layers. As meiosis is initiated, the plasmodesmatal connections between the cells of different layers (i.e., between the wall layers and the tapetum, and between the tapetum and the sporogenous tissue) get severed. But the cells of the same layer, however, continue to possess plasmodesmatal connections between themselves.

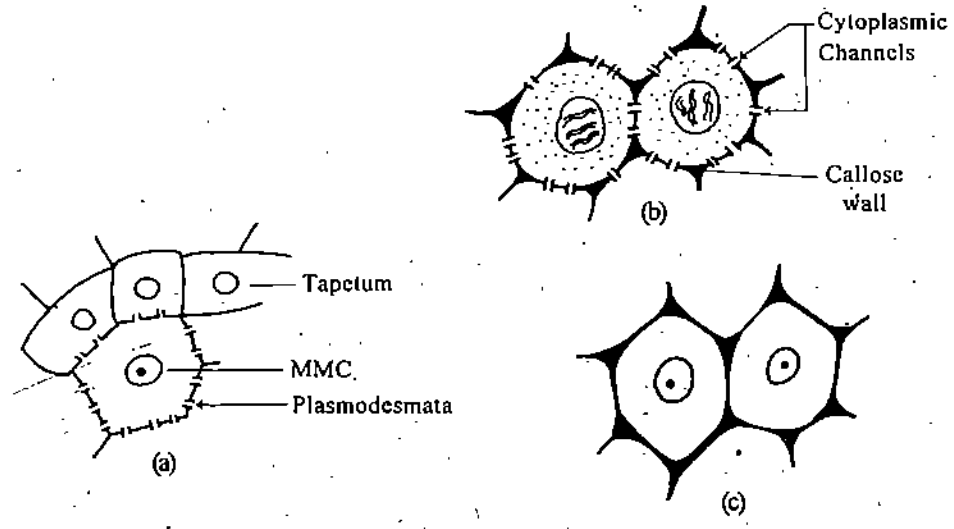


Fig. 1.9: Diagrammatic representation of the cellular connections in developing anthers, only a part of the anther is shown here. (a) shows connections between the tapetum and the MMCs,(b) the MMCs are now enclosed in thick callose walls (shown in dark colour) and are interconnected by wider cytoplasmic channels. (c) Callose wall completely surrounds the MMCs and thus isolates them.

An interesting structural change occurs in the sporogenous cells soon after meiosis starts. Each sporogenous cell develops an additional wall made up of callose (a polysaccharide made up of ß-1, 3 glucans) on the inner side of their cellulosic cell wall. The original cellulosic wall finally disintegrates. Certain plasmodesmatal connections between adjacent sporogenous cells become wider to form thick cytoplasmic bridges. Thus, each MMC becomes enclosed in a callose wall except along the massive cytoplasmic connections or channels, 1-2 µm in diameter. (Fig. 1.9b). The cytoplasmic connections are wide enough to allow the free passage of cytoplasm and organelles between meiocytes. Thus, although there are hundreds of MMCs in each microsporangium, all of them constitute a single functional entity called *syncytium*. Because of the massive cytoplasmic channels syncytium is very effective in distributing the requisite cell components amongst all the cells of the syncytium. This event is considered to regulate synchrony amongst the meiocytes of a microsporangium.

Isolation of Meiocytes and Microspores

As meiosis progresses in the MMCs, the callose wall extends even to those regions occupied by the cytoplasmic channels. This results in the interruption of the cytoplasmic channels, and thus each MMC gets completely enclosed by a callose wall (Fig. 1.9c). This happens at the end of Metaphase I and Metaphase II depending on whether the cytokinesis is successive or simultaneous. You will study about these later in the text. After the completion of meiosis and formation of four microspores from each MMC, callose wall extends to the separating walls of the microspore tetrads. Thus, individual microspores are also isolated from its neighbours. The callose wall acts as a selective barrier preventing the passage of larger molecules such as proteins and peptides. The arrangement of microspores in a microspore tetrad varies. Five different kinds of patterns have been found (Fig. 1.10).

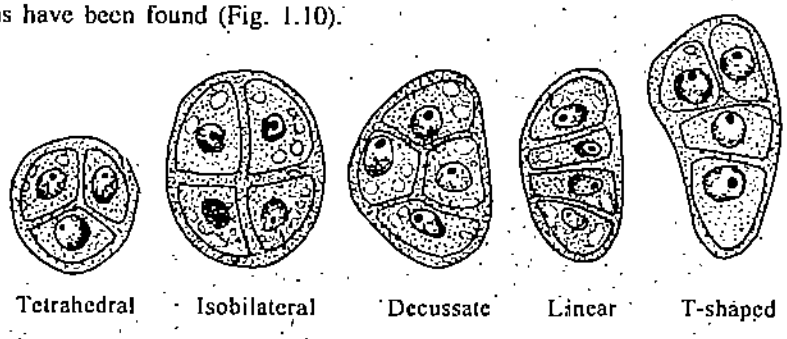


Fig. 1.10: Different arrangements of microspore tetrads in *Aristolochia elegans*. (After Johri and Bhatnagar, 1955).

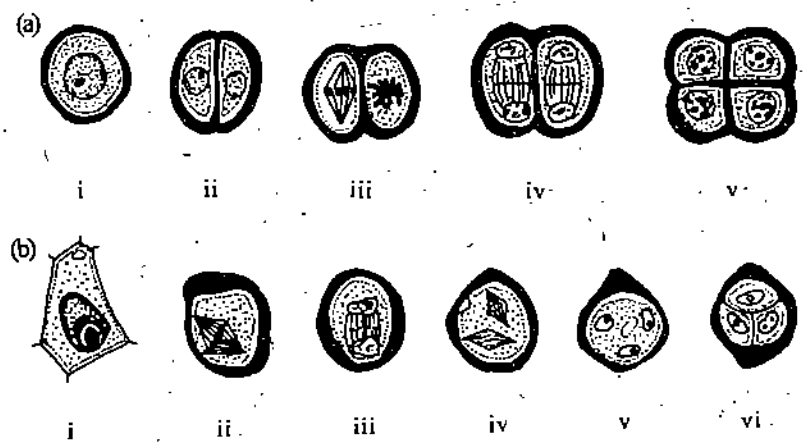


Fig. 1.11: (a) Successive type of cytokinesis in *Commelina subulata*. Note: the callose wall around the cells is represented here in dark colour. i) MMC prior to meiosis, ii) dyad stage, iii) metaphase-II, iv) telophase-II v) tetrad stage. (b) Simultaneous type of cytokinesis in *Drimys winteri*. i) MMC prior to meiosis ii) metaphase-I iii) binucleate cell iv) metaphase-II v) 4-nucleate stage vi) tetrad stage (After; (a) — Chikkannaiah, 1960; (b) — Bhandari and Venkataraman, 1968).

The tetrahedral and isobilateral types of arrangement of microspore tetrads are the most common. However, decussate, linear and T-shaped tetrads are also found. In *Aristolochia elegans* all the five types of microspore tetrads have been observed.

There is variation in the formation of the cell wall (cytokinesis) separating each microspore (see Fig. 1.11). In the successive type the two resulting nuclei formed after the first nuclear division are separated by the laying down of a wall to form a dyad, e.g., *Commelina subulata*. The nucleus of each dyad then undergoes a division, again followed by wall formation. In the simultaneous type of wall formation, which is of common occurrence, the first nuclear division is not followed by wall formation. The walls are laid down only after the formation of four nuclei.

Cytoplasmic Reorganisation

Meiosis is also associated with major reorganisation of the cytoplasm of MMCs and microspores. Microspore mother cell shows high metabolic activity. As meiosis is initiated, the synthetic activity of the cell is reduced. The rate of synthesis of RNA and proteins is drastically reduced. The cell undergoes dedifferentiation. The ribosome population of the cell comes down significantly. Even the mitochondria and plastids undergo dedifferentiation, that is, they loose most of the internal membrane system and become spherical bag-like structures. However, small pockets of cytoplasm (consisting 10-20 percent of the total cytoplasm) gets enclosed by membranes and these pockets do not undergo the reorganisation that occurs in the remaining part of the cytoplasm.

Towards the end of meiosis, the microspores restore the synthetic activity. The synthesis of RNA and proteins is initiated, the ribosome population of the cell is restored and the mitochondria and plastids redifferentiate to their normal configuration with internal membrane system and regain their original shape.

Release of Microspores

Up to the tetrad stage, there is no cellulosic wall around the microspores. As you will come to know in the next unit, a unique feature of the pollen is the ornamentation of the pollen wall. This ornamentation is seen on the outer layer of the pollen or exine. The exine is made up of sporopollenin, one of the most resistant (to physical and biological decomposition) substances known in the biological world. Pollen grains of prehistoric plants are well preserved as fossils because of their exine. There is enormous variation in the ornamentation of the exine, and it is a characteristic feature of a given species. Often pollen grains of particular group of plants can be identified on the basis of their exine pattern.

The blue print of exine, termed primexine is laid down below the callose wall after the basic structural features of the exine including the position of the germpore (a region in

the pollen wall through which the pollen tube emerges) are demarcated in the primexine. This important morphogenetic event takes place while the microspores are still enclosed in the callose wall, in groups of four or at the tetrad stage. Thus, the pattern of mature exine is laid down before the spores are set free. The callose wall seems to play an important role in the orderly deposition of primexine. You will read more about this in the next unit.

After the development of exine, the callose wall dissolves. The enzyme callase which dissolves the callose wall is produced by the surrounding tapetal cell periplasmodium. The development of the male gametophyte begins after the liberation of individual microspores.

Some Rare Features

The microspores, generally become separated shortly after meiosis. However, in some plants such as *Drimys* and *Drosera*, they do not separate but form **compound pollen grains**. In the Orchidaceae and Asclepiadaceae, all the microspores in a microsporangium remain together and form a structure called the **pollinium**. Occasionally the microspores in a tetrad undergo further divisions and produce polyads. This phenomenon is known as **polyspory**. In *Hyphaene* the number of microspores goes upto 8 (Fig. 1.12), 11 in *Cuscuta reflexa* and 22 in *Thunbergia mysorensis*.

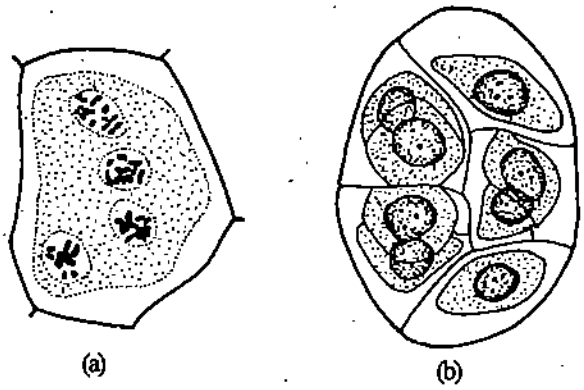


Fig. 1.12: Polyspory in *Hyphaene indica*. a) A MMC after meiosis, shows the presence of four nuclei, b) An octad that is likely to have originated from the stage of MMC as shown in figure a.

SAQ 1

Given below are a set of terms not given in any particular order. Link them in a way to form a concept: tapetum, filament, wall layers, pollen grains, endothecium, sporogenous tissue, epidermis, androecium, middle layers, anther.

Strike off the incorrect term(s) given in the bracket.

a) In (successive/simultaneous) cytokinesis, a cell plate is formed after the first as well as the second meiotic division so that there is a distinct dyad stage, whereas in (successive/ simultaneous) cytokinesis cell plates formed only after the second division.

b) A periclinal division in the (microspore mother/ archesporial) cell results in the formation of a primary parietal cell and a primary sporogenous cell.

c) The cells of (tapetum/endothecium) can be recognised easily in mature anthers on account of the presence of fibrous bands of thickenings arising from the inner tangential walls.

d) The cells of the (endothecium/middle layers) are usually short-lived and are used up quite early during the development of microspores.

e) In the (secretory/amoeboid) type of tapetum, the cell walls breakdown, and the protoplasts enter the anther sac, and by their fusion a periplasmodium is formed around the (microspore mother cells/endothecium).

f) There are numerous MMCs in a microsporangium, and they behave as a single functional entity — the (syncytium/pollinium).

1.3 OVULE

The ovule, also known as the megasporangium is the forerunner of the seed. It consists of a central mound of tissue called the **nucellus**, which is enveloped by one or two coverings — the **integuments**. An ovule ready for fertilisation consists of nucellar tissue enveloped almost completely by integument(s), leaving a small pore or opening at the apical end (Fig. 1.13). This opening, called **micropyle** is the main passage for the entry of the pollen tube into the ovule. This end of the ovule is generally referred to as the **micropylar pole** and the opposite pole where funiculus is attached is called the **chalazal pole.** The ovule is attached to the placenta through a stalk-like structure the **funiculus.** In the nucellus is present the **female gametophyte** which is commonly referred to as the **embryo sac.**

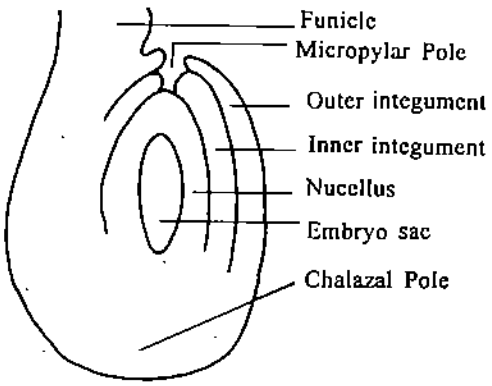


Fig. 1.13: Outline diagram of a typical ovule to show its various component parts.

1.3.1 Development

The ovule develops from a specialised region of the ovary — the placenta. Initially it appears as a small mound on the **placenta**, and is composed of homogenous tissue (Fig. 1.14a-c). Subsequently it cuts off initials for the integuments, and undergoes specific degree of curvature to develop as a mature ovule.

We shall resume discussion on the structural and developmental aspect of ovule later (in the subsection 1.3.3) when you study integuments.

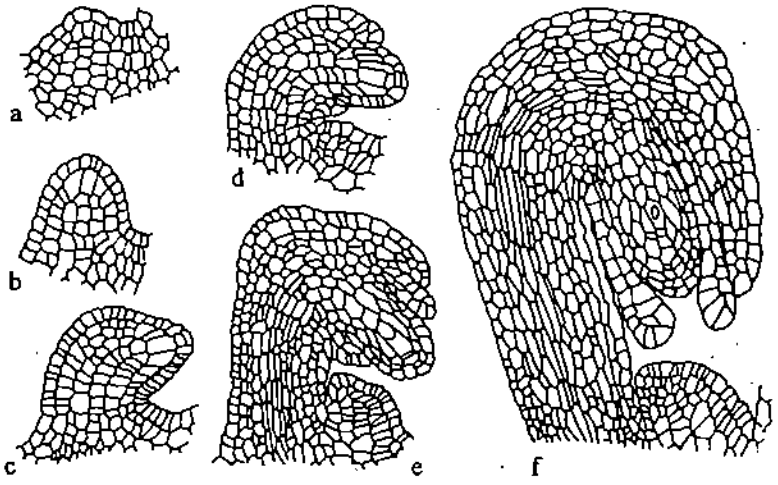


Fig. 1.14: Different stages in the development of ovule. (After Bouman, 1978).

1.3.2 Types of Ovules

Ovules undergo varied degrees of curvature during development. The position of micropyle with respect to the funiculus becomes different and constitutes the basis of classification of the ovules. Five basic types of ovules are known. These are: anatropous, orthotropous, hemianatropous, campylotropous and amphitropous (Fig. 1.15a-e).

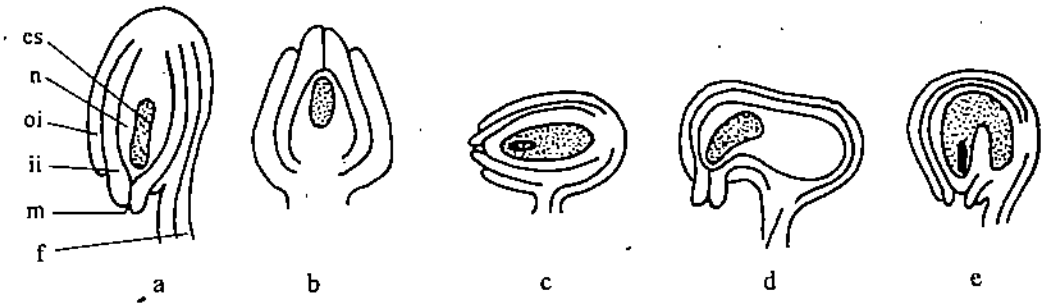


Fig. 1.15: Types of ovules. a) anatropous; b) orthotropous, c) hemianatropous, d) campylotropous, e) amphitropous. The abbreviations used in the figure are: m-micropyle; ii-inner integument; oi-outer integument; n-nucellus; es-embryo sac; and f-funiculus.

Anatropous

This is the most common type of ovule in angiosperms in which the ovule undergoes curvature such that the micropylar end comes to lie parallel to the funiculus (Fig. 1.15a).

Orthotropous

In this type of ovule no curvature occurs and the micropyle lies in a straight line with respect to the funiculus (see Fig. 1.15b).

Hemianatropous

The hemianatropous condition results from the curvature of the ovule such that the micropyle comes to lie at right angles to the funiculus (Fig. 1.15c).

Campylotropous

In campylotropous type, the ovule is curved but the curvature is less than that in the anatropous condition (Fig. 1.15d).

Amphitropous

The amphitropous ovule looks like the campylotropous ovule at the first glance. But there is a point of distinction. In this type the nucellus and the embryo sac are curved like a horse shoe (Fig. 1.15e).

Circinotropous

Besides the above basic forms, another interesting type that you should know is the circinotropous ovule (Fig. 1.15f). In the early developmental stages of such ovules the nucellar protuberance is more or less in line with the axis (see Fig. 1.16a). In the further developmental stages, due to unilateral growth, it assumes anatropous form (see Fig. 1.16c). The curvature does not stop at this, but continues until the ovule has turned over completely so that the micropylar end again points upwards. (see Fig. 1.16d-f) This kind of ovule is common in families Cactaceae and Plumbaginaceae.

1.3.3 Structure

Integuments

The integuments are the coverings of the ovule, which mature into the seed coat. The ovules may have one (unitegmic condition) or two (bitegmic condition) integuments. The Sympetalae predominantly show unitegmic condition. The bitegmic condition is common in Polypetalae and monocots.

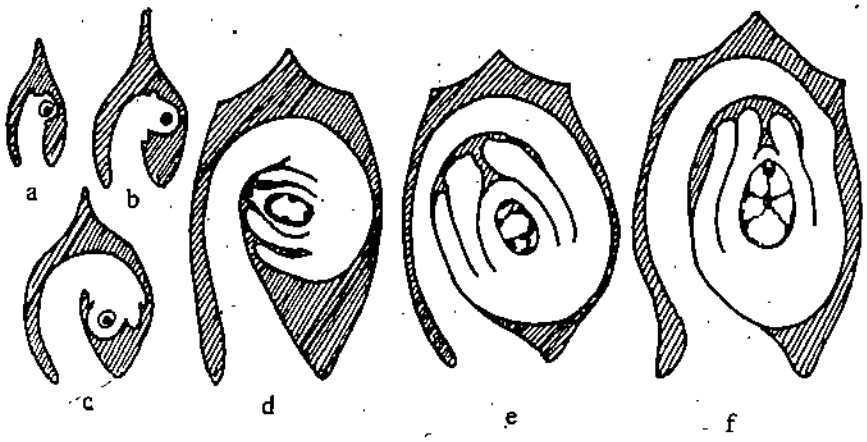


Fig. 1.16: **Development of circinotropous ovule in *Plumbago capensis*. Shaded area represents ovarian cavity (after Haupt, 1934).**

Nucellus

Enclosed within the integuments lies a homogenous mass of tissue which is termed as the nucellus. This is the tissue in which the female gametophyte differentiates and develops. At an early stage of ovule development, usually an archesporial cell or a group of cells differentiate in the hypodermal region of the nucellus. The archesporial cell may function directly as the sporogenous cell. In such situations, the sporogenous cell also remains hypodermal in position and is surrounded by a single-layer of nucellus, in the micropylar region (see Fig. 1.17). Such ovules are known as *tenuinucellate*. These are commonly seen in Sympetalae.

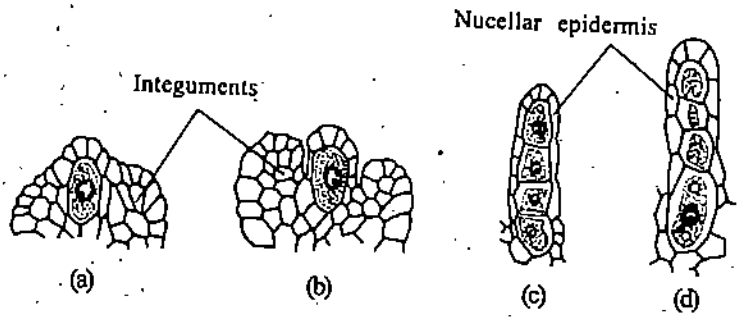


Fig. 1.17: **Tenuinucellate ovule of *Elytraria acaulis*. In c and d integuments are not shown. (After Johri and Singh, 1959).**

In some instances, the hypodermal archesporial cell divides periclinally to form an outer parietal cell and an inner sporogenous cell. The parietal cell may either remain undivided or it may undergo a few periclinal and anticlinal divisions causing the formation of several layers of cells above the sporogenous cell (Fig. 1.18).

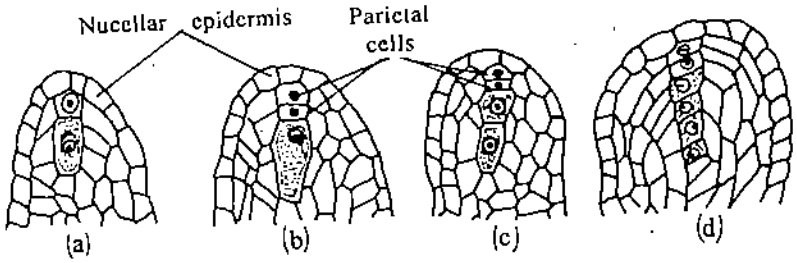


Fig. 1.18: Megasporogenesis in *Myriophyllum intermedium*. a) A division in the archesporial cell resulting in an outer primary parietal cell and inner primary sporogenous cell. b) The primary parietal cell has divided periclinally. c, d) Further divisions in the sporogenous cell (After Bawa, 1969).

Sometimes in addition to the parietal cells, the nucellar cells situated above the sporogenous tissue also divide repeatedly. Consequently, the sporogenous cell becomes deeply embedded in the massive nucellus. Such a condition of the nucellus is known as *crassinucellate* (Fig. 1.19).

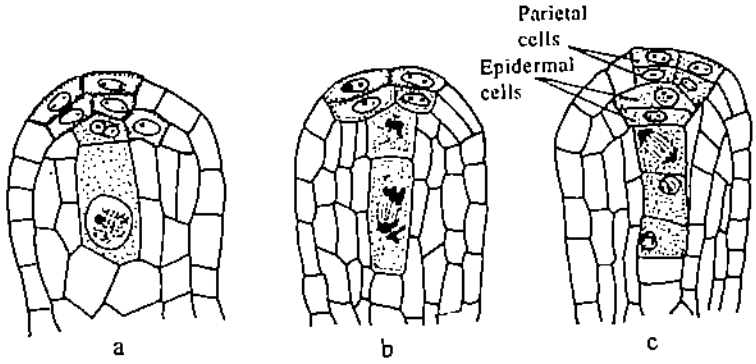


Fig. 1.19: *Nigella damascena*. A part of the ovule showing the nucellus. The megaspore mother cell becomes embedded in the nucellar tissue due to divisions in the epidermal cells of the nucellus and the formation of parietal cells. (After Vijyaraghavan and Marwah, 1969).

In the majority of angiosperms, the nucellus is consumed by the growing embryo sac or the endosperm. However, in some species, it persists in the mature seed as the nutritive tissue. This persistent nucellus is called the **perisperm**, and it mainly acts as a storage tissue. A good example of the occurrence of perisperm is black pepper (Piperaceae).

A few nucellar cells located at the base of the embryo sac, but above the vascular supply of the funiculus become differentiated from other adjacent cells (see Fig. 1.20). These may become sclerenchymatous, suberised or may remain thin walled and secretory. These cells constitute the **hypostase**. Hypostase occurs in several families and is believed to be associated with the following functions:

i) Transportation of nutrients;

ii) As a barrier or boundary for the growing embryo sac and prevents it from growing into the base of the ovule;

iii) Presumably helps in maintaining the water balance in the seed during dormancy;

iv) It is also suggested that it produces certain enzymes or hormones or plays a protective role in mature seeds;

In some species a few cells of the nucellar tip become thick-walled and differentiate into an **epistase**.

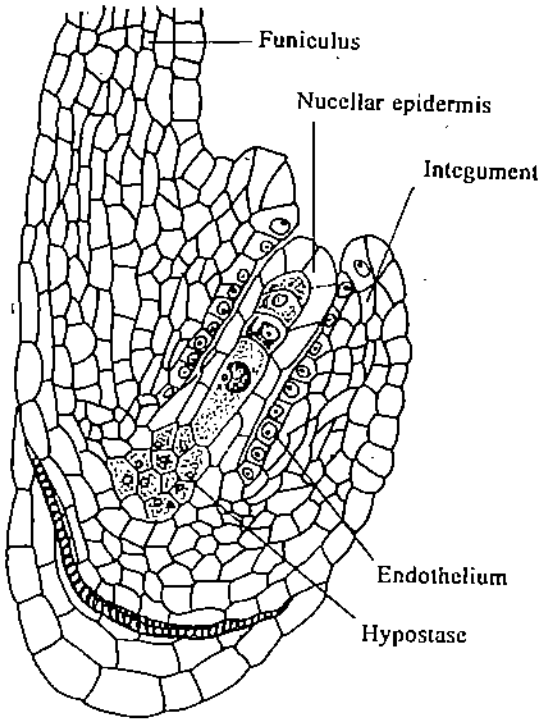


Fig. 1.20: Longitudinal section of an ovule, of *Bupleurum tenue* showing hypostase and developing endothelium (after Gupta and Gupta, 1964).

Endothelium

In plants bearing unitegmic ovules, the nucellus degenerates during early stages of ovule development and the embryo sac comes in contact with the innermost layer of the integument. The cells of this layer become specialised to supply nutrients to the embryo sac (see Figs. 1.20 and 1.21). These cells elongate radially, their cytoplasm becomes

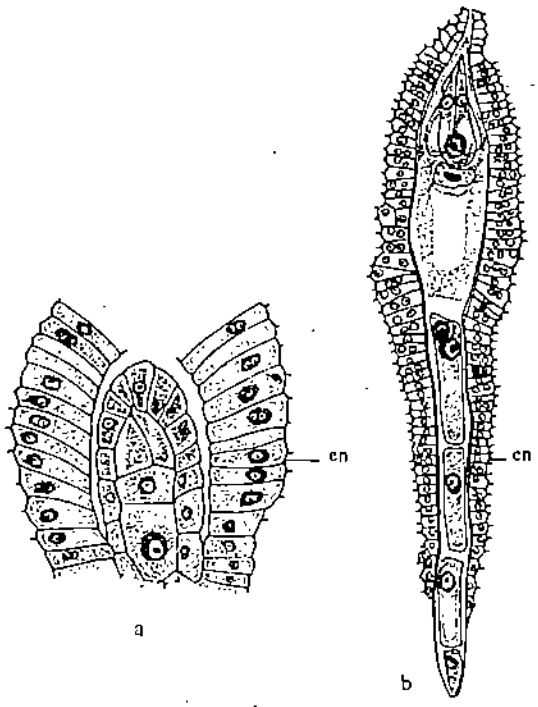


Fig. 1.21: Endothelium. (a) Note that the cells comprising endothelium (en) are uninucleate and the radially elongated. Also mark the teninucellate condition of the ovule. (b) The endothelium comprises multinucleate cells. [After: (a) Deshpande, 1964; and (b) Pullaiah, 1978].

dense and store starch and lipids. This specialised layer is called endothelium. Endothelium is also reported in some bitegmic ovules. Tapetal cells are similar to endothelium cells of anther in storing carbohydrates, proteins, RNA, ascorbic acid and other metabolites; it is secretory in function is hence referred to as the *integumentary tapetum.*

Obturator

An obturator is an outgrowth of the placenta or funicle or integument or style near the micropyle (see Fig. 1.22). It is presumed to guide the pollen tube to the micropyle. This tissue is either composed of thin-walled compact tissue or it is made up of loose hairy outgrowths. The cells of the obturator at the ultrastructural level, show dense cytoplasm containing a copious number of ER, dictyosomes, and vesicles. The cells of the obturator produce a surface exudate and provide mechanical and chemical guidance to the growing pollen tube.

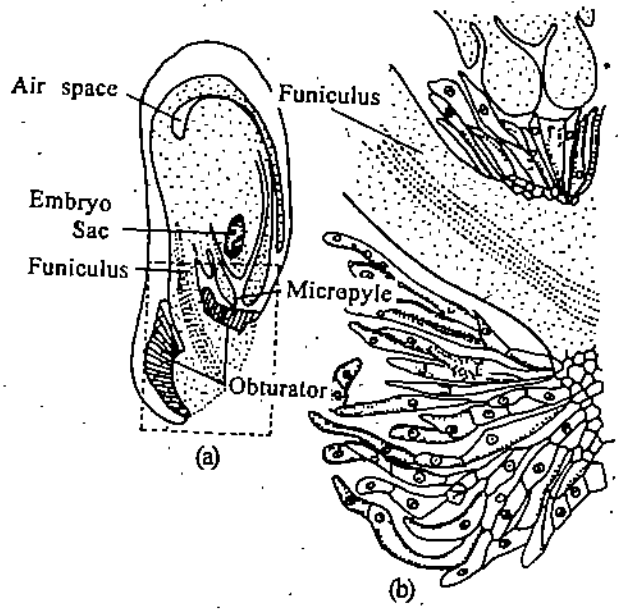


Fig. 1.22: Obturator in *Tetragonia tetragonioides*. (a) Outline diagram of longitudinal section of an ovule, showing the position of the obturator. The obturator is present on both sides of the funiculus. (b) A portion of the obturator enlarged to show that it is composed of multicellular, glandular hairs. (After Prakash, 1967).

Megasporogenesis

As mentioned earlier, one of the nucellar cells situated subjacent to the epidermis differentiates into the *primary archesporial cell.* This cell is larger than the adjacent cells has dense cytoplasm and prominent nucleus (Fig. 1.23). In tenuinucellate ovules, the archesporial cell functions directly as the megaspore mother cell (abbreviated as MgMC). In crassinucellate ovules, the archesporial cell first divides transversely to cut off a *primary parietal cell* on the outer side, and a *primary sporogenous cell* on the inner side. The primary parietal cell may either remain undivided or it may undergo a few divisions to form a few cells above the sporogenous cell.

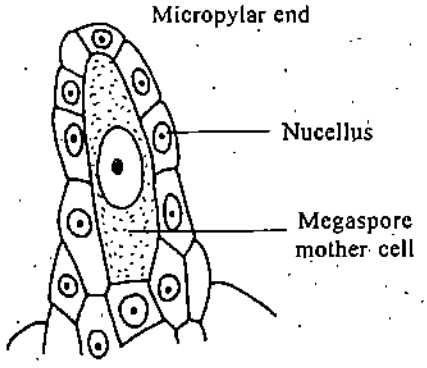


Fig. 1.23: A part of a young ovule with large megaspore mother cell.

The primary sporogenous cell usually functions as the MgMC. It has cellulosic walls and is connected to its neighbouring cells through plasmodesmata. The MgMC also known as the *megasporocyte*, undergoes meiosis and forms four haploid *megaspores*. As it happens during microsporogenesis, deposition of callose takes place inside the cell wall of the megaspore mother cell, soon after the initiation of meiosis. As a result, the plasmodesmatal connections of the MgMC get closed. Then the nucleus of the MgMC undergoes a meiosis first followed by the formation of a transverse wall. A dyad is formed (Fig. 1.24a). Each dyad undergoes meiosis II to give rise to a linear row of four cells, the megaspores (Fig. 1.24b). This is commonly known as the tetrad stage. Sometimes the megaspores are present in a T-shaped configuration. Such a situation arises due to a vertical, instead of the transverse division in the upper dyad.

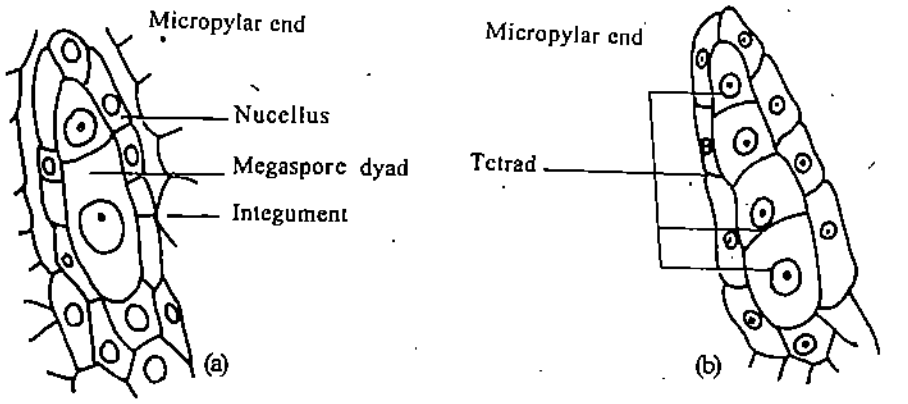


Fig. 1.24: A part of a developing ovule showing the dyad and tetrad stages of megasporogenesis.

After the formation of a tetrad, only one of the four megaspores functions and forms a female gametophyte or the embryo sac whereas the other three megaspores degenerate. Usually it is the chalazal megaspore of the tetrad that is functional.

During megasporogenesis, a notable feature is the development of callose thickenings around the nonfunctional megaspores. We shall elaborate here only the example of *polygonum type* of embryo sac, i.e., which is formed from the chalazal megaspore. In this case, callose first appears in the chalazal region of the MgMC. It then spreads over its entire surface, isolating it from the adjacent maternal tissue. Following meiosis, the callose develops around individual megaspores also. However, the callose breaks down early around the functional megaspore but is retained around the non-functional megaspores for a longer time.

Apart from callose deposition, cytoplasmic reorganisation is noticed at the ultrastructural level in the megaspores. Reorganisation of ribosomes, and plastids containing starch grains, is basically similar to that reported in microsporogenesis (see Subsection 1.2.3). This strongly suggests that isolation and insulation of sporocytes and cytoplasmic reorganisation are the two important events associated with meiosis. These events are believed to play an important role in transition from sporophytic to gametophytic generation and facilitate the expression of the gametophytic genome.

We know very little about the physiological aspects of megasporogenesis. This is mainly due to some technical difficulties. There is just one functional megaspore mother cell in an ovule as compared with numerous MMCs in the anther. A large preparation of MMCs can be easily obtained by sectioning the anther with a sharp blade and pressing it from the other end. This is however, not possible with the megasporocyte. However, whatever information is available, it suggests close similarity between microsporogenesis and megasporogenesis.

SAQ 3

Given below are a few terms. Arrange them in a way to form a continuous concept. You may use any term more than once; and also, you may use appropriate words to link the terms.

Ovule, placenta, nucellus, outer integument, inner integument, micropyle, seed coat, archesporial cell(s), sporogenous cell, tenuinucellate, crassinucellate, parietal cell.

1.4 SUMMARY

In this unit you have learnt that:

- In most angiosperms, each stamen is composed of an anther and a filament. The anther generally contains four microsporangia. The anther wall consists of four kinds of layers of cells: epidermis, endothecium, middle layers and tapetum. Inner to these wall layers lies the sporogenous tissue, made up of MMCs. The MMCs undergo meiosis and produce microspores.
- At the onset of meiosis, the meiocytes are large, rich in cytoplasm and have thin pecto-cellulosic walls. During early prophase, the meiocytes become interconnected by cytoplasmic channels and a process of dedifferentiation of cytoplasm begins. As prophase commences, a callose wall is deposited on the inner surface of the pectocellulosic wall and causes isolation of meiocytes. Meiosis yields four haploid nuclei which after cytokinesis result in four microspores. Cytokinesis may be successive or simultaneous.
- During microsporogenesis distinct changes occur in the anther wall. The middle layer(s) disintegrate. The tapetal cells enlarge and become metabolically active. Up to this stage the epidermis and endothecium remain relatively unchanged.
- The female reproductive organ, gynoecium consists of carpels. Each carpel has three parts: the ovary, style and stigma. The ovary encloses the ovules that develop from specific regions of placenta. The young ovule has a central mound of tissue, the nucellus and one or two integuments that envelop it. At the apex of the ovule, a narrow opening or micropyle is left by the integument(s). During development, the ovules undergo varying degrees of curvature. According to the position of the micropyle in relation to the funiculus, the mature ovule can be classified into ortho-, ana-, hemiana-, campylo-, and amphitropous.
- The tissues of the ovule undergo differentiation at different locations to give rise to the hypostase, endothelium and obturator, each with a specific set of functions.
- At an early stage of ovule development, one cell of the nucellus develops into MgMC. It divides meiotically to form a linear row (with rare exceptions) of four haploid megaspores. Only one megaspore persists and the remaining three degenerate. During megasporogenesis callose is deposited on the walls of MgMC and the megaspores, similar to callose deposition during microsporogenesis. After completion of megasporogenesis, the callose disappears from the walls of the functional megaspore, whereas it remains present in the walls of megaspore which degenerate.

1.5 TERMINAL QUESTIONS

1) Which of the following features make anthers a favourite material for studying meiosis?

   a) They are readily available in large quantities for experimentation.

   b) Since the meiocytes are in different stages of development, it enables one to study various stages of meiosis even in a single anther.

   c) Anthers at various developmental stages can be cultured easily on a simple, nutrient medium.

   d) Very little is known about the physiological aspect of microsporogenesis.

   e) There are only a few microspores per microsporangium, which makes their study easier.

   f) The meiocytes show synchronous development.

Choose the most appropriate answer from the choices given below:

   i) a, b, d

   ii) c, d, e

   iii) b, d, f

   iv) a, c, f

2) Given below are a set of items (a-d) grouped under the heading 'key'. A matching explanation of each item is provided in the 'statements' (i-vi). All you have to do is to pick up the matching statement for each item of the key and record your answer in the space provided.

   Key

   a) Microsporogenesis

   b) Endothecial thickenings

   c) Meiocytes

   d) Cytokinesis

   Statements

   i) The microspore mother cells which form the microspores through meiotic division.

   ii) Mitotic divisions that result in the increase in number of the microspore mother cells.

   iii) A process that separates the four haploid nuclei resulting from meiosis by the formation of cell plate.

   iv) Are made up of callose, and help in the dehiscence of the microsporangium.

   v) Involves meiotic division leading to the formation of microspores.

   vi) Are made up of α-cellulose and help in the dehiscence of anther.

Item	*Statement No.*
a)	.....
b)	.....
c)	.....
d)	.....

3) List different wall layers in a sequence starting from the epidermis inwards, in an anther. Write the salient features regarding the structure and function of each wall layer.

4) Differentiate giving examples, the secretory and t e amoeboid tapetum. Make suitable illustrations for each.

5) a) Explain the significance of microsporogenesis for the sexually reproducing plants?

b) Prepare a write up on microsporogenesis, using the following points:

meiotic stimulus — syncytium — role of callose — reorganisation of cytoplasm — release of microspores.

Illustrate your answer to explain the various points.

6) Which combination of the following statements suggest the nutritive role of the tapetum in a developing microsporangium?

a) It is ephemeral and breaks down during early stages of microsporogenesis.

b) Normal development of pollen grains fails to occur in microsporangia with poorly developed tapetum.

c) It is the innermost layer of anther wall and it has a dual origin in most angiosperms.

d) In a young anther, the tapetal cells retain continuous connection with the microspore mother cells through plasmodesmata.

e) Tapetal cells have dense cytoplasm, often become multinucleate and their nuclei become polyploid.

Choose the most appropriate set of answers from the choices given below:

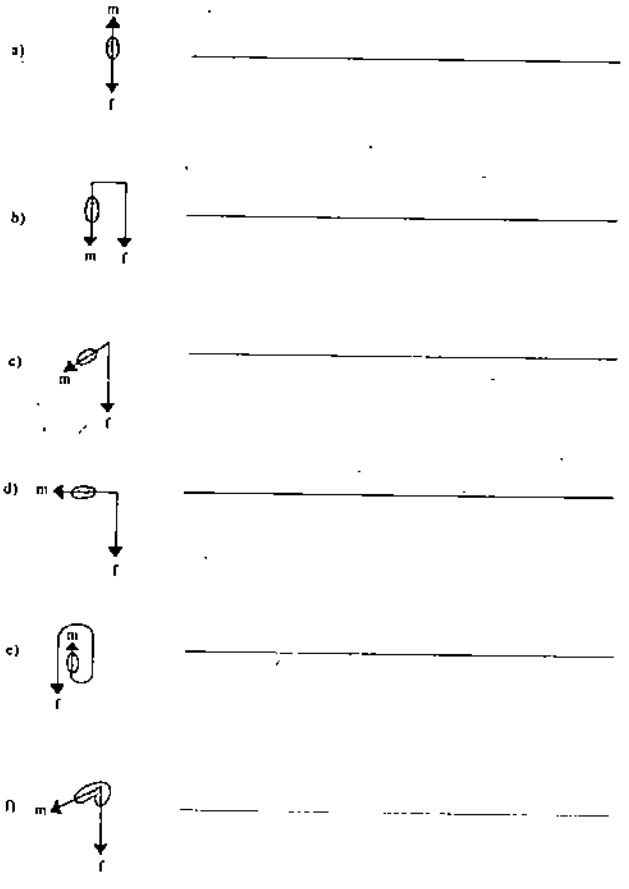
i) a, b, c

ii) c, d, e

iii) b, d, e

iv) a, c, d

7) In the set of illustrations given below, identify the type of ovule. The symbols used are: m-micropyle; f-funiculus. The rounded, oval or horse-shoe shaped structures on the lines denote the embryo sac.



8) For the items grouped under the key, find a matching statement from (i) to (viii) given below.

Key

a) micropyle

b) endothelium

c) perisperm

d) hypostase

e) obturator

Statements

i) The nucellus which perishes quite early in the development of ovule.

ii) The nucellar cells at the base of the embryo sac become specialised and obstruct the downward growth of the embryo sac.

iii) A structure of the ovule near the micropyle, that is associated with directing the growth of pollen tube toward the micropyle.

iv) The integuments form a passage for the entry of the pollen tube to the female gametophyte.

v) The nucellus being ephemeral, the role of providing nutrition to the female gametophyte is taken over by the innermost layer of the integument.

vi) A cap-like structure of cutinised cells differentiates from the nucellus in a mature ovule.

vii) The persistent nucellus in the seed, that acts mainly as the storage tissue.

viii) A group of differentiated cells at the summit of the nucellus which protect the developing female gametophyte.

9) Which of the following features of the endothelium cells suggest their presumptive nutritive role?

a) Secretory cells having high concentration of carbohydrates, proteins, RNA and other metabolities.

b) A single layer of cells, that elongate radially and become thick-walled.

c) Cells are connected with each other and with those of the integument through plasmodesmata.

d) Develops mostly in unitegenic ovules and degenerates at their early stages of development.

e) The cells often become multinucleate and polyploid.

Choose the correct answer from the choices given below:

i) a, b, d

ii) b, d, e

iii) a, c, d

iv) a, c, e

10) What is the essential difference between the tenuinucellate and crassinucellate ovules? Use appropriate illustrations to demonstrate these features.

11) Write a brief account on hypostase and obturator, touching upon the following points: location, site of origin, characteristic cellular features and functions.

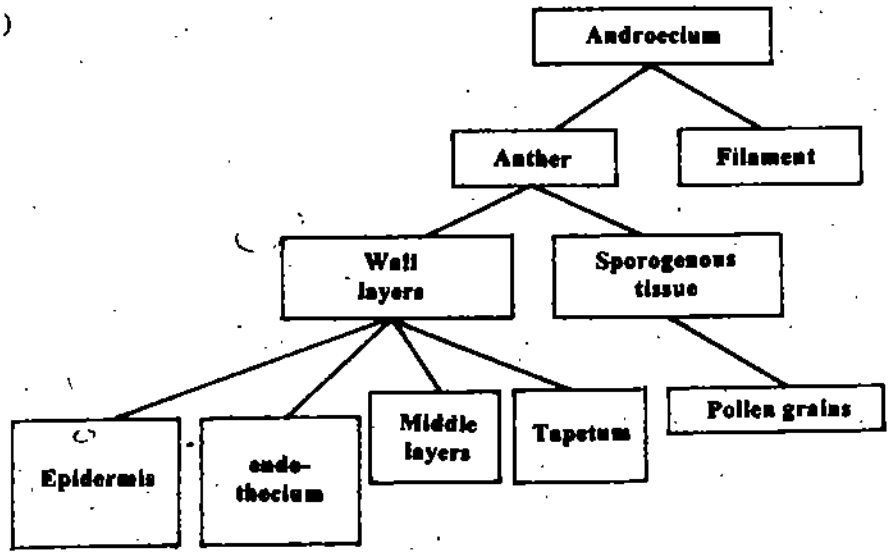
12) a) Define megasporogenesis. Why is it considered important for the sexually reproducing plants?

b) In what ways is megasporogenesis similar to, as well as different from microsporogenesis? In your answer draw parallels between the two and also enumerate the differences?

1.6 ANSWERS

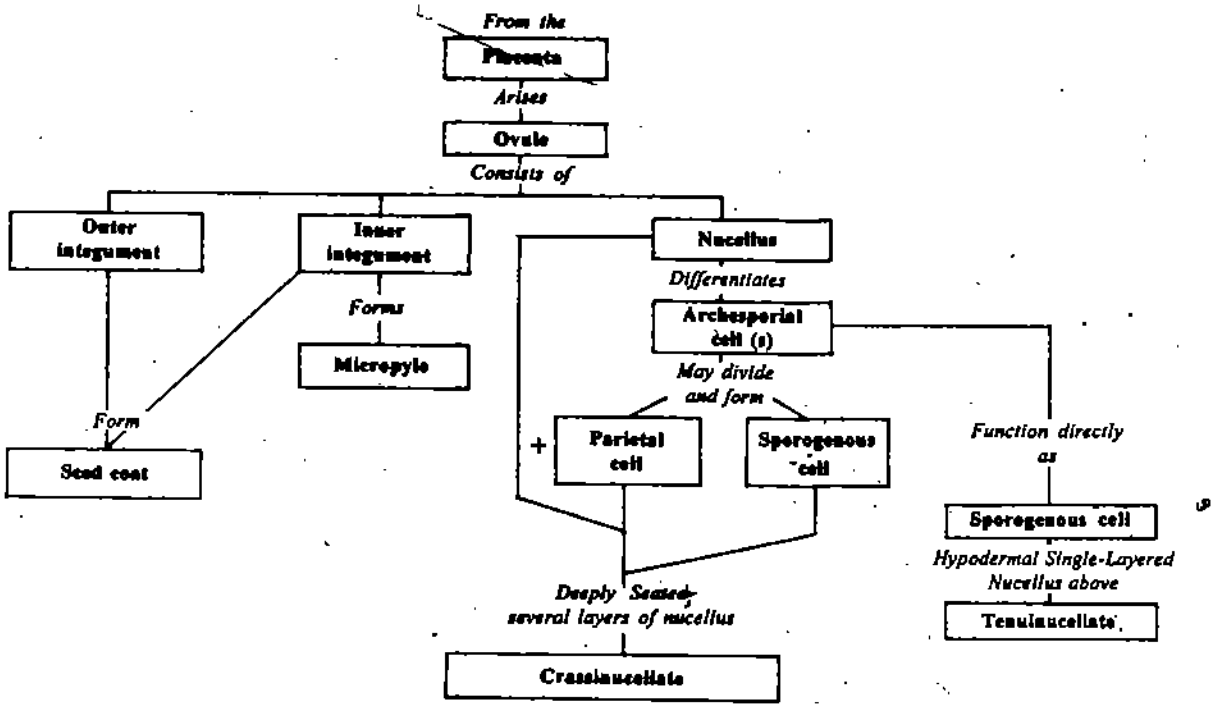
Self-assessment Questions

1)



2) a) simultaneous, successive
   b) microspore mother
   c) tapetum
   d) endothecium
   e) secretory, endothecium
   f) polyspores

3)



1) iv

2) a) v
   b) vi
   c) i
   d) iii

3) You may refer to subsection 1.2.2.

4) See subsection 1.2.2.

5) a) Hint: To maintain the ploidy level.

b) You may see subsection 1.2.3.

6) iii

7) a) orthotropous
   b) anatropous
   c) campylotropous
   d) hemianatropous
   e) circinotropous
   f) amphitropous

8) a) iv
   b) v
   c) vii
   d) ii
   e) iii

9) iv

10) You may refer to subsection 1.3.3.

11) See subsection 1.3.3.

12) a) Hint: meiotic process, and the subcellular organisation; Same as 5a.
    b) Write in your own words after reading subsection 1.2.3 and 1.3.3.

UNIT 2 GAMETOGENESIS

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2.1 INTRODUCTION

In the previous unit you have read about sporogenesis. Sporogenesis is of two types: microsporogenesis and megasporogenesis. The end products of microsporogenesis are microspores in the anthers, and the end products of megasporogenesis are megaspores in the ovules. In this unit we take you to the study of the next interesting set of events that lead to the formation of gametes. You have learnt that a "microspore" represents the first stage of the male gametophytic generation. After pollination the microspore undergoes a division to form a generative cell and a vegetative cell. As soon as the microspore undergoes the first division, it comes to be referred to as the pollen grain or the male gametophyte. The generative cell gives rise to two male gametes or sperms by a mitotic division. This sequence is classified as the "normal" course of microgametogenesis, about which you will read in section 2.2. The microspore may also take an unusual course of development. We shall discuss the unusual developmental variation of male gametophyte in sub-section 2.2.4.

In megagametogenesis, a sequence of divisions leads to the formation of an egg cell or the female gamete or megagamete. As a result of division of the megaspore nucleus and its products, eight nuclei are formed, which become organised into: a central cell bearing two polar nuclei, three antipodal cells, two synergids and one egg cell. The egg cell is the female gamete. The enlarged gametophyte is also known as the embryo sac. We shall discuss the details of megagametogenesis in section 2.3.

Objectives

After going through the unit you should be able to:

- trace the sequence of events in the development of microspore;
- exemplify deviations from normal development at various steps;
- draw a parallel between male and female gametophyte;
- account for sterility in pollen grains;
- compare with examples, the monosporic, bisporic and tetrasporic modes of embryo sac development;
- correlate the major components of a typical mature embryo sac, i.e., the female gametophyte;
- explain specific functions of various cell types in a female gametophyte.

2.2 MALE GAMETOPHYTE

You already know that a microspore represents the first stage of the male gametophytic generation. A short resting period occurs between the formation of the microspore and the division of its nucleus. The nucleus of the microspore lies in the centre to begin with. Owing to the formation of the vacuole it is pushed towards the wall.

2.2.1 Formation of Vegetative and Generative Cells

The division of a pollen grain results in two unequal cells—the vegetative cell and the generative cell. The pollen grain is hereafter known as the male gametophyte because both the cells of this structure carry a single set of chromosomes. Do you recall that gametophyte represents the haploid generation of the life cycle of a plant? The larger cell is the vegetative cell and it forms the pollen tube. The smaller cell is known as the generative cell and it produces two sperms by a mitotic division. The position of the generative cell is related to that of the microspore nucleus and the orientation of the spindle. You can see in Figure 2.1 b that before division the pollen nucleus migrates to one side of the pollen grain and comes to rest near the wall. The division involves the formation of an unusual spindle structure.

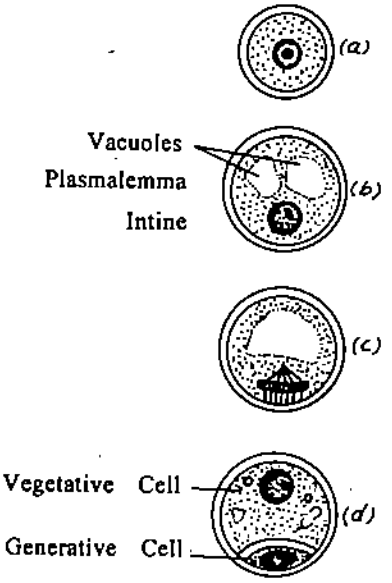

Fig. 2.1: Diagrammatic representation of stages in the formation of vegetative and generative cells; exine is not drawn. (a) Pollen soon after its release from the microspore tetrad. (b) The cytoplasm of the pollen grain has become highly vacuolated, and the nucleus has been displaced to one side. (c) Pollen mitosis; note the asymmetric spindle. (d) Two-celled pollen soon after the pollen mitosis. (e) Generative cell wall has appeared in between the plasma membranes of the vegetative cell and the generative cell. The important thing to note is the unequal division and the curved wall.

The unequal division of the microspore is considered to be related to the form of the nuclear spindle (Fig. 2.1 c). The pole next to this end of the pollen grain wall is flattened. This is termed the generative pole. Depending on the asymmetry of the spindle the phragmoplast forms a more or less curved watch-glass shaped wall which separates the generative (Fig. 2.1 d) cell from the vegetative cell. The watch-glass shape of the wall follows from the Sachs' law or Errera's law, whereby wall formation takes place in a plane which covers the minimal surface area. This wall does not fuse with the mother cell wall at the end of its centrifugal growth phase; rather it forms a lining to the mother cell wall which effectively cuts off the generative nucleus entirely (Fig. 2.2).

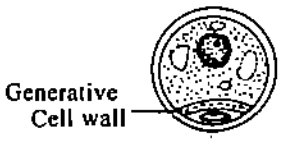

Fig. 2.2: Diagram of an idealised pollen grain at the end of the division which gives rise to the generative cell. The generative cell is enclosed within a non-rigid wall.

This wall is so thin that it is often not clearly visible. Continued development of the generative cell consists of its gradual separation from the mother cell wall. The generative cell becomes detached from its position near the wall so that it eventually

comes to lie within the pollen grain cytoplasm. At this stage it "wanders" into the vegetative cell and assumes an oval or spindle-shape because it is surrounded by a thin wall, delimited on either side by a plasma membrane.

Difference Between Vegetative and Generative Cell

The cytoplasm of the vegetative cell and the generative cell is distinctly different. The generative cell is transparent and contains almost no RNA. The cytoplasm of vegetative cell is rich in RNA. The respective nuclei of these cells differ from each other morphologically and physiologically. The nucleolus in the vegetative nucleus is usually larger than that in the generative nucleus (Fig. 2.2). The vegetative nucleus contains lower amounts of DNA, but its protein content increases after mitosis to about twice that of the generative nucleus. The protein of the vegetative nucleus is more acidic in nature. The structure of the nucleus in vegetative cell resembles a resting nucleus and it does not normally divide. The generative nucleus is small but contains a higher amount of DNA. It may divide in the pollen grain itself or in the pollen tube to form two sperm nuclei.

The pollen grains have reserve food in the form of carbohydrates, proteins and lipids. It appears that the nature of the reserve material in the pollen depends on the mode of pollination. Entomophilous pollen are rich in lipids and anemophilous pollen have mostly starch.

Box 2.1 : Food value of pollen.

The food value of pollen is extraordinarily high. It contains almost every nutrient known to man and may well contain others that no one has as yet successfully been able to analyse. We tend to eat it only in very small amount in honey but it is available in special **pollen tablets,** recently introduced in the market. One pharmaceutical company has devised a harvesting machine to collect pollen grains directly from the flowers. A process removes the outer and inner walls of the pollen as human intestine can not absorb them. This makes the remaining part of the pollen more digestible and rich in food value.

In most plants the division of the generative nucleus occurs in the pollen tube. However, there is variation in the time of division as follows:

(a) The division takes place when the pollens are still enclosed in the anther. Thus, the mature pollen grains are three-celled as in *Portulaca oleracea*.
(b) It may occur in the pollen grains after they have alighted on the stigma.
(c) Division occurs after germination of the pollen and the emergence of the pollen tube (this is the normal condition), or
(d) It may be postponed till the pollen tube has reached the embryo sac.

The division proceeds normally through the formation of a mitotic spindle and an equatorial plate (Fig. 2.3. a-c). Cell division occurs through the formation of a cell-plate, for example in, *Portulaca*. The partition may also take place by furrowing as in *Vinca* and *Vallisneria*. In *Vallisneria* and in some other plants either type of division may occur even in the same plant.

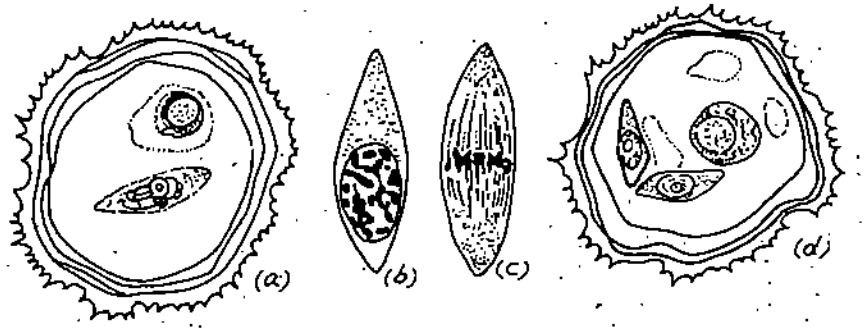

Fig. 2.3: (a–b) *Portulaca oleracea*. Division of the generative cell in the pollen grain. a) Two-celled pollen grain with spindle-shaped generative cell. b) Late prophase in generative cell. c) Metaphase. d) Pollen grain with two sperm cells.

DNA synthesis in the generative nucleus starts immediately after pollen mitosis. This is followed by a more or less prolonged mitosis. During this period the generative cell undergoes elongation and acquires a worm-like appearance. In plants with binucleate pollen grains the division of the generative cell is postponed until pollen germination. The pollen grain passes through a state of temporary dormancy "with it's nucleus in mitotic prophase."

As a result of the division in generative cell, two sperm cells are produced (Fig. 2.3 d). The two sperms formed usually remain in close association.

2.2.2 Pollen Wall Structure

The pollen wall comprises two layers; the inner intine and the outer exine. The exine is further stratified into many sublayers (See Fig. 2.4 a and b). The details of its structure have been understood by means of light and scanning electron microscope. You will learn about details later on.

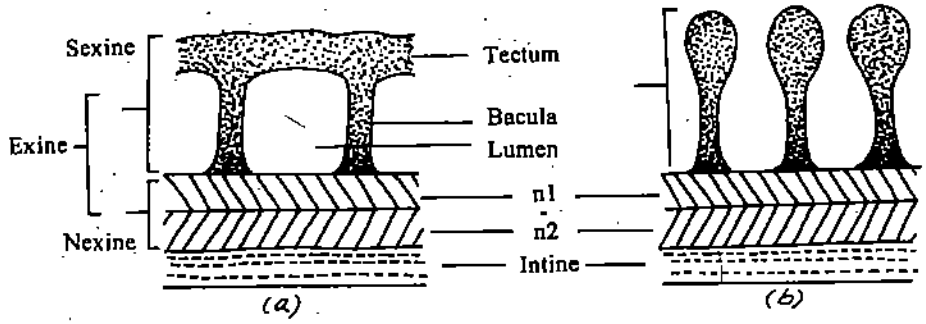

Fig. 2.4: a and b. Pollen wall architecture and terminology of wall layers. (a) Tectate exine. (b) Pilate exine.

The exine is composed of sporopollenin, a highly resistant material believed to be produced by oxidative polymerisation of carotenoid pigments and carotenoid esters. The sculptured part of the exine is made up of radially oriented rod-like *baculae*. The baculae may be enlarged above and remain free (Fig. 2.4 b) or may be fused together forming a raised wall called *tectum* (Fig. 2.4 a) often disposed in a reticulate pattern. In tectate pollen grains the lacunae open to the outside through holes termed micropores (not shown in figure).

The intine is pectocellulosic as is the primary wall of the somatic cells. Do you recall from unit 20 of the course on Cell Biology that pectocellulose is a polysaccharide typical of plant cells. It consists of cellulose mixed with pectose. A special feature of the intine is the presence of beads, ribbons or plates of enzymatic proteins, particularly in the vicinity of germpores.

2.2.3 Development of Pollen Wall

Soon after the completion of meiosis, the blue print for the exine, the primexine, is formed while the microspore tetrads are still enclosed in a wall made up of callose. Callose is β-1, 3-glucan. There are two phases in the formation of pollen wall (Fig. 2.5.). In the microspore tetrad stage the wall material is contributed by the cytoplasm of the microspore alone. In the second phase, after the callose is dissolved through enzymatic degradation and the microspores are released, then the wall materials are contributed by the tapetal cells.

The first layer of pollen wall (primexine) is cellulosic. The cellulosic microfibrils of this layer are deposited in between the convoluted plasmalemma of the microspore and the callose wall. In the cytoplasm of the microspore, just below the plasmalemma, there appear plates of endoplasmic reticulum (Fig. 2.6). The cellulosic primexine is discontinuous in these regions and the gaps thus formed are future germpores. When the cellulosic primexine has reached a certain thickness, additional gaps appear in it. As the tetrad grows, the columns of convoluted lamellae are deposited in these gaps at the surface of the plasmalemma (Fig. 2.7.c,d). These columns are called *probaculae*. Now

the spore cytoplasm synthesises the precursors of sporopellenin which are polymerised and deposited on the surface of the lamellae. The columns are now called *baculae*. The distribution and orientation of bacular columns vary according to the pattern of mature exine. Later the lower ends of the bacula spread sideways into the cellulosic layer of the primexine and coalesce to form the foot layer (Fig.2.7. c-h). The tops of the bacular column also spread sideways in all directions to form the tectum or else simply enlarge to form knob like structures. To this extent the exine is formed inside the callose wall of the tetrad. Thus, the pattern of mature exine has been laid down before the spores are set free.

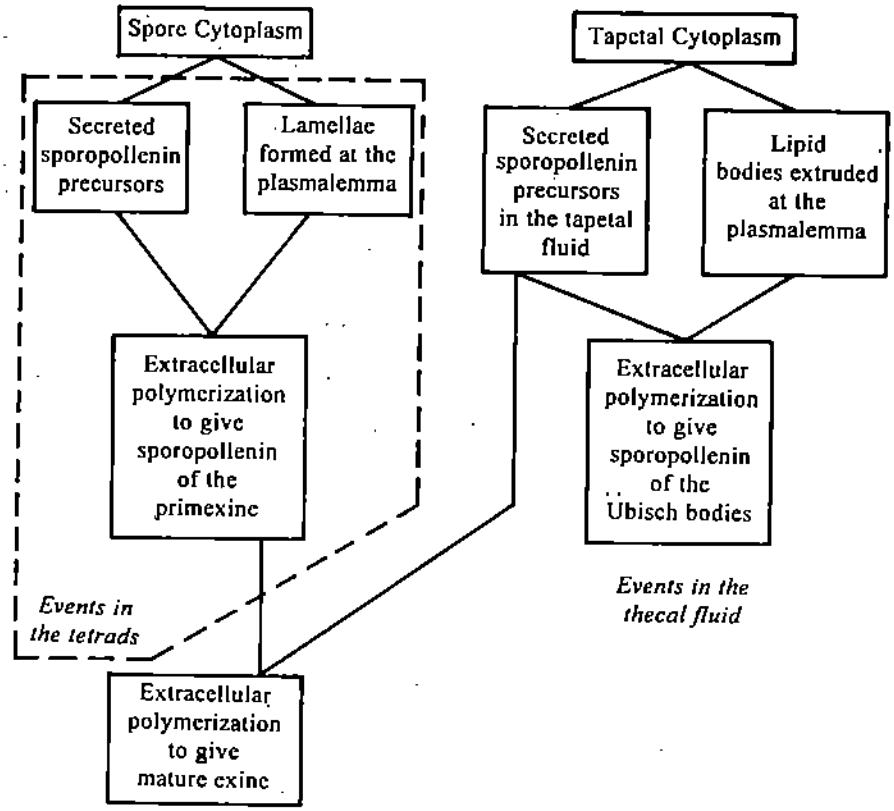

Fig. 2.5. Schematic representation of the formation of Ubisch bodies and exine development in *Lilium* (After Heslop–Harrison, 1972).

In a microspore tetrad the callose is gradually digested (Fig. 2.7 h) and the individual microspores are set free within the anther locule. In the free state, pollen grains synthesise the intine and the inner most layer of exine. (Fig. 2.7. h). The formation of the intine is by the activity of the dictyosomes in much the same manner as that of the primary wall of the somatic cells. There are two special features of intine. First, during the early growth of intine the thickening of the innermost layer of the exine (endexine) continues, and the lamellar materials and sporopollenin precursors which are contributed by the spore cytoplasm must pass through the developing intine. Secondly, certain proteinaceous plates or ribbons which are incorporated in the vicinity of the germ-pores show enzymatic activity. Intine proteins are therefore, a product of pollen cytoplasm (the male gametophyte), but the exine proteins are contributed by tapetal cytoplasm (sporophytic origin).

When the tapetal cells break down the enclosed proteins and lipids are released into the sporangial cavity as pro-ubisch bodies, and eventually become deposited in the surface depressions of the exine (Fig. 2.8). In tectate grains the protein fraction passes through the microspores of the tectum and accumulates in the spaces between the baculae, while the lipids remain on the surface of the tectum.

The intine and exine proteins of a pollen grain have gametophytic and sprophytic origin, respectively.

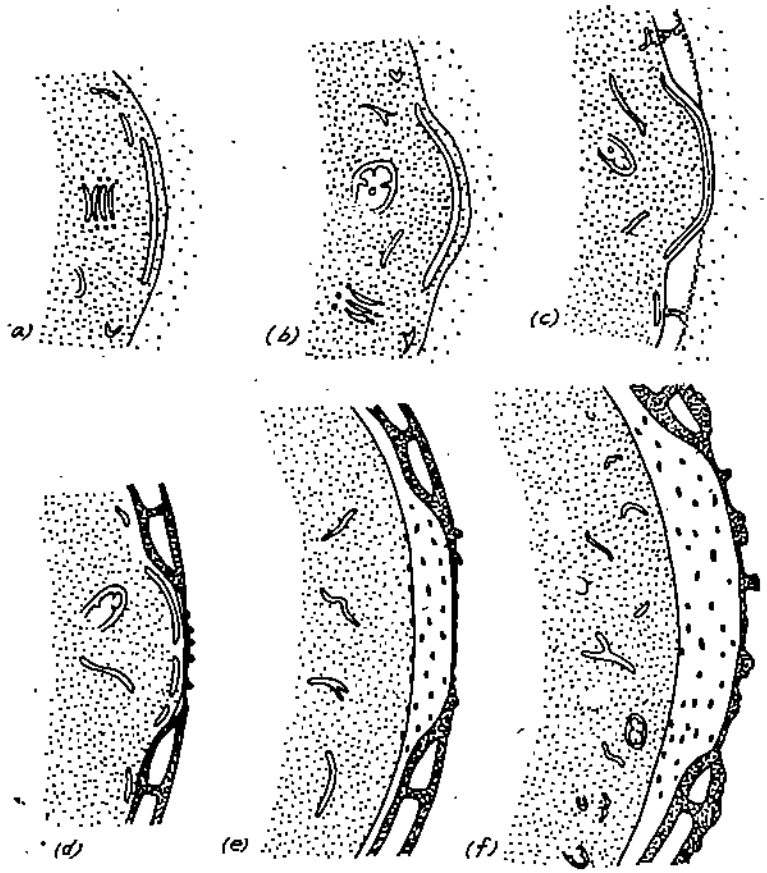

Fig. 2.6: (a-f) A Part of transverse section through microspore of *Silene pendula*. Development of pollen wall. (a-c) Stages within callose wall (cw). (d-f) Development after release (from Shivanna and Johri, 1989).

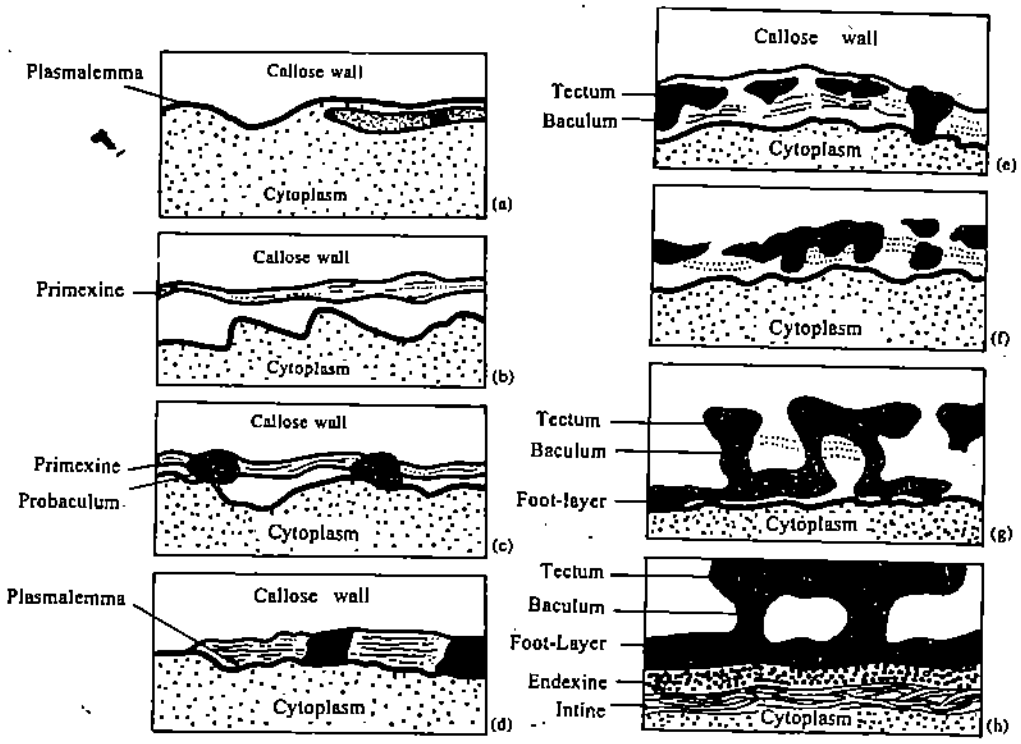

Fig. 2.7: Development of pollen wall. Only a portion of the microspore/pollen (including portions of the plasmalemma, cytoplasm and wall has been magnified in all the diagrams. (a) Plasmalemma is directly surrounded by the callose wall. (b)Cellulosic primexine has appeared between the plasmalemma and the callose wall. (c,d) Probacula have penetrated the primexine. (e)Bacula and tectum are formed. (f,g) The callose wall has disappeared and ektexine is well-developed. (h) The wall is fully developed.

The pollenkitt substances of some plants cause irritation in lung and wind pipe and other allergic reactions in sensitive persons.

Pollenkitt: The lipoidal layer found on the outside of the mature pollen grains of many insect pollinated species is known as the pollenkitt. In addition to lipids it is made up of flavonoids, carotenoids and degeneration products of tapetal proteins. The carotenoids are responsible for its characteristic yellow or orange colour. The function of pollenkitt is not clear. It is believed to act as an insect attractant, help in pollen dispersal and in protecting against the damaging effects of Uv rays. Deposition of flavonoids appears important for the normal functioning of pollen. In maize it has been shown that the pollen grains that lack flavonoids are incapable of developing tubes that are effective in fertilisation. Lipoids also help in checking water loss after anther dehiscence and discharge of pollen.

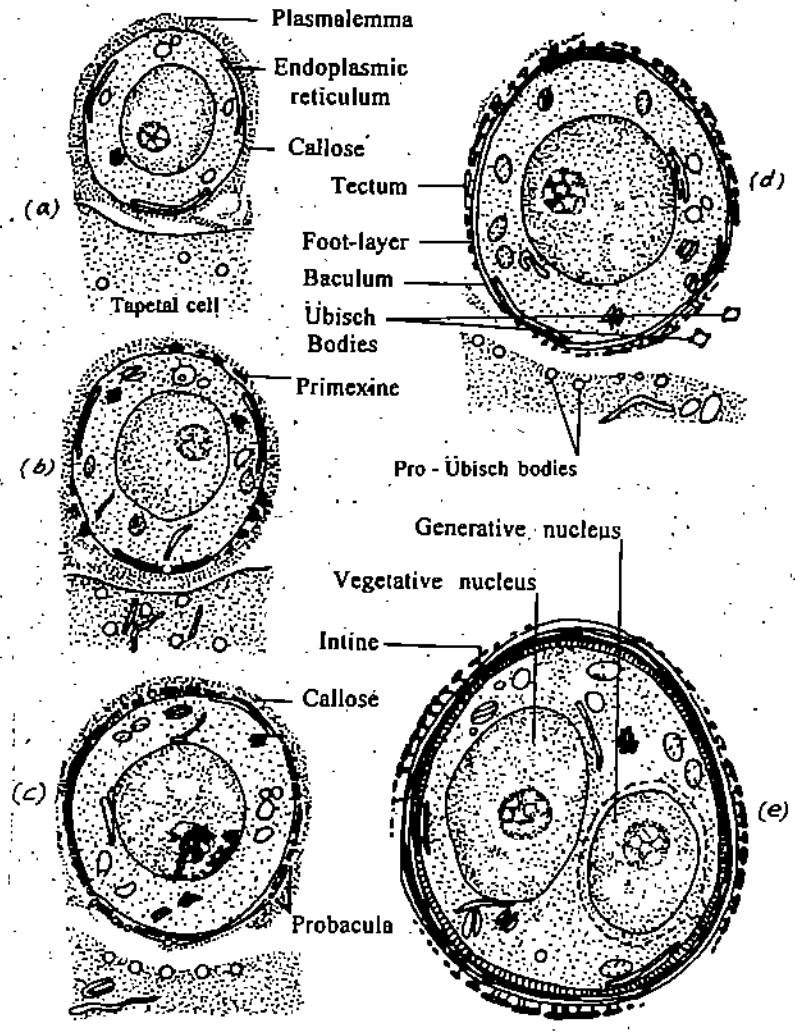

Fig. 2.8: (a-e) Formation of Übisch bodies and development of pollen wall.

Before we go on to another interesting topic–pollen variants, let us take stock of what you have learnt in this section.

SAQ 1

I) Classify the following statements as true or false. Indicate your choise by writing T or F in the boxes provided on the right hand side.

i) The pollen grain becomes male gametophyte when it divides to form a generative cell and a vegetative cell. [ ]

ii) The exine is composed of sporopollenin, whereas, the intine is made of pectocellulose. [ ]

iii) The blue print for exine and primexine, is laid down while the microspore tetrads are still enclosed in the callose wall. [ ]

iv) The columns of convoluted lamellae which are deposited in the gaps of primexine are known as probaculae. [ ]

II) Match the statements given in columns on the left side with those given in columns on the right side.

A)	The intine proteins of pollen grains are said to have gametophytic origin because	1)	they come from tapetal cells, which, like all other sporophytic cells have a diploid number of chromosomes.
B)	The exine is composed of sporopollenin, which	2)	is a resistant material. Its chemical formula is β-1, 3-glucan.
C)	The exine proteins of pollen grains are said to have a sporophytic origin because	3)	is a highly resistant material produced by oxidative polymerisation of carotenoid pigments and carotenoid esters.
D)	Microspore tetrads before being released into anther locule are enclosed in callose, which	4)	they are contributed by the microspore cytoplasm. The microspore has a haploid number of chromosomes.

III) Fill in the blanks with appropriate words:

i) The position of generative cell in a pollen is usually fixed for a genus and it is often near the inner wall, and towards the ..... of the tetrad.

ii) The unequal division of the microspores is because of the asymmetric form of ..... , as a result the phragmoplast forms a more or less curved watch glass-like structure.

iii) As a result of mitosis in generative cell of pollen, two ..... are produced which usually remain in close association, forming a pair, for a considerable period of time.

iv) The distribution and orientation of bacular columns varies according to the pattern of mature ..... .

2.2.4 Pollen Variants

So far you have studied the sequence of events in the normal course of development of male gametophyte. Let us discuss some instances in which male gametophyte ends up forming variant structures, such as: abortive pollen; sterile pollen; pollinia and massulae; pollen embryo sacs; and sporophytes. In this unit we shall discuss the first three aspects, only.

Development of Pollen in Cyperaceae: In all members of the Cyperaceae out of the four nuclei formed after meiosis, only one functions, the others degenerate. The functional nucleus remains in the centre and the three non-functional nuclei are pushed to a side. In *Cyperus*, *Kyllinga*, and *Scirpus* the non-functional nuclei are separated from the functional nucleus by a wall (Fig. 2.9 a,b). Walls are also laid between the nonfunctional nuclei. The functional nucleus divides to form a vegetative cell and a

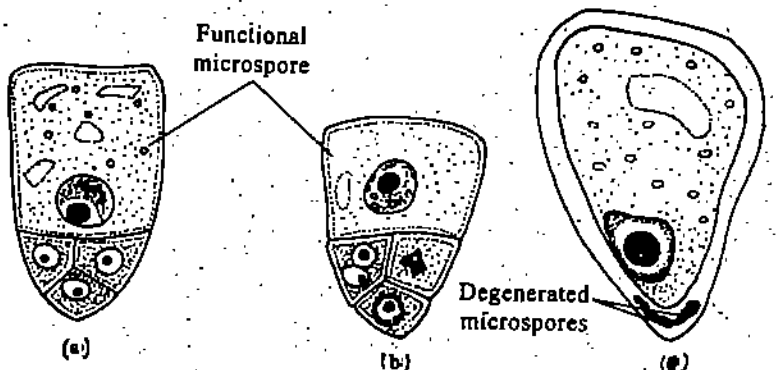

Fig. 2.9: (a-c) Pollen development in *Cyperus*. (a) The non-functional microspores have been cut off on one side. The functional spore is large and contains a prominent nucleus. (b) Two of the non-functional spore nuclei have divided. (c) The non-functional microspores have degenerated.

generative cell. The non-functional nuclei may also begin to divide but the divisions are abortive, and they eventually degenerate (Fig. 2.9c). Thus in a tetrad, only one pollen grain is functional. This feature is similar to the behaviour of megaspores in the development of monosporic embryo sac as you will study in the next section.

Pollen Sterility: Among several causes of male sterility we will discuss only the one in which non-viable pollen grains are produced due to malfunctioning of the spore mother cells.

Sterility in angiosperms may arise due to several causes. Either the anther may not be formed, or anthers are formed but meiosis may be abnormal.

A. In situations where anthers are not formed, they may take abortive forms and appear like petals or sepals. These conditions are referred to as **petaloidy and phyllody** respectively. There are even cases where anther formation may be suppressed altogether. These cases of pollen sterility have premeiotic determination.

B. There are other examples in which anthers are produced but meiosis is abnormal resulting in the formation of non-viable pollen. We shall come back to this aspect a little later and study it in detail.

C. Sometimes normal pollen grains are formed but they are not released. This results from failure of anther dehiscence. Such a situation is also described by the term **contabiscent** anther.

D. Sometimes meiosis is normal but abortion can take place due to premature dissolution of callose. This can occur at any stage during microsporogenesis.

Let us come back to cause B, i.e., aberrant meiosis. Male sterility in which nonviable pollen are produced due to malfunctioning of meiocytes is termed gametophyte-determined sterility (a). Alternatively, non-functional pollen may be produced because of some causes/factors emnating from sporophytic tissue. This is termed sporophyte-determined sterility (b).

(a) Gametophyte-determined sterility is usually due to the following two causes:

  (i) Meiotic irregularities. In polyploid plants meiocytes divide resulting in unequal distribution of chromosomes in the daughter cells. As a result, meiosis produces genomically imbalanced spores. Another example is of plants heterozygous for a gene or plants having a minor deficiency which is lethal in the haplophase. They will produce nonviable pollen.

  (ii) abberrant gametophyte is a condition exhibited in some plants, which produce normal viable pollen but gametophyte development is incomplete.

(b) Sporophyte-determined sterility resulting in the formation of non-viable spores may be due to several factors.

We are here concerned with only the second cause of male sterility in which non-viable pollen grains are produced due to malfunctioning of spore mother cells or gametophyte, or it may be due to some sporophytic factor. In the former case (type B-a) an anther may contain a mixture of fertile and sterile pollen grains whereas in the latter case (type B-b) the entire population of pollen grains within an anther would be sterile.

Some plants invariably produce 2-3 types of pollens, which are morphologically different from each other. It appears that they distribute functions namely, attracting the pollinator, providing food for pollinator and carrying out fertilisation of the female gamete. Production of different forms of pollen to perform different functions is known as **pollen polymorphism.**

Gametophyte-determined pollen sterility is usually due to meiotic pollinator and carrying out abnormalities, such as meiosis in polyploids which often results in unequal distribution of chromosomes in the daughter cells. Plants heterozygous for a gene or minor deficiency which is lethal in the haplophase will also produce non-viable pollen.

Sporophyte-determined pollen sterility is due to genic, cytoplasmic, or environmental factors. It may become operative at any stage of anther development. Genic and cytoplasmic pollen sterility is widespread among angiosperms. The latter is of considerable importance in plant breeding. Genic pollen sterility is due to recessive genes. Maize shows genic as well as cytoplasmic pollen sterility.

Certain plant growth regulators such as auxins, maleic hydrazide, mendok (sodium salt of 2,3-dichloro isobutyric acid) and dalapon (2, 2-dischloropropionic acid), induce male sterility.

In most cases the pollen sterility which manifests itself through the malfunctioning of the tapetum. Some of these irregularities are:

1. Inhibition of normal RNA synthesis and increase in DNA content.
2. Hypertrophy of the tapetal cells. They enlarge acquiring supranormal cytoplasmic RNA and become multinucleate. The cells invade the anther locule, and crush the meiocytes or spores.
3. Premature degeneration of the tapetum causing disruption in the nutrition of developing spores.

Pollinia and Massulae: In most cases the pollen grains of each tetrad become separated from one another and they lie freely in the pollen sac. In some plants, mostly members of Ericaceae, the pollen grains remain in tetrads even when mature. In certain plants, such as *Acacia*, the tetrads are stuck together as groups, which may contain as many as 64 pollen grains. In some other plants, such as members of the Asclepiadaceae, all the pollen grains of a sac are united in a single compact mass such a mass is termed a **pollinium.** In some members of the family Orchidaceae the anthers produce groups of pollen grains which are loosely jointed among themselves, usually by means of viscin threads. These groups are called as **massulae.**

This completes our discussion on development of male gametophyte. Let us understand the development of the female gametophyte. But before we start, let us turn to the subsection 2.2.5 and try the following SAQ.

SAQ 2

I. Fill in the blanks using appropriate words.

i) In members of the family ..... , out of the four microspores formed as a result of meiosis, three abort and only one becomes functional.

ii) Pollen sterility in plants may be either gametophyte-determined or sporophyte-determined. The gametophyte-determined pollen sterility is usually due to .... whereas the sporophyte-determined pollen sterility is due to ..... , ... or ..... factors.

iii) Environmental factors affect sporophyte-determined pollen sterility in most cases through malfunctioning of ..... .

2.3 FEMALE GAMETOPHYTE

In this part of the text we shall be using the terms embryo sac and female gametophyte interchangeably, because in ontogenic terms embryo sac represents the female counterpart of the haploid generation of the angiosperms, just as the pollen represents the male gametophyte.

In the previous unit you have studied, up to the formation of four megaspores (Subsection 1.3.3). Let us pick up the thread from there and study the development of a typical megaspore into a female gametophyte. The development of a female gametophyte is initiated with the enlargement of one of the megaspores (usually the one close to the chalaza in a linear tetrad) followed by three mitotic divisions. Thus, the female gametophyte, also called the embryo sac, is almost always a 7-celled or 8-nucleate structure (Fig. 2.10). The eight nuclei thus formed organise into the egg apparatus, central cell with two polar nuclei and three antipodal cells. This mode of embryo sac development occurs in the majority of flowering plants.

There are variations from this mode of female gametophyte development. In order to visualise these deviations let us recall the formation of megaspore tetrad. Out of the four megaspores, three degenerate and only one participates in the formation of embryo sac. This mode of development of female gametophyte is known as the **monosporic** type. In

some plants, two megaspores out of four participate. For example, if after meiosis I, the lower or the upper dyad produces the embryo sac and the other degenerates, the development is termed **bisporic**. Sometimes, cell plate formation does not occur after any of the meiotic divisions and all the four megaspore nuclei contribute to embryo sac formation. This is called **tetrasporic** type of development. Depending upon the orientation and subsequent behaviour of the megaspore nuclei and their derivatives, the tetrasporic gametophyte may be of several types.

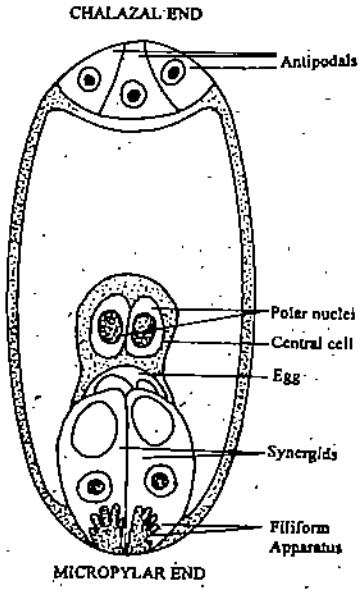

Fig. 2.10: Diagram of an organised embryo sac.

2.3.1 Types of Female Gametophyte

From the following account you would realise that depending on the number of megaspore nuclei involved in the formation of gametophyte, the embryo sac development may be classified as monosporic, bisporic or tetrasporic types. Each of these type has more than one variant, generally named after the genus in which it was first described (Fig.2.12).

MONOSPORIC EMBRYO SACS

The monosporic embryo sac is that which is derived from only one megaspore, as in the genus *Polygonum*. All the nuclei in such an embryo sac are genetically alike as they are derived from mitosis of a single nucleus. There are two types of monosporic embryo sacs:

1. *Polygonum type*: The embryo sac is formed from the chalazal megaspore in the tetrad and is eight-nucleate.

The development of the embryo sac begins with elongation of the functional megaspore. Initially, the megaspore cytoplasm is non-vacuolate but later small vacuoles appear which fuse to form a large vacuole. The spindle of the nuclear division in the megaspore is oriented along the long axis of the cell. Wall is not formed after the nucleus divides. A large central vacuole appears between the two daughter nuclei. It expands and pushes the nuclei towards the opposite poles of the cell (Fig. 2.11 a). Both the nuclei divide to form four nuclei, two at each pole (Fig. 2.11 b-c). By a further division an eight nucleate condition is reached (Fig. 2.11c-d). This is followed by cellular organisation of the embryo sac (Fig. 2.11 e). Out of the four nuclei at the micropylar end of the sac, three organise into egg apparatus and the fourth is left free in the cytoplasm of the central cell as the upper polar nucleus. Similarly, three nuclei of the chalazal quartet form three antipodal cells; the fourth one functions as the lower polar nucleus. Eventually, the latter comes to lie close to the upper polar nucleus.

2. *Oenothera type*: This type of embryo sac is derived from the micropylar megaspore of the tetrad and is four nucleate and is characteristic of the family Onagraceae. The mature embryo sac has an egg apparatus and a uninucleate central cell. (Fig. 2.12 b).

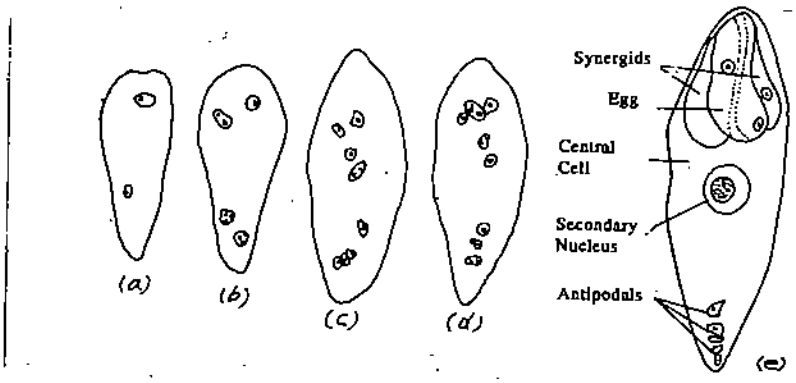

Fig. 2.11: Stages in megagametogenesis (a) Megaspore after first post-meiotic division. (b) 4-nucleate stage. (c) 8-nucleate stage. (d) older 8-nucleate stage, showing 3+2+3 distribution of the nuclei. (e) Mature embryo sac; it comprises 3-celled egg apparatus, 3 antipodal cells, and a large central cell with a secondary nucleus, the fusion product of upper and lower polar nuclei.

BISPORIC EMBRYO SACS

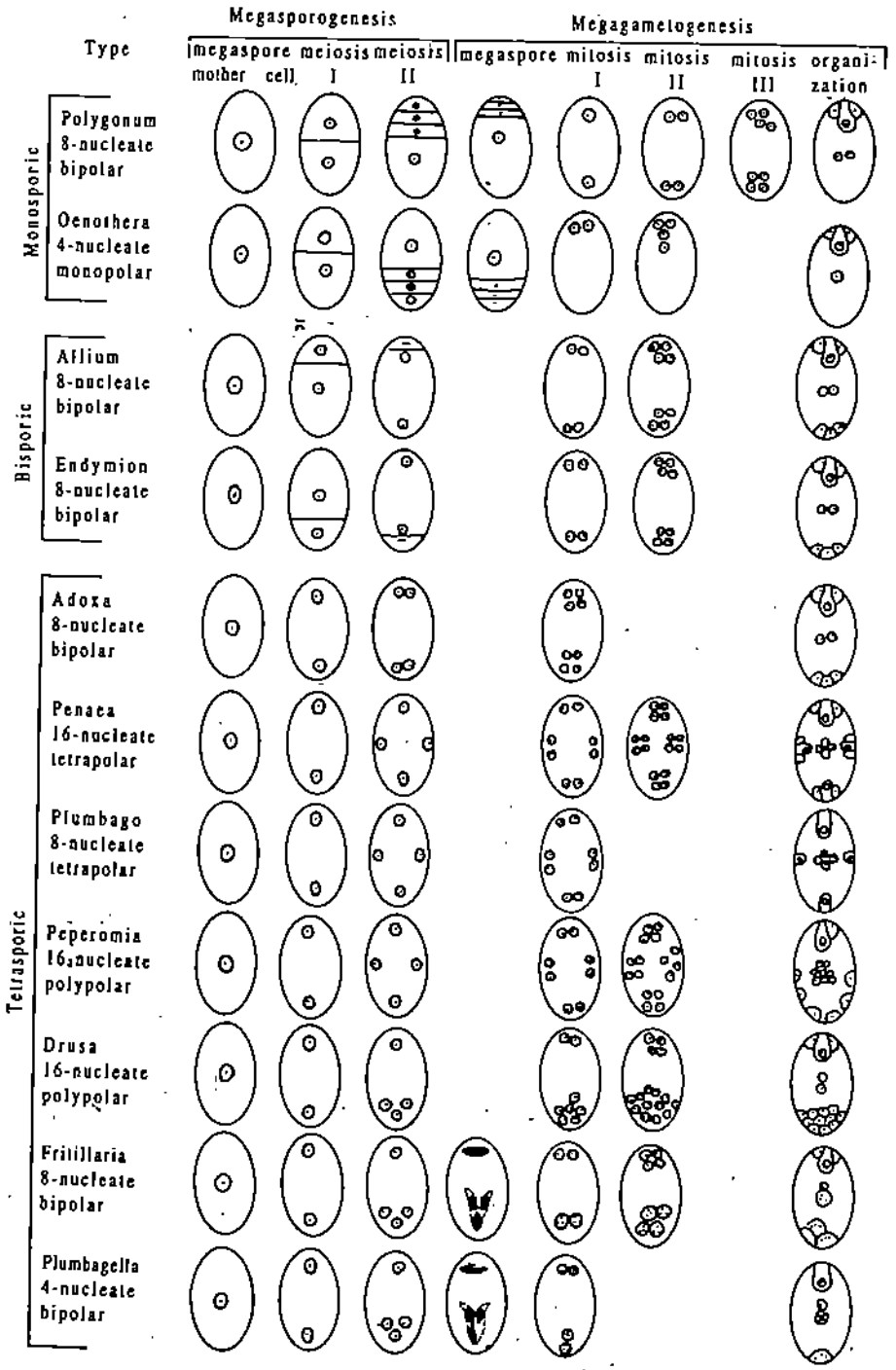

Fig. 2.12: Diagrammatic representation of various types of embryo sac development.

As already explained, the bisporic embryo sac is derived from one of the dyads formed after the first meiotic division, whereas the other dyad subsequently degenerates. In the functional dyad cell wall formation does not occur after the second division and thus both the megaspore nuclei undergo two mitotic divisions forming eight nuclei. The final organisation of embryo sac is similar to the Polygonum type. (Fig. 2.12 c, d).

Since a bisporic embryo sac is derived from two meiotic products, their nuclei have two different genetic constitutions; four nuclei are of one type and the other four of a different type. Bisporic embryo sacs are of two types:

1. Allium type: Derived from the chalazal dyad cell.
2. Endymion type: Derived from the micropylar dyad cell.

TETRASPORIC EMBRYO SACS

In this group neither of the meiotic divisions is accompanied by wall formation so that at the end of meiosis all the four haploid nuclei remain in a common cytoplasm forming what is termed a coenomegaspore. A tetrasporic embryo sac is more heterogeneous than a bisporic embryo sac because the four products of meiosis involved in its formation are genetically different.

The nuclear behaviour in tetrasporic embryo sacs is quite variable. The arrangement of the four nuclei in the coenomegaspore, before the beginning of post-meiotic mitosis, is of three types: (a) 2+2 arrangement (Fig.2.12 e); two nuclei at the micropylar end and two of the chalazal end (b) 1+1+1+1 arrangement (Fig. 2.12 f, g, h); one nucleus at the micropylar end, one at the chalazal end, and two placed laterally, one in each side, and (c) 1+3 arrangement (Fig. 2.11 i, j, k); one nucleus at the micropylar end and three at the chalazal end. Depending on whether nuclear fusion occurs or not, the number of the post-meiotic mitosis in the coenomegaspore and final organisation of the embryo sac, tetraporic embryo sacs are of many types. For explanation see Fig.2.11.

SAQ 3

Fill in the blank spaces with appropriate words.

i) The development of embryo sac is classified into various types depending on the number of megaspores participating in its formation. If out of four megaspores three degenerate and one forms the embryo sac, it is ..... type of development. If two megaspores degenerate and the remaining two form the embryo sac, it is ..... type of development and, if all the four megaspores take part in the formation of the embryo sac it is termed ..... type of development.

ii) In tetrasporic embryo sacs the nuclei may orient themselves in three ways. Before the beginning of post-meiotic mitosis the four nuclei may arrange into: either (a) ..... arrangement, (b) ..... arrangement, or (c) ..... arrangement.

2.3.2 Mature Embryo Sac

At maturity a typical embryo sac comprises the following components: synergids, an egg cell, a central cell with two polar nuclei, and tree antipodal cells. The two synergid cells along with one egg cell constitute the egg apparatus. There is much variety in the number and arrangement of nuclei/cells in the mature embryo sacs.

Synergids — As seen in the longitudinal section of the embryo sac the synergids may be hooked or beaked. They usually show a vacuole at the chalazal end and the filiform apparatus and nucleus at the micropylar.

The wall around the synergids is incomplete. There is a distinct wall around the micropylar one-third of synergid cell which thins towards the chalazal end and, finally, disappears (Fig.2.13). As a result, the chalazal one-third of the cell lacks a wall. In this part the protoplast of the synergids is separated from that of the central cell by double membranes; one of the synergids and the other of the central cell. This description of wall is true for cotton and most other species investigated. A prominent structure, called *filiform apparatus*, is present at the micropylar end of the synergids (Fig. 2.13). Transmission electron microscopic study has shown that the filiform apparatus is a

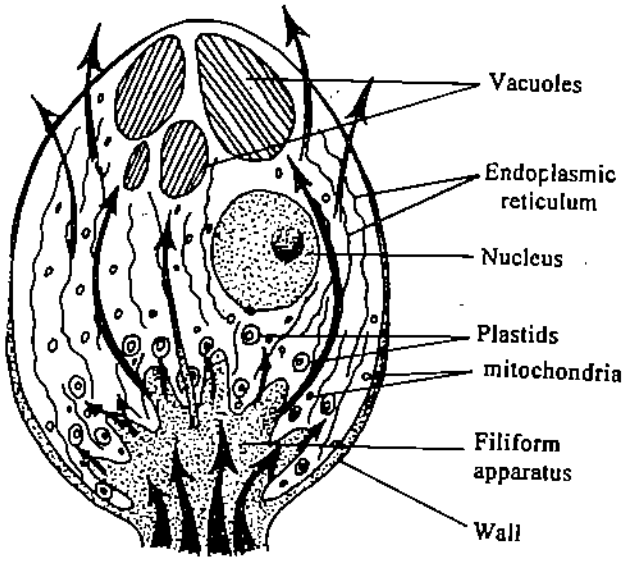

Fig. 2.13: A sectional view of mature synergid of cotton (diagrammatic). Most of the cell organelles are concentrated round the filiform apparatus. The arrows depict the proposed direction of flow of nutrients into the embryo sac through the synergid.

mass of finger-like projections of the wall into the cytoplasm (Fig. 2.14 a). Structurally, each projection of the filiform apparatus has a core of tightly packed microfibrills (possibly cellulosic) enclosed by a non-fibrillar sheath. They are rich in polysaccharides. The structure of filiform apparatus resembles the spongy wall of the "transfer cells" associated with short distance transport across membrane.

Transfer cells: These are associated with the flow of solvents and solutes, where there is large disparity in surface to volume between donor and the recipient compartments. The cell develops wall ingrowths and the membrane is thrown into folds.

The cytoplasm of a synergid is strongly polarised. The chalazal region of the cell is occupied by one large or many small vacuoles (Fig. 2.14 a). In cotton these vacuoles appear to be rich in calcium salts and carbohydrates. Large amount of cytoplasm and a prominent nucleus are present in the micropylar half of the cell. The cytoplasm is rich in mitochondria, endoplasmic reticulum and dictyosomes which are specially concentrated near the filiform apparatus (Fig. 2.14. b).

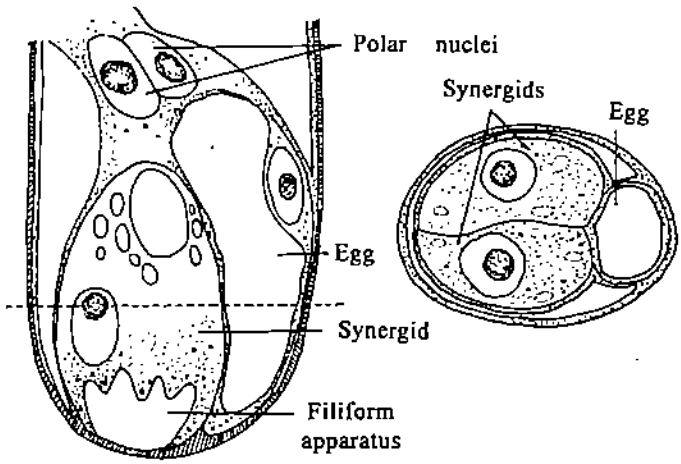

Fig. 2.14: (a) Diagram of the micropylar end of a mature embryo sac of cotton. (b) Transverse section of embryo sac at the level marked in a.

Synergids are short-lived structures. In embryo sacs of most species one of the two synergids degenerates before the entry of pollen tube into the embryo sac. The other, often called **persistent synergid**, that degenerates shortly afterwards.

In very rare instances synergids assume a haustorial appearance. In *Quinchamalium chilense*, a member of the family Santalaceae, the synergid elongates and reaches a length of up to 1,200 µm (Fig. 2.15). You will read about other types of haustoria in Subsection 2.3.3.

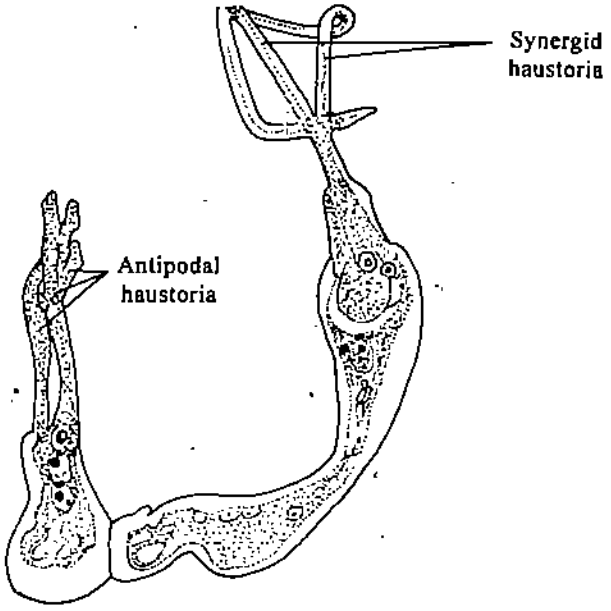

Fig. 2.15: Mature embryo sac of *Quinchamalium chilense* showing synergid, and antipodal-haustoria.

Functions of Synergids

Ultrastructurally the structure and concentration of cell organelles reveals that synergids are metabolically active. The following three functions are attributed to synergids.

1. Directing of pollen tube growth by secreting some chemotropically active substances.
2. The degenerating synergid forms the site for pollen tube discharge in the embryo sac.
3. The filiform apparatus may aid in the absorption and transporation of materials into the embryo sac from the nucellus (Fig.2.13).

Egg: The three cells of the egg apparatus are arranged in triangular fashion with the egg sharing a common wall with the two synergids and the central cell. In the egg the wall is thicker at the micropylar end and is absent at the chalazal end. At this end the lateral walls of the egg cell appear to join the central cell wall. The egg cell wall is traversed by plasmodesmata on the sides of the two synergids and the central cell but not at its outer face.

The egg cell becomes highly polarised early in its development. Polarity is expressed by the aggregation of cytoplasmic elements at the chalazal end of the cell. The micropylar end of the cell is occupied by a large vacuole. Thus, the distribution of the vacuole and cytoplasm in the egg cell is just the opposite of that in the synergids.

The ultrastructure of the egg cytoplasm indicates that it is inactive. The mitochondria show only a few cristae. But for *Zea mays*, dictyosomes are either absent (*Epidendrum*) or are only a few in number. Where present, the dictyosomes exhibit an inactive state. Plastids are present in the egg; they often contain a striking difference from the male gametes. The egg cytoplasm is rich in ribosomes.

In *Plumbago capensis*, where the embryo sac lacks syngergids. However, many finger-like wall projections arise at the micropylar end of the egg cell. They resemble the filiform apparatus of the synergid. In this plant the egg cell seems to have taken over the role of synergids in addition to its own gametic function.

Antipodals: The antipodals show a great deal of variation among the components of the embryo sac. Usually they degenerate just before or soon after fertilisation. In many plants antipodals are persistent and show some structural and cytological features suggesting their possible role in the nutrition of the embryo sac.

In grasses the antipodals undergo mitotic divisions to form as many as 300 cells. In *Zea mays*, during additional divisions in antipodals, the walls of many cells remain

incomplete resulting in the formation of a multinucleate protoplast or a syncytium. Antipodal nuclei may also become polyploid due to endopolyploidy or polyteny.

The antipodal cells in maize have abundant mitochondria, plastids and multicisternal dictyosomes. The cytoplasm has numerous small vesicles derived from the endoplasmic reticulum or the dictyosomes. An interesting feature shown by some of these cells is the presence of papillate wall outgrowths projecting into the cytoplasm. These outgrowths, which are restricted to the cells bordering the nucellus, appear similar to the filiform apparatus. Similar wall projections have also been noticed in the antipodal cells of the poppy plant.

Histochemical tests have revealed that the antipodal cells are rich in protein, peroxidase, cytochrome oxidase, ascorbic acid and sulfhydril compounds. They show very low concentrations of RNA and polysaccharides.

There are several instances in which antipodal cells act as haustoria. In *Argemone mexicana* the antipodal cells are very large and they persist up to the heart-shaped stage of the embryo. They consume 8–10 layers of the nucellar cells at the chalazal end.

The role of antipodals is not clearly known. A nutritive role has been presumed for those antipodals which are persistent. The nature of their cytoplasm suggests that they are highly active cells. They resemble cells of secretory anther tapetum and integumentary tapetum in having a high DNA content. The presence of wall projections in antipodal cells of certain plants indicates that they may be actively associated with the nutrition of the embryo sac.

Central Cell: It is the largest cell of the embryo sac, and the mother cell of the endosperm. The enlargement of the embryo sac after the last nuclear division is mainly due to the inflation of the large central vacuole of the central cell. The vacuole in the central cell is presumably the reservoir of sugars, amino acids and inorganic salts.

The nuclei of the central cell, also called the polar nuclei, are very large, and are characterised by a conspicuous nucleolus. They are present either in the centre of the cell, suspended by cytoplasmic strands, or in the cytoplasm close to the egg apparatus (Fig.2.10). In the latter case the chalazal portion of the embryo sac is occupied by a large vacuole. The two polar nuclei fuse before or during double fertilisation to form the secondary nucleus.

The cytoplasm of the central cell is rich in plastids, mitochondria, numerous dictyosomes, and ribosomes or small polysomes. This cell appears to be the centre of intensive synthetic activity in the embryo sac.

The thickness of the central cell wall is variable. It is thickest in the regions in contact with the nucellus. Where the central cell touches the egg and synergids, it shows the common feature of partial wall. It becomes thinner towards the chalazal end of the egg apparatus, and finally, in the chalazal region there is no wall between the plasma membranes of the central cell and those of the egg and the synergids. The central cell is connected with the egg, synergids, and the antipodals through plasmodesmatal connections.

2.3.3 Haustorial Behaviour of Embryo Sac

There are instances in which the entire embryo sac may grow beyond the ovular tissue. The central cell may also form multicellular projections from micropylar and chalazal ends which may penetrate into the carpellary tissues and function as haustoria.

In the family Loranthaceae which is characterized by semi-parasitic plants, the embryo sacs are usually highly elongated. In one plant of this family, *Moquiniella rubra* the tip of the embryo sac has been observed to enter the style, touch the stigma and even grow down downwards. The maximum length of such embryo sacs is about 48 mm.

2.3.4 Nutrition of Embryo Sac

The morphology of the ovule suggests that the chalazal end is the main route for the entry of nutrients. The funicular vascular supply terminates at the base of the

integuments. The nutrients reach the embryo sac via nucellus. Often a patch of specialised tissue called **hypostase** is present in between the funicular vascular supply and the chalazal end of the embryo sac. The hypostase is also presumed to have a role in nutrition supply.

In some cases the nucellar tissue is completely consumed during the development of the embryo sac. The inner integument becomes a glandular endothelium, and absorbs nutrients from outer tissues and supplies them to the embryo sac. Because of its structural and functional similarity to anther tapetum the endothelium is also called **integumentary tapetum** (see Fig. 1.21, Unit-1).

In this unit you have learnt the main aspects of gametogenesis. In the next unit we will discuss pollination and fertilisation, two important events in life cycle of a plant. How about solving an SAQ before we summarise this unit?

SAQ 4

I. Match the items given in the column given on the left hand side with those of the right hand side.

A. The synergid which degenerates first	i) takes up the role of absorbing nutrients from outer tissue and supplying them to the embryo sac.
B. The vacuole in the central cell	ii) forms the seat for pollen tube discharge.
C. Besides the embryo sac haustoria, plants may even	iii) develop synergid and antipodal-haustoria, to nourish the embryo sac.
D. Sometimes the entire nucellar tissue is used up. In such cases the inner integument	iv) serves as reservoir of sugars, amino acids inorganic salts for enlargement of embryo sac.

II. Fill in the blank spaces using appropriate words.

i) A prominent structure present at the micropylar end of the synergids, appearing like a mass of finger-like projections of the wall, is known as ..... .

ii) The wall thickness of the central cell of embryo sac is highly variable. It is thickest in the regions against the nucellus and thins out towards the ..... end of the egg apparatus.

iii) The cytoplasm of the synergids is strongly polarised. The chalazal region of the cell is occupied by one large or many small ..... .

III. State whether the following statements are true or false. Indicate your choice in the space provided.

i) The lateral walls of egg cell appear to join the central cell wall at the micropylar end. Actually, the end wall of the egg cell is the wall of the central cell. [ ]

ii) The central cell is connected with the egg, the synergids and the antipodals through plasmodesmatal connections but no such connections exist between the central cell and the adjacent nucellar cells. [ ]

iii) In embryo sacs with two synergids, one degenerates before the entry of pollen tube into the embryo sac. The other synergid degenerates shortly after the embryo sac has received the pollen tube. [ ]

iv) That the synergids are involved in the function of transport of nutrients may be assumed by the texture of the filiform apparatus which is spongy, similar to that noted in "transfer cells". [ ]

v) The wall around the synergids is incomplete. The chalazal one third of the cell lacks a cell wall. [ ]

4.4 SUMMARY

- This unit describes the events in the formation of gametes from the spores, i.e., gametogenesis. The male gametophyte gives rise to sperms and the female gametophyte bears the egg.
- The abnormal course of development of the male gametophyte shows that microspore and megaspore can at times follow similar sequence of cell divisions to produce structures that appear alike.
- Egg and sperms, the key cells in gametogenesis differ widely from each other in their size, form and contents.
- Normally there are two polar nuclei in the central cell, which fuse to from the secondary nucleus. It is connected with the egg cell, synergids and the antipodals through plasmodesmatal connections.
- Haustoria arising from synergids, antipodals, or sometimes from the entire embryo sac procure additional sources of nutrition to the embryo sac.

2.5 TERMINAL QUESTIONS

1. What are the differences between vegetative cell and the generative cell?
2. What are the similarities and differences between male and female gametophyte?
3. Name the three functions of synergids.

2.6 ANSWERS

Self Assessment Questions

1) I i) T
   ii) T
   iii) T
   iv) T
   II A 4
   B 3
   C 1
   D 2
   III i) centre
   ii) nuclear spindle
   iii) sperms
   iv) exine

2) i) Cyperaceae
   ii) meiotic abnormalities, genic, cytoplasmic, environmental
   iii) tapetum

3) i) monosporic, bisporic, tetrasporic,

ii) 2+2, 1+1+1+1, 1+3

iii) fusion

4) I A ii

B iv

C iii

D i

II i) filiform apparatus

ii) chalazal

iii) vacuoles

III i) F

ii) T

iii) T

iv) T

v) T

Terminal Questions

1)

	Criterion	Generative Cell	Vegetative Cell
(i)	Size	Smaller	Larger
(ii)	Quality of cytoplasm	Hyaline, contains almost no RNA,	Dense, rich in RNA
(iii)	Nucleus	Size smaller with low DNA content but high in the amount of proteins	Size large, high DNA content but low in protein content
(iv)	Proteins in the nucleus	Less acidic proteins	More acidic proteins
(v)	Reserve foods	There are no reserve foods	All types of reserve foods are present
(vi)	Products	Divides to form two male gametes.	Does not divide, gets consumed as it nourishes the gametes.

2) **Similarities**

Both produce gametes; have single set of chromosomes and are haploid; may sometimes produce structures similar to each other and undergo similar course of cell division.

Differences

Feature	Male gametophyte	Female gmetophyte
The first state is known as	Microspore	Megaspore
The mature gametophyte	has two nuclei, one of vegetative cell and the other of the generative cell	has generally nuclei-organised into two polar nuclei, three antipodal cells two synergids and one egg cell.

The gamete(s) (the end product)	commonly known as sperms (2 in number) produced by the division of the generative cell	egg generally, (one cell) or, Occasionally four	Gametogenesis

3. The synergids play an important role in directing the pollen tube growth by secreting chemotropically active substances. The degenerating synergids form the seat of pollen tube discharge in the embryo sac.

   The filiform apparatus helps in absorption of materials from nucellus and its transport to the growing embryo sac.

UNIT 3 POLLINATION AND FERTILIZATION

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3.1 INTRODUCTION

The reproductive structure of angiosperms including the details of formation of male and female gametophytes were considered in the earlier units. You may recall that as a result of meiosis, haploid pollen grains and egg cell are formed. After the formation of the gametophytes, the next two essential steps of sexual reproduction, i.e., pollination and fertilisation take place. As a result zygote is formed, which eventually develops into the embryo. Before fertilisation can occur, the pollen must be transferred from the stamen to the stigma of the carpel. The transfer of pollen is known as pollination and it may be accomplished by a number of agencies such as wind, water or animals. Double fertilisation, a process unique to flowering plants, follows pollination. In this unit you will become familiar with the types of pollination, some of the important adaptations exhibited by plants for successful pollination, details of the structural features of the pistil, pollen-pistil interaction, double fertilisation, incompatibility and apomixis.

Objectives

After studying this unit you should be able to:

- explain the process of sexual reproduction in flowering plants;
- describe the methods that the plants adapt to disperse the pollen grains for effective pollination;
- interpret as to why in certain cases hybridization fails;
- define the methods to overcome incompatibility;
- analyse how apomixis operates to ensure survival in certain plants;

- relate the control mechanism that plants have developed to avoid indiscriminate sexual reproduction.

3.2 POLLINATION

Pollination refers to the transfer of pollen from dehiscing anthers to the pistil. Unlike animals, plants cannot move to their mates for sexual reproduction. Hence, they need some external device or agency for the transfer of pollen grains from the male parent to the stigma of the female parent. Exceptionally, in *Vallisneria*, an aquatic plant, complete male flower may be transported to the female flowers. The physical (wind and water) and biological (insects, birds and bats) agencies promote cross-pollination. The dehiscence of anthers and the transfer of pollen are the prime requirements of pollination.

Anther dehiscence: Anther dehiscence simply means the release of pollen grains from dry and mature anthers. It involves the rupturing of anther wall due to the mechanical pressure developed by the fibrous thickenings of endothecial cells along the stomium (the area where mechanical layer does not differentiate). If endothecium is lacking, the mechanical role is passed on to epidermal cells. In most of the angiosperms the stomium is a narrow strip along the entire length of the anther lobe. It may, however, also be restricted to a lid or valve (Berberidaceae) or pores (*Solanum, Cassia, Polygala*).

Pollen Transfer: The transfer of pollen can be **autogamous** (self-pollination) in which the pollen grains of an anther reach the stigma of the same flower. In cross-pollination, the pollen of one plant reach the pistil of some other plant of the same species. If the pollination occurs between two flowers of the same plant it is termed **geitonogamy** and if it is between two flowers on different plants it is **xenogamy**.

3.2.1 Types of Pollination

Self-Pollination

Self-pollination refers to the transfer of pollen grains from the anther to the stigma of the same flower. In **chasmogamous** flowers the mature anthers and stigma are exposed to pollinating agents. In **cleistogamous** flowers fertilization is accomplished without exposing the sex organs (cleistogamy) to the atmosphere.

Commelina benghalensis produces both chasmogamous (aerial) and cleistogamous (underground) flowers. Conversion of chasmogamous flowers to cleistogamous type depends on environmental conditions, such as temperature.

Cross-pollination

In this kind of pollination the pollen from anther of one individual is transferred to the stigma of another individual of the same species. The process is mediated by physical or biological agents that include wind, water, insect, birds or mammals. Whereas, cross-pollination is obligatory in unisexual flowers, the bisexual flowers may have adaptations that prevent self-pollination. These adaptations include: self sterility, dichogamy, herkogamy and heterostyly. These have been discussed in Subsection 3.2.2. We shall now take up some of the common agents that mediate cross-pollination.

(a) **Anemophily:** It is also commonly referred to as wind pollination, i.e., the pollen grains are carried through wind currents. To ensure good pollination the anemophilous plants produce astronomical number of small, dry, light and smooth pollen grains that are released preferably on warm and dry days. Flowers in such plants are unisexual with reduced sepals and petals so as to effectively position the long and feathery stigma for pollen interception. Stamens have long filaments and are exposed to facilitate convenient pollen dispersal. Palms, grasses, millets, bamboos are common examples.

(b) **Hydrophily:** All hydrophytes are not necessarily pollinated by water. In fact, most of the aquatic plants are anemophilous, e.g., *Myriophyllum, Potamogeton* or entomophilous, e.g., *Alisma, Nymphaea*. Like anemophilous plants floral envelops

are highly reduced or absent in hydrophilous plants. Hydrophily may involve underwater pollination referred to as hyphydrophily, e.g., *Ceratophyllum, Majus, Zostera.*

A unique example of this type is *Zostera nariana* (a submerged marine perennial) in which pollen grains are long (up to 250μm) and needle-like resembling pollen tubes. Because of the specific gravity of these pollen they freely float at any depth, and when they come in contact with the stigma they coil around it.

In some taxa ephydrophily operates. In these plants pollination is brought about at the surface of water. The classical example is the submerged dioecious plant, *Vallisneria*. The male and female flowers are produced under water but on maturity the males get detached from the stalk and float on the surface while the female flowers attached to thin, spirally coiled, long, slender stalks are brought to the surface at the time of pollination. Pollination is achieved through water currents when male flowers come in contact with pistillate flowers. After pollination the flowers are dragged down to the bottom by the recoiling of the stalk. The fruits thus develop under water.

(c) **Entomophily:** It involves insects to carry the pollen to achieve pollination. *Salvia* exhibits a specialised 'tumapipe' floral mechanism that signifies classic adaptation for bee pollination. In *Salvia* the corolla is bilipped and the stamens are attached to corolla tube. Only one-half of each anther is fertile, the other half being sterile joins together to form a sterile plate of tissue placed above the lower lip at the mouth of the flower. However, the fertile part lies under the hood of the upper lip of the corolla. When a bee visits the flower for nectar it pushes against the sterile plate which consequently brings down the fertile anthers on its back dusting it with pollen. When the bee visits another flower, the forked stigmas picks up the pollen from the back of the insect.

Plants that are pollinated by insects often have predominently yellow or blue petals. Among insects bees and butterflies do not perceive colour in the same manner as the humans. They are able to see in the ultraviolet range of the electromagnetic spectrum, an area that is invisible to human eye. They see blue and yellow flowers differently than humans. Red appears black to them. Consequently, flowers that are pollinated by insects are not usually red. Many insect-pollinated flowers have dramatic ultra-violet markings, that are invisible to us but direct the insect to the flower, where pollen or nectar may be located. Insects have a well developed sense of smell.

Some flowers have developed "fly-trap mechanism" for their pollination, by emitting unpleasant odours, e.g., *Rafflesia* (rotten meat), *Arum* (human excreta), and *Aristolochia* (decaying tobacco and humus).

In the orchid, *Ophrys speculum* a highly specialized type of pollination has evolved. It is pollinated by a hairy wasp, *Colpa amea*. As the appearance and smell of female wasp matches with that of orchid flowers, the male wasp mistakes the flower as its female partner and carries our pseudo-copulation. In this process the transfer of pollinia from one flower to another takes place.

An obligate symbiotic relationship has been observed between a moth *Tageticula* and the plant *Yucca*. The moth cannot complete its life cycle without the association of *Yucca* flower and in turn *Yucca* has no other pollinator. The female moth lays her eggs in the ovary. Neither would be able to reproduce successfully without the other. In case, one species were to become extinct, the other would also become extinct eventually.

(d) **Ornithophily:** In tropical areas, the birds dominate over insects as important pollinators. The most common among them are humming-birds, sun-birds and honey-eaters. Flowers pollinated by birds are usually red, orange or yellow. Birds see well in this region of visible light. Birds do not have a strong sense of smell; consequently, bird-pollinated flowers usually lack much scent. Characteristic features of ornithophilous flowers are their tubular (*Nicotiana glauca*), cup shaped (*Callistemon*), or urn shaped (some members of Ericaceae) form, bright colour, excess of pollen and nectar. As humming-birds (they occur only in The New World)

are able to extract nectar while hovering over the flower, they do not need any heavy platform to land when they visit pendant flowers for pollination. In contrast, sun-birds (old world inhabitants) can perch in any position and suck out nectar even if they visit erect flowers.

(e) **Chelropterophily:** Pollination brought about by bats is called cheiropterophily. Bats which feed at night and do not see very well, are frequent pollinators in the tropics. Bat-pollinated flowers have dusky, dull-coloured petals. The flowers of these plants produce a strong scent, usually of fermented fruit. Bats are attracted to the flowers by the scent and they lap up the nectar. As they move from flower to flower, pollen is transferred. To facilitate the visit of bats, the flowers in cheiropterophilous plants are borne singly or in clusters quite away from the branches and foliage. A bat clasps the flower with its claws and during nectar lapping its back becomes dusted with pollen grains. Examples pollinated by bats include the sausage tree (*Kigelia pinnata*), Baobab tree (*Adansonia digitata*).

3.2.2 Self- vs Cross-Pollination

A major advantage of self-pollination is its certainty. Continued self-pollination over many generations, however, results in weaker progeny. This is referred to as **inbreeding depression.** From the evolutionary point of view, self-pollination is a disadvantage as there is no scope of genetic recombination.

Cross-pollination brings pollen grains from other plants which are genetically different. Genetic heterogeneity is advantageous for the plant in many respects. The offspring are more vigourous and better adapted for survival even under adverse environmental conditions. Thus cross-pollinated species show wider distribution when compared to self-pollinated species. Thus cross-pollination is favourable for evolution. The main disadvantage of cross-pollination is its uncertainty. It also involves considerable expenditure of resources by the plants as they have to produce an enormous amount of pollen, as compared to self-pollinated plants, to compensate for wastage. Further, when the pollinating agent is an animal, the plant should also provide adequate rewards for the pollinating agent in the form of pollen or nectar. These disadvantages are offset by the advantages mentioned above.

Because of the specific benefits of cross-pollination, flowering plants have evolved many devices to prevent self-pollination and to encourage cross-pollination. The most common ones are discussed below.

(a) **Dichogamy:** In many species the anthers and the stigma come to maturity at different times. That is, the dehiscence of anthers and the receptivity of the stigma of a flower do not coincide. In the sunflower plant, the anther dehisces before the stigma becomes receptive and thus self-pollination cannot occur. This condition is called **protandry**. In *Mirabilis*, and *Magnolia* the stigma becomes receptive before the anthers dehisce. This condition is called **protogyny**.

(b) **Herkogamy:** Some species show structural adaptations to prevent pollen grains from coming into contact with the stigma of the same flower. In many herkogamous species the relative position of the anthers and the stigma is such that self-pollination cannot occur. For example, the stigma in many plants projects beyond the level of anthers and as a result the pollen of the same flower cannot land on the stigma. Similarly the pollinia (pollen in sacs) of orchids and *Calotropis* cannot reach the stigma of the same flower.

(c) **Self-sterility:** In many species, self-pollination does not result in fertilization. This is because pollen germination on the stigma or the growth of pollen tubes in the stigma or style is inhibited. For effective fertilization, pollen has to come from another plant. Self-sterility is widespread in flowering plants. It is estimated that about half the total number of species of flowering plants exhibit this phenomenon. It is genetically controlled and is considered a primitive character. It seems to have evolved very early in the evolution of flowering plants as an effective mechanism for outbreeding.

(d) **Dicliny:** In these species flowers are unisexual. Male and female flowers are borne either on the same plant (e.g., many cucurbits). This condition is referred to as

monoecious. When male and female flowers are borne on different plants (eg., date palm, mulberry, cannabis) the condition is called dioceous. Since pollination in these, including the monoecious plants, involves two different flowers, it is considered as cross-pollination.

SAQ-1

Put a tick mark on the correct word given in the bracket.

(a) The (autogamous/allogamous) condition involves transfer of pollen from the anther to the stigma of the same flower.

(b) (Geitnogamy/Xenogamy) refers to cross-pollination involving flowers on different plants.

(c) In (chasmogamous/cleistogamous) flowers, both pollination and fertilization take place within the unopened flower.

(d) (Cross/Self) pollination is obligatory in unisexual flowers.

(e) The (anemophilous/ornithophilous) flowers, produce a copious amount of pollen grains that are small, smooth, dry and light and their stigmas are long and feathery.

(f) The species that undergo (self/cross) pollination show wider ecological distribution, as they are better adapted to adverse environmental conditions.

3.3 FERTILIZATION

The ultimate aim of pollination is to lead to successful fertilization through the fusion of male and female gametes. In flowering plants, there are a number of barriers which must be overcome. The barriers start immediately after pollination with pollen-stigma interaction.

3.3.1 Pollen Stigma Interaction

The Stigma

After landing on the stigma pollen grain germinates, and produces a pollen tube that carries the male gametes. The stigma has been classified into two principal types depending on the presence or absence of stigmatic exudate at the time of pollination: (i) the **wet stigma** is covered by a sticky secretion, e.g., *Aegle marmelos* and *Petunia hybrida*, and (ii) the **dry stigma** lacks any secretion, e.g., cotton. EM observations indicate that the exudate is secreted by the ER and it is extruded by exocytosis. The diagrammatic representation of the mechanism involved is shown in Figure 3.1. In some plants such as *Lilium*, the stigma is non-secretory. The exudate which is present on stigma is, in fact, secreted by the stigmatic papillae and those present in the stylar canal emanate from the style. Figure 3.2 represents diagrammatic sketch of a papilla.

Wet Stigma: *Petunia* shows several randomly distributed 2-celled papillae on its surface. In a developing stigma, the epidermis is covered by a continuous, thin cuticle and the subepidermal cells are densely cytoplasmic, without any intercellular spaces. In a mature stigma, the cells of the subepidermal zone elongate to form a secretory zone with large schizogenous cavities filled with a lipoidal secretion. The secretory zone is delimited from the basal part of the stigma by a storage zone. The cells of the stigma contain numerous amyloplasts and a large amount of lipid globules which gradually coalesce and migrate to the peripheral part of the cytoplasm and eventually out of the cell. The lipoidal exudate accumulates between the cell wall and the cuticle. In the secretory zone the exudate fills the large intercellular, schizogenous cavities. At the time of anthesis, the epidermis becomes disorganized, the cuticle is discarded in the form of flakes and the accumulated exudate spreads over the entire surface of stigma. A very thin layer of water is also trapped.

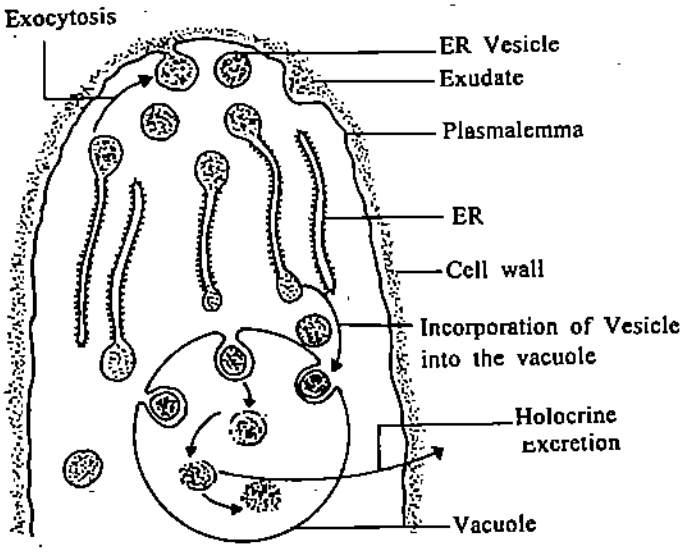


Fig. 3.1: Postulated secretion pathways in stigmatic papillae of *Aptenia* (After Kristen et al. 1979).

The exudate is a highly viscous, refractive and adhesive substance. It appears in the form of tiny droplets due to high surface tension and is a complex mixture of lipids and phenolic compounds. The lipid compound protects the stigma from desiccation, and regulates the availability of water to pollen. The phenolic compounds occur as esters or glycosides, and protect the stigma from insects and other pests. Enzymes diffusing out of pollen grains on the stigma probably release free sugars from phenolic glycosides which then provide proper osmotic conditions. Reducing sugars (glucose, fructose, and sucrose) are also present in the stigmatic exudate.

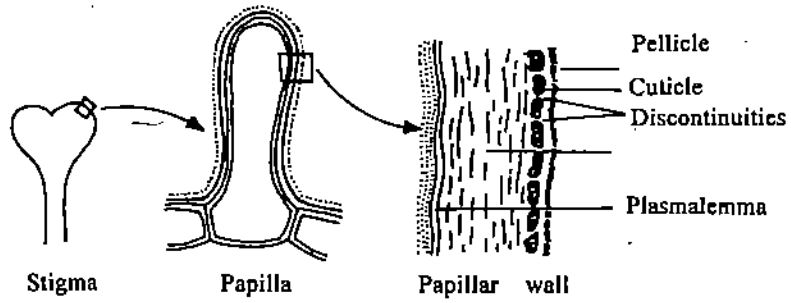


Fig. 3.2: Stigmatic Papilla (After Shivanna, 1977).

Dry Stigma: The Cotton (*Gossypium hirsutum*) stigma is covered with long unicellular hairs. At the time of pollination, the stigmatic hairs show a distinct and continuous cuticle which is closely pressed to the thin wall. The pellicle that represents extracellular proteins are present on the dry stigma. In addition, the stigmatic surface also carries lipids and phenolic substances. Immediately below are several layers of a thin-walled parenchymatous tissue with large intercellular spaces. The size of the intercellular spaces gradually decreases until no such space is present and the cell wall thickens with a heavy pectin content. This tissue provides a connecting link with the transmitting tissue.

The Style

The style has been distinguished into two types: (i) in **open styles** a stylar canal is present which is lined with a well-developed glandular epidermis (monocotyledons), (ii) in **closed styles** a compact core of transmitting tissue is present (dicotyledons, especially in Gamopetalae). A correlation between the type of style with that of composition of the stigmatic exudate has been observed. The solid style exudate is usually rich in polysaccharides, lipids and proteins whereas, open style exudates only have polysaccharides.

Open Style: *Aegle, Fritillaria, Lilium* spp. have variable number of stylar canals depending on the number of carpels. The epidermal cells of stylar canal divide actively

and become papillate in acropetal succession. In *Lilium* each cell contain 1-5 nuclei which later fuse. The stylar canal thus becomes lined with highly glandular and secretory cells which are dome-shaped with a thick outer tangential wall (canal cells). The wall towards the canal is smooth but is highly convoluted towards the interior of the cells. In *Citrus* the inner tangential wall of canal cells is thick and made up of fibrillar homogeneous and granular nonhomogeneous material.

The canal cells have a large nucleus and often become multinucleate. Cytoplasm is rich in organelles such as mitochondria, dictyosomes, free ribosomes or polysomes, smooth and rough ER and occasional amyloplasts. It is believed that a major portion of the secretion product is transported to the canal cells from the neighbouring parenchyma cells through the numerous plasmodesmatal connections.

The golgi apparatus of canal cells of *Lilium regale* and *Lilium devidii* secrete a non-cellulosic and amorphous polysaccharide containing mucilage during the bud stage. This is easily transported to the outer walls of canal cells. In *L. longiflorum*, the secretion products of the canal cells are retained with the help of a thin and continuous layer of cuticle until after pollination (Fig. 3.3 a-c). The stigmatic papillae of *Lilium* lack the characteristic secretory zone seen in canal cells and the stigmatic exudate is known to appear before pollination. The stigmatic exudate, may, therefore be a secretion product of the canal cells transported through the intercellular spaces. The stylar exudate is produced in two phases in *Lycopersicon*; the first contains carbohydrate and the second proteins.

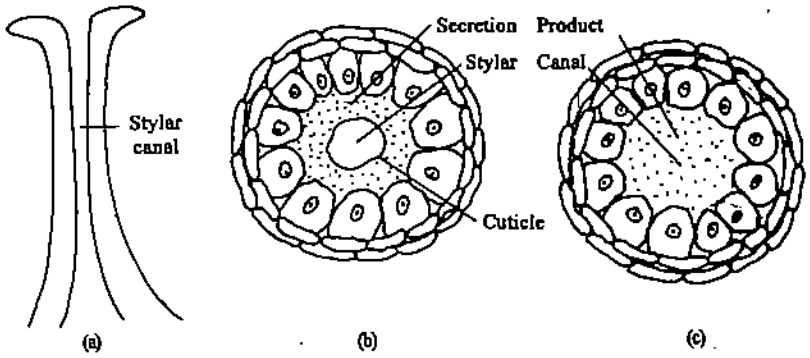


Fig. 3.3: The structure of hollow style. a) Longisection, note continuous stylar canal. b. c) Transection, the secretion product accumulates between the cuticle and the canal cells (b); the cuticle is later disrupted (c).

Pistils of *Lilium longiflorum* secrete large quantities of an exudate which accumulates on the surface of the stigma in the form of droplets. The stylar canal is also filled with this secretion which is an aqueous solution of high molecular weight protein-containing polysaccharides—galactose, arabinose, rhamnose, glucoronic acid, galacturonic acid, and monosaccharides. The composition is similar to plant gum exudates. The polysaccharide gum exudates play an important role in sealing of wounds and it is quite likely that the large amount of acidic polysaccharides found in the exudate of *Lilium* may be involved in protecting the fragile pollen tubes during their growth, in addition to providing a source of carbohydrate residues for pollen tube wall biosynthesis.

Closed (Solid) Style: Cotton shows an epidermis with stomata, a cortex of thin-walled parenchyma with several vascular bundles and strands of transmitting tissue. The cells of transmitting tissue have thin transverse walls but lateral walls are thick and consist of several distinct and concentric layers. The innermost wall layer 1, is composed of pectic substances and hemicellulose, surrounding this is wall layer 2 which is darker, thinner and similar to wall layer 1 in composition with a large hemicellulose content. Wall layer 3 is loosely textured, rich in pectin substances and contains small amounts of noncellulosic polysaccharides and cellulose but poor in hemicellulose. Wall layer 4 is represented by the middle lamella region and is primarily pectic in nature. Small amounts of protein is also present in layers 3,4 (3 also contains masses of small vesicles). The cells of transmitting tissue contain many mitochondria and active vesicles forming dictyosomes. The plastids are large with numerous amyloplasts, polysomes and abundant rough ER. Transmitting tissue cells have a spherical or slightly ellipsoidal vacuole. Nuclei are large and frequently lobed indicating their active metabolic state. EM studies of transmitting tissue in *Petunia*, *Lycopersicon* and *Nicotiana* and some other taxa show that the cells in general have thin walls traversed with plasmodesmata.

There are hardly any cell divisions during the growth of transmitting tissue from the very young stage; cell elongation, however, does take place. As seen in transections, the cells are circular and separated from one another and are surrounded by intercellular substance of different electron density. It is more complex than middle lamella and comparable to the secretion fluid of stylar canal (Fig. 3.4 a-c). It contains protein in *Lycopersicon*. Along the transmitting tissue only carbohydrate, peroxidase and acid phosphatase is detectable.

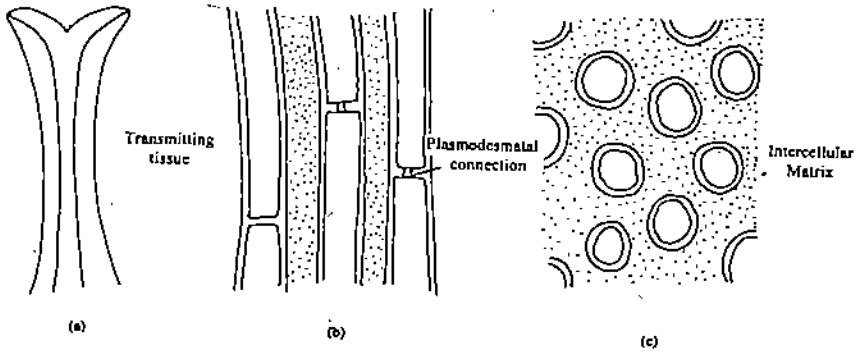


Fig. 3.4: Diagrammatic representation of solid style. a) Longisection. b, c) Transmitting tissues in longitudinal (b), and transection (a). Note the plasmodesmatal connections.

3.2.2 Pollen Germination—Events on Stigma and in Style

As you have learnt, the stigma provides appropriate conditions for the retention and germination of pollen grains and the subsequent growth of pollen tube (Fig. 3.5). Receptivity of the stigma is generally limited to a short period (before and after anthesis) and varies from species to species. The stigma supports pollen adhesion, hydration and germination.

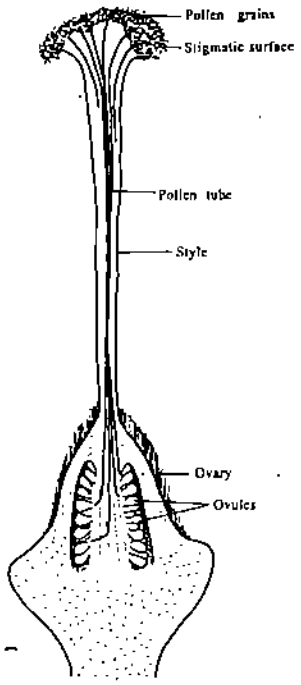
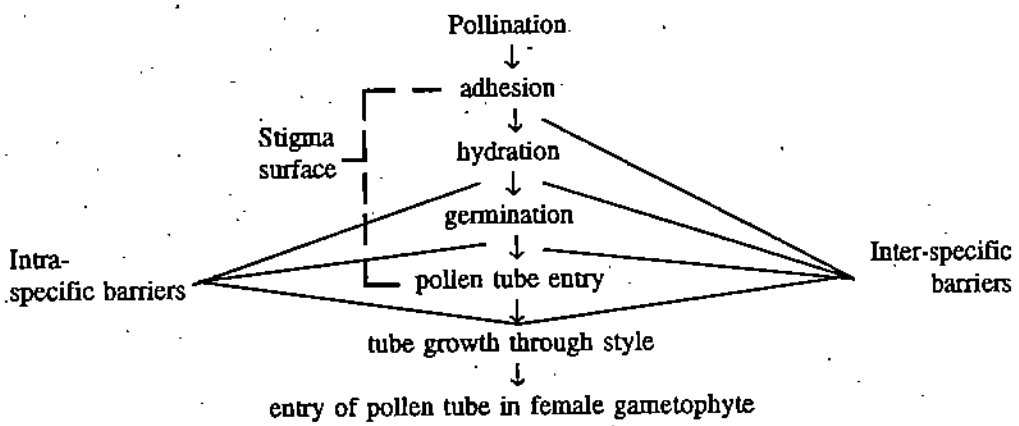


Fig. 3.5: L.S. pistil showing pollen tube passage and entry into the ovule.

Adhesion is accomplished by various means and is determined by the stickiness of pollen and stigma, exine ornamentation, composition of pellicle, amount of surface-coat substances, electrostatic forces and more importantly specificity between the two parents. Pollen hydration is achieved by the moisture provided on the stigmatic surface. On a dry stigma, hydration is gradual. Hydration triggers the release of pollen-wall proteins and subsequent interaction (compatibility/incompatibility) between the two parents.

The events that follow pollination are depicted below:



In plants having dry stigma with solid style, enzyme cutinase present in the pollen tube digests the stigmatic cuticle at the point of contact. The tube penetrates the pectocellulosic wall of the papillae and then traverses through the intercellular spaces of the stigma and style. Finally, it travels through the intercellular matrix of the transmitting tissue. In plants with dry stigma and hollow style, the cuticle over the papillae is continuous throughout the stylar canal. The pollen tube grows through the subcuticular mucilage. In plants with wet stigma and closed style the pollen tube enters the intercellular matrix of the stigma before making its way into the style. In plants with hollow style, the pollen tube grows on the surface of the stigma and then enters the stylar canal.

The extra-cellular proteins contributed by tapetum localised in the pollen grain wall (sporophytic) contribute to pollen germination, penetration of pollen tube and its early growth. Other fractions are involved in recognition responses which control inter-and intra-specific incompatibility. Gametophytic proteins are injected into the intine from microspore cytoplasm and are probably involved in the germination and early nutrition of the grain and in gametophytically controlled incompatibility systems. Sporophytic enzymes leach out within seconds after pollination but gametophytic enzymes are slower to move and are detected after several minutes.

In the mustard family, the stigmatic papillae are completely covered by a cuticle layer and no exudate is produced. Pollen grains breakdown the cuticle enzymatically and come in direct contact with the stigmatic papillae. Pollen grains are then able to absorb water from the turgid cells of the stigmatic surface and germinate readily. The walls of the stigmatic papillae of *Brassica nigra* consist of an outer layer of cuticle, a thin intermediate pectic-layer and an inner layer of pectin and cellulose. After cross-pollination the pollen tubes penetrate the cuticle and the intermediate pectic layer and actually grow in between the cellulosic lamellae of the innermost pectin-cellulose layer by dissolving only pectic constituents of the wall. In *B. oleracea*, the stigmatic papillae are covered by an additional waxy layer and only after piercing this layer that the pollen comes in contact with the cuticle. Enzymes for the breakdown of cutin and pectin have been demonstrated in pollen grains.

The Passage of Pollen Tube

In cotton, the pollen produces a tube within an hour which grows on the surface of the stigmatic hairs, and then between the cells of the stigma at the bases of hairs and beyond. The cytoplasm of the stigmatic hair degenerates; no exudate is secreted. The tube continues growth through the intercellular spaces of the thin-walled cells of transmitting tissue. After reaching the thick-walled cells of the main strand, it actually grows through wall layer 3. It has been reported that pollen tube of *Petunia* grows within the compact matrix of the middle lamella of the transmitting tissue by enzymatically creating a pipe-like path in front.

By the increased dictyosome activity the cells become thicker. Callose is deposited in the pit fields on the transmitting tissue after the passage of pollen tube. The pollen tube passage probably changes the permeability of cells and callose is formed as a wound response and as a reaction against cell leakage. Once the pollen germinates and the pollen tube has penetrated the stigmatic tissue, the path of the pollen tube through the

rest of the stigma and style appears to be determined by the nature and structure of the cell walls and the morphology and distribution of the transmitting tissue.

The nutritive role of the transmitting tissue was recognised early. Pollen tubes of *Lilium*, *Petunia* and *Oenothera* are shown to draw nourishment (sugar and amino acids) from the stylar tissue. Growth of tubes through style causes an increased inflow of carbohydrates into the pistils. In *Aegle marmelos* the cells surrounding the stylar canals show an optimal concentration of starch just before pollination, subsequently as the starch is digested, the canal cells and the basal portions of the stigmatic papillae show reducing sugars which also disappear within 3 days after pollination. Disappearance of stylar starch has also been observed in *Fritillaria, Zephyranthes* and *Pavonia*.

Metabollsm of Pollen Tubes

Pollen grains contain auxins, and gibberellins which are known to be involved in post-pollination enlargement of the ovary and the development of the fruit. Pollen from unrelated species, nonviable pollen or even pollen extracts can prevent abscission and cause swelling of the ovary and formation of near-normal but seedless fruits. The initial and small amounts of auxins such as IAA or other auxin-like substances and GA supplied by the germinating pollen to the pistil serve to initiate some minimal growth and metabolic processes as a result of which enzymes liberate additional amounts of auxin from the tissue of the style and the ovary, e.g., *Nicotiana tabacum.* The auxin released initiates growth also in the fertilised ovules which later produce appreciable amounts of auxins and gibberellins in the endosperm, and auxins and cytokinins in the developing embryo. Thus, the initial supply of auxins and gibberellins from germinating pollen not only triggers the development of the fruit but is also responsible for the subsequent release and production of additional amounts of plant growth regulators in the pistil tissues.

Respiration: In the unpollinated pistils of *Hippeastrum hybridum* very high O_2 tension exists from stigma down through most of the style. During pollen tube growth a marked drop in O_2 tension takes place in the region of style containing the tips of pollen tube and this drop in tension moves down progressively with the growth of the pollen tubes. However, after the passage of the tube, the original high level is restored, though not completely. It appears that the tube grows aerobically through the stigmatic and stylar tissues and only in the lower most region of the style and ovary that the tube confronts anaerobic condition.

Pollen Tube Structure

The pollen tube in the stigma is filled with cytoplasm containing numerous mitochondria and dictyosomes. The number of dictyosome cisternae is reduced in the tubes. Large vesicles associated with dictyosome seem to be incorporated in the tube wall. Abundant ER and polysomes which are either in free form or attached to ER can also be seen. The pollen tube wall in the stigma and style show two distinct regions: the outer part of the wall (PAS positive), and the inner portion which is thicker, more homogeneous (much less reactive to PAS), and rich in callose. The dense cytoplasm contains vesicles of various sizes, ER, ribosomes, and a few poorly-developed plastids with swollen outer membranes. Dictyosomes are quite numerous with 4 or 5 cisternae, and produce vesicles. The vesicles appear to fuse with the plasma membrane of the pollen tube. A very large population of small, spherical vesicles are scattered throughout the pollen tube cytoplasm (Fig. 3.6).

The ER in the pollen grains and during early growth of the pollen tube has extended cisternae and apparently serves as a storage site for proteins. As the pollen tube grows down the style, the ER shows the common variety of narrow cisternae indicating that protein present is being gradually utilized during tube growth. The ultrastructure of the distal region of the pollen tube and the wide variety of cell organelles are indicative of active carbohydrate and protein metabolism. The part of the tube immediately behind the tip region shows less dense cytoplasm and more dispersed organelles. The more mature parts of pollen tube contain only a thin layer of cytoplasm closely appressed to the wall and a large vacuole occupies the rest of the space. Plugs of the wall material, mostly callose serve to separate the older parts of the pollen tube from the growing distal region. The plugs originate as rings on the inner side of the wall and grow inwards like the closing of an iris diaphram.

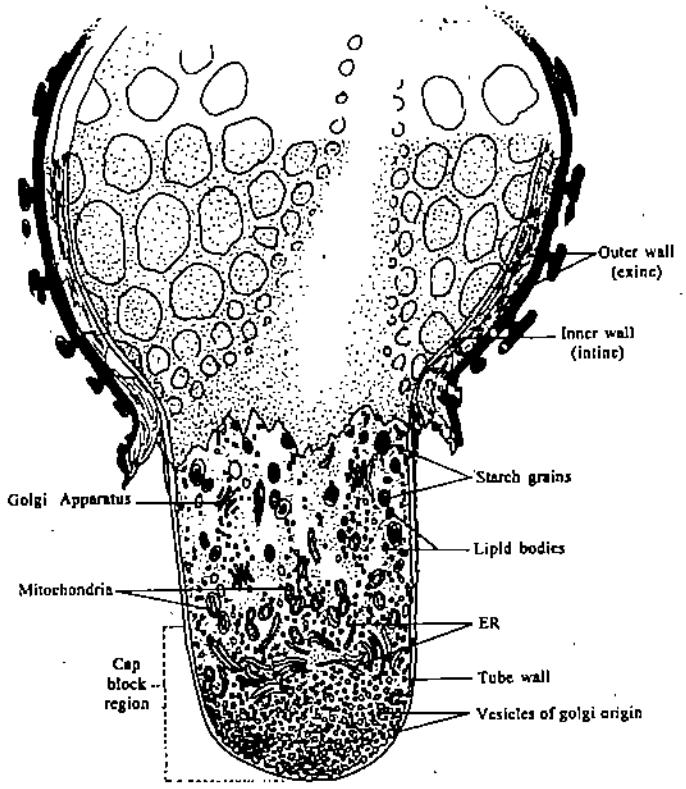


Fig. 3.6: Representative fine-structural diagram of a growing pollen tube (adapted from Iwanami et al, 1988).

Pollen Tube Growth

There are significant differences in the fine structure of the tips of pollen tubes in compatible and incompatible pistils such as in *Lilium*. Tubes growing in compatible pistils show deep embayments but no compartments (tubes in incompatible pistil have a compartmented cap). The compatible tubes undergo a transition from autotrophic nutrition (characterized by compartmented cap of golgi derived vesicles) to heterotrophic condition in which the secretion product from the stylar canals enter the pollen tubes through the deep embayments (tubes growing in incompatible pistils are unable to make this transition and thus are unable to continue growth due to the exhaustion of endogenous food reserves).

The pollen tube wall is composed mainly of polysaccharides. Since the pollen grain carries limited food reserves and a relatively large amount of new pollen wall material is synthesized during growth, it has been assumed that at least a part of the substance needed for pollen tube wall is contributed from the polysaccharides present in the pistil tissues. It has been shown that the polysaccharide component of the stigmatic exudate of *L. longiflorum* is incorporated into the cytoplasm of the growing pollen tubes and later a specific fraction of the incorporated exudate is extensively metabolised before being utilized for pollen tube wall biosynthesis.

3.2.3 Pollen Tube Growth *in Vitro*

Pollen tubes of a few species can be grown in culture. Lily pollen tubes grow to a maximum length of only about one cm in vitro whereas the lily pistil is 10 times longer. Obviously then, the pistil provides appropriate conditions for tube growth. Also, a constant nutrient medium is employed in culture experiments. The environment of the tube may be changing as it grows through the pistil. Many amino acids and hormones have been reported to stimulate the pollen tube growth in vitro.

High relative humidity is the most essential requirement for pollen germination. Other factors important for *in vitro* pollen germination are:

1. Carbohydrate—sugars control osmotic pressure and serve as respiratory substrates. Sucrose is most effective.
2. Boron—most pollen are deficient in boron content and this is made up by its presence in stigma and style. Boron reduces bursting of pollen tube and helps in the

translocation of sugars. It also has direct or indirect effect on enzymatic steps that are involved in the biosynthesis of carbohydrates.

3. Calcium—population effect is mediated through Ca^{++} ions. The growth of the tube is more vigorous and they are more straight and rigid. Permeability of the tubes is also controlled. Calcium antagonises the inhibitory effects of certain heavy ions. Calcium effect is dependent on the presence of a suitable osmotic milieu, O_2 and borate. It is enhanced by a methyl donor and other inorganic cations especially Mg^{++}, K^+, Na^+ and H^+.
4. Enzymes—cellulase, pectinase and callase are present in pollen grains. They increase the rate of tube elongation when present in the medium.
5. Plant hormones-tube growth is promoted by auxins and gibberellins.
6. Germination Medium

Sucrose	100 mg/l
H_3BO_3	100 mg/l
$Ca(NO_3)_2$	300 mg/l
$MgSO_4$	200 mg/l
KNO_3	100 mg/l

Media containing raffinose often yield better growth. Cobalt, Zn and other minerals have occasionally been reported to stimulate pollen tube growth. Calcium was found to be insignificant in easter lily. Growth of lily pollen *in vitro* is stimulated by Co^{++}. It is accumulated in the pistils from where the growing tubes can apparently accumulate it. Co^{++} activates an aminoacylase found in pollen.

7. Physical factors—temperature (20-30° C).

Fine Structure of Pollen Tubes Grown *in vitro*: The growth in pollen tubes is exclusively restricted to the tip. Cytochemical analysis reveals the pollen tip zone to be rich in RNA and protein. This zone has numerous vesicles and an elaborate network of smooth membranes. The vesicles appear to rise from the ends of dictyosome cisternae. They coalesce with one another and ultimately contribute their membrane and contents to the compartmented cap covering the growth zone at the tip. The cap and the vesicles contain pectin and the RNA resides in the smooth membranes. In the region behind the tip the tube contains those organelles present in pollen before germination, as well as numerous amyloplasts. The tube wall is thin (tubes growing in pistil have complex wall). Cellulose is the primary wall component. The cytoplasm of pollen tubes growing in vitro lack microtubules. EM shows that the generative cell is surrounded by its own distinct wall and the cytoplasm is different from the grain proper. It contains a small number of poorly developed organelles which possess little storage material.

Thus, the pollen chemistry and growth studies provide the following general conclusions:

1. Metabolic pathways in pollen are those common to most non-green tissues;
2. The overall composition and balance of pollen chemical constituents vary with species, plant nutrient level and environment during development;
3. Enzymes or some chemical constituents rapidly diffuse out of pollen;
4. Chemicals diffusing out of pollen or pollen surface can interact with the pistil tissues;
5. Tube growth can be modified by certain chemicals;
6. Tube extension occurs by the addition of pectin and hemicellulose via addition of vesiculated membrane-like components at the tip, cellulose is probably added after the initial tube membrane is formed;
7. Decreased pollen viability after dehiscence is generally related to enzyme activities metabolising, endogenous substrate.

3.3.4 Syngamy and Triple Fusion

After traversing through the stylar region, the ultimate destination of the pollen tube is to reach the female gametophyte and release the male gametes that can ensure fertilization. In angiosperms, **double fertilization** is an important feature which involves fusion of one male gamete with the egg (syngamy) to from the *zygote*, the progenitor of next generation and the other male gamete fuses with the fusion product of the polar nuclei (secondary nucleus) resulting in **triple fusion** (haploid male gamete + two haploid polar nuclei = 3n), the primary endosperm nucleus.

Entry of Pollen Tube into the Embryo Sac: The pollen tube enters the embryo sac through the filiform apparatus of one of the synergids. Generally, one of the synergids degenerates before the entry of pollen tube and the tube invariably enters through such synergid. In *Plumbago*, where synergids are absent and the egg has the filiform apparatus, the tube enters directly into the egg. In several taxa, however, both the synergids remain healthy until the entry of pollen tube and the one that receives the pollen tube starts degenerating. It has been proposed that the degeneration of synergid and also of pollen tube cytoplasm is essential to prevent male gametes from a rejection or antigen-antibody type of reaction.

The choice of the male gamete involved in syngamy and triple fusion has long been a subject of speculation. Embryologists were curious to know which of the two male gametes participated in syngamy. Transmission electron microscopy and scanning electron microscopy have revealed that in certain plants the two male gamates are unequal in size and in the number of plastids and mitochonoria they contain. The plastid rich sperm seems to be preferentially involved in syngamy and the one with poor plastids fuses with the secondary nucleus (Fig. 3.7). However recent studies indicate that heteromorphic sperms are not seen in other plants.

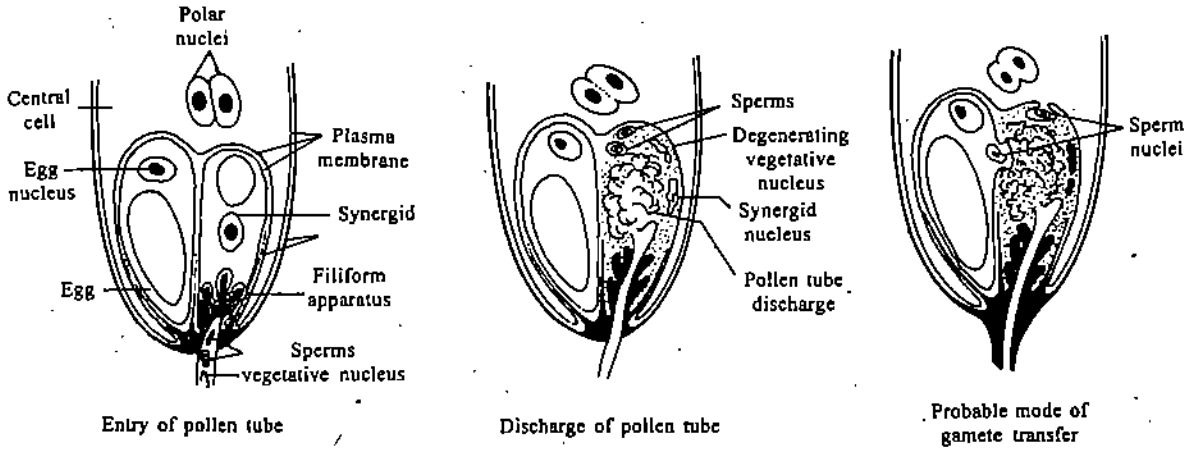


Fig. 3.7: Diagrammatic representation of fertilisation (After Jensen, 1973).

Syngamy: The pollen tube grows to a very limited extent in the synergid. It releases the contents either through a terminal or a subterminal pore. The contents include the two male gametes alongwith the accompanying cytoplasm, some reserve nutrients and perhaps vegetative nucleus. One of the sperms enters the egg and the other to the central cell. The distance that the male gamete has to travel to come in contact with the egg or secondary nucleus is insignificant.

The male gamete comes in contact with the plasma membrane of the egg that forms a bridge through which the male gamete enters. Nuclear fusion is initiated by the joining of the outer membrane of the two nuclei. The inner membrane also fuses at localized areas forming a small bridge between the two nuclei. Enlargement of bridge causes eventual fusion of the male and egg nuclei (syngamy). Depending upon the stage of the male gamete at the time of nuclear fusion, three types have been recognized: (a) in pre-mitotic type—the male nucleus fuses with the egg before reaching the mitotic interphase, (b) in post-mitotic type the male nucleus undergoes interphase while in contact with the egg and the fusion is postponed until the initiation of the first mitosis in both, and (c) in intermediate cases, the fusion occurs when male nucleus is still at interphase. Generally, the male cytoplasm does not take part in fertilization.

However, there are plants in which biparental inheritance of plastids has been demonstrated.

Triple Fusion: The fusion process between the other male gamete and the secondary nucleus follows the same pattern as syngamy. In most plants, the polar nuclei are only partially fused when the male gamete approaches. The fusion of the male gamete with one of the polar nuclei completes the fusion process of all the three nuclei (triple fusion). Interestingly, initiation of syngamy starts before triple fusion, but triple fusion is completed first.

SAQ 2

Fill in the blank spaces with appropriate word(s).

a) The growth of the pollen tube is confined to the ..... region.

b) The enzymes namely ....., ... and ..... that are present in the pollen grains, aid in pollen tube growth in the style.

c) The stigmas which secrete exudates are called ..... stigmas, e.g., ... and those which do not are called ..... stigmas, e.g., ...

d) The stigmatic exudates are extruded from a stigmatic cell by ..... .

e) The ..... component of stigmatic exudate protects it from desiccation and also regulates the available water, whereas, the ..... components protect it from pathogens.

f) The exudates in ..... styles are rich in polysaccharides, lipids and proteins whereas those of ..... style have mostly polysaccharides.

g) The .... are associated with open styles and the .... are associated with the solid styles.

h) The ..... enzymes are leached out within seconds after pollination, whereas the ..... enzymes are slower to come out, and they can be detected several minutes later.

i) The ..... and ..... present in the growing pollen tubes are involved in post-pollination enlargement of ovary and the development of fruit.

j) In angiosperms, the two products of double fertilization are: one, the ..... and second, the ..... .

3.4 INCOMPATIBILITY

Plants growing under natural conditions have a preference for their mating partners. The stigma of the female parent receives all kinds of pollen. However, the choice of pollen of the desired parent that would accomplish fertilization is finally determined by both, pistil and the pollen. This gametophyte (pollen) and sporophyte (pistil) interaction leads to recognition (acceptance or rejection) of mating partners. In angiosperms, the female gamete is located in the ovule present in the ovary. Therefore, pollen grains carry male gametes through pollen tubes that travel from stigma to ovary to affect fertilization. The pollen tube has to traverse through the tissues of the stigma and style. A situation in which fertile pollen fails to accomplish fertilization process so that a viable embryo/seed is not formed is sexual incompatibility. Self-incompatibility means the inability of the plant producing functional gametes to set seed upon self-pollination. It may operate between the individuals of the same species—**intraspecific or self-incompatibility** or of different species—**interspecific incompatibility**.

3.4.1 Intraspecific Incompatibility

The majority of flowering plants are fertilized successfully only by the pollen of other plants. Various floral adaptations have evolved to prevent self-pollination. These include, dichogamy, herkogamy and unisexuality about which you have studied in Subsection 3.2.2. On the basis of morphology alone, self-incompatibility can be categorized into:

Heteromorphic Types: Plants of the same species produce flowers that differ in morphology. This involves two (distyly) or three (tristyly) morphologically distinct types of flowers showing similar breeding behaviour (mating type) within a species. The difference in the mating types lies in the position of stigma and anthers (heterostyly). Distyly is under the control of a single gene complex with two alleles, S (for short style-dominant) and s (for long style-recessive). Long style individuals are, therefore, homozygous recessive (ss) and short style are heterozygous (Ss). This ensures approximately equal ratio of short and long-styled individuals from compatible crosses. Tristyly refers to three floral morphs with individuals having either long, mid- or short-styled flowers. Each type has stamens of two heights corresponding to the height of stigma in the other two forms. Successful pollination results only between forms having stigma and stamens of the same height (Fig. 3.8). Two genes, M and S with two alleles each control tristyly. Gene S is epistatic to M. Homozygous recessive for both the genes (ssmm) determine long style, mid-style have ssMM or ssMm and short-styled flowers carry the genes SsMm or Ssmm or SsMM. In both di- and trimorphic forms sporophytic incompatibility operates.

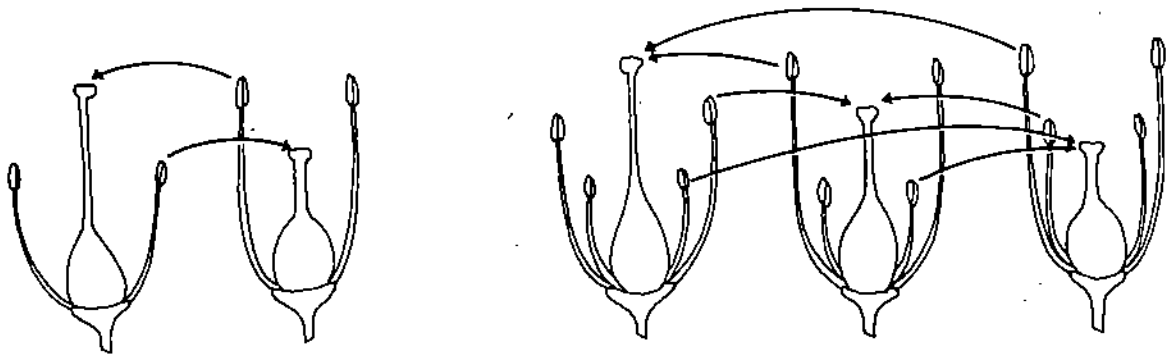


Fig. 3.8: Heteromorphic intraspecific incompatibility.

The S-gene has been suggested to be a super gene complex with several linked genes. It is supposed to have at least six (may be more) closely-linked genes which determine length of style (gene G), surface of stigma (S), pollen incompatibility (I'), stylar incompatibility (I"), pollen size, and/or shape (P), and stamen height (A). Being closely linked they are inherited together but because of crossing over, can be separated. In addition heteromorphic incompatibility can be further characterized by—(a) sporophytically-determined incompatibility reaction, b) inhibited growth of incompatible pollen tube in the style, and c) expression of dominance between alleles of the incompatibility genes in both, pollen and style.

It has been proposed that physiological incompatibility is superimposed by heteromorphism. The two supplement each other in controlling unwanted matings. Rejection through morphological variations is mechanical and relates to interspecific incompatibility. The physiological control is similar to homomorphic mechanism.

Homomorphic Types: It is characterized by morphologically indistinguishable mating types within a species. A proper breeding is required for their recognition. This kind of incompatibility operates in more than 250 genera belonging to 71 families of angiosperms. It operates through multiple alleles (as many as 45 alleles are already reported from 500 plants) of S-gene. Pollen grains with one S-allele common with one

or both S-allele/s of pistil is incompatible. It is therefore conceivable that on pollen side the haploid gametophyte controls incompatibility. The S-allele may express a relationship of dominance or independence in pollen and/or pistil.

Basis of Self-Incompatibility: Very few taxa have been worked out to explore the genetics of incompatibility. In many taxa it is controlled by one gene, in Poaceae by two genes (S and Z) each with many alleles and in Chenopodiaceae, Brassicaceae and Ranunculaceae by 3 or more genes.

East and Mangelsdorf in 1925 proposed "Opposition S-alleles" hypothesis, about the genetic control of self-incompatibility. According to this hypothesis, a single gene, the S-gene with several alleles controls the incompatible reactions. Pollen grains having S-allele common to any one of the two alleles present in the pistil will not be functional on that particular pistil. For example, a plant having S_1 and S_2 alleles in its sporophytic cell, including the pistil (Fig.3.9), two types of pollen one half having S_1 and the other half with S_2 allele will be produced during microsporogenesis. Neither of these pollen types (S_1 or S_2) will be functional in this plant because in the stylar cells also S_1 and S_2 alleles are present. However, if this plant were to be pollinated with pollen from a plant of S_2, S_3 genotype, pollen grains carrying only S_3 allele would be able to bring about fertilization. The other half with S_2 allele would be nonfunctional. One hundred percent pollen grains would be functional from a plant having S_3 S_4 on the pistil of a S_1 S_2 plant as none of the alleles is common between these two plants. In all such cases it is the S-allele of the pollen or male gametophyte which controls the incompatible reactions (Gametophytic self incompatibility-GSI).

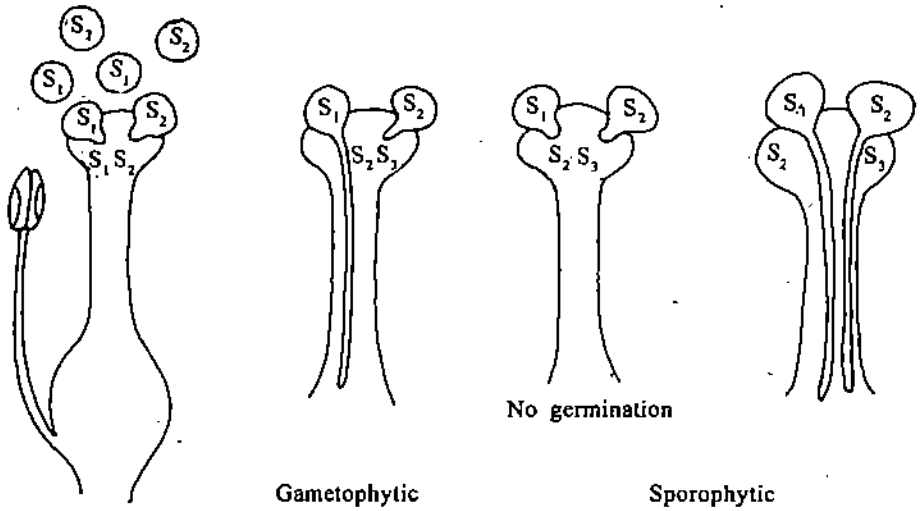


Fig. 3.9: The operation of gametophytic and sporophytic incompatibility. The S_1 and S_2 pollen are inhibited in both types of incompatibilities. S_1 S_2 plants pollinated with S_2S_3 (not shown in figure) will function in the gametophytic system because S_1 pollen have a matching allele in the pistil but S_2 pollen is inhibited. In sporophytic system the S allele may express independently, or show dominance of one over the other in pollen and/or pistil. When S_2 and S_3 have independent action or S_2 is dominant in pollen both are inhibited. However, S_1 is dominant over S_2, both are functional. S_2 S_3 alleles in the pistil are considered to have independent action.

However, in SSI-systems (sporophytic self incompatible) the pollen behaviour is same for all irrespective of the S-allele they carry. For example a plant carrying S_1, S_2 alleles would be completely incompatible to plants carrying S_1 S_2, S_1 S_5 or S_2 S_4, S_2 S_5 and so on but a plant carrying S_3 S_4 or S_3 S_5 and so on would show one hundred per cent compatibility (see Fig.3.9).

Based on the difference in time of S-gene action, the two types of incompatibility are further explained. Because S-gene is activated before the completion of meiosis in sporophytic systems, products of both the genes are incorporated in all the microspores. Nonetheless at least in some plants, S-allele specific substances are produced in the tapetum and then incorporated into pollen exine. Thus both tapetal and pollen components are involved in controlling sporophytic incompatibility. In gametophytic

systems since S-gene action is delayed, two microspores receive the product of one S-allele and the other two microspores receive another S-allele.

Studies have been carried out to assess the biochemical nature of the incompatibility factors. Antisera produced in rabbits against pollen extracts of some plants precipitate in the presence of diffusates of pollen. In certain plants S-allele specific antigens are identical in pollen and pistil.

Through immunodiffusion tests it has been possible to demonstrate the presence of a unique protein for each S-allele in *Brassica*. S-allele specific antigens could also be detected from diffusates of intact stigma. Findings related to the nature of self-incompatibility proteins in relation to phenotypic expression of self-incompatibility in F_1 and F_2 of cross $S_2\ S_2 \times S_{21}\ S_{21}$ provides interesting insight. All F_1 progenies showed protein concentration intermediate to those of parents, whereas F_2 individuals showed high, intermediate and low levels of S-protein in the ratio of 4:7:4. The results indicate that the quantity of S-protein is directly proportional to the intensity of the incompatibility reaction.

Barriers to Fertilisation: Incompatibility can occur any where from pollination to syngamy and consequently obstructing fertilisation. The pollen fails to germinate or the pollen tube is inhibited to penetrate the stigma. The **progamic barriers** to fertilisation may be on the stigma or in the style at any level from pollen germination to the discharge of male gamete in the vicinity of egg. On the other hand, **syngamy barriers** include the inhibition of the entry of pollen tube to the ovary, ovule or within embryo sac. At the stage of pollination, the barriers to fertilisation are of morphological and ecological types, whereas, at progamic phase and syngamy physiological barriers occur.

Physiology and Biochemistry of Incompatibility: On the basis of recognition and rejection reaction, the type of incompatible process can be distinguished.

Recognition reaction: The compatibility of pollen grains is decided at the molecular level by the pistil. In SSI systems acceptance or rejection of a pollen grain is decided on the stigmatic surface. Certain GSI systems also operate recognition reaction on stigma though normally it occurs in the style.

Rejection reaction: For rejection reaction the physiological and biochemical processes are set in the pistil by the recognition reaction specific to the type of pollen that lands on stigma. In contrast to systems where inhibition can occur on stigma itself thus preventing germination of pollen or its entry into style, in GSI system it may occur in style leading to either inhibition of pollen tube growth or its bursting.

The pollen wall and its protein content plays an important role in pollen stigma interaction. Through cytochemical studies it has been observed that the pollen wall is perforated by protoplasmic strands giving the appearance of a living physiological structure playing a very responsible role in the process of interchange between the pollen grain and the substrate.

The incorporation of proteinaceous substances in the exine and the intine have been demonstrated by EM studies of the pollen wall during development. A good amount of mobile proteins of the pollen grains is S-gene specific. The recognition proteins of incompatibility are held extra-cellularly in the pollen wall. In GSI plants, these proteins are present in the intine while in SSI plants they occur in the exine.

The Exine Layer: It consists of two layers, inner (nexine) which is continuous and an outer (sexine) the sculptured one. The outer layer has ornamentations comprising rod-like bacula showing terminal expansion which sometimes fuses to form a roof like tectum perforated by micropores. During pollen development, the tapetum releases a mixture called tryphine, consisting of carotenoid lipid droplets and fibrogranular proteins into the thecal cavity. These fibrogranular proteins are enclosed in membrane bound cisternae. In tectate pollen, the released substances of tapetum become incorporated on the surface of pollen grains where cisternae membrane ruptures and protein released through micropores enter the tectum and accumulate in the interspaces of bacula. Pollenkitt is the pigmented lipid fraction left on the surface of the tectum. Unlike intine, the exine held proteins exhibit only one enzymatic activity. The hydration of pollen is described to be stimulatory in releasing the proteins held in the pollen wall

layers. The calculated time taken by the exine held proteins of sporophytic origin to pass out is 30 seconds while the intine of gametophytic origin takes a few minutes. This time difference in the protein release has made it easy to collect both samples separately. In SSI systems therefore the rejection reaction is faster than in GSI system.

The Intine Layer: As soon as the tetrads release the microspores, the inner layer of the pollen wall (intine) is formed. Proteinaceous lamellae are embedded in the matrix of the intine, concentrating around the germpore. The proteins incoporated into the intine are contributed by the cytoplasm of the gametophyte, i.e., pollen cytoplasm. In members of the Malvaceae and several other plants, the proteinaceous lamellae are dispersed in the intine without coming in contact with the exine or pollen cytoplasm.

Sporophytic and Gametophytic Self-Incompatibility: Besides the categories based on morphology, self incompatibility can be further classified into sporophytic or gametophytic types depending on the origin of factors that determine the mating types on the pollen side. i) **Gametophytic self-incompatibility (GSI)** is governed by the genotype of pollen (male gametophyte) itself, e.g., Poaceae, Liliaceae, Solanaceae, Fabaceae, and Commelinaceae. **ii) Sporophytic self-incompatibility (SSI)** the genotype of the sporophytic tissue which donates the pollen controls the incompatibility process, e.g., Asteraceae, Brassicaceae and Convolvulaceae. Other characteristics of GSI and SSI systems are described in Table 3.1 (See p. 68) and 3.2. A correlation has been established between the type of self-incompatibility, pollen cytology, and the site of inhibition. Generally, species that shed pollen at the 2-celled stage are credited with gametophytic incompatibility (inhibition occurring in style) while those shedding 3-celled pollen show sporophytic incompatibility (zone of inhibition on stigma). Additionally, taxa having dry type of stigma are associated with sporophytic incompatibility whereas those with wet stigma show gametophytic incompatibility. There are a few exceptions to these correlations.

Table 3.2: The incompatibility factors, and the site of action

	Pollen	Pistil	Inhibition site
Gametophytic systems	intine wall and/or pollen cytoplasm	surface of stigma and/or transmitting zone of the stigma and upper region of the style	surface of stigma, transmitting zone of the stigma, upper region of the style
	pollen cytoplasm	stylar canal secretion	stylar canal
Sprophytic systems	exine layer and probably pollen cytoplasm	surface of stigma	surface of stigma

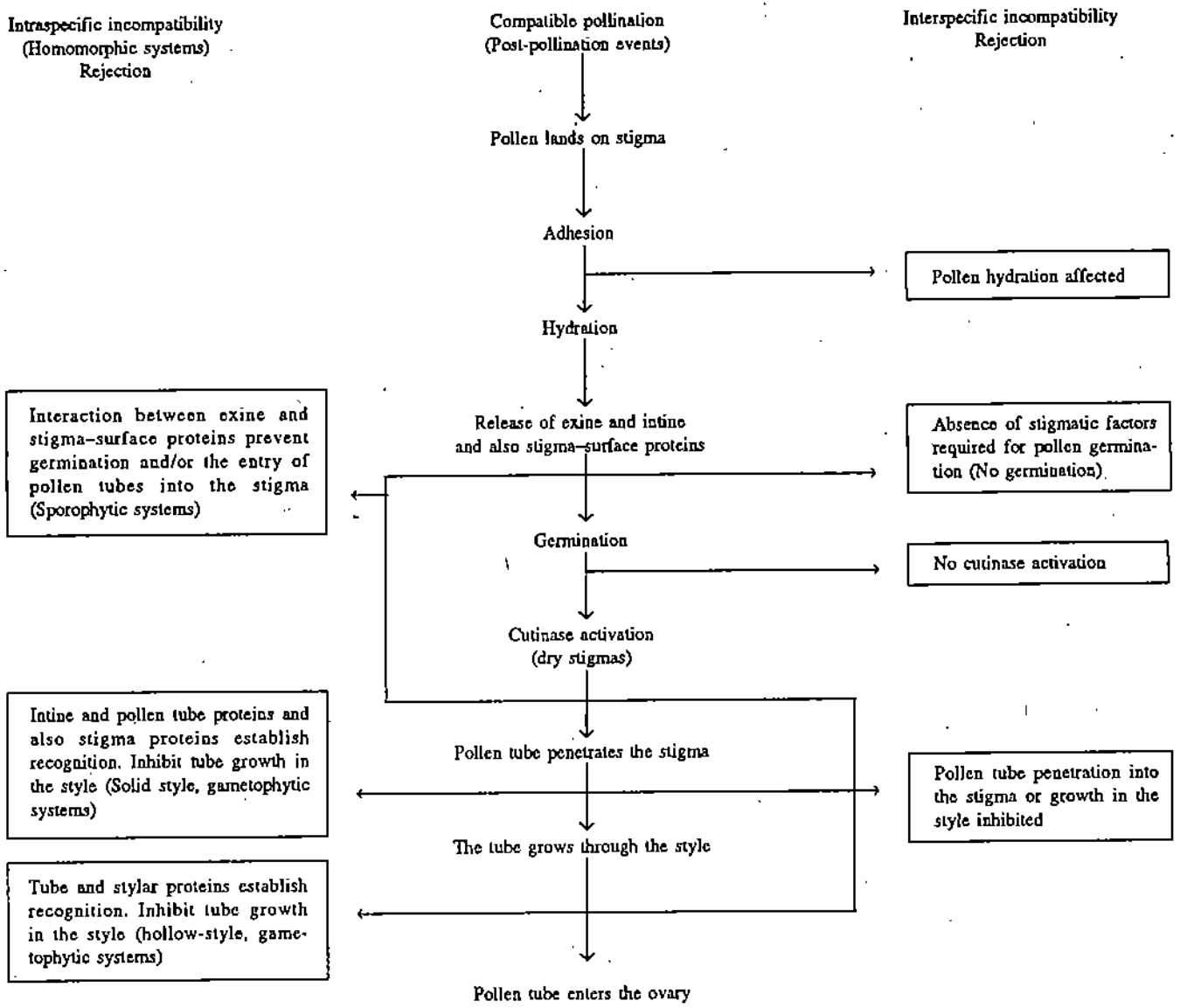
Sporophytic Incompatibility: The recognition and rejection reactions in a SSI system occur on the stigma surface, placing barriers for pollen germination or penetration of pollen tube into the stigma. Thus the pollen grains either fail to germinate or the small tube they put out is inhibited by callose deposition at the tip (Fig. 3.10). The papillae at the stigma also develop a lenticular callose plug within 10 min. after pollination. Infact, in incompatible cases, inhibition of varying degrees operates at every level starting from pollen adhesion, hydration, germination and tube entry into the stigma.

In some plants stigma is covered with a cuticular layer such that the incompatible pollen tube fails to penetrate. Various studies have proved the necessity of enzyme cutinase in eroding the cuticle. This enzyme is activated only by the compatible cases.

Incompatible stigmas may lack copious exudate, but dry stigmas have a hydrated layer called pellicle over the cuticle. This pellicle consists of a lipid layer where a mosaic of proteins floats. The pellicle probably originates by the protrusion on the surface of the papillae through discontinuities in the cuticle. As soon as pollen is received by the stigmatic surface the papillate cells exude moisture. The incompatibility is the result of interaction between protein fractions of exine and stigma.

In incompatible pollination a number of irregularities observed in the behaviour of male gametophyte are: (1) the pollen does not germinate (2) if it germinates, the pollen tube

Table 3.1: Sequence of recognition, acceptance and rejection during pollen-pistil interaction, (After Shivanna 1979).



does not grow (3) the inhibition of one tube leads to the emergence of another tube (4) tip of the pollen tube swells like an appressorium (5) the most distinguishing response for incompatibility is the development of a callosic plug between the plasma membrane and pectocellulosic layer of the stigmatic papillae, just below the point of contact with the pollen. Likewise a plug also develops at the tip of pollen tube. Consequently, growth of the pollen tube ceases.

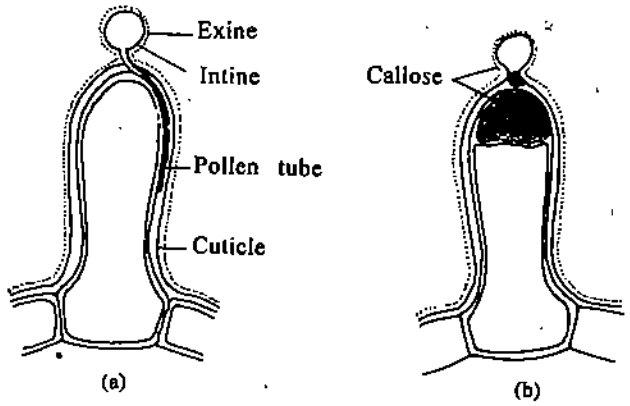


Fig. 3.10: Stigma interaction in sporophytic systems. a) compatible, b) incompatible; the deposition of callose plug between the plasmalemma and the cell wall in the stigmatic papilla (After Shivanna, 1982).

The inhibition reaction between pollen and stigma is extremely localised and does not interfere with the growth of other pollen grains lying on the same stigma. Detection of incompatible pollen can be made by the callose deposition at the rejection site. It is thus obvious that in incompatible reactions the exine borne proteins are involved. Such proteins when isolated and applied directly on stigma also respond similarly, as do intact pollen. Fragments of anther tapetum also behave likewise when placed on stigma surface.

The pistillate factors in this type of incompatibility are therefore localized only on the surface of the stigma. Other distinguishing features are: (a) presence of S-allele specific proteins on the stigmatic surface alone,(b) isolation of characteristic specific proteins from the stigmatic leachates, and (c) overcoming incompatibility by organic solvent treatment of stigma.

Experimental evidences show that recognition factors are synthesised in the stigma during its maturation. Bud pollination serves as one of the possible methods to overcome self-incompatibility as S-allele specific proteins are absent or present in insignificant amounts, at this stage. This indicates that factors that inhibit germination and tube growth are built up gradually in the pistil.

Gametophytic Incompatibility: In GSI systems callose deposition is not evident on the stigma but is very conspicuous in the pollen tube. Sometimes the callose deposition occurs even in the germpore, inhibiting pollen germination. In the Poaceae, the rejection reaction is completed within a few minutes after pollination and subsequent rejection or acceptance takes 10 minutes.

With the exception of Poaceae and *Oenothera*, the recognition/rejection reaction in GSI system occurs after the pollen tube has grown about two third the length of the style. Genetically the recognition factor on the male side has been attributed to the male gametophyte, the proteins incorporated during pollen wall development in the intine are involved. Since in GSI systems the rejection reaction is comparatively delayed, is also possible that the synthesis or activation, and release of the recognition substances are also delayed and they occur in the pollen tube. In some species the tubes show reduced level of carbohydrates and starch.

3.4.2 Interspecific Incompatibility

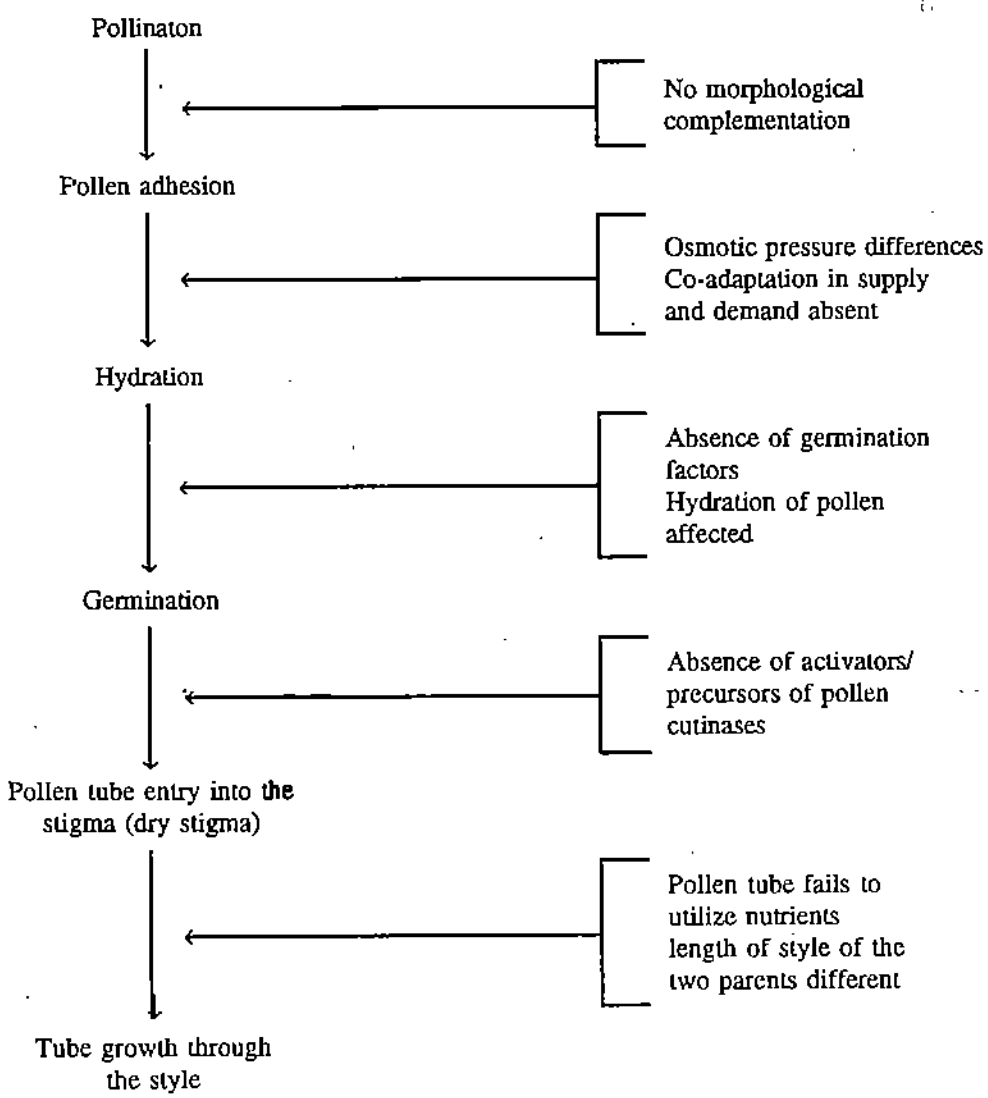
This type of incompatibility is characterised by the prevention of fusion of gametes between members of different species. The incompatibility factors also operate after fertilization and cause eventual breakdown and failure of embryo development. The factors that prevent seed set following interspecific crosses have not been worked out

It is however apparent that rejection reaction may operate at any level. In *Petunia* the morphological abnormalities of the pollen tube are similar in both self and inter-generic incompatibility. The inhibition may result from unilateral incompatibility, or incongruity or a passive rejection may operate (see Table 3.3).

Interspecific incompatibility involves non-proteinaceous substances besides the stigma surface proteins. Phenolics and carbohydrates also seem to play an important role. It is believed that more than one gene at different loci controls the interspecific incompatibility. In crosses between incompatible species either fertilisation does not occur or the hybrid embryo aborts due to either inadequate development of endosperm or lack of support from endosperm.

Unilateral Incompatibility: This relates to incompatibility that operates in one direction. The most common way to ascertain unilateral incompatibility is to perform crosses between a self-incompatible species and a self-compatible species. The cross is successful only when the self-compatible species serves as the female parent. This kind of incompatibility also operates between species of different genera. Unilateral incompatibility can be explained on the following basis:

Table 3.3: Interspecific incompatibility - Passive rejection and possible mechanisms.



3.4.3 Biological Significance of Incompatibility

Both interspecific and intraspecific incompatibility determine the degree of inbreeding and outbreeding of plants. Self-incompatibility is the natural way to circumvent the extensive selfing that promotes homozygous individuals and eventual inbreeding depression (accumulation of lethal recessives).

Sexual incompatibility also serves as a hindrance in crop improvement programmes. Until the technique of androgenic haploidy was evolved, continuous self pollination was the only way to obtain homozygous individuals. Even now where haploid production is not possible through anther culture, a successful approach can only be through self-pollination. Even interspecific incompatibility prevents distant hybridization. Therefore, various methods have now been adopted to overcome the inter-and intra-specific incompatibility.

3.4.4 Methods to Overcome Incompatibility

It has been possible to facilitate the germination of incompatible pollen by extracting pollen wall proteins from compatible pollen and supplying these during interspecific crosses. The pellicle, which lies over the cutinised wall of the stigmatic papillae is functionally important in the capture and hydration of pollen grains and may also be the site of the recognition reactions.

In *Oenothera* stylar grafting experiments led to pollen tube growth in incompatible styles. Treatment with Ca^{++} resulted in pollen tube growth in incompatible style to some extent in *Oenothera*. Emasculation of immature flowers in *Petunia* reduced pistil elongation and weakened the incompatibility barrier. In general relatively high temperature has been found to inactivate the incompatibility mechanism and the site of activation has been the style. Self-incompatibility in *Lilium* could be overcome by an interaction of temperature and 1% solution of naphthaleneacetamide-in lanolin. Heating of intact and detached style in water at 50° for 6 min before pollination could also overcome incompatibility. The stigmas of some plants with sporophytic incompatibility have a cuticular layer which serves as an incompatibility barrier. Pollen of such plants possess a cutinase enzyme system for destroying the cuticular layer. More specific methods useful in overcoming self-incompatibility are mentioned below.

1. Use of mentor Pollen: There are various ways to overcome incompatibility by the use of mentor pollen. In a mixture of mentor (compatible) pollen and incompatible pollen the recognition protein from compatible pollen masks the inhibition reaction and allows incompatible pollen to germinate and penetrate the stigma. Mentor pollen seem to provide a pollen growth promoting (PGS) or regulating substance which permits incompatible pollen to sustain tube growth. The other mechanism proposed is that it involves the signals which stimulate mentor pollen tube to provide substances critical for sustained growth of ovules, ovary and other fruit tissues.

2. Bud Pollination: In *Petunia axillaris* early self-pollination of buds leads to normal fertilization.

3. Stub Pollination: Incompatibility can also be overcome by either removing the stigma or the upper part of style, if the inhibition factor lies on stigma. Sometimes length of the style also hinders the process. To overcome self-incompatibility in *Ipomoea trichocarpa*, the stigmatic lobes or a part of the stylle are cut off to make an easy way for pollination. Stylar length in *Nicotiana tabacum* is greater than that in *N. rustica* and *N. debney*. By removing a major portion of style from *N. tabacum* and smearing its cut surface with agar sucrose medium suitable for germination, it is possible to achieve fertilization with *N. rustica* and *N. debney* pollen.

4. Intra-ovarian Pollination: Incompatibility occurring on stigma or in the style can be overcome by intra-ovarian pollination that refers to injection of pollen suspension in the ovary and its subsequent germination inside the ovary.

   In this technique ovary is first surface sterilized with ethanol. Then two holes one for injecting pollen suspension into ovary and other for allowing ovarian air to eject out are made. These holes are sealed with petroleum jelly and pollen are allowed to germinate inside the ovary and bring about normal fertilization.

5. Test tube Pollination and Fertilization: The excised ovules or pistils are dusted with pollen grains and allowed to grow on a nutrient medium. This technique favours pollen germination followed by fertilization and development of seeds.

   Self-incompatibility in *Petunia hybrida* and *Brassica campestris* can be broken by placental pollination.

Attempts have been made through placental pollination for interspecific, intergeneric and interfamily crosses. Seeds with viable embryos develop in crosses between *Melandrium album, M. rubrum, Viscaria vulgaris, Silene schafta* and *Nicotiana alata.*

Hybrid embryos also develop by pollinating the excised ovules of *Nicotiana tabacum* with the pollen grains of *Hyoscyamus niger.*

6. Modification of Stigmatic Surface: Application of the lectin, concanavalin A, to stigma is also helpful in overcoming incompatibility in *Brassica*. Likewise, prior treatment of stigma with detergent (Tritox X-100), and organic solvent (hexane) helps to modify the incompatibility reaction. Pretreatment of stigma with NaCl (15g/l) in *Brassica campestris* blocks the incompatibility reaction.

7. Heat Treatment of Style: In *Lilium longiflorum* pre-treatment of the style at 50° C for 6 min. could prevent self incompatibility. In rye, even 30° C is effective in inhibiting the incompatibility reaction.

8. Irradiation: Incompatibility in *Lycopersicon peruvianum* could be overcome by gamma rays. The higher seed-set per plant achieved by this method is ascribed to prevention of flower abscission at early stage. Irradiation seems to be fruitful only in plants where incompatibility is under the control of gametophyte.

9. Chemical Treatment: When premature abscission is the barrier to compatibility, application of p-chlorophenoxyacetic acid to the pedicel enhances the life span of arrowroot flower.

   Similarly, it is possible to overcome the premature flower abscission in *Ipomoea batatas* by applying 100 ppm of 2,4-D that helps early embryo development.

   Application of p-chloromercuribenzoate and kinetin have proved useful in *Lilium longiflorum* and in *Oenothera organensis* respectively.

10. Increased CO_2 Level: In *Brassica* species, raising of atmospheric level of CO_2 by 100 fold, at 100% relative humidity helps to overcome self incompatibility. This method is an important tool to maintain the inbred parental lines of *Brassica* for the production of S_1 hybrid seeds.

11. Parasexual Hybridization: As it involves the hybridization of somatic cells (protoplast) to overcome sexual incompatibility this is known as "parasexual hybridization".

    The process involves three steps: 1. isolation of protoplasts 2. fusion of isolated protoplasts and 3. culture of hybrid protoplast to regenerate whole plants.

SAQ 3

Which of the following statements are not true?

(a) In the dimorphic and trimorphic forms sporophytic self incompatibility operates.

(b) The syngamy barrier refers to any barrier on the stigma or in the style, in other words, from pollen germination to the liberation of male gamete near the egg cell.

(c) In the GSI plants, the recognition proteins are present in the intine, whereas in the SSI plants, these occur in the exine.

(d) On pollen hydration, the proteins of gametophytic origin are first to leach out, whereas the proteins of sporophytic origin take longer time.

(e) Sexual incompatibility may be between individuals of different species—interspecific, or between the individuals of the same species–intraspecific.

(f) Interspecific incompatibility involves non-proteinaceous substances such as phenolics and carbohydrates in addition to the stigma surface proteins.

(g) Parasexual hybridisation can be successfully used to overcome sexual incompatibility.

(h) In nature a balanced inbreeding and outbreeding of the plants is regulated by intraspecific and interspecific incompatibility.

3.5 APOMIXIS

The formation of sporophyte from the gametophyte without sexual process signifies apomixis. It relates to the replacement of alternation of a reduced gametophyte and an unreduced sporophyte by an unreduced gametophyte and sporophyte (summarized in Fig. 3.11, Table 3.4). It occurs in relatively in about 35 families. The most common being Asteraceae and Poaceae. Apomixis can be classified into two types, recurrent (in non-reduced embryo sacs) and non-recurrent (in reduced embryo sacs). The later relates to development of haploid embryos without the actual fusion of gametes.

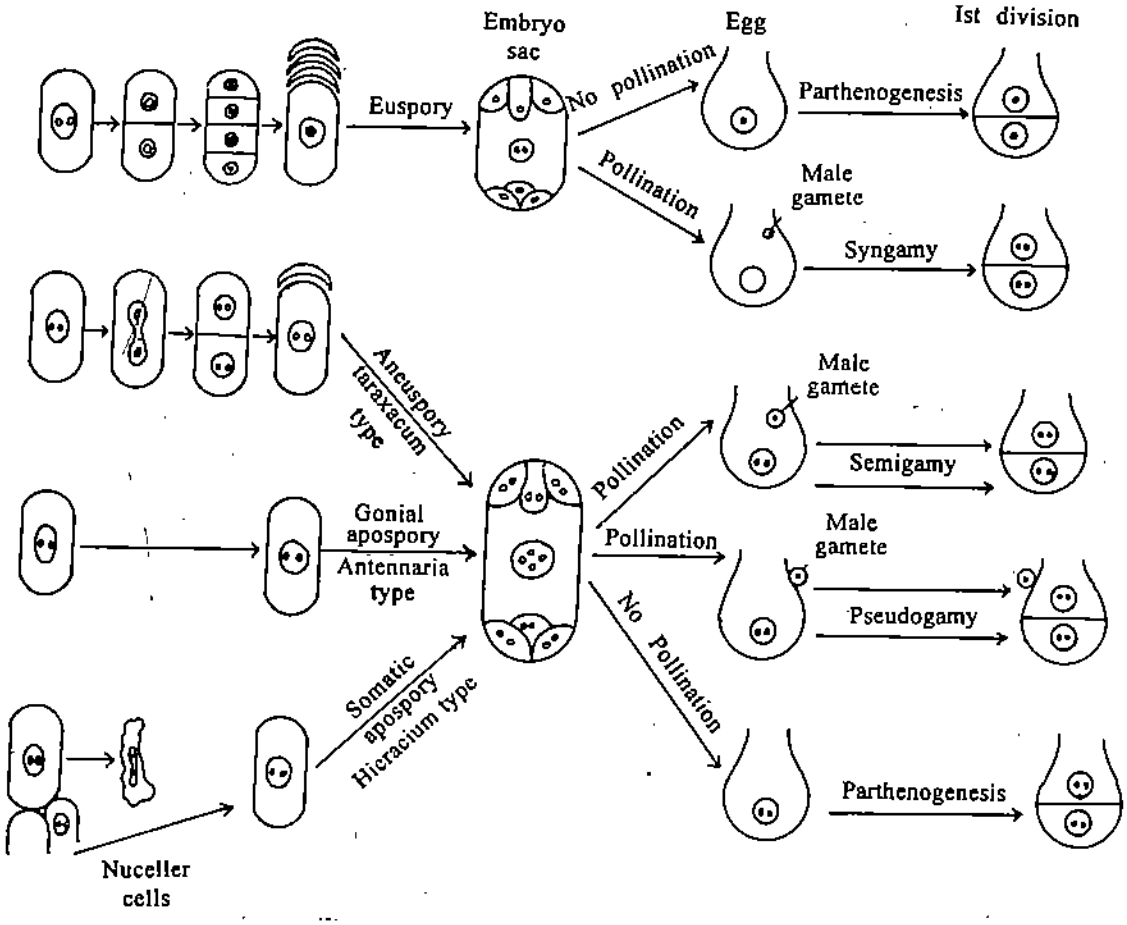


Fig. 3.11: Diagrammatic representation of Apomixis types.

3.5.1 The Recurrent Type

In this type, euspory (seen in normal cases) is replaced by aneuspory (Diplospory) because of irregular meiosis. When the spore mother cell functions directly as the embryo sac initial it is termed gonial apospory and when one or more of the somatic cells of the nucellus or chalaza act as embryo sac initial it is called somatic apospory.

A. Aneuspory (Diplospory): Includes cases in which during spore formation the meiotic process is irregular. Depending upon the extent of irregularity in meiosis in various plants, aneuspory is further categorized into various types.

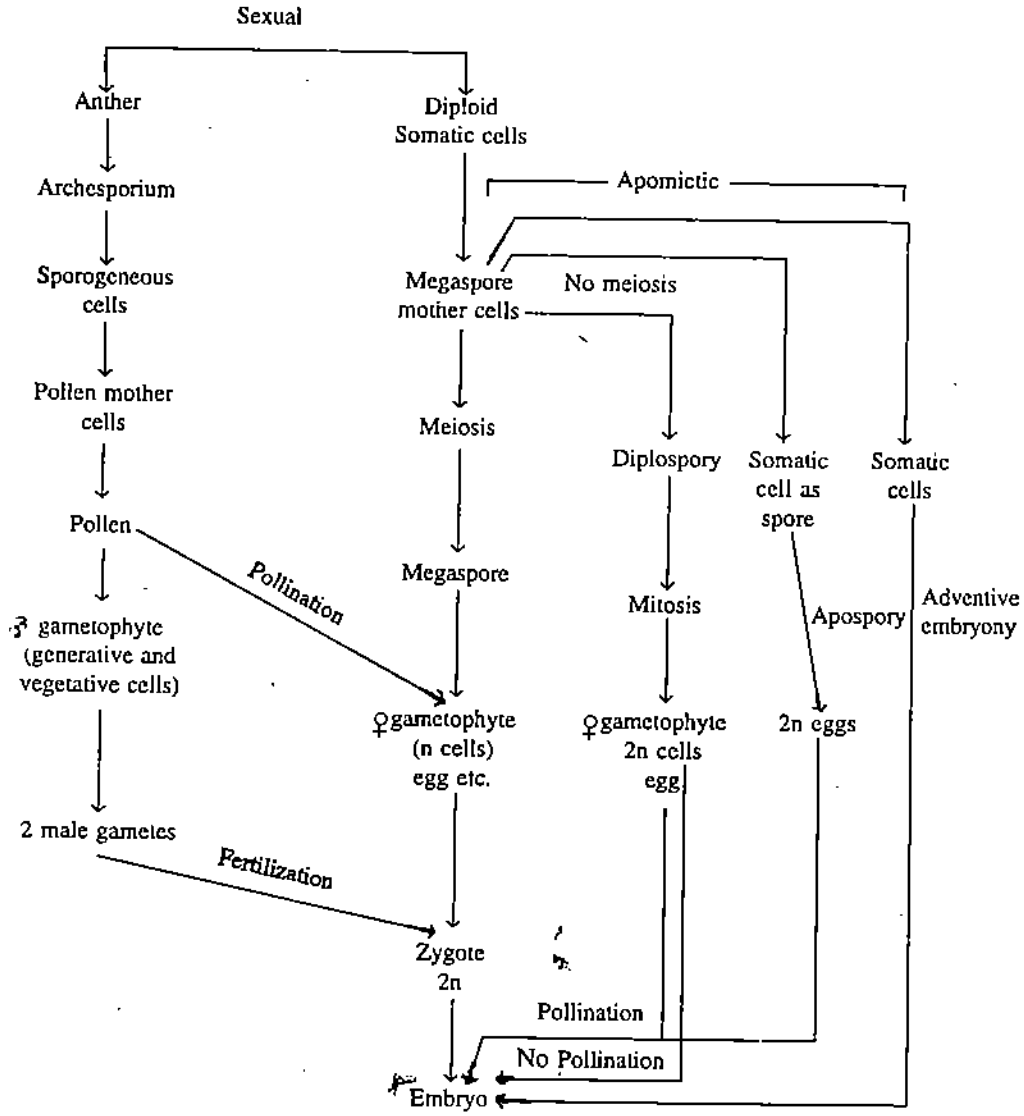
   i. Datura type — the two unreduced nuclei undergo two mitotic divisions to produce an 8-nucleate embryo sac.

   ii. Taraxacum type — first meiotic division ends in a restitution nucleus. Meiosis II produces unreduced cells. Ultimately an 8-nucleate embryo sac is formed. The egg directly forms the embryo.

iii. Ixeris type — it is like Taraxacum, but a single binucleate gynospore is formed. Two divisions lead to the formation of an 8-nucleate embryo sac.

iv. Allium type — a premeiotic-endomitotic doubling makes meiotic prophase to start with double chromosome number. The result is an unreduced embryo sac.

Table 3.4: Schematic Representation of Apomixis.



B. Gonial Apospory: There is no meiosis. The megaspore mother cell enlarges, a small vacuole appears above and below the nucleus. The cell thus becomes 1-nucleate embryo sac. Three successive mitotic divisions result into an 8-nucleate embryo sac. In *Brachyocome*, the polar nuclei function directly as the first 2 endosperm nuclei.

C. Somatic Apospory: Meiosis is apparently normal but megaspores play no part in the embryo sac formation. Either during or soon after meiosis one or more sporophytic cells (nucellar or chalazal) enlarge and invade the nucellar lobe destroying and replacing the megaspore. After 3 successive nuclear divisions each such cell becomes differentiated into an aposporic 2n embryo sac with normal organization. Thus, gametophytic generation is completely eliminated.

Organization in non-reduced embryo sacs

The fate of a nucleus in the embryo sac depends upon its position. Many irregularities in the disposition of nuclei in the early polarization have been recorded. The mature embryo sac otherwise shows a normal organisation with two synergids, three antipodals, an egg and proendospermic cell.

Embryogenesis in non-reduced embryo sacs

Embryo development in these embryo sacs can take place as a consequence of pollination. Those associated with pollination are called **eugamous, semigamous** or **pseudogamous** and those that develop without pollination stimulus are termed **parthenogenic.**

Eugamy: Normal fertilization of the apomictic eggs takes place to produce the zygote.

Semigamy: The male gamete penetrates into the egg but does not fuse with the egg nucleus. Both the nuclei multiply independently but the division of the male nucleus stops early.

Unreduced Pseudogamy: The male gamete degenerates either inside or outside the embryo sac. Thus, the egg develops without fusion taking place.

3.5.2 Non-recurrent Type

As mentioned earlier it refers to embryo development in reduced embryo sacs. The mechanisms identified for such cases are:

i. Reduced Pseudogamy: The reduced egg develops in a pseudogamous, and parthenogenic manner. The pollen tube enters normally but the male gamete fails to fuse with the egg and disintegrates in the cytoplasm of egg.

ii. Reduced Parthenogenesis: Embryo development is accomplished by heat or cold treatment.

iii. Androgenesis: The egg nucleus degenerates, the sperm nucleus functions in the cytoplasm of the egg and produces the embryo.

3.5.3 Endosperm Development in Apomicts

Endosperm development is usually poor and without preceding the fusion of polar nuclei, hence diploid.

3.5.4 Anthers of Apomicts

Meiosis is abnormal or there is total failure of meiosis. Formation of plasmodial microspore mother cells is reported. Polyads are also noticed. Usually, only 2 microspores of a tetrad are normal. The generative nucleus rarely divides. Thus pollen remains at 2-celled stage.

3.5.5 Causes of Apomixis

Apomictic species are generally hybrids or polyploids, as a consequence, there is irregular meiosis. Apomixis appears to be controlled by a set of genes. The trait is genetically inherited. The genes controlling sexual reproduction are non-allelic to those of apomixis. Accordingly, any line of dissent carrying the genes for apomixis will produce both types, apomicts as well as sexually reproducing plants.

It has been proposed that apomixis is governed by recessive genes. The three genes (AABBCC) determine the breeding behaviour. In homozygous condition *a* forms unreduced eggs, *b* prevents fertilization, and *c* promotes egg development without fertilization. Thus, aaBBCC will have unreduced egg but cannot develop without fertilization, AAbbCC produces reduced egg but no embryo development take place because fertilization is prevented, and AABBcc will show normal sexual behaviour because the gene C has no effect in the presence of A and B.

As a consequence of apomixis, genetic variability in such species is frozen as they have the same genotypes as parents. However, facultative apomicts have an advantage as they have retained both kinds of reproduction.

3.5.6 Parthenogenesis

The diploid egg produced in the embryo sacs during diplospory and apospory develops into an embryo without fertilization, thus maintaining the sporophytic level of chromosomes. This process of embryo development from an unfertilized egg is called

parthenogenesis. The stimulus to form embryos may be pollination dependent. For example, in grasses pseudogamy operates that involves pollination stimulus while in apomictic taxa of Asteraceae and Rubiaceae no such stimulus is required.

Pseudogamy has been credited with: (i) supply of male nucleus for endosperm development, (ii) activation of growth of ovule and ovary, and (iii) stimulation of parthenogenesis.

Pollination even otherwise is reported to initiate the development of adventive embryos of *Citrus*. Likewise, parthenogenetic development of embryo proceeds in apomictic grasses but normal embryos result only when endosperm is also formed.

Parthenogenesis can be distinguished into reduced and unreduced, accordingly, the developed embryo can be haploid or diploid, (see Fig. 3.11).

3.5.7 Significance of Apomixis

Apomixis offers the possibility of indefinite multiplication of specially favourable biotypes without any variation due to segregation or recombination. In obligate apomicts such as mangosteen (*Garcinia mangostana*) this advantage is enjoyed at the cost of long term evolutionary flexibility which is advanced chiefly through sexual reproduction. However, in facultative apomicts or groups of plants where sexual and apomictic members co-exist, the phenomenon is of special significance.

There is much interest currently to induce apomixis in important hybrids to fix the vigour and to save the cost of hybrid production. There is already some success in forage grasses.

SAQ 4

Part-1 Which of the following features characterise the recurrent type of apomixis?

a) formation of non-reduced embryo sacs due to irregular meiosis.

b) egg nucleus of unreduced embryo sac degenerates, and the sperm nucleus in the cytoplasm of the egg forms the embryo.

c) megaspore mother cell acts as embryo sac initial.

d) on subjecting the unfertilised egg cell to heat treatment.

e) somatic cells of nucellus function as embryo sac initial.

Select the right answer from the choices given below.

i) a,b,c

ii) c,d,e

iii) a,c,e

iv) b,d,e.

Part-2 Which combination of the following features signify the non-recurrent apomixis?

a) reduced egg nucleus degenerates and sperm nucleus function in the cytoplasm of egg, to form the embryo.

b) development of reduced egg by pseudogamy, and in parthenogenic manner.

c) development of embryo from reduced egg cell by heat/cold treatment.

d) the nucellar cell forms embryo.

e) the egg cell is diploid due to irregular meiosis.

Select the correct answer from the choices given below:

i) a,b,c

ii) b,c,e

iii) c,d,e

iv) a,c,e

3.6 SUMMARY

In this unit you have learnt that:

- In sexually reproducing plants, pollination and fertilisation follow gametogenesis.
- Pollination is the transfer of pollen from anthers to the stigma. Flowering plants have evolved a number of strategies for pollination. A large number of plants undergo self-pollination, others have evolved contrivances to ensure cross pollination although their flowers are bisexual.
- The events that follow pollination are: pollen adhesion, its hydration, germination, pollen tube entry into the style, growth of pollen tube through the style, and into the female gametophyte.
- On reaching the female gametophyte, the two male gametes are released from the pollen tube. One fuses with the egg (syngamy) to form the zygote and other fuses with the nucleus of the central cell to form the primary endosperm nucleus (triple fusion). This process termed 'double fertilization', is unique to angiosperms.
- In nature, the stigma is exposed and can receive a large variety of pollen grains, but not all of them succeed in germinating, and effecting fertilisation. The plants have different devices that allow the pollen of only the right mating type to function normally, the others are discarded. One of the most exciting aspects of reproduction is how plants register recognition at the cellular level. If a pistil carrying functional ♀ gamete(s) fails to set seeds following pollination with viable and fertile pollen, capable of bringing about fertilisation in another pistil, the two are said to be incompatible and the phenomenon is known as sexual incompatibility. Sexual incompatibility may be inter-or intraspecific. Morphological, genetical and physiological mechanisms are involved on excersing avoidance of selfing and providing cross pollination. It is also possible to overcome self incompatibility by several surgical and chemical methods.
- Apomixis is a type of reproduction in which an unfertilised egg develops into an embryo without sexual fusion. Apomixis may be of recurrent type or non-recurrent type. Apomicts show irregular meiosis in their anthers, and have poorly developed endosperm. Apomixis is controlled by a set of genes, and the trait is genetically inherited.

3.7 TERMINAL QUESTIONS

1) What are the merits and demerits of self and cross-pollination? Present your answer in tabular form.
2) List the common mechanisms devoloped by plants to prevent self-pollination, and promote cross pollination write five lines about each.
3) Match the items given in key with the descriptions given below:

Key

a) Anemophily

b) Hydrophily

c) Entmophily

d) Ornithophily

e) Cheiropherophily

i) flowers with highly reduced perianth, slender pollen grains with low specific gravity.

ii) red or orange coloured, vessel-like flowers that produce large quantities of pollen grains and nectar, found mostly in the tropical regions.

iii) flowers having peculiar strong odour, and are borne singly or in clusters quite away from the branches and foliage.

iv) pollen grains small, light, smooth, produced in enormous amounts by mostly the unisexual flowers with reduced perianth. Female flowers with long, feathery stigmas.

v) flowers with showy corolla, often modified, having odour, and nectar.

4) How does the open style differ from the closed style in terms of structure and the nature of exudates?

5) What is a transmitting tissue? Where is it found? List its salient characters.

6) What are the factors that govern the adhesion of pollen grains on the stigmatic surface?

7) Why is pollen hydration a crucial step in pollination?

8) Differentate between gametophytic and sporophytic incompatibility.

9) What structural changes do the open styles and the closed styles undergo as a result of pollination?

10) What are the salient structural differences seen in the tips of the pollen tubes growing in compatible and incompatible pistils?

11) What is meant by double fertilisation?

12) How does syngamy differ from triple fusion? What are the end products of the two and their ploidy levels?

13) What are the major differences between the heteromorphic and homomorphic self incompatibility?

14) Discuss the genetic basis of self-incompatibility.

15) The pollen wall and its protein contents play an important role in pollen-stigma interaction. Explain.

16) Why is rejection reaction faster in SBI systems than the GSI systems?

17) What are the features that enable you to ascertain whether it is sporophytic or gametophytic self incompatibility.

19) List and write briefly about the important methods to overcome incompatibility in plants.

20) Differentiate between recurrent and non-recurrent type of apomixis.

21) Explain in genetic terms the phenomenon of apomixis.

22) Of what ecological significance is apomixis over vegetative reproduction?

3.8 ANSWERS

Self-assessment Questions

1) a) autogamous

b) xenogamy

c) cleistogamous

d) cross

e) anemophilous

f) cross

2) a) tip

b) cellulase, pectinase, callase

c) wet, *Petunia*, dry, cotton

d) exocytosis

e) lipid, phenolic

f) solid, open

g) canal cells, transmitting tissue

h) sporophytic, gametophytic

i) auxins, gibberellins

j) zygote, primary endosperm nucleus

3) b, d,

4) Part-1 (iii)

Part-2 (i)

Terminal Questions

1) You may refer to subsection 3.2.2.

2) See subsection 3.2.2.

3) a) iv

b) i

c) v

d) ii

e) iii

4) Hint: Open styles lined by glandular and seccretory cells, exudates contain mainly polysaccharides; closed style have a compact core of transmitting tissues, their exudates contain lipids, proteins and polysaccharides.

5) See subsection 3.3.1

6) Hint: Stickiness of pollen and stigma, exine pattern, composition of pellicle, surface coat substances, electrostatic forces and specificity between the two parents.

7) Hint: Hydration triggers the release of pollen-wall proteins which subsequently govern the compatibility/incompatibility interaction between the two parents.

8) You may refer to subsection 3.3.2.

9) See subsection 3.3.2.

10) See subsection 3.3.2, 'Pollen Tube Growth.'

11) Hint: fusion of one male gamete with egg, and the other one with the secondary nucleus.

12) You may refer to subsection 3.3.4.

End products: syngamy—zygote and embryo, triple fusion primary endosperm nucleus-endosperm.

Ploidy levels: zygote—2n; primary endosperm nucleus-3n.

13) See subsection 3.4.1.

Hint: Heteromorphic forms have morphologically different flowers. This kind of incompatibility can be predicted by examining the different morphs. It operates at the morphological and physiological levels.

Homomorphic—It consists of flowers that are morphologically indistinguishable. This kind of incompatibility can be identified by proper breeding experiments. It involves multiple alleles.

14) See subsection 3.4.1.

15) You may refer to subsection 3.4.1 — Physiology and Biochemistry of incompatibility.

16) Hint: Usually, the proteins of sporophytic origin are situated in exine and these pass out first while those of gametophytic origin situated in intine take a longer time.

17) See Tables 3.1 and 3.2.

18) Hint: It prevents the formation of homozygous individuals through self-fertilisation. These individuals have low survival rate. It helps in introducing genetic diversity to enable them to adapt better in diverse ecological situations.

19) See subsection 3.4.4.

20) See subsections 3.5.1 and 3.5.2.

21) See subsection 3.5.5.

22) See section 3.5.

UNIT 4 ENDOSPERM

Structure

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4.1 INTRODUCTION

In the previous units you have read about anther and ovule, gametogenesis, pollination and fertilization. In this unit you will study endosperm in some detail. Flowering plants are unique in exhibiting double fertilization. In these plants two male gametes are delivered into the embryo sac, by the pollen tube, of which one fuses with the egg cell (female gamete) and the other fertilizes the polar nuclei or the secondary nucleus (the fusion product of the two polar nuclei). The fertilised egg cell i.e. zygote (2n) develops into the embryo and the central cell, with the fertilised secondary nucleus i.e. primary endosperm nucleus (3n), develops into the endosperm. The endosperm is predominently a triploid tissue. However it is diploid in all the members of the family Onagraceae (evening primrose family) and pentaploid in *Fritillaria*. The products of double fertilisation i.e. the zygote and the primary endosperm nucleus follow different patterns of growth and have different destinies. The former develops into a well-organised embryo—the progenitor of future plant, while the latter gives rise to an unorganized tissue—the endosperm which stores reserve material and has limited growth. As the seed ripens, the embryo generally attains its full structure and the endosperm is consumed either entirely or partially by the embryo. Thus, the endosperm serves as the chief nutritive tissue for the embryo. The endosperm of angiosperms is comparable to the female gametophyte of the gymnosperms. However, it is important to note that in the latter, the endosperm is a gametophytic (haploid) tissue and differentiates before fertilization. Thus the endosperm of both plant groups differs markedly.

Objectives

After reading this unit, you will be able to:

- describe the development and nature of endosperm;
- list various types of endosperm;

- distinguish the endosperm haustoria;
- describe the important functions of endosperm in the development of embryo;
- explain the cytology, structure and fate of endosperm;
- identify the morphological nature of endosperm.

4.2 DEVELOPMENT

In a fertilized embryo sac, the primary endosperm nucleus is generally observed below the zygote (Fig. 4.1). It divides, and further divisions of its products give rise to an endosperm.The nutritive role of endosperm has long been recognized. It nurses the embryo from the proembryo stage until it becomes self-sufficient, and completes its development. The endosperm tissue is the source of growth regulators such as gibberellins and cytokinins. Coconut water and sometimes the extract of corn endosperm, at the milk stage are added to the nutrient media of tissue cultures to stimulate growth. The developing endosperm derives nutrients from the food reserves stored in nucellus and integuments. In several families the development of chalazal, micropylar or secondary endosperm haustoria leads to partial or entire absorption of integuments. Based on the mode of development, three main types of endosperms have been recognised in angiosperms: (1) Nuclear (2) Cellular and (3) Helobial.

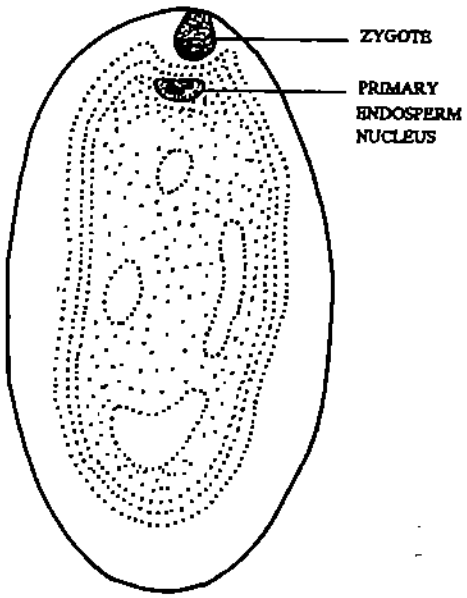
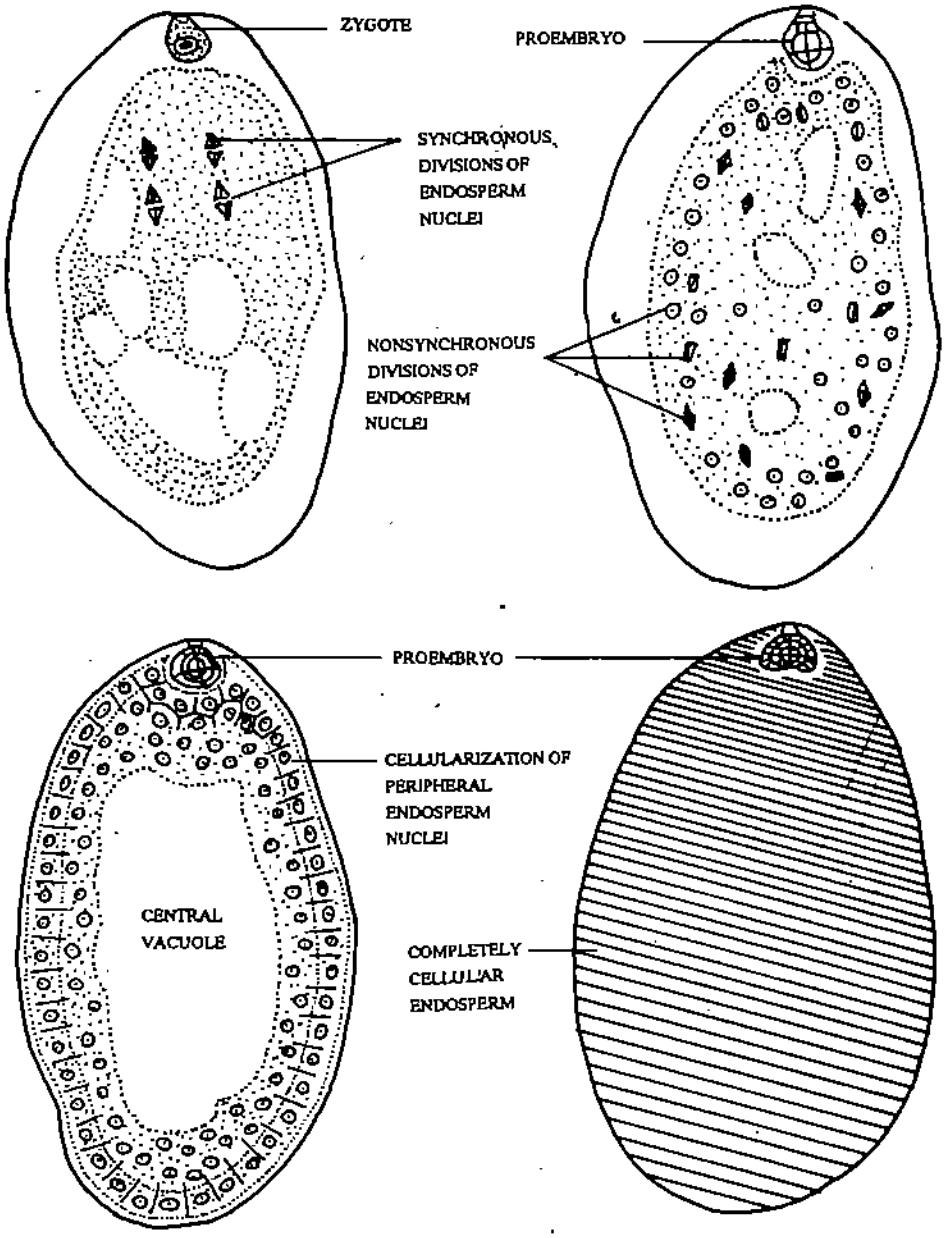


Fig. 4.1: A fertilized embryo sac showing the primary endosperm nucleus situated below the zygote.

4.2.1 Nuclear Type

The primary endosperm nucleus divides. The cell wall is not laid. These nuclei and their division products form a large number of free nuclei. The first few divisions are synchronous (Fig. 4.2) as a result nuclei are seen in multiples of two i.e. 4, 8, 16, 32 and so on. Later the nuclear divisions are non-synchronous i.e. the nuclei may be seen in different stages of divisions (Fig. 4.3) and the number of endosperm nuclei are not in multiples of two. The free nuclei thus formed remain suspended in the cytoplasm of the embryo sac. After some time the nuclei become gradually pushed towards the periphery by an expanding central vacuole (Fig. 4.4). A large number of nuclei accumulate towards the micropylar and chalazal ends. The nuclei may increase in size either by the fusion of two or more or by their independent growth. The process of cell formation starts with the centripetally growing walls from the periphery proceeding towards the centre of embryo sac or from the apex progressing towards the

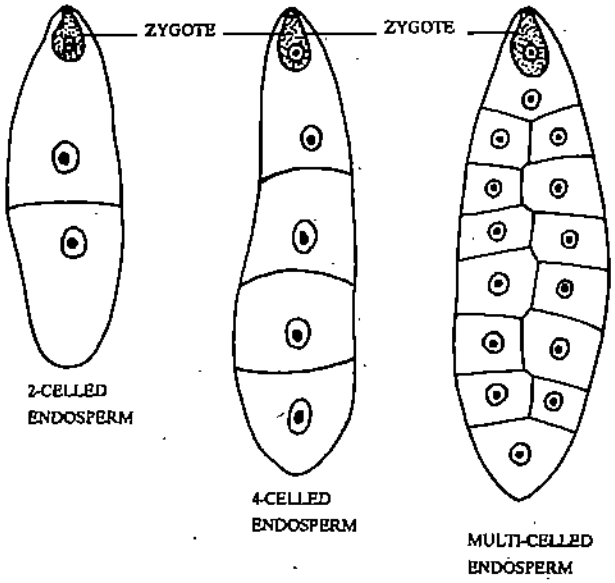
base. To begin with a single layer of uninucleate cells is formed (Fig. 4.4). Subsequent anticlinal and periclinal divisions of these cells lead to complete cellularization of the endosperm (Fig. 4.5). In some plants only one or two peripheral layers of endosperm cells may develop and the entire embryo sac may remain in the free nuclear state or cell formation may be restricted only to the micropylar end of the embryo sac. In a few plants wall formation may not take place at all and the endosperm has free nuclei. Normally the endosperm cells are only uninucleate; sometimes more than one nucleus may be enclosed within a cell. The number may further increase by nuclear divisions. The development of endosperm in coconut is interesting. When the fruit is young the embryo sac is filled with a clear fluid containing numerous free endosperm nuclei. Later, the periphery becomes jelly-like, containing several cells. As the fruit matures, and the cellular endosperm along the periphery becomes very massive, the central part contains a sweet liquid with a large number of nuclei. The cellular endosperm constitutes the edible copra rich in stored fat. In the betel nut and fruits of several other palms, the cellular endosperm becomes very hard and woody.



Figs. 4.2-4.5: Nuclear endosperm. Fig. 4.2: Embryo sac showing synchronous divisions of endosperm nuclei. Fig. 4.3: Embryo sac showing nonsynchronous divisions of endosperm nuclei. Fig. 4.4: Periphery of embryo sac. Cellularisation has started. Fig. 4.5: Endosperm has become fully cellular, embryo is heart shaped.

4.2.2 Cellular Type

In this type, as the name indicates, the division of the primary endosperm nucleus is immediately followed by the laying down of a wall, which is usually transverse (Fig. 4.6) but may be sometimes vertical or oblique. Subsequent nuclear divisions are immediately followed by wall formation (Figs. 4.7, 4.8). As a result the embryo sac contains cellular endosperm from the very beginning and no free nuclear stage occurs (Figs. 4.6, 4.8).

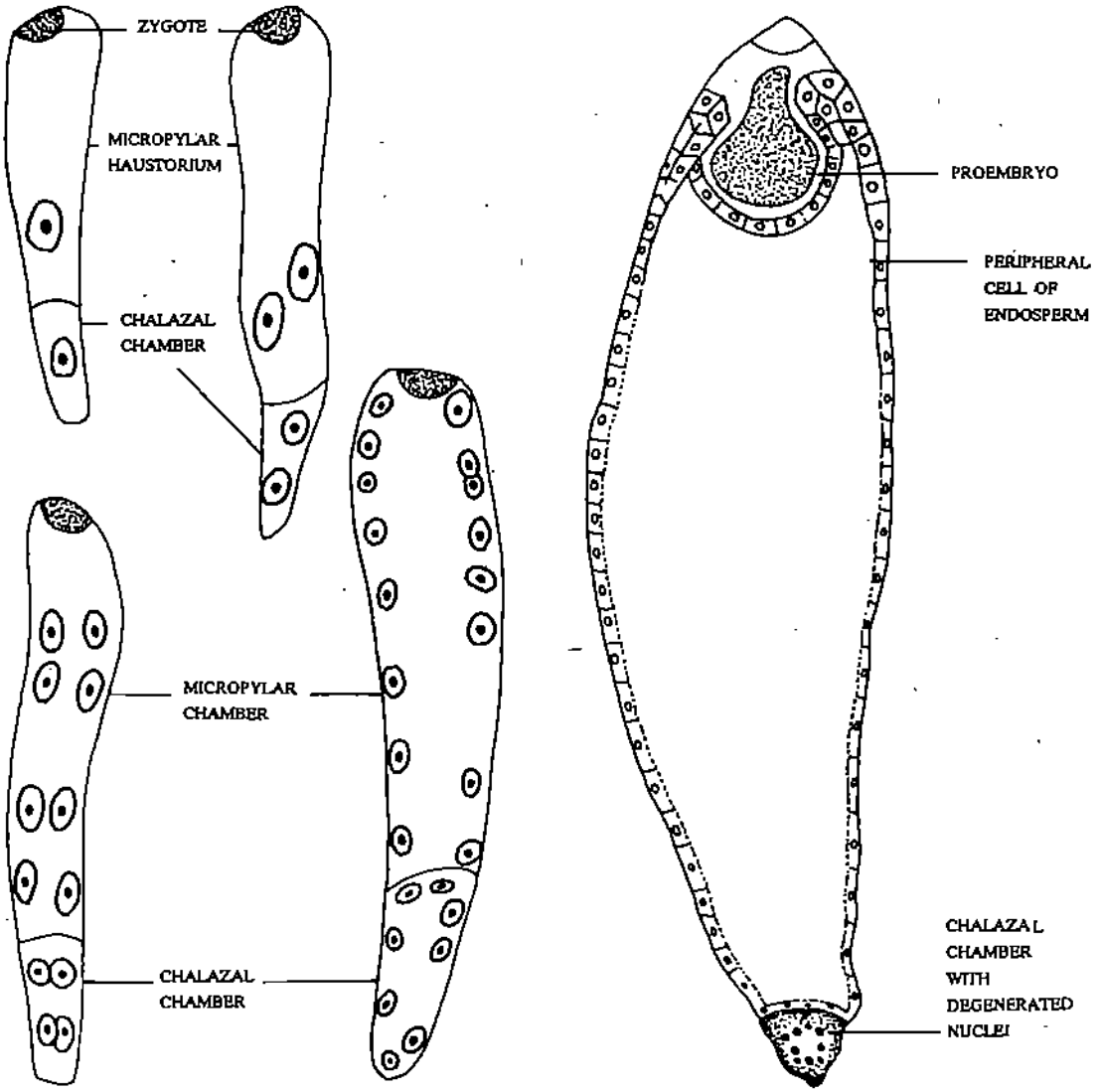


Figs. 4.6-4.8: Cellular endosperm. Fig. 4.6: Two celled endosperm formed by transverse wall. Fig. 4.7: Four-celled endosperm. Fig. 4.8: Multicellular endosperm.

4.2.3 Helobial Type

This type of endosperm is intermediate between the nuclear and the cellular types. The division of the primary endosperm nucleus is followed by the formation of transverse wall that divides the embryo sac into two chambers—the micropylar and the chalazal (Fig. 4.9). The micropylar chamber is larger and undergoes active nuclear divisions to form several free nuclei (Figs. 4.9-4.12). The chalazal chamber is smaller and its nucleus may not divide or may undergo only a few divisions. Later on wall formation normally takes places only in the micropylar chamber which forms the main endosperm (Fig. 4.13). The chalazal chamber eventually becomes crushed and its nuclei disintegrated. According to Swamy and Parameshwaran, the formation of true Helobial endosperm is confined only to the monocotyledonous families. If it occurs in dicotyledonous families, it is only a modification of the cellular or nuclear type of endosperm. However, typical helobial type of endosperm has been observed in dicotyledonous families, such as Santalaceae and Saxifragaceae.

A natural question that may arise in mind is "which of the three types of endosperm is primitive?" One can derive the Helobial from the Nuclear, and Cellular from the Helobial type. These three types are randomly distributed in the primitive and advanced families of angiosperms, as is also true of several other embryological characters. Generally, the Nuclear type is common in the Polypetalae, Cellular in the Sympetalae, and Helobial in the Monocotyledons. However, as the Nuclear type of endosperm has been observed in a larger number of taxa than the other two types, it may be considered to be primitive.



Figs. 4.9-4.13 : Helobial Endosperm. Fig. 4.9 : Two-celled endosperm with large micropylar chamber and small chalazal chamber. Figs. 4.10-4.13 : Later stages of Helobial Endosperm.

SAQ 1

a. What is meant by single fertilisation and double fertilisation?

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b. What are the products of double fertilisation? What are their destinies?

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c. What is unique about the endosperm in coconut?

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d. Name a plant in which mature endosperm is hard and woody.

.....

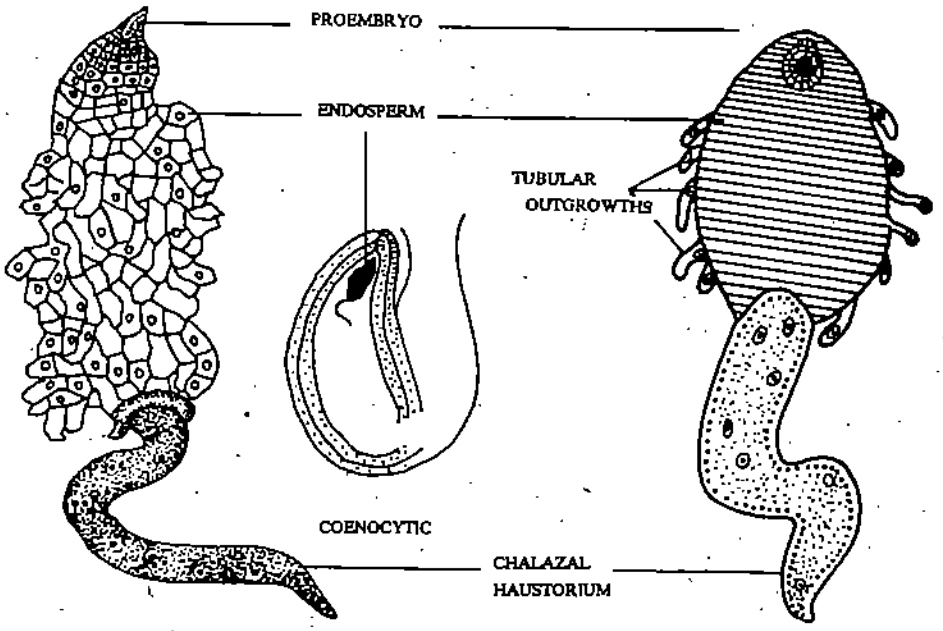
.....

4.3 ENDOSPERM HAUSTORIA

All the three types of endosperms described above may develop special structure called haustoria, which elongate considerably and invade the tissue in the seed and placenta. Haustoria are believed to absorb energy sources and metabolise them for the developing endosperm. A few interesting examples of endosperm haustoria are given below.

4.3.1 Endosperm with Chalazal Haustorium

In *Grevillea robusta*, a member of Proteaceae the endosperm is of the free nuclear type. The upper part of endosperm becomes cellular, whereas the lower part develops into a coenocytic, coiled worm-like structure called the 'vermiform appendage' (Figs. 4.14, 4.15). It serves as an aggressive haustorium, invades the chalazal tissue an transports nutrients to the main endosperm. The occurrence of a chalazal haustorium is also reported in several other plants viz. *Macadamia ternifolia*, *Magnolia obovata*, *Iodinia rhombifolia* etc. The longest endosperm haustorium is found in *Echinocystis lobata* of the family, Cucurbitaceae. In *Lomatia*, besides the chalazal haustorium, numerous single-celled, finger-like projections arise from the cellular endosperm (Fig. 4.16). These penetrate the nutritive nucellar tissue and help in increasing the absorptive surface of the endosperm.



Figs. 4.14-4.16 : Endosperm with chalazal haustorium. Fig. 4.14: L.S. Ovule showing vermiform chalazal haustorium in *Grevillea*. Fig. 4.15: Endosperm and proembryo is magnified to show coenocytic chalazal haustorium. Fig. 4.16: Endosperm of *Lomatia* showing chalazal haustorium and several uninucleate tubular outgrowths.

4.3.2 Endosperm with Micropylar Haustorium

A very prominent and aggressive micropylar haustorium is seen in *Impatiens*. Here the division of the primary endosperm nucleus is followed by a transverse wall, to form an upper smaller chamber and a larger lower chamber. The terminal/distal part of the upper chamber develops into an extensive, much branched haustorium (Fig. 4.17). Its branches extend deep into the funiculus and derive nutrition. Micropylar haustoria are present in many other plants such as *Nemophila* and *Hydrocera*.

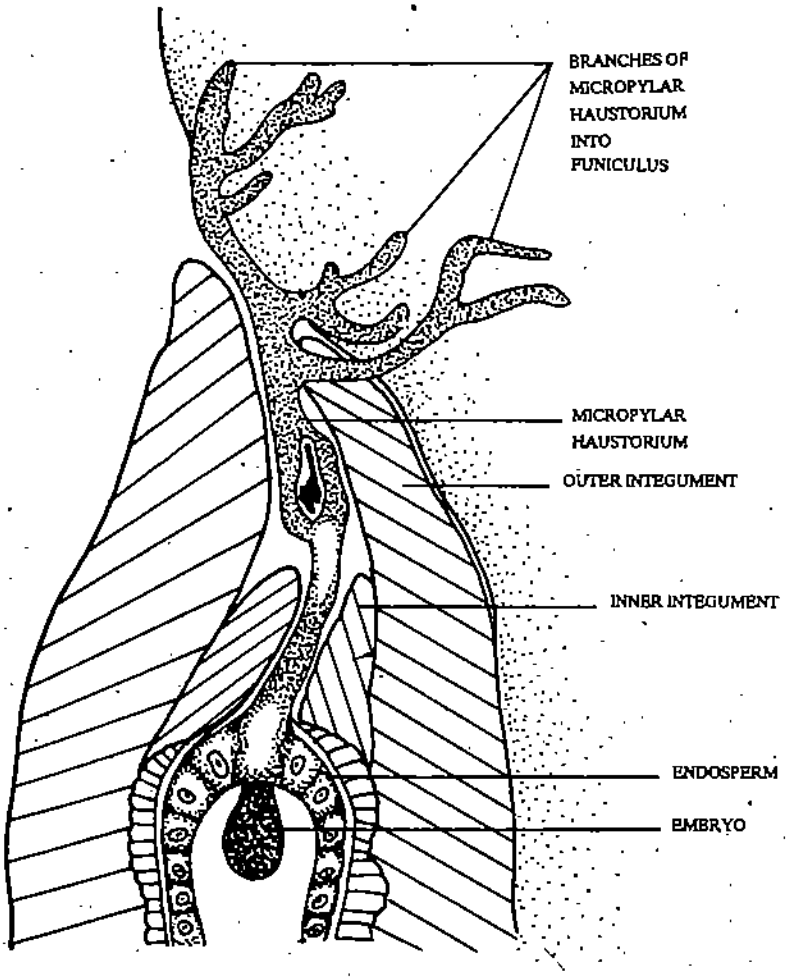
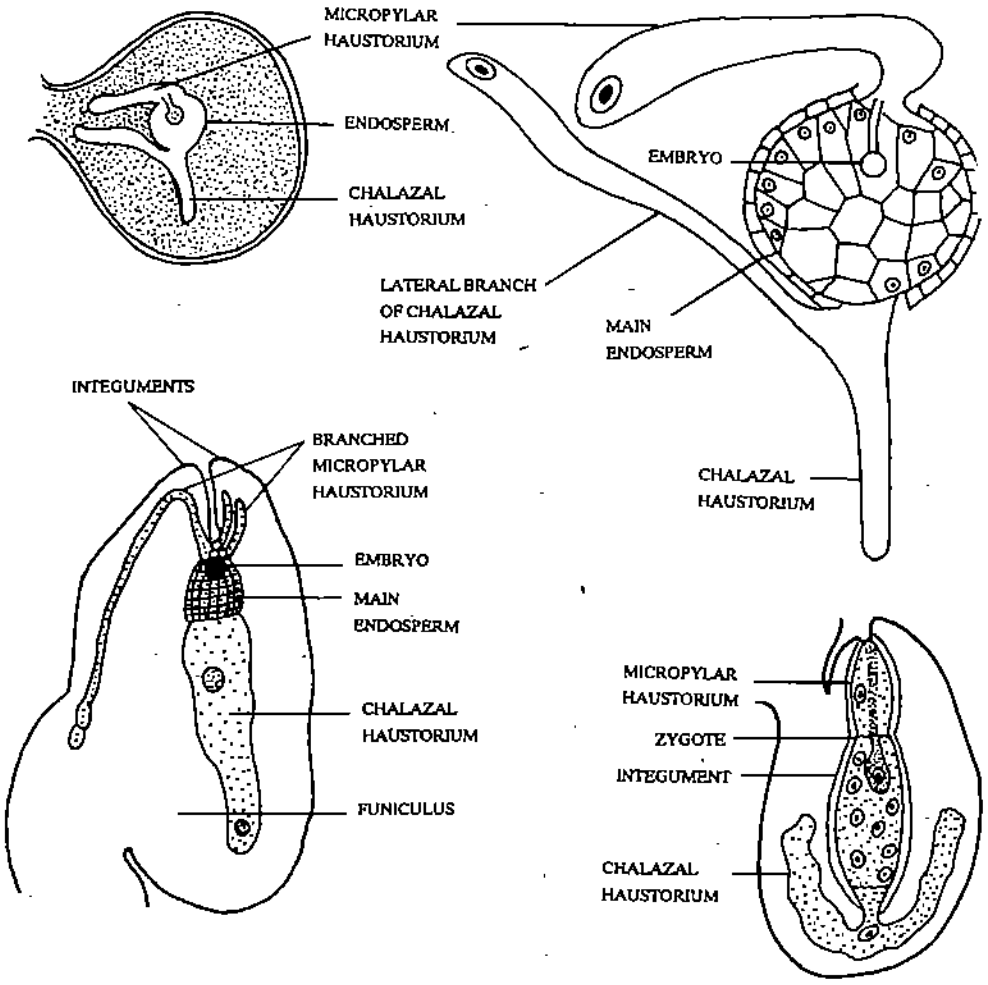


Fig. 4.17: L.S. Upper part of ovule of *Impatiens* showing endosperm with micropylar haustorium; note the penetration of branches of the haustorium into tissues of funiculus.

4.3.3 Endosperm with Micropylar and Chalazal Haustoria

Some plants develop haustoria from both micropylar and chalazal ends of endosperm. In *Nemophila* aggressive haustoria arise from micropylar and chalazal ends. The chalazal haustorium sometimes gives out a prominent lateral branch which grows towards the funiculus so as to come in direct contact with the starchy tissue of the placenta (Figs. 4.18, 4.19). In *Melampyrum lineare*, the micropylar haustorium comprises a single cell with many tubular processes which enlarge considerably and invade the tissue of the integument and funiculus. The chalazal haustorium is short and confined to the nucellar tissue only (Fig. 4.20). In *Klugia notoniana* the chalazal haustorium grows laterally and terminally, consuming the sub-epidermal cells of the integument (Fig. 4.21). The micropylar haustorium starts functioning after the activity of the chalazal haustorium declines.



Figs. 4.18 - 4.21: Endosperm with chalazal and micropylar haustoria. Fig. 4.18: L.S. Ovule of *Nemophila*. Fig. 4.19: Endosperm enlarged to show micropylar and chalazal haustoria; note the lateral branch from chalazal haustorium. Fig. 4.20: L.S. Ovule of *Melampyrum* showing chalazal and micropylar haustorium and one of its branches which has entered the funiculus. Fig. 4.21: L.S. Ovule of *Klugia* showing micropylar and branches of chalazal haustorium that enter the integuments.

4.3.4 Endosperm with Secondary Haustoria

In *Centranthera*, (family Cucurbitaceae) the micropylar and chalazal haustoria are ephemeral. A certain number of cells of the endosperm close to the micropyle develop tubular outgrowths and extend considerably into the tissue of nucellus and serve as secondary haustoria (Fig. 4.22).

4.3.5 Endosperm with Lateral Haustoria

In *Monochoria*, in which the endosperm development is of helobial type, the haustorium is neither chalazal nor micropylar but lateral. The chalazal chamber does not grow further and contains only a few nuclei but the micropylar chamber shows active nuclear divisions and develops two lateral outgrowths, one on either side of the chalazal chamber (Fig. 4.23). These grow downwards and function as active haustoria invading the tissue of chalazal. Later, the main body of the endosperm enlarges considerably and fuses with the haustoria to form a compact mass of endosperm.

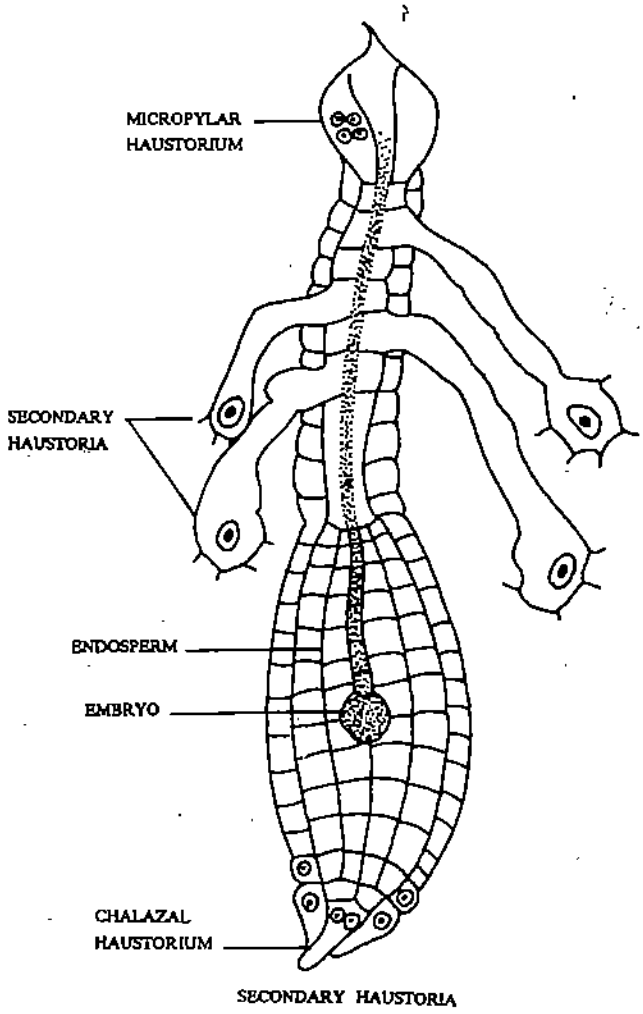


Fig. 4.22: Endosperm with aggressive secondary haustoria and poorly developed chalazal and micropylar haustoria.

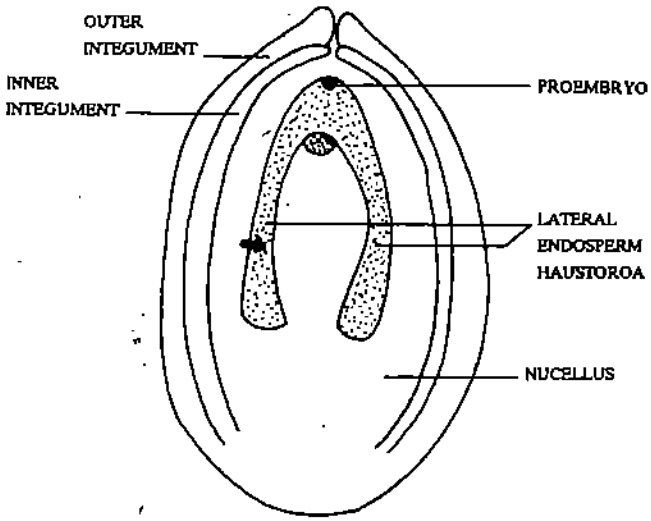


Fig. 4.2.3: L.S. Ovule of *Monochoria* showing endosperm with lateral haustoria.

SAQ 2

a. What are endosperm haustoria? What is their function?

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b. Name the different types of endosperm haustoria and draw labelled diagram of each.

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c. List the various tissues invaded by endosperm haustoria.

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d. In which family of flowering plants is the longest chalazal endosperm haustorium recorded?

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e. Name a plant with vermiform chalazal haustorium.

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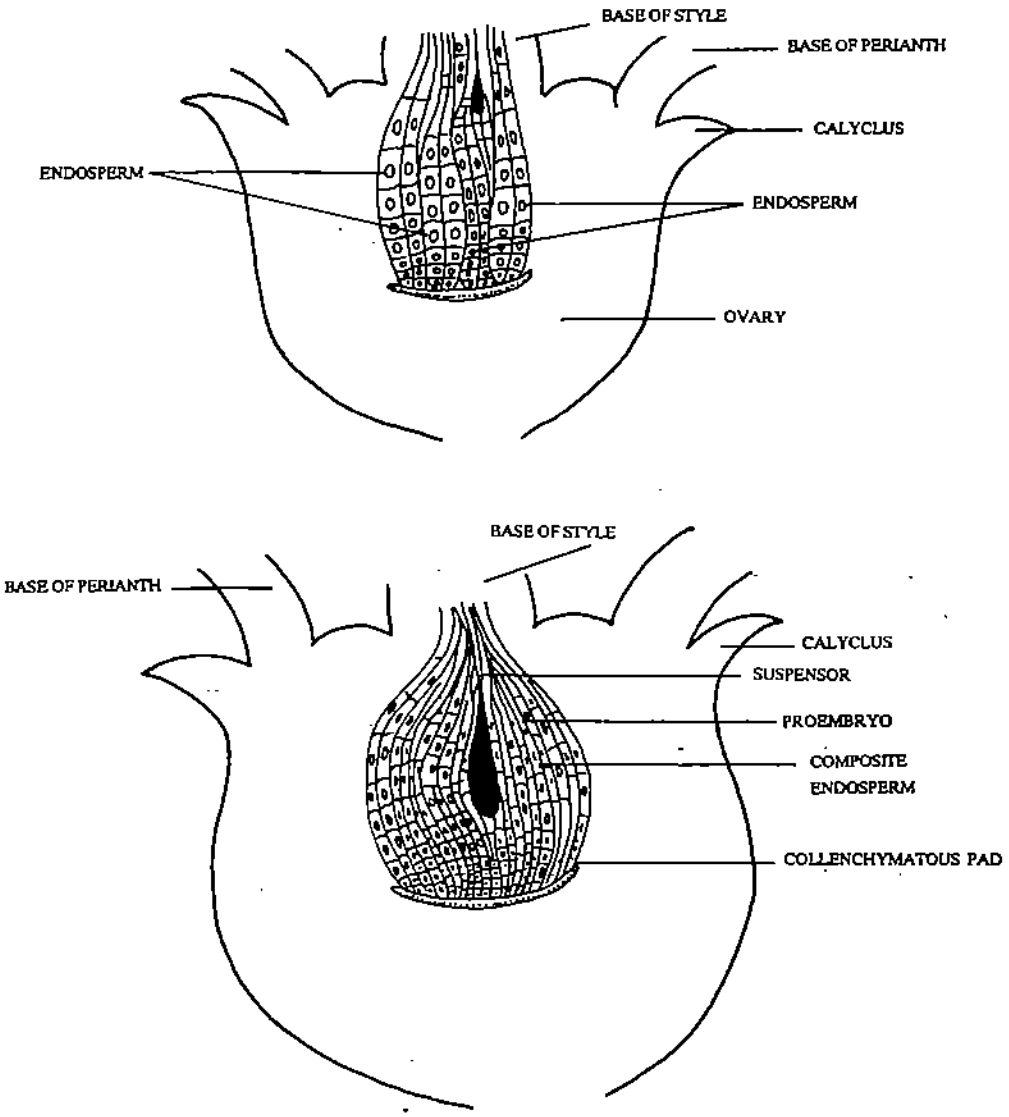
4.4 VARIANTS OF ENDOSPERM

The mode of development of endosperm discussed above conforms to one of the three basic types: Nuclear, Cellular and Helobial. However, variations arise at a later stage of development, such as composite endosperm, ruminate endosperm and mosaic endosperm.

4.4.1 Composite Endosperm

In the family Loranthaceae, the development of endosperm is unique. The ovary lacks ovules. The sporogenous tissue located at the base of ovary develops several embryo sacs which elongate considerably, some of them even entering the style. After fertilization, the primary endosperm nucleus of each embryo sac moves to the basal part where it divides to form cellular endosperm (Fig. 4.24). During further development, the endosperms of all the embryo sacs in an ovary enlarge and become fused to produce a composite endosperm mass (Fig. 4.25). Several proembryos belonging to individual embryo sacs with long suspensors develop but only one survives and attains maturity.



Figs. 4.24-4.25: Composite endosperm in Loranthaceae. Fig. 4.24: L.S. Ovary showing several embryo sacs with their independent endosperm; note proembryo is one of the endosperms. Fig. 4.25: L.S. Ovary; all the endosperms have fused to form a composite endosperm; note the proembryo with elongated suspensor embedded in the endosperm.

4.4.2 Ruminate Endosperm

In certain plants the surface of the mature cellular endosperm shows a high degree of irregularity and unevenness, giving a ruminated appearance (rumination means as if chewed). It is caused either by the activity of the seed coat or by the endosperm itself. Ruminate endosperm is found in about 32 families of Angiosperms. On morphological basis, Periasamy (1962) distinguishes seven types: *Annona, Passiflora, Myristica Spigelia, Verbascum* and *Coccoloba* and *Elytraria*. In all these types except *Elytraria* irregularities occur in the growth of integuments which bring about the rumination of endosperm. In *Elytraria* during the development of seed, localized regions in the peripheral layers of cellular endosperm show active growth causing rumination.

4.4.3 Mosaic Endosperm

In some plants patches of two different colours appear in the tissues of the endosperm providing a mosaic design. In maize, red and white patches of tissues are sometimes seen in the grain. The occurrence of such endosperm has also been reported in *Petunia, Lycopersicön* and *Acorus* etc. Several theories have been advanced to explain the development of mosaic endosperm but none of these has been cytologically demonstrated. The most appealing explanation for the development of such endosperm is said to be the aberrant behaviour of the chromosomes during mitosis or somatic mutations.

SAQ 3

a. What is composite endosperm? Name the family which is characterised by it.

.....

.....

b. Name the type of endosperm against each plant listed below:

i) *Loranthus* .....

ii) *Annona* .....

iii) *Lycopersicon* .....

iv) *Passiflora* .....

v) *Acorus* .....

vi) *Petunia* .....

vii) *Verbascum.* .....

4.5 FUNCTIONS OF ENDOSPERM

The tissue of young endosperm is rich in food materials and various growth hormones. It regulates the precise mode of embryo development and nourishes the developing embryo. During seed germination, the reserve food materials stored in mature endosperm are digested and utilized for the growth of the seedling until the later develops chlorophyll and is able to manufacture its own food. In some plants, the seed coat and the fruit wall are consumed by the endosperm, which ultimately becomes exposed to sunlight and develops chlorophyll for photosynthesis. Rarely the outermost layer of such exposed endosperm takes on a protective function. In the absence of endosperm, the embryo usually aborts.

Endosperm development is a characteristic feature of all the families of angiosperms with the exceptions of Podostemaceae and Trapaceae. In the Orchidaceae endosperm degenerates quite early. Endosperm may be used up by the embryo as such the mature seed has no traces of it (exalbuminous seed). In most monocotyledons it persists (albuminous conditions). In the main food plants such as wheat, rice, maize and sorghum it is the starchy endosperm that forms the bulk of the grain. In many legumes the mature seed has food reserves in the cotyledons rather than in the endosperm. In the castor seed endosperm is laden with fatty substances.

The cereal endosperm is made of very different tissues at maturity. The outer aleurone layer consists of living cells. The endosperm usually occupies the bulk (87%) of the grain and about 10% of the endosperm dry weight is aleurone. The aleurone layer stores lipid (about 90% of total endosperm lipid) and also contain 20% of protein. During germination, hydrolytic enzymes are produced in the aleurone layer and these are released into starchy endosperm where the reserves are hydrolysed.

When the barley grains are soaked in water gibberellins (GA_3 and GA_1) are released from scutellum of embryo and diffuses into the endosperm. The largest tissue for this hormone is the aleurone, which responds by breaking down its own protein reserves and by secreting enzymes (mostly hydrolytic) into the starchy endosperm. Some of these enzymes are newly synthesized (e.g. α-amylase) and some are (e.g. β-glucanase) pre-existent Probably all cereals except sorghum, have aleurone that responds similarly.

Thus endosperm has a very important role in the development of the embryo. In most of inter varietal and interspecific crosses, embryos fail to form because of failure of endosperm formation.

SAQ 4

a. What is the function of endosperm?

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b. Name the families of angiosperms which lack endosperm.

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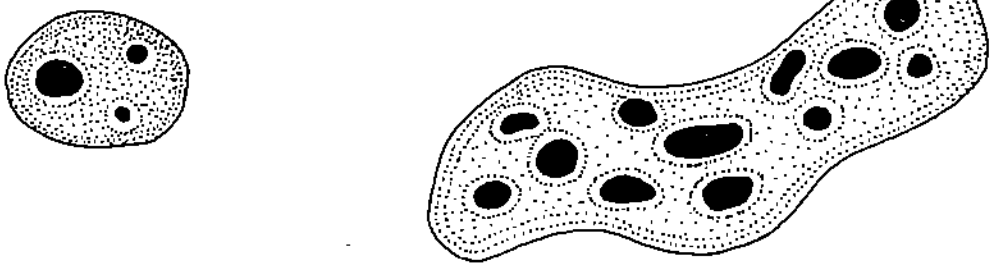
4.6 CYTOLOGY OF ENDOSPERM

Normally the young endosperm is triploid as it is formed by the fusion of three haploid nuclei (male gamete + upper polar nucleus + lower polar nucleus). However, in some plants it shows ploidy of different levels due to variation in the number of polar nuclei which may be 1, 2, 4 or 8, depending upon the type of embryo sac. The number of polar nuclei contributing to the formation of endosperm is one only in *Oenothera* leading to the formation of diploid endosperm while it is 8 in *Peperomia* so the endosperm is 9n. During further development, the cells of endosperm may undergo further polyploidization due to endomitosis and nuclear fusion. The highest level of ploidy is reported in *Arum* in which the nucleus of endosperm becomes 24576 n. The size of nuclei and the number of nucleoli also exhibit enormous variation (Figs. 4.26, 4.27).



Figs. 4.26-4.27: Endosperm nuclei. Fig. 4.26: A small endosperm nucleus with three nucleoli. Fig. 4.27: A large endosperm nucleus with several nucleoli.

SAQ 5

a. Give an example of a diploid endosperm.

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b. What would be the ploidy level in the primary endosperm nucleus of *Peperomia*?

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.....

c. Name the plant with highest level of ploidy in the endosperm.

.....

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d. What type of nuclear divisions lead to polyploidy in endosperm cells?

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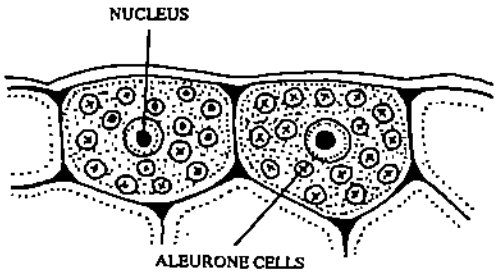
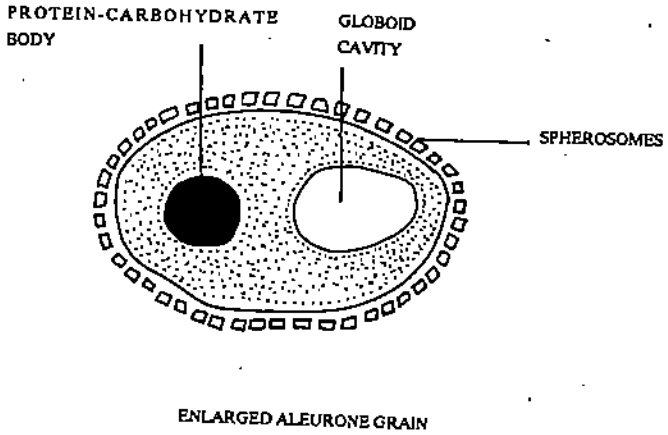
.....

4.7 STRUCTURE AND FATE OF ENDOSPERM

The cells of endosperm are usually thin-walled, large, isodiametric and devoid of pits and store large amount of food materials. The starch alongwith other food substances like oils and proteins are gradually accumulated in these cells. On account of heavy deposition of food materials, the nuclei become disorganised and deformed. In mature dryseeds, the endosperm represents a physiologically inactive tissue. In any plant such endosperm constitutes the edible part of seed/fruit (cereals, coconut) and source of commercial oils (castor, coconut).





Figs. 4.28-4.29: Aleurone cells of a cereal with aleurone grains. Fig. 4.29: A single aleurone grain enlarged.

Usually the endosperm is non-chlorophyllus. However, in some members of the Amaryllidaceae such as *Crinum* seed coats as well as fruit wall are absorbed during seed development and the endosperm which becomes exposed to sunlight turns green. In some cases the outermost layer of such naked endosperm becomes suberized and protective in function. In the Gramineae, one or a few outer layers of the endosperm become highly specialized and constitute the aleurone tissue. To begin with, the cells of these layers are meristematic which contribute thin-walled cells towards inside. These newly-cut cells later become deposited with starch. During maturation of seed, the cells of the outer most peripheral layer, lose their meristematic activity, become enlarged and develop thick walls. The seeds become gorged with aleurone grains (Fig. 4.28). Each aleurone grain is surrounded by a single membrane which is closely associated with spherosomes (Fig. 4.29). Structurally, the aleurone grains contain two types of inclusions in their ground substance—(i) Globoid cavity containing phytin and lipids and (ii) Protein carbohydrate bodies. The ground substance which contains the above bodies also possess as a high concentration of protein. Thus, the chief chemical components of the aleurone grains are protein, carbohydrates, phytin and phospholipids. During seed germination, the cells of aleurone layer secrete certain hydrolytic enzymes viz., amylases

and proteases which convert the stored food materials of endosperm so as to make it suitable for the germinating embryo.

Regarding the final fate of the endosperm in mature seeds, two conditions are noted. In some plants such as coconut, castor, wheat and maize the endosperm persists as massive storage tissue and the seed is called endospermous or albuminous. In plants like pea, gram and bean, the endosperm is entirely absorbed by the developing embryo so that it is absent in mature seed. Such seeds are known as non-endospermous or ex-albuminous.

SAQ 6

a. Describe the structure of endosperm cells and their storage materials.

.....

.....

b. Normally endosperm is enclosed in the seed. How does it become naked in some plants?

.....

.....

4.8 MORPHOLOGICAL NATURE OF ENDOSPERM

The morphological nature of endosperm in angiosperms has been a subject of much discussion in evolution. The endosperm in gymnosperms is a gametophytic (haploid) tissue as it develops directly by the continued free nuclear divisions of the functional megaspore. In angiosperms, however, it develops from the primary endosperm nucleus which is normally formed by the fusion of two polar nuclei and a male nucleus and hence it is neither haploid nor diploid but generally triploid. Some workers have suggested that the endosperm in angiosperms is a gametophytic tissue just like those of gymnosperms, the only difference being that its development remains arrested till the entry of the pollen tube into the ovule. Other embryologists have considered it as a second embryo or a maimed embryo. The most agreeable view regarding the morphological nature of the endosperm in angiosperm is that it is an undifferentiated tissue which shows different degrees of polyploidy and becomes physiologically subservient to the embryo.

By suppressing the growth of the embryo in a seed, it has been possible to induce triploid shoot bud development in the endosperm. However, truly triploid plants have not been obtained so far.

4.9 SUMMARY

- Endosperm development is a characteristic feature of all families of angiosperms except Orchidaceae, Podostemaceae and Trapaceae.
- On the basis of mode of development, endosperm has been classified into three main types:
  - i) Nuclear type
  - ii) Cellular type
  - iii) Helobial type.
- All of these types of endosperm can develop haustoria which transports food materials to the endosperm proper.
- Some variant types of endosperms are: composite endosperm, ruminate endosperm and mosaic endosperm.
- The main function of endosperm is nourishment of the developing embryo.

Endosperm may be used up by the embryo such that there is no trace left of it in the mature seed.

- Normally the endosperm is triploid, but different ploidy levels from diploid to polyploid are found in various plants.
- Histologically endosperm has thin walled isodiametric cells, which store large amounts of reserve food materials. Seed in which the endosperm persists as a massive storage tissue are known as albuminous seeds.Seeds in which endosperm is entirely used up are termed ex-albuminous seeds.

4.10 TERMINAL QUESTIONS

1. How does the endosperm of angiosperms differ from that of gymnosperms?
2. Why is it difficult to state whether the endosperm is nuclear, cellular or helobial type by only looking at the persistent cellular endosperm in a mature seed?
3. What are endosperm haustoria? Name the types of haustoria and describe them.
4. How is mosaic endosperm formed?
5. What is meant by ruminate endosperm? Explain the two ways in which ruminations can develop, with one example for each.
6. What is meant by the following statement "Without the endosperm the world would be starving".
7. Explain the hormonal relationship between endosperm and embryo.
8. Name some albuminous and ex-albuminous seeds which you come across in your daily life.

4.11 ANSWERS

Self Assessment Questions:

SAQ 1

a. In lower plants and gymnosperms, the male gamete fuses with female gamete to form the zygote (2n). This process is called **fertilisation**. In angiosperms, two male gametes are brought to the embryo sac by the pollen tube. one of this fuses with the egg cell to form the zygote (2n), and the second male gamete fuses with the secondary nucleus in the central cell forming the primary endosperm nucleus (3n). This process is called double fertilisation.

b. Zygote (2n) and a primary endosperm nucleus (3n) is the product of double fertilization. Zygote develops into an embryo. The products of the primary endosperm nucleus gives rise to a nutritive tissue known as the endosperm. It stores reserve materials for the growing embryo and usually has a limited growth.

c. In a young coconut fruit the embryo sac is filled with a clear fluid which is the free nuclear endosperm. Its volume is so enormous that it is actually consumed by people. Gradually the peripheral part becomes cellular to yield the white edible part.

4. Betel nut—*Areca catechu*.

SAQ 2

a. Endosperm haustoria are elongated structures that invade the tissues of seeds and sometimes reach as far as the funiculus. The haustoria absorb food materials from various tissues and supply them to the developing endosperm.

b. You may refer to Section 4.3

c. i) Tissues of seeds

ii) Placenta

c. Cucurbitaceae

d. *Grevillea robusta* (Proteaceae)

SAQ 3

a. See Section 4.4.1. Family Loranthaceae is characterised by composite endosperm.

b. i) Composite type
   ii) Ruminate type
   iii) Mosaic type
   iv) Ruminate type
   v) Mosaic type
   vi) Mosaic type
   vii) Ruminate type.

SAQ 4

a. See Section 4.5

b. Orchidaceae, Podostemaceae and Trapaceae.

SAQ 5

a. *Oenothera* sp.

b. Endosperm is 9n in *Peperomia*

c. *Arum* —It is 24567n.

d. See Section 4.6.

SAQ 6

a. The cells of endosperm are usually thin-walled isodiametric, large and without any pits. These cells are rich in reserve food materials. The stored material is mainly in the form of starch, alongwith other substances like oils and proteins.

   Note: As a special case you may describe the endosperm of any member of family Gramineae.

b. In some members of the family Amaryllidaceae such as *Crinum*, the seed coat as well as fruit wall are absorbed during the development of seed and eventually the endosperm becomes exposed to sunlight and turns green and thus becomes naked. In some plants this naked endosperm become suberised and serve the protective function.

Terminal Questions

	Gymnosperms	Angiosperms
1.	The endosperm of gymnosperms is haploid.	The endosperm of angiosperm is generally triploid.
2.	It is differentiated before fertilization.	It is differentiated after fertilization because it is the fusion product of two polar nuclei.

2. By looking at the persistent cellular endosperm in a mature seed it is difficult to state whether endosperm is nuclear, cellular or helobial because all these three types in their later stages may become cellular.

3. See Section 4.3.

4. See Section 4.4.3.

5. In some plants the surface of the mature cellular endosperm shows a high degree of irregularity and unevenness giving a ruminated appearance or achewed appearance. This type of endosperm is called ruminate endosperm.

   The ruminations are caused by:

   i) Activity of seed coat, e.g., *Elytraria*

   ii) Activity of endosperm , e.g., *Annona*

6. Endosperm plays an important part in the development of embryo. The tissue of young endosperm is rich in reserve materials and various growth hormones. It regulates the precise mode of embryo development and also provides it nourishment. The grains of our main food crops such as wheat, rice, maize and sorghum, are made up of starchy endosperm. In some seeds, fatty substances are found in bulk in the endosperm. The endosperm also supports seed germination, the reserve materials stored in mature endosperm are utilised for the growth of the seedling until the seedling becomes chlorophyllous and independent. Thus without endosperm the embryo will not grow. There will be no food reserves in grains and as a consequence the world will starve.

7. See Section 4.5.

8. Albuminous—wheat, sorghum, maize, barley, castor seed, coconut.

   Exalbuminous—pea, gram, bean.

UNIT 5 EMBRYOGENESIS

Structure

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5.1 INTRODUCTION

In the previous units 1, 2, 3 you have studied all the aspects upto the fertilization. In this unit you are going to study embryogenesis in detail. The process of double fertilization culminates in the fusion of one of the male gametes (discharged by the pollen tube) with the egg and of the second male gamete with the fusion nucleus in the central cell. As you have already studied in units 3 and 4, the fusion of a male gamete nucleus with the polar nuclei results in transformation of the central cell into the primary endosperm cell. It is this cell which gives rise to the nutritive tissue called the endosperm in the seed. The fertilized egg, (zygote) develops into the embryo and is the forerunner of the sporophyte. The embryo through embryogenesis develops into mature embryo.

Objectives

After studying this unit you should be able to:

- explain the stages through which an embryo is formed starting from zygote,
- distinguish between a monocotyledonous and dicotyledonous embryo,
- describe how the embryo derives its nutrition.

5.2 ZYGOTE

The fertilized egg or zygote is situated at the micropylar end/pole of the embryo sac, its basal (micropylar) end is attached to the embryo sac wall and apical (chalazal) part projects into the central cell. The zygote usually undergoes a period of rest during which it shrinks. This resting period of the zygote varies with different species and is to some extent dependent on environmental conditions e.g. in *Theobroma cacao* the zygote divides 14 to 15 days after fertilization, in *Oryza sativa* zygote divides about 6 hours after fertilization. A complete cell wall is formed around the zygote. (You would recall that the egg has only plasmalemma and no wall at its apical part). A TEM picture shows that zygote cytoplasm show a more polarized appearance and that is the two poles appear different, micropylar part is vacuolate and chalazal part with a prominent nucleus (Fig. 5.1).

Fig. 5.1: *Gossypium hirsutum*, zygote after four hour of fertilization—highly polarized zygo clustering of plastids and mitochondria around the nucleus at the chalazal pole. (After Jansen 1968)

A marked increase occur in the density of cytoplasmic organelles such as mitochondri dictyosomes and plastids. Endoplasmic reticulum becomes more extensive and the density of ribosomes and polysomes increases indicating intense metabolic activity. Nc we will study early embryogenesis in embryo.

5.3 EARLY EMBRYOGENESIS

In a majority of angiosperms the zygote divides by a transverse wall (Fig. 5.2) resultir in a smaller apical cell (usually designated as *ca*) and a relatively large basal cell (designated *cb*). The division of the zygote is exceptionally veritcal or oblique, (as in members of the Loranthaceae and the Piperaceae). Beginning from the 2-celled stage t to the initiation of cotyledons the young sporophyte is commonly called the proembryc In the 2-celled proembryo the basal cell *cb* usually divides transversely to form two cells *m* and *ci* (Fig. 5.3A). The apical cell *ca* may divide vertically or transversely, so that the 4-celled proembryo has either a linear (all four cells in a row) (Fig. 5.3B) or : T-shaped (Fig. 5.3C) configuration. In the linear proembryo the two daughter cells of (l' & l') may undergo two vertical divisions at right angles to each other to give rise tc an octant having two superposed tiers (l & l') of four cells each (Fig. 5.3D).

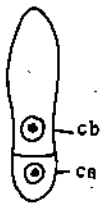

Fig. 5.2: Division of zygote by transverse wall.

In the T-shaped proembryo, a vertical division at right angles to the first vertical division in the apical cell can produce a quadrant *q*. (Fig. 5.3E). A transverse division each cell can then produce an octant similar to the one produced by the linear 4-celled proembryo. (Fig. 5.3F). The T-shaped proembryo can also give rise to an octant by vertical tangential divisions in the four cells of tier *q* so that all the eight cells are included in the same tier (Fig. 5.3G).

The cells derived from *ci*, *m* and *q* divide further and differentiate to form the differen parts of the mature embryo. The early development of the proembryo is similar in bott monocotyledons and dicotyledons. After the octant stage, the destiny of the various cells of the proembryo differs in these two major groups. Because of these later

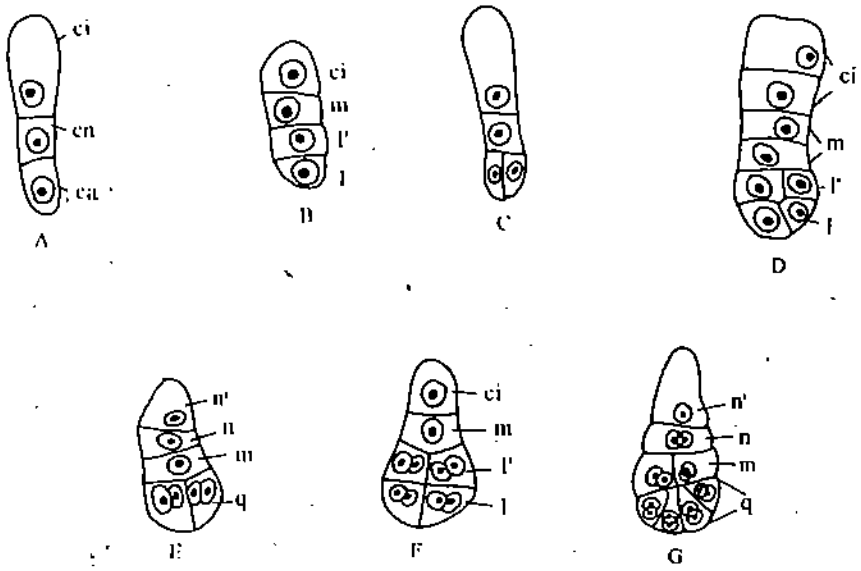

Fig. 5.3: Division of angiospermic embryo.

differences the mature dicot embryo possess an apical shoot apex and two lateral cotyledons (Fig. 5.4), whereas the monocot embryo has only one cotyledon and a somewhat laterally placed shoot apex (Fig. 5.5). The structure of the dicot and the monocot embryos would be described in greater detail later in this unit after giving you a general account of the development of the proembryo.

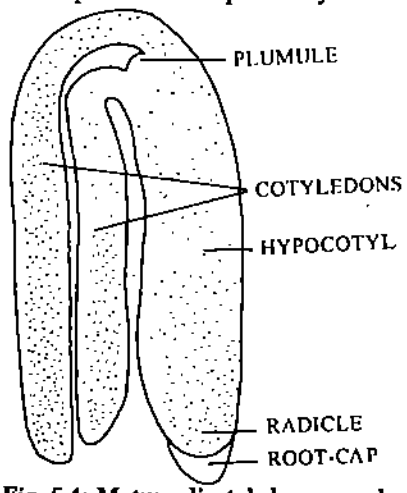

Fig. 5.4: Mature dicotyledonous embryo.

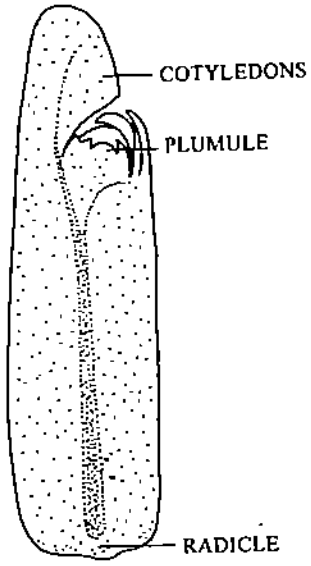

Fig. 5.5: Mature monocotyledonous embryo.

Types of Embryogeny

On the basis of the plane of division of the zygote and of the cells of the 2-celled proembryo, and also taking into account the relative contributions of the cells of the 4-celled proembryo to the mature embryo, six cheif types of embryogeny have been recognised (Johansen, 1950; Maheshwari, 1950).

A. Division of the zygote is vertical — Piperad type (e.g., Loranthaceae, Piperaceae).

AA. Division of the zygote is transverse.

B. Apical cell of the 2-celled proembryo divides vertically to form a T-shaped, 4-celled proembryo.

C. Basal cell plays no role at all or only an insignificant role in subsequent development of the proembryo—(Crucifer or Onagrad type (e.g., Ranunculaceae, Brassicaceae).

CC. Basal cell and apical cell both contribute to the development of the embryo — Asterad type (e.g., Asteraceae, Violaceae).

BB. Apical cell of 2-celled proembryo divides transversely so that the 4-celled proembryo is usually linear.

D. Basal cell does not participate or only contributes a little to development of embryo proper.

E. Basal cell usually forms a suspensor — Solanad type (e.g., Solanaceae, Linaceae).

EE. Basal does not divide further and the suspensor, if present, is derived from the apical cell — Caryophyllad type (e.g., Caryophyllaceae, Crassulaceae).

DD. Basal cell and apical cell both divide and contribute to formation of the embryo — Chenopodiad type (Chenopodiaceae, Boraginaceae).

SAQ 1

State whether the following statements are true or false. Write either T or F in the boxes provided.

a) Zygote usually has a densely-cytoplasmic basal (micropylar) part and a vacuolate apical (chalazal) part. [ ]

b) Zygote has a complete cell wall around it. [ ]

c) Division of the zygote is nearly always vertical or oblique. [ ]

d) Early development of the proembryo is similar in monocots and dicots. [ ]

e) In Caryophyllad type of embryogeny the basal cell forms a well developed suspensor. [ ]

5.4 HISTOGENESIS AND ORGANOGENESIS

After the octant stage numerous cell divisions occur in various planes. The proembryo become globular or bulb-shaped. As a general rule, some tangential divisions occur in the cells of the octant so that three cells layers are differentiated—the outer **dermatogen** which later forms the epidermal covering, middle **periblem** which gives rise to the cortex of the stem and root, and inner **plerome** that is responsible for the vascular tissue and pith. Such a process of differentiation of cell layers is termed **histogenesis**.

Organogenesis or differentiation of the initials of various organs begins in the globular proembryo in such a way that cotyledons (which form the first leaves), **epiphysis** (which gives rise to the stem apex) and **hypophysis** (that forms the root cortex and cap) are produced. Since organogenesis differs in dicots and monocots, we may discuss this aspect separately for the two groups of angiosperms with the help of suitable examples.

5.5 DICOTYLEDONOUS EMBRYO

The development of a dicotyledonous embryo may be illustrated with the help of the familiar classical example of *Capsella bursa-pastoris*. Embryogeny in this species conforms to Crucifer or Onagrad type, in the classification that you have just studied. Division of the zygote is transverse resulting in a basal cell **cb** and a terminal cell **ca** (Fig. 5.6 A, B). Basal cell divides transversely, whereas the terminal cell divides longitudinally. The 4-celled proembryo, thus, has an inverted T-shaped appearance (Fig. 5.6 C). Each of the two terminal cells next divides by a vertical wall oriented at right angle to the first, forming a quadrant (Fig. 5.6 D). The quadrant cells divide transversely to form an octant (Fig. 5.6 E). By carefully tracing the course of subsequent development, it is possible to conclude that the derivatives of the lower four cells of the octant give rise to the stem tip and cotyledons whereas the cells derived from the upper four cells of the octant form the hypocotyl. All the eight cells divide periclinally (Fig. 5.6 F).The outer derivatives form the dermatogen, while the inner undergo further divisions (Fig. 5.6 G) to form the cortical, vascular and pith regions. At this stage, the proembryo may be said to be in globular stage of development. While these developments are taking place in the terminal cell, the derivatives of *ci* and some cells derived from the intermediate cell *m* of the 4-celled proembryo divide to form a row of 6 to 10 suspensor cells (Fig. 5.6G). The upper most cell *v* of the suspensor becomes vesicular and serves a haustorial function. Some derivates of *m* contribute to the suspensor. The lower most cell which is placed between the suspensor and the embryonal mass is generally referred to as hypophysis or *h* (Fig. 5.6 H).

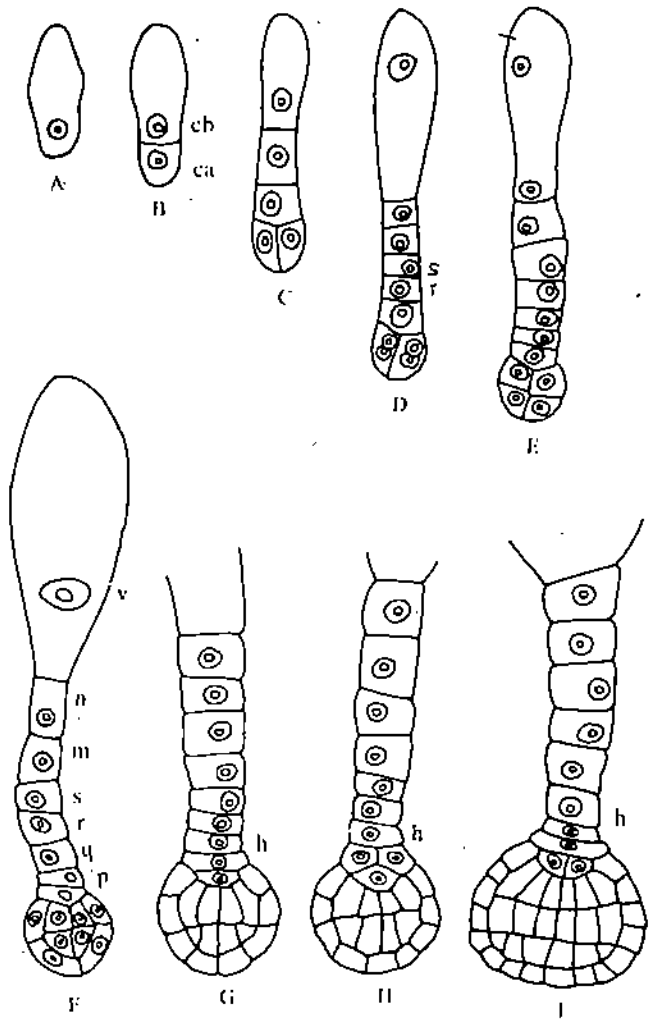

Fig. 5.6: Embryo development in *Capsella bursa -pastoris*.

This cell *h* undergoes a transverse division followed by two longitudinal divisions at right angles to each other in both the derivates. This results in a group of eight cells, of which the inner four give rise to the initials of the root cortex and the outer (toward the suspensor) four form the root cap and root epidermis.

The globular proembryo undergoes further cell multiplication, especially at the two points which are destined to form the cotyledons. At the stage when cotyledons are initiated the embryo is cordate or heart-shaped (Fig. 5.7A). Between the two cotyledons i.e., at the tip of the embryonal a wedge shaped group of cells is cut off which represents region the epiphysis or forerunner of the shoot tip. The hypocotyl and the cotyledons elongate so that the embryo become torpedo-shaped. (Fig. 5.7 B). During further development in *Capsella* the elongating cotyledons become curved like a horse shoe (Fig. 5.7C). However, in a majority of dicotyledons the mature embryo is straight.

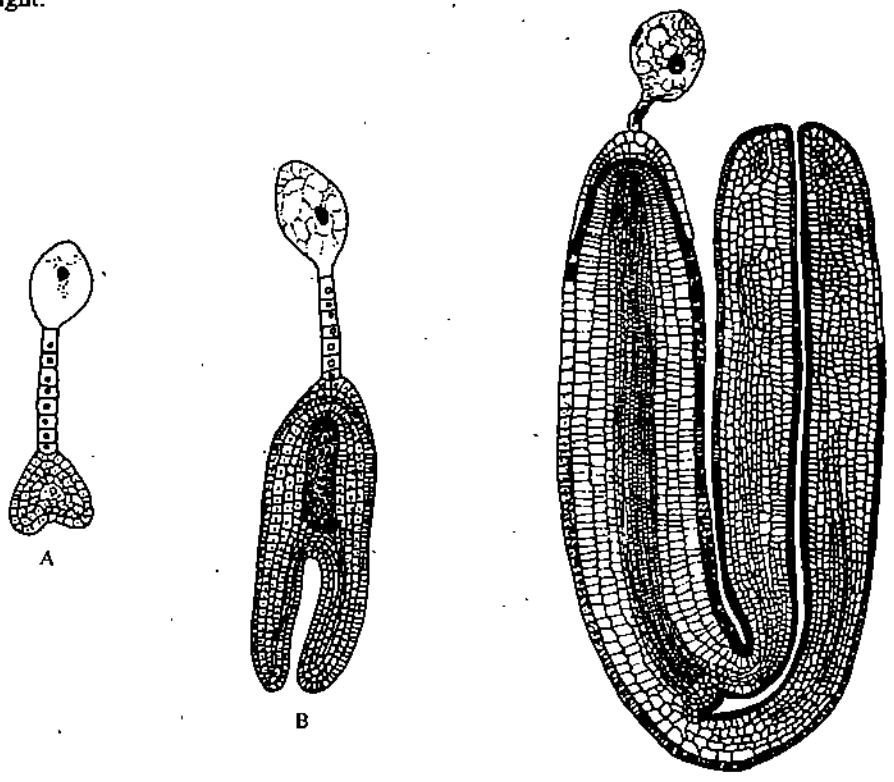

Fig. 5.7: Later stages of development of *Capsella embryo.*

5.6 MONOCOTYLEDONOUS EMBRYO

The early development of the proembryo in monocots follows the same pattern as in the dicots. However, at the time of differentiation in the globular proembryo certain fundamental differences arise. In monocots one half of the terminal cell and its derivatives have retarded growth, whereas the other half grows rapidly to form one cotyledon. As a result of this assymetric growth, in later stages the stem tip, which is also derived from the terminal cell, appears to be lateral in position.

The major differences between the dicot and the monocot embryos arise due to disparity in the number and position of the cells of the terminal quadrant of the proembryo which contribute to the formation of the cotyledonary and epicotyl regions. In the dicotyledons derivatives of the two opposite cells of the terminal quadrant give rise to the two cotyledons (Fig. 5.8 A, B). Among the monocotyledons the number of cells of the quadrant that contribute to the cotyledons varies (Fig. 5.8 C, D, E).

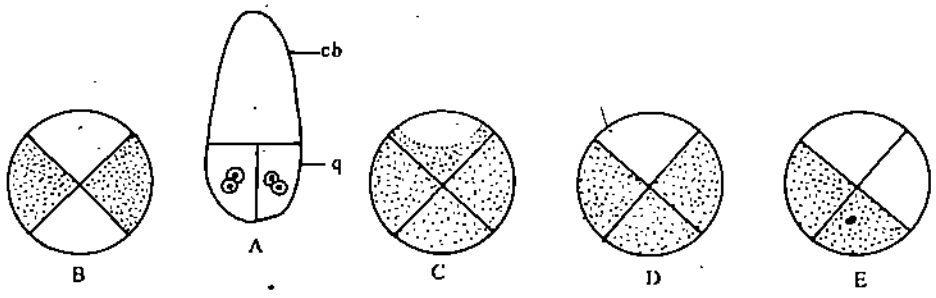

Fig. 5.8: Derivation of cotyledons in monocotyledonous and dicotyledonous. A. Quadrant proembryo. B. Development in dicotyledons. C-E. Development in various monocotyledonous taxa.

5.7 MATURE EMBRYO

A typical dicotyledonous embryo, as seen in a median longitudinal section, consists of an embryonal axis having two broad cotyledons. The portion of embryonal axis above the level of cotyledons is termed epicotyl which terminates in the plumule or stem tip. The cylindrical portion below the level of cotyledons is called the hypocotyl which terminates at the lower end in the radicle or root tip. The root meristem is covered by a well defined root cap.

The embryo of monocotyledons, possesses only one cotyledon. The grass embryos is highly specialized and has received a great deal of attention. It has a single cotyledon in the form of scutellum, which appears to be laterally attached to the embryonal axis (Fig. 5.9). At its lower end the embryonal axis has the radicle and root cap, enclosed in an undifferentiated part of the embryo called *coleorhiza*. On one side the *coleorhiza* is given out a small outgrowth called the epiblast. The portion of embryonal axis above the level of attachment of the scutellum is termed epicotyl. It has a shoot apex with some leaf primordia, enclosed in a hollow foliar structure called the coleoptile.

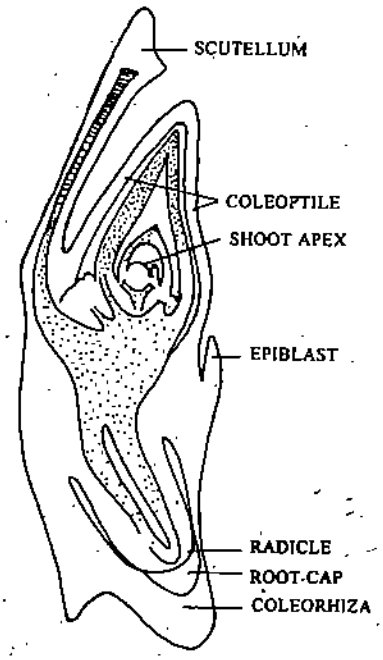

Fig. 5.9: Median longitudinal section of mature embryo of *Triticum*.

As you may be already aware, the embryo remains embedded in the endosperm in the seed, which in turn is enclosed in the fruit. The seed is dispersed by the plant and serves as a unit of propagation. The seed can also perennate in the soil till appropriate conditions are available for its germination. On germination of the seed, the cotyledons

expand and appear leaf-like, the epicotyl grows with the help of the meristem at its tip to form the stem axis, and the radicle or root meristem forms the primary root.

SAQ 2

In the following statements, tick the correct ones (√) and cross the wrong ones (X):

a) Development of early embryo takes place in the same, precise manner in all the dicotyledons. [ ]

b) Dermatogen divides anticlinally to form the epidermal cells of the embryo. [ ]

c) Initials of the root cap and root cortex are derived from hypophysis. [ ]

d) In the monocotyledons embryo, the stem tip is not derived from the terminal cell. [ ]

e) The epicotyl terminates in the plumule which forms the primary root. [ ]

5.8 MODIFICATIONS OF SUSPENSOR

In the early part of this unit much of the attention was focussed on the development of the terminal part of the proembryo which produces the embryo proper. However, you would recall that the basal cell also divides contributes to the formation of a suspensor. The suspensor grows relatively fast in the early stages and usually attains the maximum size at globular or the heart-shaped stage of the embryo. Later it degenerates and at maturity only vestiges of the suspensor will be seen attached to the embryo.

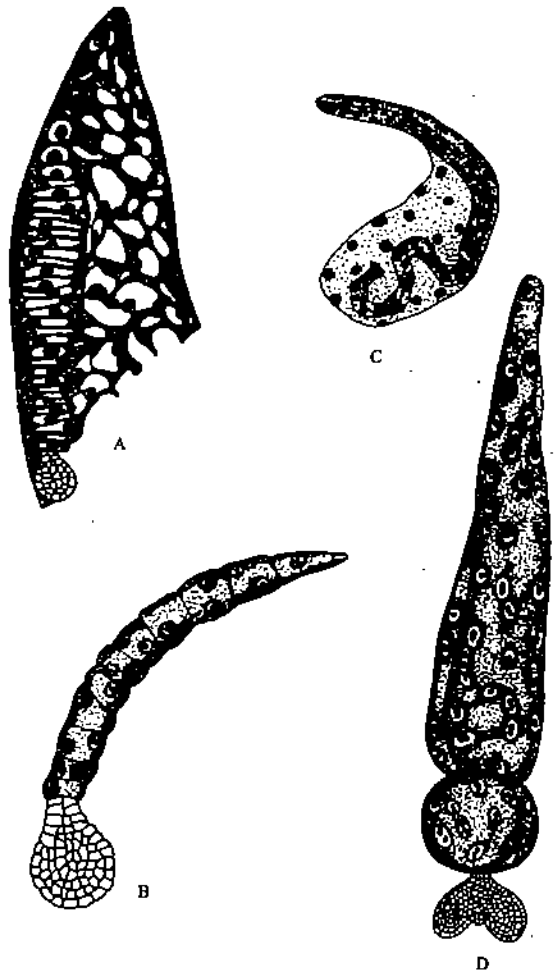

Fig. 5.10: Various modifications of suspensor cells.

It was believed earlier that the function of the suspensor was merely to hold the embryo proper and push it into the nutritionally rich endosperm. However, detailed investigations cytochemistry and ultrastructure of the suspensor have shown a more active role for the suspensor. The diversity in size, shape, longevity and cytological characteristics of suspensor observed among different taxa relate to the mechanism of function of suspensor in the nutrition of the embryo.

In some flowering plants (e.g., *Viola* and *Tilia*) there is no suspensor and in many others (e.g., *Euphorbia* and *Bryonia)* the suspensor is highly reduced. It is obvious that in such plants the suspensor could play little or no role in the nutrition of the embryo. However, in several families (e.g., Brassicaceae and Loranthaceae) a long filamentous suspensor is present. In the Fabaceae the suspensor displays considerable diversity. While in some legumes the suspensor is poorly developed in others there is a uniseriate (Fig. 5.10 A) or biseriate (Fig. 5.10 B) filamentous suspensor. In *Cytisus laburnum* the cells of the suspensor are clustered like a bunch of grapes (Fig. 5.10 C) and in *Pisum sativum* the suspensor is composed of four large, multinucleate cells (Fig.5.10 D). Suspensors with such large, multinucleate cells are considered to be haustorial as they are believed to derive nutrition from the surrounding cells in an aggressive manner.

You have already read that the Orchidaceae, Podostemaceae and Trapaceae lack endosperm formation. In the absence of endosperm, the embryo usually develops an elaborate suspensor haustorium. In the Orchidaceae the suspensor shows various modifications. It may be:

a) single celled, vesicular or sac-like (e.g., *Dendrobium*, Fig. 5.11 A);

b) a uniseriate filament of 5-10 cells which form haustorial branches in the placental tissue (e.g., *Ophrys*, Fig. 5.11 B);

c) like a bunch of grapes (e.g., *Epidendrum*, Fig. 5.11 C);

d) consisting of eight cells, formed by three vertical divisions in the suspensor initial, elongating downward and enveloping almost half of the embryo (e.g., *Vanda*; Fig. 5.11 D);

e) an irregular mass of 6-10 cells, of which some cells situated toward the micropylar end elongate and form tubular structures (e.g., *Cymbidium*, Fig.5.11 E).

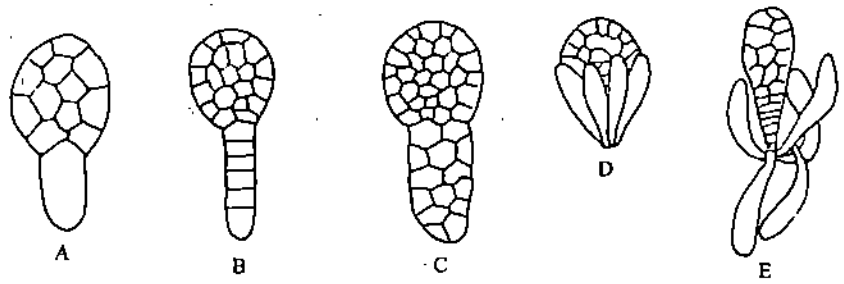

Fig.5.11: Some Types of suspensor cells found in orchids.

In the Podostemaceae (*Dicraea*; Fig. 5.12), the basal cell of the 2-celled proembryo enlarges and contains two hypertrophied nuclei. As the proembryo grows, the basal cell gives out a number of thin-walled haustorial branches which grow in the space between the two integuments of the ovule.

Suspensor haustoria which grow into the tissues of the ovule are also found in Rubiaceae, Fumariaceae, Crassulaceae, Tropaeolaceae and some other families.

The suspensor serves as a conduit for transfer of nutrients from the ovular tissues into the embryo. In *Capsella bursa-pastoris* and *Diplotaxis erucoides* for instance, the embryo has a uniseriate suspensor in which the micropylar cell is large and haustorial. The wall of this cell shows finger like wall ingrowths which expand the surface area of plasmalemma to promote greater intake of nutrients (Fig. 5.13). The cell walls separating individual suspensor cells are transversed by plasmodesmata. Cytoplasm of the suspensor cells has well developed endoplasmic reticulum and a large number of ribosomes, dictyosomes, mitochondria and plastids. Often the suspensor cells also show a high degree of endopolyploidy or polyteny. You will recall that these are characteristic

features of transfer cells which are involved in short distance transport of metabolites. Hence, the suspensor participates in absorption nutrients and providing them to the morphogenetically more important part of the embryo. When the developing pods of *Phaseolus coccineus* are supplied with C-14 labelled sucrose, then the radioactivity is observed first in the suspensor and later in the embryo proper (Yeung, 1980). This demonstrates that the suspensor is the site of uptake of nutrients in the young embryo. At later stages the cotyledons themselves absorb nutrients from the endosperm. The suspensor thus acts as a temporary 'embryonic root' for nutrition of the embryo.

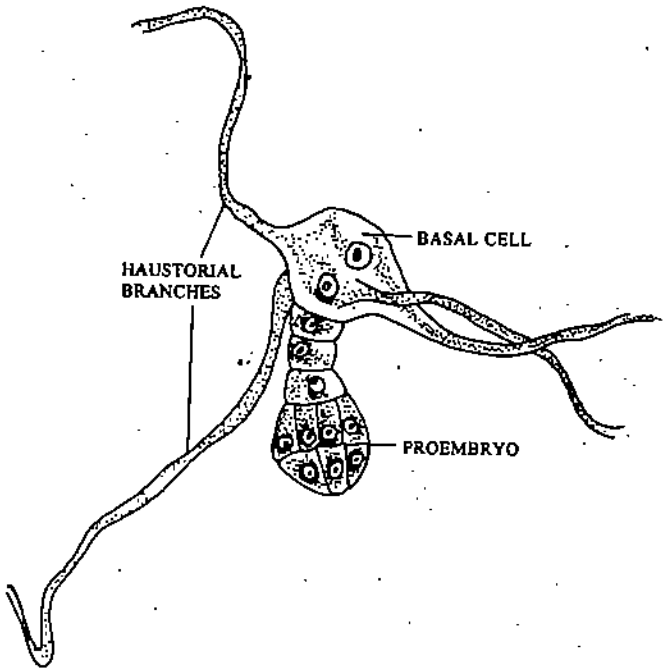

Fig. 5.12: Globular proembyo of *Dicraea*: The basal cell enlarges and gives out haustorial branches (after Mukkada, 1962).

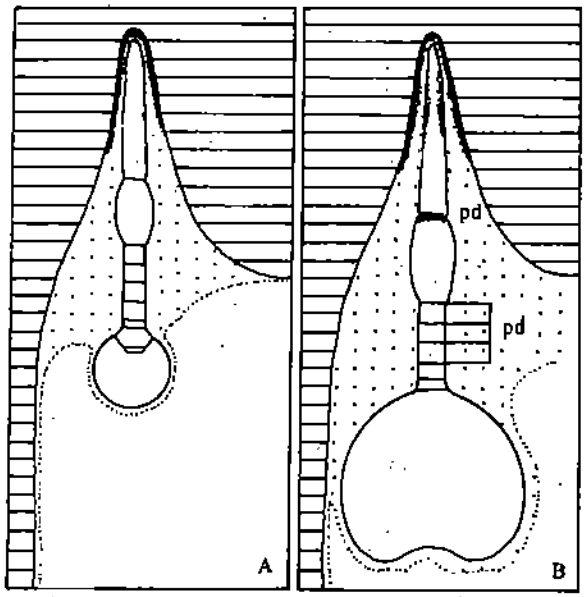

Fig. 5.13 A, B: *Diplotaxis erucoides*, diagrammatic representation of globular proembryo (A) and heartshaped embryo (B). Plasmodesmata (pd) occur on the transverse walls separating individual suspensor cells. (After Simoncioli 1974).

The suspensor starts degenerating from the heart-shaped stage of the embryo onwards. This is manifested by the disorganisation of cytoplasm and rupture of vacuolar membranes.

The suspensor is also considered as an important source of growth regulators. Suspensor cells have significantly amounts of plant growth regulators (PGRs) such as gibberellins, auxins and cytokinins. PGRs are believed to be supplied at specific stages

to the embryo proper for regulating development. It has been observed in *Phaseolus* and *Eruca* (Corsi, 1972; Yeung & Sussex, 1979) that when a young embryo is cultured in an artificial medium without its suspensor, growth is retarded. However, at later stages of embryo removal of suspensor has no effect on the growth of cultured embryos. After the formation of cotyledons the level of gibberellin in the suspensor is reduced dramatically and there is a significant rise in its level in the embryo proper. This indicates that gibberellin has been transferred from the degenerating suspensor cells to the embryo proper. Nagl (1973) has aptly compared the embryonal suspensor in plants to the mammalian trophoblast which acts as a supply line for the nutrition of the foetus.

SAQ 3

Given below are some statements regarding the formation and function of suspensor. Put a (√) mark against those statements which are correct and cross (X) those which are wrong in the boxes provided:

a) The basal cell of the 2-celled proembryo generally contributes to the formation of the suspensor. [ ]

b) Growth of the suspensor is generally maximum after heart shaped stage of development of embryo. [ ]

c) In developing seeds of members of Fabaceae and Orchidaceae, a well developed suspensor helps in pushing the embryo deeper into the nutritive endosperm. [ ]

d) Suspensor cells often display a high degree of polyploidy. [ ]

e) Suspensor is rich in hormones that influence the growth and differentiation of embryo. [ ]

5.9 NUTRITION OF EMBRYO

Now you will study ***in vivo*** **and** ***in vitro*** studies in nutrition of embryo.

5.9.1. *In Vivo* Studies

The young proembryo derives its nutrition from ovular tissues with the help of suspensor. As the embryo develops its suspensor degenerates. Later the chief source of nutrition of the embryo inside the developing seed is the endosperm. By the time the proembryo attains a late globular state, and its suspensor becomes disfunctional, the endosperm is generally already a cellular tissue surrounding the embryo on all sides. The developing seed is a powerful sink for nutrients. Food materials received through the funicular vascular supply, are absorbed by the endosperm and passed on to the embryo for its growth and development. The central cell wall has transfer cells for absorption of nutrients. Sometimes persistent antipodal cells or special structures such as hypostase and postament of the ovule (about which you have studied in unit 2) help in translocation of food to the endosperm. After the embryo is fully grown, the endosperm persists in a large number of plants, particularly monocotyledons, storing starch or oil or protein or all the three. These are used up during seed germination. In other plants no endosperm may persist in the mature seed as the cotyledons store food reserves. Legumes are the best examples and have been the subject matter of research on storage proteins in recent times.

5.9.2 *In Vitro* Studies

Studies involving culture of embryos excised of various stages of development (i.e., early globular proembryo, late globular proembryo or heart shaped and torpedo shaped embryos) in media containing various combinations/concentrations of nutrients and growth regulators have been very helpful in understanding the growth requirements of embryos.

Experiments using embryos of *Datura*, *Capsella* and a few of other plants have shown that the mature embryo can develop into a normal seedling when cultured in a nutrient medium. Torpedo-shaped embryos require salts of essential minerals dextrose and certain amino acids, vitamins and PGRs for successful growth. However, late globular or heart-shaped embryos can be cultured only on addition of coconut water (liquid endosperm of coconut) to the above medium. So far it has not been possible to isolate and grow the zygote or a few celled proembryo on an artificial medium. Young proembryos when cultured become formless and do not acquire the typical structure of a mature embryo. Raghavan (1966) recognised two phases of embryo development based on nutritional requirements.

i) Heterotrophic phase: During this phase, which may last up to the globular stage, the proembryo is dependent upon the endosperm (or the ovular tissues).

ii) Autotrophic phase: This may begin at the late heart-shaped stage when the embryo becomes fairly independent for its nutrition. As a result, isolated older embryos require only a simple nutrient medium (containing sucrose and minerals) to develop into an organised embryo.

5.10 POLYEMBRYONY

Presence of more than one embryo in a seed is termed polyembryony. The phenomenon, first discovered in orange seeds by Leeuwenhoek (1719), attracted considerable attention because of its potential for application in and horticulture. Polyembryony is broadly classified into simple and multiple, depending on whether the supernumerary embryos arise in one or more embryo sacs in the ovule. Simple polyembryony may be sexual or asexual. In sexual polyembryony embryos may originate from the fertilized egg and a synergid, or by budding or cleavage of the proembryonal cells or suspensor of the zygotic embryo. Asexual embryos are produced within the embryo sac without fertilization. Embryos may also originate from diploid nucellar or integementary cells of the ovule and grow into the embryo sac. These embryos are termed adventive or sporophytic. Multiple polyembryony involves production of accessory embryos from two or more embryo sacs in the same ovule. For example, in the sea Island cotton, *Gossypium barbadense*, fertilization of egg in one embryo sac stimulates the induction of embryo from an unfertilized egg in the adjacent embryo sac within the same ovule. Thus, diploid-haploid twin embryos are produced in the seed.

Some of the more common methods of formation of additional embryos in the seed are discussed below:

5.10.1 Embryos from Synergids

The synergids, which usually degenerate prior to or soon after double fertilization, are reported to give rise to embryos in *Argemone mexicana, Tamarix ericoids, Dioscorea composita* (Fig.5.14).

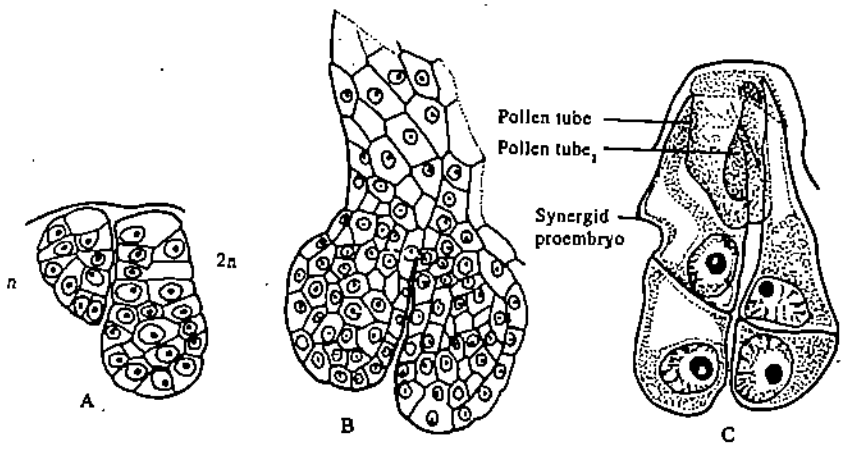

Fig. 5.14: Synergid polyembryony: A : *Argemone mexicana* B : *Tamarix ericoids* C : *Dioscorea composita.*

The synergid embryos grow along with the zygotic embryo in the embryo sac. In *Najas major* the synergid and the egg cell are fertilized and the resulting embryos resemble each other. Fertilization of the synergid is usually on account of entry of additional pollen tube in the embryo sac. Sometimes the unfertilized synergid is also stimulated to divide and form an embryo like structure. In the normal course only the zygotic embryo attains maturity and the haploid or diploid synergid embryo degenerates.

5.10.2 Zygotic or Suspensor Polyembryony

Cleavage of the apical cells of the globular or filamentous proembryo produced by the zygote may result in two or more embryos in a seed. Such (examples *Cocos nucifera* and *Primula auriculata*). This mode of polyembryony is also common among the orchids. Fig. 5.15A shows a group of cells produced from the zygote in *Eulophia epidendraea* which have developed into three distinct proembryos. In Fig. 5.15B an embryo is seen budding from a globular proembryo. In *Zygophyllum fabago* (Fig. 5.15C) and in several members of the Acanthaceae buds or new embryos arise from the uniseriate suspensor of the young proembryo.

The multiple embryos that arise from proembryonal or suspensor cells are diploid.

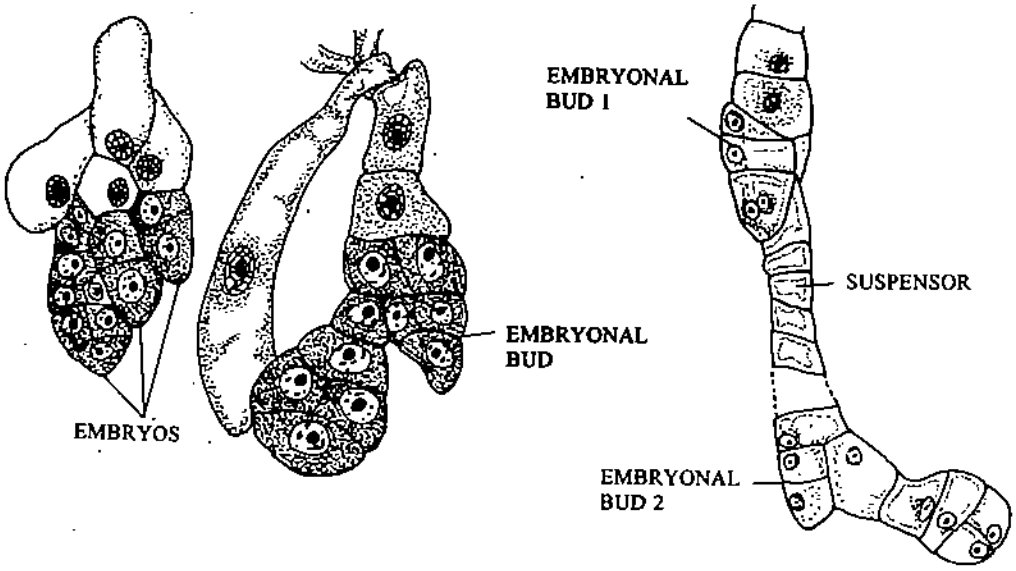

Fig. 5.15: Zygotic or Suspensor polyembryony. A. Zygote has produced a group of cells three of which have divided to form independent embryos. B. Budding of an embryo from a globular proembryo C. Proembryo with embryonal bud arising from uniseriate suspensor.

5.10.3 Nucellar Polyembryony

Members of families, Rutaceae, Anacardiaceae, Cactaceae, Myrtaceae and Orchidaceae, have a marked tendency for nucellar polyembryony. For example, in *Citrus microcarpa*

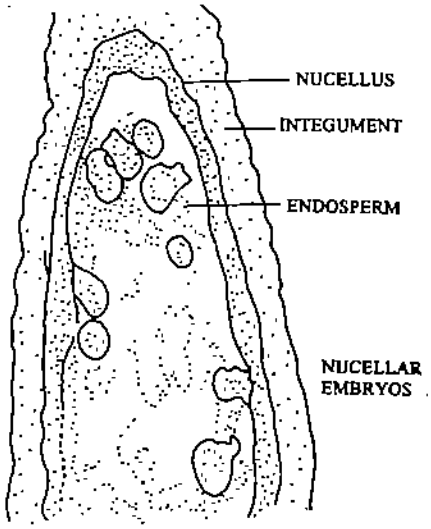

Fig. 5.16: Nucellar polyemb[illegible]

in certain nucellar cells in the micropylar region become conspicuous by their denser cytoplasm and large nuclei. These cells divide repeatedly to form embryonal structures which project into the embryo sac. There can be 9-21 nucellar proembryos in an embryo sac (Fig. 5.16), which has a normal zygotic proembryo and endosperm resulting from double fertilization. The nucellar or adventive embryos pass through globular, heart-shaped and torpedo-shaped stages before undergoing complete differentiation as dicotyledonous embryos.

In the various species of *Citrus*, nucellar embryos are of great horticultural importance as the plantlets they produce are more vigorous, virus-free and endowed with well-developed tap root system in comparison with the shoot cuttings of the mother plants. Moreover, the seedlings are more uniform than those obtained through seeds.

In mango, *Mangifera indica* polyembryonate seeds may contain as many as 50 embryos. The embryos originate from nucellar cells in the micropylar region and grow into the embryo sac (Fig.5.17). When zygotic embryo is also present, it is difficult to distinguish it morphologically from the nucellar embryos. The varieties which form adventive embryos can be propagated by seeds which give seedlings that are of the same quality as the parent stock.

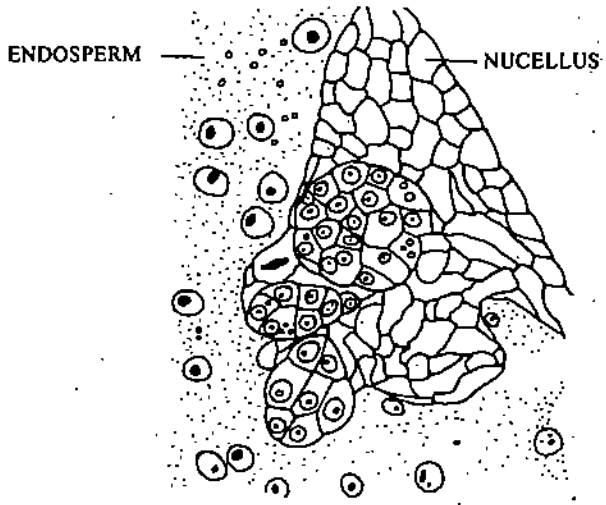

Fig. 5.17: Adventive proembryos.

5.11 USES OF PLURAL EMBRYOS

Although the basic that trigger causes polyembryony are not fully understood, there has been no dearth of interest in exploiting supernumerary embryos. The multiple embryos within a seed can be haploid, diploid or triploid. Haploid seedlings can be utilized for obtaining homozygous diploid forms by doubling the chromosome complement by a polyploidising agent. Such haploids and double haploids or homozygous diploids are of immense use in breeding superior crop varieties and hybrids. Adventive embryos are useful in agriculture and horticulture because they are genetically uniform and generally disease free.

In view of the utility of polyembryony, there have been several attempts to induce it artificially in those plants which are not normally polyembryonate or where the phenomenon occurs only occasionally. Environmental factors are often responsible for induction of polyembryony. However, application of PGRs has not proved successful in inducing polyembryony.

During the last three decades tissue in culture technology, involving excision of a desired tissue or organ and growing it on a nutrient medium under sterilized conditions, has been highly successful. It has helped in large scale propagation of plantlets obtained from vegetative or reproductive parts of several plants of agricultural and horticultural importance. Plantlets can now be obtained from the diploid tissues of nucellus or embryo or even from haploid micropores and triploid endosperm. Because of the wider application of tissue culture methodology, early interest in induction of polyembryony in field grown plants has died down.

SAQ 4

Fill in the blanks in order to convey a correct meaning of each sentence.

a) Formation of more than one embryo in a seed is termed .....

b) Asexual embryos arise without the intervention of .....

c) In *Argemone mexicana* additional embryo may arise from .....

d) Cleavage polyembryony is characteristic of the family .....

e) Nucellar cells destined to produce embryos have denser cytoplasm and ..... nuclei.

f) Plantlets from adventive embryos are superior to vegetative cuttings in having ..... root system.

5.12 SUMMARY

- You have studied in this unit that the zygote, gives rise to the embryo. The zygote undergoes a period of rest during which it shrinks, develops a complete wall around it and becomes polarized. Prior to division, it has a high rate of metabolic activity.
- With rare exception the zygote invariably divides transversely. Derivatives of the basal cell generally gives rise to the suspensor and those of the apical cell contribute to formation of different parts of the mature embryo. The four-celled proembryo is linear or T-shaped.
- Based on the plane of the first two divisions in the zygote, and the relative contribution of the daughter cells to different parts of the embryos, six embryogenic types are recognised — Piperad, Onagrad, Asterad, Solanad, Caryophyllad and Chenopodiad.
- Tangential divisions in the cells of the octant bring about histogenic stratification into three distinct layers — dermatogen, periblem and plerome.
- Precursors of various organs — cotyledons. The first change in the globular embryo is the initiation of cotyledons subsequent to which hypocotyl, stem apex and root tip — become evident in the organized embryo during maturity following globular stage of development.
- Proembryo receives nourishment through the suspensor. At later stages the embryo derives it from the endosperm.
- The suspensor undergoes various modifications in certain groups of plants, specially those which lack endosperm, to make it an effective source for absorption and transport of food materials for the embryo proper.
- A seed may have more than one embryo. Polyembryony may result from synergids or by cleavage of zygotic embryo. Adventive embryos also arise from the proliferation of nucellar or integementary cells. Nucellar embryos are of horticultural importance as they are true to the material parent and possess several describable features.

5.13 TERMINAL QUESTIONS

1) List the ultra structural changes that occur in the egg as a result of syngamy
2) On what basis is embryogeny classified into six major types?
3) The 4-celled proembryo in *Capsella* has inverted T-shaped configuration. Describe the further course of development which leads to formation of a mature dicotyledons embryo.

4) Highlight the differences in development of a dicotyledonous embryo and a monocotyledonous embryo.

5) What are the parts of a fully organised dicotyledonous embryo? Trace their destiny.

6) Describe some features of the suspensor which make it suitable for absorpiton and transfer of nutrients to embryo.

7) Enumerate some modifications of the suspensor.

8) What is the role of endosperm in nourishment of the embryo?

9) What is polyembryony? Describe the important types of polyembryony in flowering plants.

10) Name the applications of polyembryony in horticulture

5.14 ANSWERS

SAQ 1

a) F, b) T, c) F, d) T, e) F.

SAQ 2

a) X, b) √, c) √, d) X, e) X.

SAQ 3

a) √, b) X, c) X, d) √, e) √.

SAQ 4

a) polyembryony

b) fertilisation

c) synergid

d) Orchidaceae

e) larger

f) tap.

Terminal Questions

1) In the fertilised egg or zygote a complete cell wall is formed. Cytoplasm becomes more polarized. There is an increase in the density cytoplasmic organelles. Refer to Section 5.2.

2) Six types of embryogeny are recognised on the basis of:

   i) plane of division of the zygote and of the cells of the 2-celled proembryo;

   ii) relative contribution of the derivatives of the 4-celled proembryo the formation of suspensor and parts of the mature embryo.

3) In the 4-celled proembryo of inverted T shape, each of the two terminal cells is divided by a vertical wall to form a quadrant. The quadrant cells in turn divide transversely to form an octant. Derivatives of the lower four cells of the octant give rise to the stem tip and cotyledons, whereas cells derived from the upper four cells of the octant form the hypocotyl. Derivatives of *ci* and some cells cut off from the intermediate cell *m* of the 4-celled proembryo divide to form a row of 6-10 suspensor cells. The lowest cell derived from *m* is referred to as hypophysis. It divides transversely to produce initials of the root cortex and of the root cap and epidermis.

4) Initial development of the proembryo is similar in dicotyledons and monocotyledons. However, during subsequent development in monocotyledons one half of the

half of the terminal cell and its derivatives have retarded growth. Derivatives of the other half grow rapidly to form one cotyledon. As a result, the stem tip which is also derived from the terminal cell appears to be lateral in position. According to Lakshmanan (1972) two adjacent cells of the terminal quadrant of the proembryo in Amaryllidaceae and Potomogetonaceae, three cells of the quadrant in Iridaceae and Potomogetonaceae, and all four cells except a few derivatives of one of them in Philydraceae contribute to formation of one cotyledon. The remaining portion or cells of the quadrant give rise to the stem tip.

5) A dicotyledonous embryo has an embryonal axis having two cotyledons, which expand into a pair of leaf like structures of the seedling. The portion of the embryonal axis above the level of cotyledons is termed the epicotyl which terminates in the plumule or stem tip. The cylindrical portion below is known as hypocotyl. It terminates at the other end in the radicle or root tip, covered by a well defined root cap.

6) The vesicular micropylar cell of the suspensor has transfer cell like structure. Cell walls separating individual suspensor cells have plasmodesmal connections. Cytoplasm of the suspensor cells has well-developed endoplasmic reticulum and a high density of ribosomes, mitochondria and dictyosomes. Suspensor, cell nuclei often show high levels of polyploidy. These characteristics make the suspensor highly suitable for absorption of nutrients from ovular tissues and transferring these to the embryo.

7) In *Pisum sativum* the suspensor consists of four large, multinucleate cells. In *Cytisus laburnum* cells of the suspensor are clusterd like a bunch of grapes. Such modified suspensors in Fabaceae are considered haustorial in nature.

Among the Orchidaceae the suspensor may be:

i) single-celled, vesicular (e.g., *Vanda*)

ii) branched, filamentous (e.g., *Ophrys*)

iii) like a bunch of grapes (e.g., *Epidendrum*)

iv) in the form of eight finger-like extensions enveloping the upper half of the embryo (e.g., *Vanda*).

v) Irregular mass of cells of which some micropylar cells elongate and form tubular extensions (e.g., *Cymbidium*) In number of the Rubiaceae, Fumariaceae, Crassulaceae and Tropaeolaceae also the suspensor cells form aggressive haustoria which grow into the tissues of the ovule.

8) Food materials received from the funicular vascular supply is absorbed by the endosperm from the ovular tissues and passed on to the embryo for its growth and development. In certain plants endosperm is fully used up during such development. In many others even after the embryo is fully grown, the endosperm stores large quantities of food materials in the form of starch or lipids or protein bodies or all of these. This reserve food is utilized by the embryo during germination of the seed.

9) Presence of more than one embryo in a seed is termed polyembryony. Polyembryony is simple if additional embryos arise in the same embryo sac, and multiple if they are formed in more than one embryo sac in the ovule. In sexual polyembryony embryos may originate from the fertilized egg and synergid (e.g., *Najas*), or by budding or cleavage of the suspensor (e.g., *Diptera-canthus*) or proembryonal cells (e.g., *Eulophia*) of the zygotic embryo. Asexual embryos are produced in the embryo sac without the process of fertilization. More commonly multiple embryos may develop from the diploid nucellar (e.g., *Citrus* and *Mangifera*) or integumentary cells of the ovule. Such embryos are termed adventive embryos.

10) Multiple embryos can be haploid or diploid depending on whether they originate from an unfertilized gameotophytic cell such as a synergid or an egg, or a sporophytic tissue such as nucellus or integument. The haploid, double haploid and homozygous diploid obtained from it, are of immense use in breeding of super crop varieties. Adventive embryos are useful in horticulture because they can generate uniform and disease free seedlings.

UNIT 6 SEED AND FRUIT

Structure

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6.1 INTRODUCTION

Sexual reproduction in flowering plants requires two processes—pollination and fertilization, about which you have studied in Unit 3. Following double fertilization, the zygote develops to form the embryo. The Primary endosperm nucleus divides and the resulting nuclei further proliferate to eventually give rise to the nutritive tissue—endosperm (Unit 4). Meanwhile the ovule undergoes a series of changes that transform it into a protective container of the young sporophyte (Unit 5). In this unit you will study various aspects of the development of seeds and fruits. A seed may be defined as the unit of reproduction which has the embryo and commonly food-laden endosperm enveloped by a seed coat derived from the integument(s) of the ovule. Beginning with pollination, the ovary is activated to form the fruit, which encloses the developing seeds. The fruit and the seed not only protect and nurture the young sporophyte, but also serve the function of dispersal. Seeds of many plants remain viable for long periods in the soil. They may even have a certain period of dormancy to ensure germination only when conditions (temperature and moisture in particular) become suitable. Incidentally, the food stored in the fruit wall and seed are also the main source of nutrition for man, wild and domestic animals, bacteria and fungi.

Objectives

After studying this unit you should be able to:

- explain the structure and development of the seed;
- describe the various modifications in seed structure for effective dispersal;
- know the nature of reserve materials in the seed for the nutrition of the young sporophyte during seed germination and seedling establishment;
- trace the changes that occur as the ovary develops into a fruit.

- classify the various types of fruits;
- elucidate how some fruits such as banana develop without having seeds;
- describe the relatively rare but interesting phenomenon of vivipary, which involves *in situ* germination of the seed while still enclosed and attached to the parent plant.

6.2 SEED

A seed is a mature ovule enclosing an embryonic plant, stored food material (in endosperm, persistent nucellus or embryo itself) and a seed coat formed by one or two integuments. In a broad sense the term seed is also applied to small one-seeded, dry fruits (e.g., grains of wheat or barley which are in fact made up by fusion of fruit wall and seed coat) or other disseminules (fruits with attached bracts, inflorescences or even vegetative structures such as tubers and bulbils).

The size, shape, colour and surface of the seed show innumerable variations. Most orchids have minute seeds like dust particles. Seeds of a majority of flowering plants are a few millimetres in diameter (e.g., mustard, guava and poppy) or extend to a length of about a centimetre (e.g., castor, cucumber and groundnut). Some tropical trees and lianas have fruits with very large seeds. The double coconut, *Lodoicea maldivica* has bilobed seeds as large as 10 cm weighing nearly 6 kg.

The seed surface may be smooth, wrinkled, striated, ribbed, furrowed or it may have a variety of patterns on it. The surface may be glossy (as in linseed and castor), fleshy or pulpy (in *Magnolia*) or covered with hair (in cotton).

Parts of a Seed

Seed is attached to the fruit by a stalk, the funiculus (funicle). The prolongation of the funiculus running along the seed and terminating at the chalaza is called raphe (Fig. 6.1). The funicular vascular supply is responsible for the flow of food reserves. When the seed is separated from the funiculus a scar is left at the point of attachment which is termed hilum.

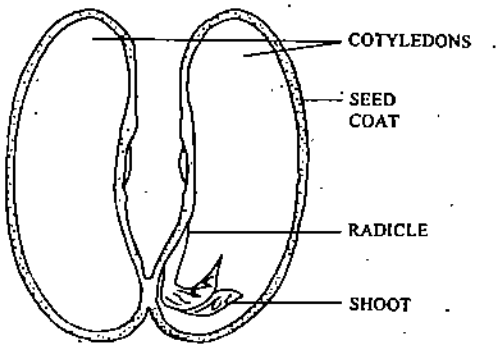


Fig. 6.1: Various parts commonly present in the seeds

How Seed Develops?

Corresponding with the development of embryo and endosperm the ovule, the integument(s) and the nucellus also embark on certain changes which eventually result in the formation of mature seed. The usual alterations are described with the help of a few examples.

Nucellus: In a large majority of flowering plants the nucellus is gradually utilized by the endosperm or embryo. In leguminous seeds, for example, the nucellus degenerates completely. Sometimes, as observed in *Euphorbia spp.*, nucellar cells near the micropyle (termed epistase) and chalaza (hypostase) survive longer and may even persist in the mature seed. In the black pepper fruit the bulk of the volume is occupied by the persistent nucellus (Fig. 6.2), which is also the chief food storage tissue (endosperm is relatively little). Such persisting nucellus in the seed is designated perisperm. In *Daphniphyllum himalayense* the seed has copious endosperm surrounded by perisperm, which is characterized by the presence of oil droplets and even protein crystals (Fig. 6.3).

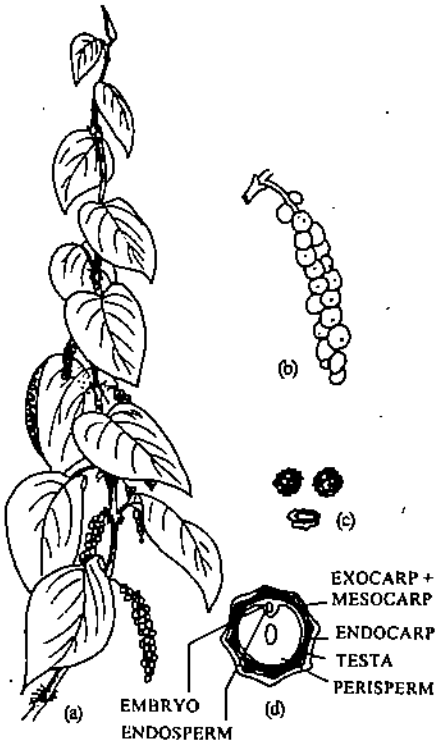


Fig. 6.2: Black pepper—A fruiting branch of *Peper nigrum*. B—a pendulous dense spike. C — whole and split fruits D — Fruits with seed cut in longitudinal section.

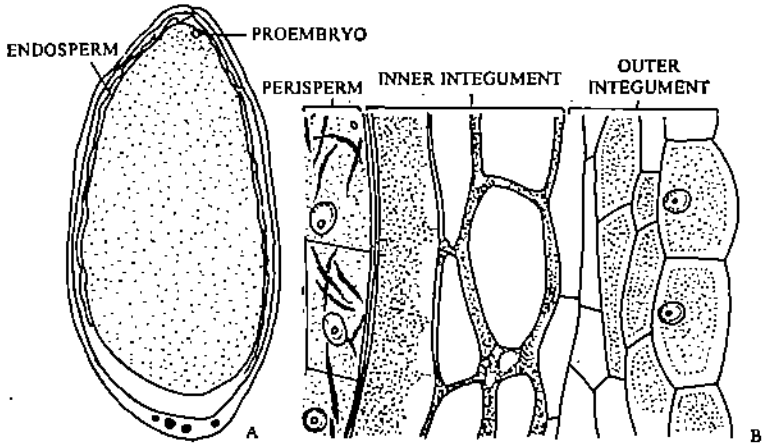


Fig. 6.3: Endosperm and seed coat of *Daphniphyllum*. A. L.S. of seed at globular proembryo stage. B. Magnified to show scelerification of tegmen and presence of crystalline protein reserves in perisperm.

Integuments: The ovule has usually one or two integuments. After fertilization, there may be initially a proliferation of cell layers in one or both the integuments. An integument in which the cell layers increase is described as multiplicative. If the number of cell layers in the integument remains the same as in the mature ovule then the integument is regarded non-multiplicative. Alternatively, a process of disorganisation of cells may begin at an early stage. In either case, as the seed matures, most of the cell layers degenerate and get compressed. At the same time, some particular cell layers in one or both the integuments persist and may become hard to form a protective sheath. Cells of the protective layer often enlarge in the anticlinal (perpendicular to the seed surface) plane, and their walls become lignified and even cutinized. The characteristic layer, if present, is described as sclerotic, mechanical, palisade or Malpighian layer. Some seed biologists prefer to call the palisade-like cells as a layer of macrosclereids.

In a seed developing from a bitegmic ovule, the persisting outer integument is termed testa and the inner integument tegmen. In seeds originating from unitegmic ovules the

seed coat is loosely termed testa. Seeds with characteristic testa are called testal and those with prominent tegmen are described as tegmic. Seeds in which outer part of the outer integument constitutes the mechanical layer are designated exotestal, and those having hardened inner portion are endotestal. Similarly, seeds with outer part of the inner integument modified as sclerotic zone are exotegmic and those with inner layers forming the protective sheath are endotegmic. In some plants which have a stony or tough fruit wall, the seed coat may be thin and soft (coconut and almond).

The histological changes that lead to the formation of the seed coat may be studied with the help of examples of cotton, melon, mustard and bean.

In cotton (*Gossypium spp.*) the ovule has two integuments (Fig. 6.4) and both participate in formation of the seed coat. At mature embryo sac stage the outer integument is 4-6

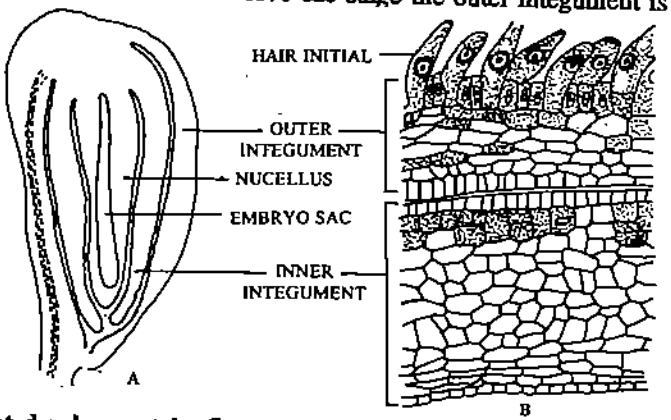


Fig. 6.4: Seed coat development in *Gossypium herbaceum* . A L.S. of ovule having mature embryo sac. B. Portion of integuments from ovule after 2-3 days of pollination.

cells thick and inner is 8-15 cells thick. The inner integument is multiplicative. Six days after pollination the outer integument can be distinguished into three zones (Fig. 6.5): (i) outer epidermis; (ii) outer pigmented zone of 2-5 layers having some tannin and starch-filled cells; (iii) inner epidermis. In the inner integument, cells of the outer epidermis start elongating radially. These epidermal cells enlarge many times their original size and their walls become thick (Fig. 6.5). This layer forms the sclerotic layer of the mature seed coat. In the mature seed the inner integument has four zones: outer palisade layer; a pigmented zone of 4 or 5 layers, inner colourless zone of 9 or 10 layers; inner epidermis. Thus, the mature seed coat has seven distinct zones made up by both the integuments.

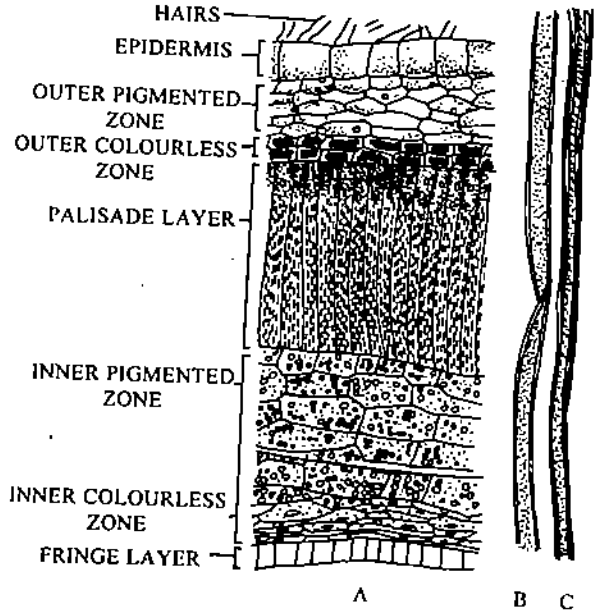


Fig. 6.5: Structure of integuments of *Gossypium herbaceum*. A—Mature seed coat. B—A lint hair C—A fuzz hair.

During development of seed coat in cotton, some of the outer epidermal cells of the outer integument enlarge and then elongate outward to form hairs. These hairs, the cotton of commerce, are single celled, thin-walled and attain a size of up to 40 mm. Lint hairs are longer with characteristic twists, whereas fuzz hairs are short and without twists.

In the gourd family, cucurbitaceae the ovules are bitegmic but the outer integument alone forms the seed coat. In *Luffa spp.* at mature embryo sac stage the outer integument is 10-15 layers thick and inner is 2 or 3 layered (Fig. 6.6). During seed development the inner integument degenerates. The outer epidermal cells of the outer integument elongate radially and develop rod-like thickenings on their radial walls (Fig. 6.6). A few layers of small pitted cells lie beneath the epidermis. The innermost layer of this zone comprises radially elongated cells. A single layer of large, radially elongated and somewhat bone-shaped cells occur next, constituting the palisade or mechanical layer. Inside the palisade layer is a region of spongy parenchyma having thin walls and intercellular air spaces.

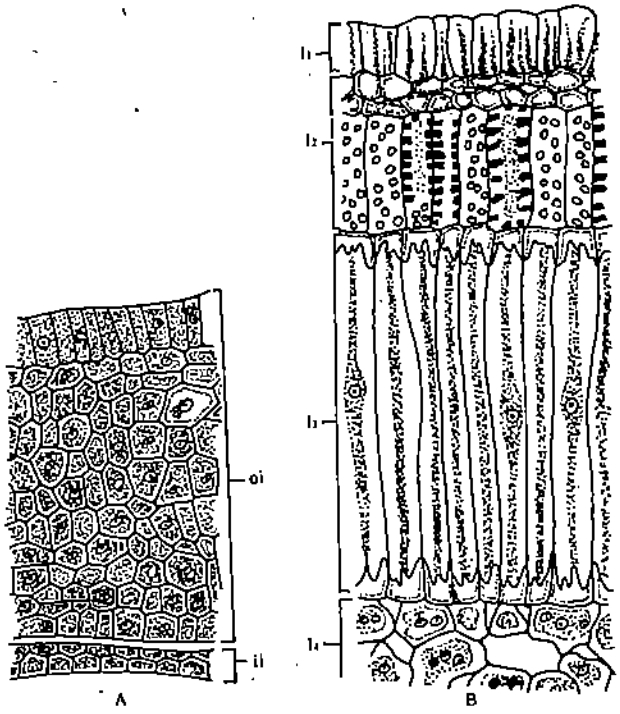


Fig. 6.6: Seed-coat development in *Luffa*. (ii), inner integument; oi, outer integument; l_1, epidermis, l_2, hypodermis; l_3, sclerenchymatous layer; l_4, aerenchyma).
A. Portion of longisection of integuments before fertilization in *L. hermaphrodita*.
B. Portion of longisection of mature seed-coat in *L. graveolens*; chlorenchymatous zone is not drawn.

In mustard, *Brassica campestris* the outer integument has 2-5 layers of cells and the inner has up to 10 layers. Cells of the outer epidermis of outer integument become large and filled with mucilage (Fig. 6.7). The subepidermal layer has tangentially elongated cells which are gradually crushed. Inner epidermis of the outer integument forms the sclerotic layer. The inner integument gets obliterated, except for its inner epidermis which forms a pigmented layer.

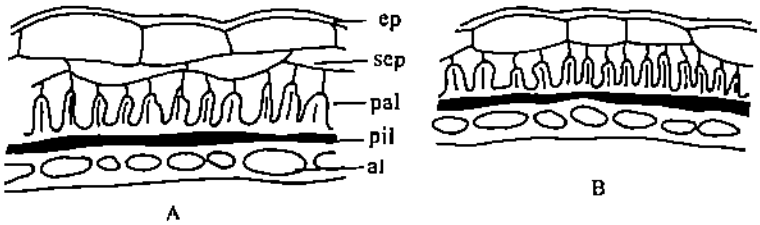


Fig. 6.7: T.S. of testa of *Brassica sp.* ep—epidermis, sep—sub epidermis, Pal—palisade; pil—pigment layer; al—aleurone layer of endosperm.

In leguminous seeds, such as those of *Phaseolus lunatus*, also the seed coat is derived from the outer integument whereas the inner integument degenerates. The outer

epidermis of the testa forms the palisade layer (Fig. 6.8). Its cells have a characteristic light line running tangentially along the middle or close to the outer walls. The light line is the result of intense refraction in the particular region of epidermal walls. Orientation of microfibrils that constitute the wall thickening is responsible for the varying refraction. The subepidermal layer of cells differentiates into hour-glass or funnel-shaped cells. Below this is the region of thin-walled parenchymatous tissue with vascular bundles. The outer part of this tissue has well-developed intercellular spaces.

The region of hilum in leguminous seed has an extraordinary organisation. The attachment of funicle forms a disc-shaped structure which fits into the depression of the hilum. The outer layer of cells of the head of the funicle also forms a palisade layer (termed as counter-palisade), which is attached to the palisade layer of the testa (Fig. 6.8). Both palisade and counter-palisade are interrupted in the centre by a narrow groove which serves as an air passage in the ripening seed. The groove also leads to a group of tracheids in the seed called the tracheid bar. On either side of the bar is aerenchyma. Since the testa is impermeable to water, the tracheid bar also serves as a 'hygroscopic valve' for absorbing moisture during seed ripening and germination.

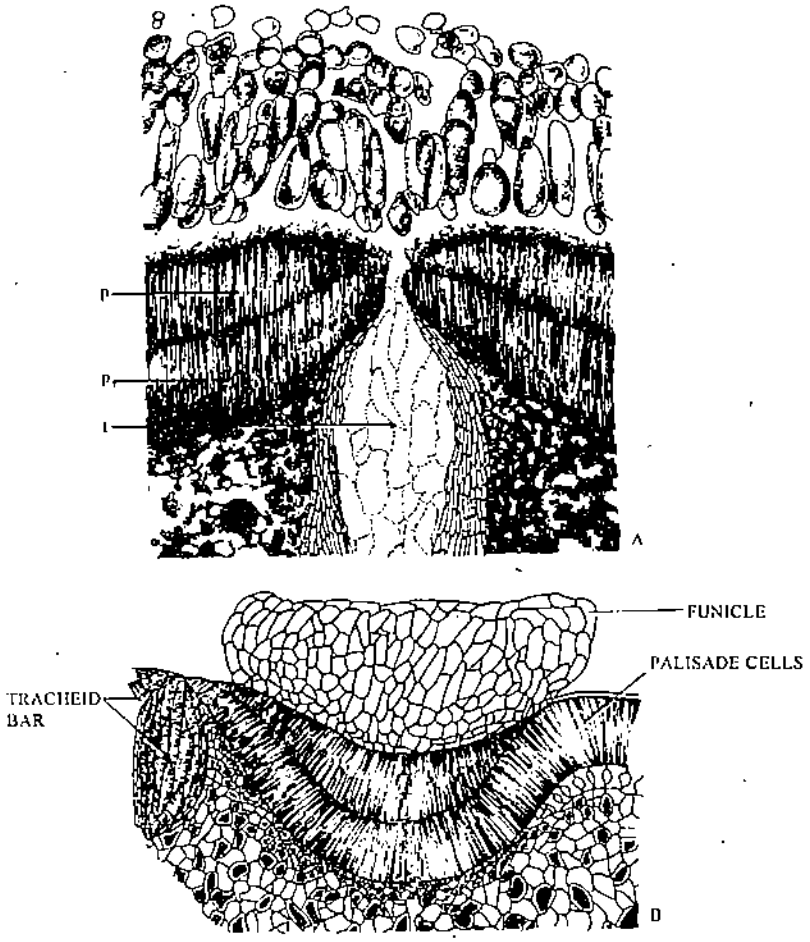


Fig. 6.8: Median L.S. of seed coat through hilum of *Phaseolus aureus*. A—photographic representation of the region of hilum. B. Diagrammatic representation of funicle region.

The seed coat of certain plants is fleshy or juicy. In the pomegranate, *Punica granatum*, for example, the outer epidermal cells of the testa enlarge and become filled with a sweet sap under considerable turgor pressure. This layer forms the juicy edible part of the seed (Fig. 6.9). The inner part of the testa is hard and the tegmen is membranous. In *Magnolia* spp. the inner integument forms the hard shell, whereas the outer integument is fleshy and brightly-coloured. Pulpy and fleshy testa can be termed **sarcotesta**.

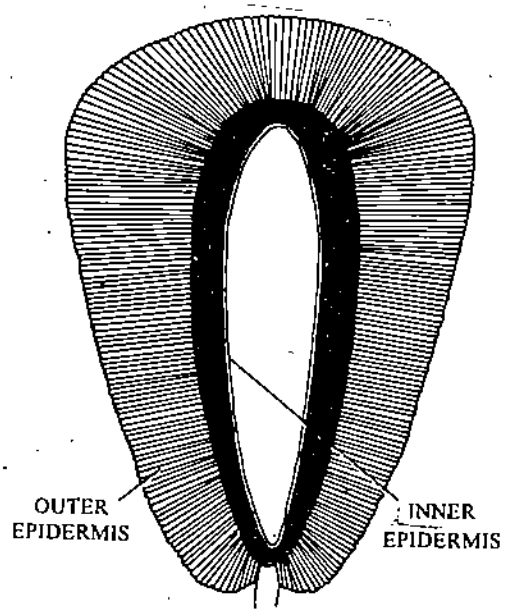


Fig. 6.9: L.S. of seed (Diagrammatic representation) of *Punica granatum*. Outer epidermis of testa has radially elongated cells. These cells form fleshy part of seed. Solid black portion is inner portion of outer integument and is made up of sclerenchyma.

SAQ 1

State whether the following statements are true or false. Write either T or F in the boxes provided.

a) Seeds of flowering plants are always enclosed in a fruit. [ ]

b) Micropyle of the ovule is represented in the seed as hilum. [ ]

c) A true seed is formed from a ovule and it encloses embryo formed with or without fertilization. [ ]

d) In the majority of seeds the nucellus constitutes the nutritive tissue. [ ]

e) Malpighian layer of seed coat has radially elongated cells with thick wall impregnated with lignin or cutin. [ ]

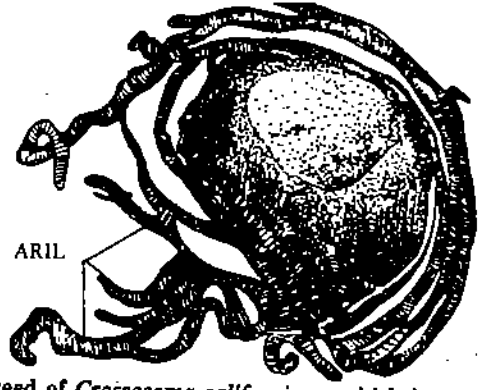
f) The seed of cotton is exotegmic. [ ]

g) Leguminous seed is endotestal. [ ]

6.3 SEED APPENDAGES

Seeds of certain plants have specialized outgrowths or envelopes. These structures develop following fertilization from parts of the ovule or funicle. Here you will study the origin and structure of some seed appendages. In a later part of this unit you will learn more about how these seed appendages help in effective seed dispersal.

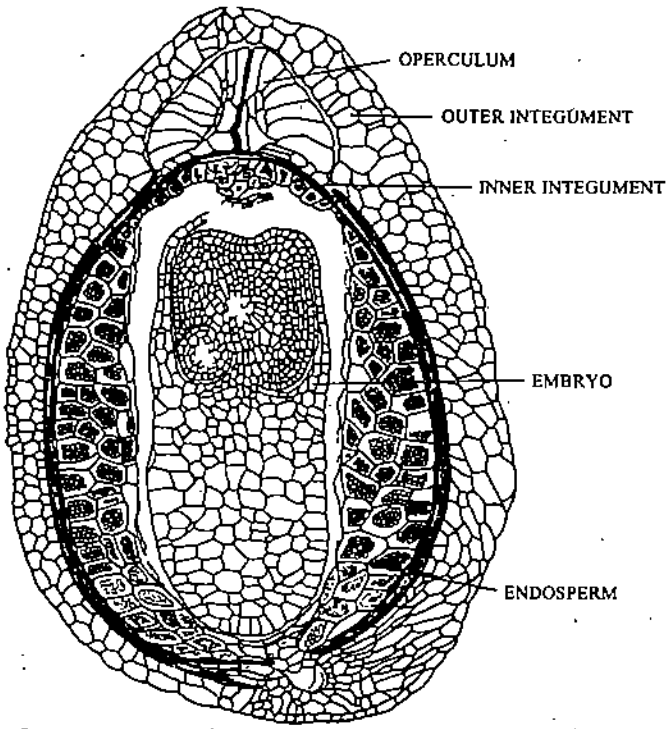
1. **Aril.** It is an outgrowth that arises from the funicle or the testa near the raphe and covers the seed partially or completely. It is often referred to as the third integument. It is often fleshy or brightly coloured. The edible part of litchi fruit is aril, which envelops the hard brown seed coat. In *Myristica fragrans* the hard seed (nutmeg of commerce) is covered by a thin, irregular and bright-orange aril (that gives us the precious spice mace). Seeds of *Pithecellobium dulce*, a leguminous tree, have a fleshy red aril that partially surrounds the seed. In *Crossosoma californicum* a fimbriate aril covers the seed on the sides (Fig. 6.10). Cells of the aril contain oils, starch, sugars, pigments and aroma containing compounds. The appendage is mostly an attraction for birds, which consume the aril and scatter the seeds. Seeds of white water lily, *Nymphaea alba* have a spongy aril that provides buoyancy for dispersal of seed by water.



6.10: Mature seed of *Crossosoma californicum* which is surrounded by imbriate aril.

aruncle. This is a white, collar-like structure borne on the micropylar end of the ed in many members of the Euphorbiaceae, such as castor, *Ricinus communis*. The ft outgrowth capping the hard seed is formed by proliferation of the cells at the of the outer integument. It is rich in starch and sugars. The violent opening of e fruit (this is termed gun-shot mechanism) causes seeds to be thrown away a few et. It is believed that ants consume the caruncle and in the process carry the seed rther away. The caruncle is also considered to be hygroscopic and it may be lpful in absorbing moisture for seed germination.

perculum. The term operculum is applied to a plug-like structure formed in the icropylar portion of the seed by proliferation of cells at the tip of the inner tegument or the nucellus. An operculum has been observed in seeds of many onocotyledonous families, such as the Commelinaceae, Musaceae, Lemnaceae and ngiberaceae, and a few dicotyledonous families like Bignoniaceae and ymphaeaceae. In *Lemna paucicostata*, cells at the tip of the inner integument dergo a remarkable expansion after fertilization and form a dome-shaped, stopper- e operculum (Fig. 6.11). Cells of the operculum are thick-walled and contain an ange-red substance. During germination of the embryo the operculum becomes tached from rest of the seed and facilitates emergence of the embryo.



.11: L.S. of mature seed of *Lemna paucicostata*. A prominent operculum can be seen at the micropylar end. (After Maheshwari and Kapil, 1964).

ings and hairs. Seeds of certain plants have epidermal outgrowths or the eguments themselves may form folds and projections that present a wing-like pearance. In the tree *Oroxylon* sp. a thin, transparently white, circular or oval, c-like wing spreads all around the two-lobed seed (Fig. 6.12). The seed of urbit *Zanonia macrocarpa* has wings measuring up to 10 cm. In the drumstick nt *Moringa oleifera* the seed has three equidistant wings. Seed wings provide ge surface area, with optimum strength, combined with a minimum of

material. A surprisingly large number of winged seeds can be arranged compa in a fruit. Wings help the seed to propel, sail or spin some distance away wh released from the fruit.

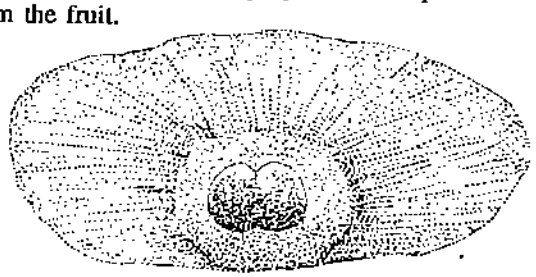


Fig. 6.12: Winged seed of *Oroxylon*.

Have you observed some tiny structures with lustrous white hairs floating around air, specially in early summer? These are seeds which travel long distances with t help of hairs. Seeds of milkweed, *Calotropis procera* have a tuft of hairs at one p (Fig. 6.13). Those of *Adenium* sp. of the Apocyanaceae have hairs at both ends. I cotton and poplars the seeds have hair all over the seed coat surface. Hairs provid seed a large surface area without a corresponding increase in weight, thus helping dispersal by air. In some aquatic plants such as *Nymphoides* spp. the seed hairs ar filled with air and provide buoyancy to the seed to float and become distributed o large area.

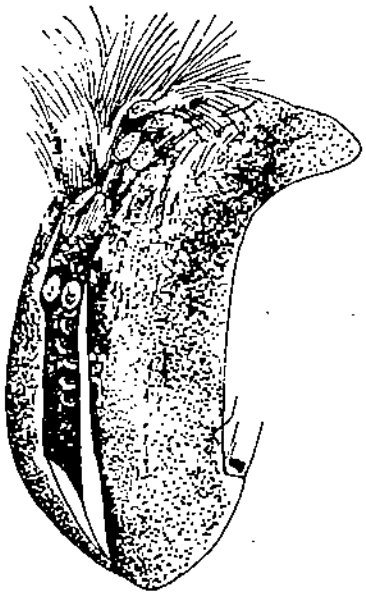


Fig. 6.13: Mature and dehisced fruit of *Calatropis*, showing hairy seeds at one end

SAQ 2

Given below are some incomplete statements regarding seed appendages. Fill in th blanks using appropriate words:

a) The sweet, edible part we relish in a litchi fruit is ..... .

b) Caruncle is involved in seed dispersal by ..... .

c) Aril is also known as the ..... .

d) In the drumstick fruit the seed has ..... wings.

e) Operculum is produced by proliferation of cells at the tip of ..... or ..... .

f) ..... and ..... are two families in whic members have seeds hairs.

g) In *Oroxylon* the seed has a ..... for dispersal.

h) Several members of the family ..... bear carunculate seeds

6.4 STORED METABOLITES

In a large majority of seeds food is stored in the cells of the endosperm. In coconut, wheat and castor bean for example, it is the endosperm which stores the bulk of the food reserves. Food stored in endosperm is utilized by the embryo during development and seed germination. In Unit 4 you have already learnt that endosperm is usually a triploid tissue derived from the fusion product of a male gamete (brought by pollen tube) and two polar nuclei in the central cell of the embryo sac. The endosperm surrounds the embryo all around and is ideal for nurturing the embryo till the seedling begins to photosynthesize and becomes autotrophic.

A second seat/site of nutritive tissue in some seeds is the perisperm, which represents the persisting nucellus. Nucellus is observed in some monocot families, such as the Zingiberaceae (to which turmeric and ginger belong), and a few dicotyledonous families, including the Piperaceae (e.g., black pepper) and the Nymphaeaceae (e.g., lotus). In *Canna* the chalazal cell of the ovule divides repeatedly to form a starch-containing tissue called chalazosperm. Mature seeds containing persistent endosperm or perisperm are called albuminous and those lacking them exalbuminous.

Mature leguminous seeds lack endosperm. In pea, gram (chick pea) groundnut and a whole range of pulses the large cotyledons of the embryo take up the function of storage of food. In cotton seed also the large, lobed and folded cotyledons (Fig. 6.14) are the main repository of nutrients.

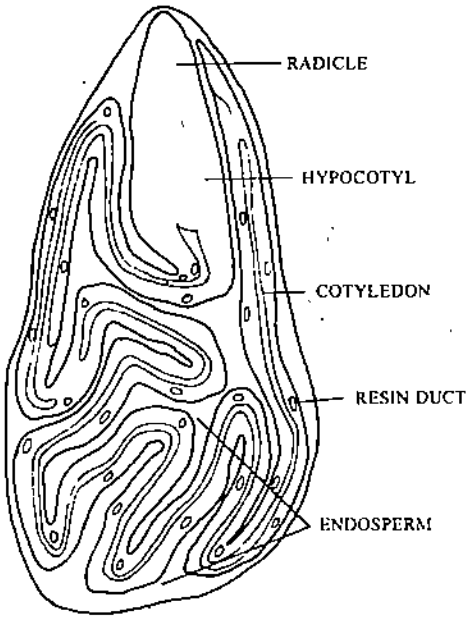


Fig. 6.14: L.S. of cotton seed kernel showing embryo with intricate folds of the cotyledons dark dots are glands.

The embryo in a germinating seed has critical requirements to mobilize food reserves. It needs a source of carbon skeletal precursors, and a source of energy to assemble the precursors for building complex compounds. Energy is not only provided by carbohydrates but also by the stored lipids. Their oil content varies from 30% of dry weight in sunflower to 50% in castor and groundnut and even higher in coconut and oil palm.

Carbohydrates may be stored in the seed by way of thickened walls of the endosperm (as in the date palm and coffee beans) or cotyledons (in balsam and garden nasturtium). More often, cells of endosperm and cotyledons contain starch grains. Cereal grains such as wheat and rice contain 70-80% starch, mainly in the endosperm. In beans, cotyledonary cells may have as much as 50% starch.

Seeds are often described as starch type (cereals) or oil type (castor, linseed). However, nearly all seeds have in addition protein reserves for supplying nitrogenous compounds to the young seedling till it becomes capable of absorbing nitrogen from the soil with the help of roots. Protein reserves are also needed for rapid synthesis of enzymes

required for digestion of starch at the time of germination. Storage proteins occur in the form of discrete protein bodies, often termed aleurone grains. These grains also contain some minerals. Protein bodies occur in the endosperm and embryo in most plants. Soybean seeds are particularly rich in protein content. However, in some others such as the cereal grains these are concentrated in the specialized outer layer of the endosperm called the aleurone layer. The aleurone protein bodies become active during seed germination, triggered by the gibberellins released by the embryo. This results in the production of the enzyme α-amylase which digests starch present in rest of the endosperm. Grain legumes are rich in reserve proteins. For example, groundnut contains about 25% and soybean nearly 40% protein.

SAQ 3

State whether the following statements are true or false by indicating T or F in the box provided.

a) In a majority of seeds the endosperm is the main reservoir of nutrients. [ ]

b) Leguminous seeds have well-developed perisperm. [ ]

c) Castor seeds are exalbuminous. [ ]

d) Food reserves in the seed are meant for tiding over the period of dormancy. [ ]

e) Proteins occur in only those seeds which lack starch and lipids. [ ]

f) In wheat barley grains proteins occur mainly in the cells of the outermost layer of the endosperm called the aleurone layer. [ ]

g) During seed germination, enzymes responsible for digestion of starch are produced in the seed itself. [ ]

6.5 DEVELOPMENT OF FRUIT

Concurrent with the development of the seed(s), the ovary is transformed into a fruit. The fruit protects the seeds and allows their release or germination. In primitive families such as the Magnoliaceae the fruit opens while still on the plant and the seed itself is the unit of dispersal. However, in most of the flowering plants the function of dispersal is at least partly transferred to the fruit.

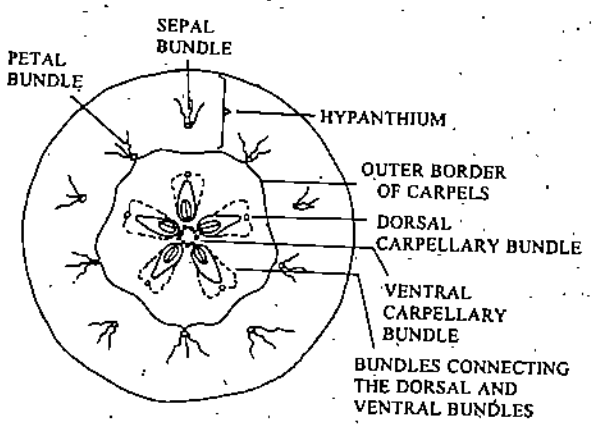


Fig. 6.15: Cross-section of the fruit of *Pyrus malus* (diagrammatic representation).

A true fruit develops from the carpel, specifically from its ovary. However, in many so-called fruits, organs or tissues in addition to those of the ovary participate in protection and dispersal of seed. Examples of accessory tissues or organs contributing to fruit formation are many. In strawberry, *Fragaria* spp. the floral receptacle extends to form the fleshy edible part of the fruit. In apple, *Pyrus malus* the floral tube formed by the floral organs and the receptacle around the inferior ovary, together constitute the bulk of the fruit (Fig.6.15). In both these instances the edible fruit is product of carpellary and accessory tissues. On the other hand, in jackfruit, *Artocarpus integrifolia*

the perianth and in pineapple, *Ananas comosus* the bracts surrounding the flowers in an inflorescence proliferate to contribute to formation of the fruit. Where organs other than gynoecium participate in forming a fruit, the fruit is termed a false fruit or pseudocarp.

The wall of a true fruit is termed pericarp. The mature pericarp is often made up of three distinct regions. In mango, for instance, the outer skin or peel represents the exocarp or epicarp. The fleshy and juicy middle portion is the mesocarp. The inner shell or stone is formed by the endocarp.

Fruits of different plants display a rich diversity in size, shape, structure, and hardness. Chemical constituents and dispersal mechanisms. From the morphological standpoint they are classified into a few types based on two criteria. The main criterion is the degree of hardness of the fruit wall or pericarp whether it is dry and hard or soft and fleshy. The second criterion is the ability of the fruit to dehisce or remain intact after ripening. Based on these criteria some important types of fruits are classified in Table 6.1.

Table 6.1: Types of Fruits

Dry fruits

Indehiscent fruits

Developing from a single carpel

1. Achene. Contains only one seed which is loose inside the fruit (buttercup).
2. Caryopsis. It is like an achene but the pericarp and the testa of the single seed become fused (wheat, corn).
3. Samara. A winged one-seeded fruit (maple).

Developing from a compound gynoecium with several carpels

4. Nut. A single-seeded fruit that develops from an ovary that originally contains several carpels of which all but one degenerate. Mature nut has one carpel and one seed (walnut).

Dehiscent Fruits

Developing from a single carpel

5. Follicle. A pod-like fruit that splits open on the ventral side (larkspur).
6. Legume. The pod splits open on both ventral and dorsal sides (pea, beans).

Developing from a syncarpous ovary with two or more carpels.

7. Capsule. The dehiscence is along fusion lines of carpels (*Hypericum*), along dorsal bundles or lines of dehiscence in each carpel (*Iris*), by splitting transversely into top and bottom portions (*Primula*) or by small pores which develop in the pericarp (below the persistent but dried stigma poppy).
8. Siliqua. A pod-like fruit of two carpels with a false septum dividing the locule. When the fruit ripens the two valves separate and the seeds remain attached to the septum (mustard).

Fleshy Fruits

9. Berry. Exocarp, mesocarp and endocarp are distinguishable but soft or fleshy. The fleshy pericarp encloses one or many seeds (grapes, tomato).
10. Drupe. Like a berry except that endocarp is thick and hard (peach, mango).
11. Pepo. Like a berry, but the exocarp is hard forming a rind (pumpkin, squash).

Schizocarpic Fruits

There are fruits that develop from multilocular ovaries that separate when ripe into individual achenes, each representing a carpel (*Malva, Abutilon*).

Development of fruit can be studied by selecting a few representative examples of follicle, caryopsis, legume, capsule, berry and drupe.

1. Follicle. In larkspur, *Delphinium* sp. the exocarp develops from the outer epidermis and sometime the hypodermis of the ovary wall. It consists of thick-walled cells (Fig. 6.16). Mesocarp is parenchymatous. The endocarp, formed from inner epidermis, consists of thick-walled cells. At maturity the pericarp dries up and the follicle opens along the line of fusion of the carpels.

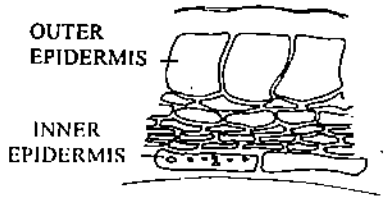


Fig. 6.16: T.S. of the follicle wall of *Delphinium*.

2. Caryopsis. In cereals each carpel has one ovule and therefore the mature fruit has, just one seed. During maturation, very little or no cell divisions are necessary in the ovary wall. The pericarp and the remains of the integuments of the seed get completely fused. In wheat caryopsis three main regions can be distinguished: (i) the caryopsis wall which includes the pericarp, seed coat and remains of nucellus; (ii) endosperm; (iii) embryo (Fig. 6.17). The pericarp can be distinguished into five layers (Fig. 6.18): (i) epidermis; (ii) hypodermis; (iii) zone of thin-walled cells; (iv) cross cells; (v) tube cells. The outer epidermis and hypodermis together form the exocarp, having thick-walled compressed cells. Inside the exocarp are one or a few layers of thin-walled parenchymatous cells. These are followed by the cross cells that have thick walls with characteristic pits elongated transversely to the cell. The tube cells constitute the inner epidermis of the pericarp. These cells have thinner walls than cross cells but these too are pitted. In the mature caryopsis testa is destroyed but tegmen is discernible along with one or two layers of nucellus.

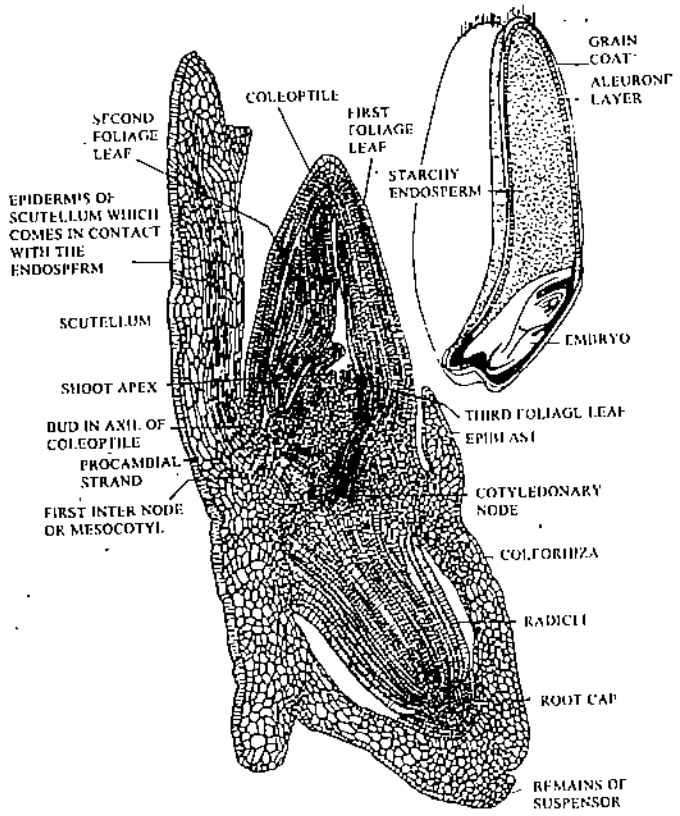


Fig. 6.17: A L.S. of Grain of *Triticum* B. L.S. of embryo of *Triticum*.

3. Legume. Outer epidermis of the ovary usually forms the exocarp of the leguminous pod. Next few cell layers constitute the mesocarp with thick-walled parenchyma. The endocarp consists of sclerenchyma on the inside of which is epidermis or a few layers of parenchyma and epidermis. In *Astragalus macrocarpus*, for example, inside the sclerenchymatous part of endocarp there is a thin-walled hypodermis and an

epidermis (Fig.6.19). Vascular bundles are located in the mesocarp accompanied by some sclerenchymatous cells.

The two valves of the dried legume usually twist, resulting in their opening and scattering of seeds. The twisting and opening of valves is brought about by shrinkage of the sclerenchymatous cell layers which are obliquely oriented.

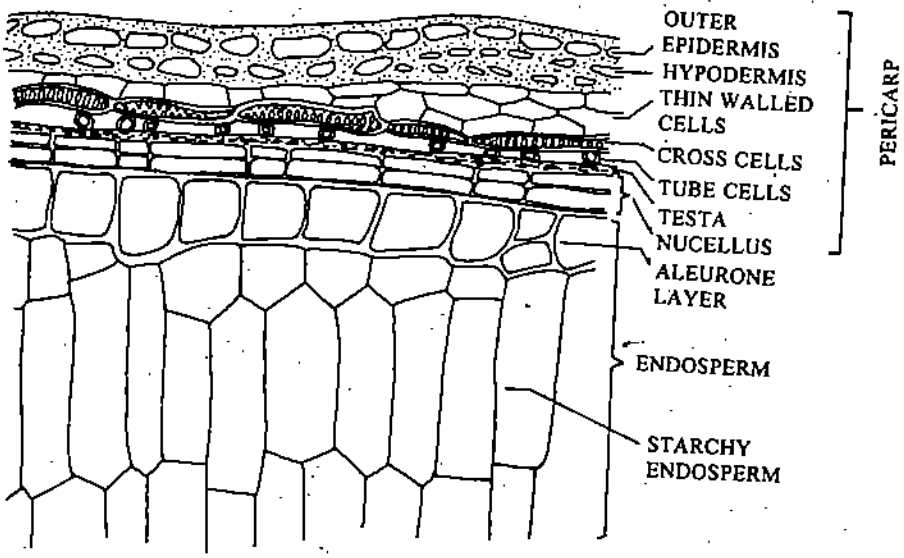


Fig. 6.18: Portion of C.S. through the caryopsis of *Triticum* at mature stage of development.

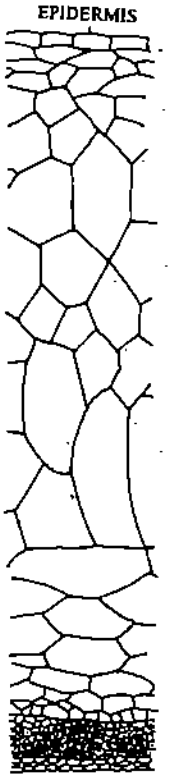


Fig. 6.19: *Astragalus macrocarpus* (oblique section).

4. *Berry.* In a berry much of the pericarp is fleshy or juicy. In tomato, *Lycopersicon esculentum* even the placenta on which the seeds are borne is fleshy (Fig. 6.20). The exocarp consists of an epidermis and 3 or 4 layers of collenchymatous cells. The epidermis is covered by a cuticle. Mesocarp consists of large, thin-walled cells with abundant intercellular spaces. There is a large increase in cell volume in this zone of pericarp. With the development of ovules, after pollination, the parenchymatous tissue of the placenta grows around the funiculi. This parenchymatous tissue continues to proliferate further and envelops the seeds completely.

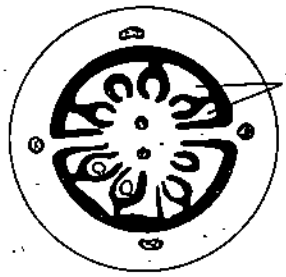


Fig. 6.20: Diagrammatic representation of T.S. of *Lycospersion esculentum* after fertilization.

5. Drupe. In the drupe of peach, *Prunus persica* most of the cell divisions occur in the ovary wall before fertilization or soon after it. Further growth of the fruit is accomplished mainly by cell enlargement. To begin with, cell enlargement occurs in all directions, but later expansion is mainly in radial direction. This is more so in the inner portion of the mesocarp. At maturity the outer epidermis has thick cuticle and unicellular hairs. Mesocarp has loose parenchyma. Endocarp has sclereids and forms the stone of the fruit.

SAQ 4

State whether the following statements are true or false by indicating T or F in the box against each.

a) A true seed develops within a carpel. [ ]

b) Apple is called a false fruit because it has no fertile seeds. [ ]

c) In mango the entire pericarp is fleshy and the seed coat is hard. [ ]

d) In the caryopsis of wheat the pericarp and seed coat are fused together. [ ]

e) A follicle opens along the line of marginal fusion of the carpel . [ ]

f) In a tomato fruit only the endocarp is edible. [ ]

6.6 DISPERSAL OF SEEDS

A plant usually bears many fruits and innumerable seeds. If all the seeds produced by a plant were to germinate in the immediate vicinity, this will have several disadvantages. The resulting seedlings would compete intensely among themselves for space, light, water and minerals. They will be more vulnerable to attack by pests and pathogens. Moreover, there will be greater chances for backcrossing, in the resulting progeny, rendering them genetically inferior.

To overcome these problems most plants have evolved one or another mechanism for dispersal of seeds over a wide area. Fruits of some plants have built-in mechanism for dispersing their seeds to considerable distances (autochory). Other plants depend on external agencies such as air (anemochory), water (hydrochory) and animals (zoochory) to disseminate their fruits or seeds.

1. Autochory. This mechanism of self-dispersal is based on forceful expulsion of the seed from the fruit because of desiccation or turgidity of the cells of the pericarp. In balsam, *Impatiens* spp., for example, the fruit is a cylindrical capsule formed by the fusion of five carpels (Fig. 6.21 A). The fruit wall comprises three regions of which the middle is made up of radially elongated cells with high turgor pressure (Fig. 6.21 B). This zone is termed expansion zone. In the dry, ripe capsule the cells of the expansion zone are in a state of high tension. However, the inner portion of the pericarp consists of 2 or 3 layers of collenchyma which offer resistance. At this stage even a mild touch or jerk results in separation of the carpels at the base (Fig. 6.21 C). The five carpels instantly curl inward throwing the seeds at a distance of about 2 metres.

In *Arcenthobium* sp. the fruit is a pseudoberry enclosing a single seed. The mesocarp is formed by viscin cells. When the fruit is detached from its stalk the cells of the viscid

layer generate a high pressure and the pericarp contracts to hurl the seed out with great force (Fig. 6.22).

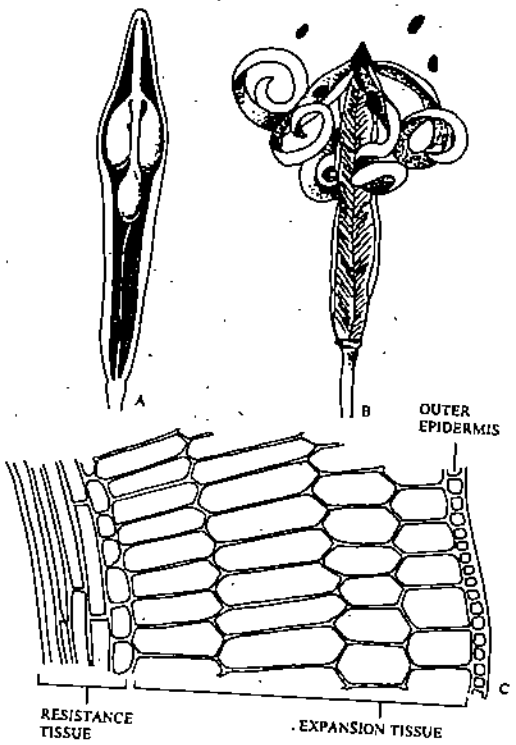


Fig. 6.21: A L.S. of closed fruit of *Impatiens*. L.S. through pericarp showing elongated cells of middle region with high turgor pressure. A fruit the valves of which have curled inwards and thus have ejected the seeds.

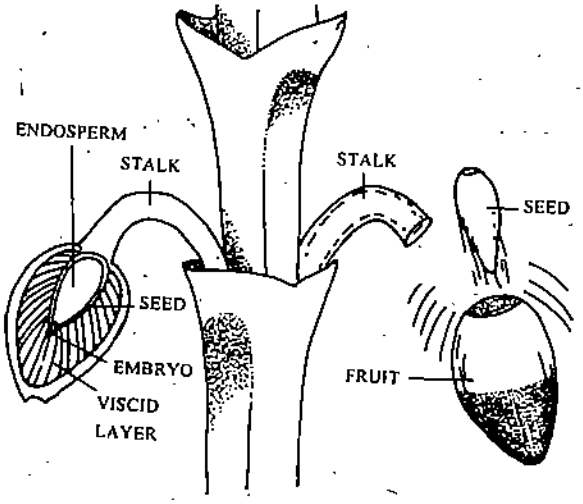


Fig. 6.22: Diagrammatic representation of explosive seed discharge in *Arcenthobium*.

2. Anemochory. Seeds that are dispersed by air currents are usually light or are provided with special structures to help them remain air-borne for long periods. Since a large proportion of seeds are wasted, anemochorus plants usually produce a profuse quantity of seeds per plant. Some of the orchids, for example, produce as much as seven hundred million seeds per plant and the seeds are so tiny that these are blown away like dust particles. The orchid seeds have an undifferentiated embryo and lack endosperm (Fig. 6.23).

You have studied earlier in this unit about seeds and fruits of several species with hairs or wings that propel them even in mild wind.

Winged fruits and seeds are a characteristic feature of some tall trees. In maple the wings represent expansions of the pericarp.

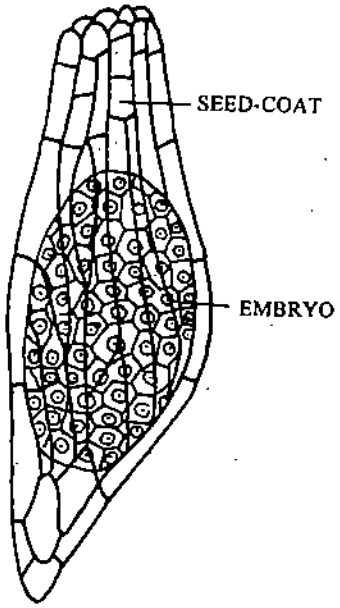


Fig. 6.23: A seed of *Cypripedium*. Embryo can be seen without endosperm through transparent seed-coat.

3. Hydrochory. Plants that grow in or along the bank of water bodies often utilise water as an agency for dissemination of fruits and seeds. Coconut, *Cocos nucifera* is an excellent example of a fruit which has spread to different continents because of its ability to float over hundreds or even thousands of kilometres. The fruit has a smooth, waterproof exocarp, followed by a fibrous and air-filled mesocarp and a hard endocarp. The seed with a thin seed coat retains its viability for more than three months. During this period the fruit can travel as long as 4300 km. Seeds of lotus and some cypresses have air-filled cortical tissues.

4. Zoochory. Some fruits are eaten by animals and the seeds are passed out with the excreta (endozoochory). Plums, *Lantana*, grapes, figs and guava are examples of some of the fruits eaten by birds. Fleshy part of the fruit is digested in the gut of the bird and the seeds are expelled along with the droppings. Using the excreta as manure, many of such seeds germinate. It is believed that the seeds of *Ficus* species germinate only after they have passed through the gut and have been subjected to the scarification in the gullet by small pebbles and digestive fluids in the alimentary canal.

In plants like neem, maulsari etc., the seeds are too large. The birds eat the pulp and discard the seeds below their perch. Fruits and seeds of many other plants are carried by animals externally (exozoochory), sticking to their body or mouth parts. Seeds of mistletoe, (*Viscum album*) are dispersed widely because they adhere to the beaks of birds feeding on its fruits. Fruits of many members of the Asteraceae have spines (e.g., *Xanthium*) or hooks (*Bidens*) with the help of which they stick to the bodies of animals and get distributed over a large area.

Mammals like squirrels, monkeys and even goats (these are used by farmers to encourage germination of seed species of *Acacia nitotica* (feeding pods) play an important role in dispersal. Humans serve as active agents of deliberate distribution of seeds to raise plants useful to them.

6.7 PARTHENOCARPY

It is generally observed that the fruit develops after fertilization and it has fertile seeds inside it. However, this is not always so. Fruits of certain varieties of plants, such as edible banana (*Musa* sp.), tomato (*Lycopersicon esculentum*), orange (*Citrus* sp.) and

grapes (*Vitis vinifera*), develop without seeds. In the seed-bearing bananas the three locules of the berry are occupied by large seeds, whereas in the parthenocarpic varities, the ovules degenerate and the cells of the pericarp and septae proliferate to form the pulp regions (Fig. 6.24). The phenomenon of formation of fruit without fertilization is known as parthenocarpy. Parthenocarpic development may require pollination (stimulative parthenocarpy), or it may occur even without pollination (vegetative parthenocarpy).

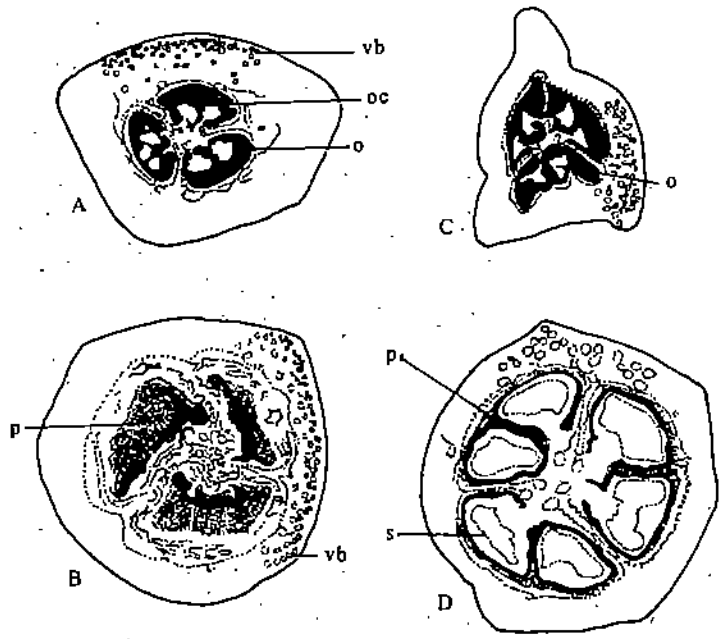


Fig. 6.24: T.S. Fruits of developing parthenocarpic and seed bearing varieties of banana. (a) Parthenocarpic fruit at the time of emergence of the inflorescence. (b) Parthenocarpic fruit 8 weeks after emergence, showing the pulp (p) invading the ovarian cavity (oc) or locule. (c) Seeded fruit at the time of emergence (d) Seeded fruit 8 weeks after emergence. Very little pulp is present around the locules, which are occupied by the enlarging seeds. o, ovule; s. seed; vb, vascular bundle.

Three types of parthenocarpy are generally recognised: (i) genetical, (ii) environmental, (iii) chemically-induced.

Genetical parthenocarpy is observed in many species cultivated for their fruit. It arises due to mutations or hybridization. Generally parthenocarpic varieties have sterile pollen so that the pollination stimulus is available, but fertilization does not take place. As a result, a fruit is produced which has no seeds. The famous navel orange has arisen from a normal seeded *Citrus* variety through mutation in an axillary bud, which formed a branch bearing seedless fruits. Several cultivated varieties of grapes and cucumber have also resulted from bud mutations.

Environmental parthenocarpy is the result of some environmental conditions such as frost or low temperature which interferes with the normal reproductive process. In tomato, for instance, cultivation under low temperature and high light intensity can induce parthenocarpy. Under these conditions pollination is so poor that seeds are not produced but ovary is activated to form the fruit.

Induced parthenocarpy involves treatment of the flowers with certain plant growth regulators. Auxins and gibberellins at low concentrations (about 10^{-7} - 10^{-6} M) have been successfully utilised for induction of parthenocarpy in a large number of plants which otherwise bear seeded fruits. Seedless guava, tomato, and strawberry fruits have been obtained by this method.

Parthenocarpy is important in horticulture because seedless fruits are more convenient to consume and are particularly suitable for the industry manufacturing jams, jellies and fruit juices. Gibberellins also cause fruit enlargement (in grapes which are considered commercially beneficial for packaging and marketing, and also cause looseness of bunches).

6.8 VIVIPARY

In flowering plants the seed or fruit generally is dispersed and germination occurs when the conditions are congenial for growth. However, in some plants growing along the sea shores or in mangrove areas the soil is too saline or salty for seed germination. Moreover, in such places the seed and the young seedlings are likely to get washed away by the tide. Mangrove plants (e.g., *Rhizophora* spp.) have, therefore, evolved a unique adaptation for ensuring seed germination and nurturing of the young sporophyte. The young seedling grows out of the intact fruit and hangs with its pointed radicular end facing downward. Once the seedling attains a large size it drops down and penetrates the soil. Roots are produced rapidly by the radicle and the seedling is fixed firmly in the soil. By this time the seedling would have developed an extensive photosynthetic tissue to ensure its establishment and has been compared to the similar state observed in mammals. Certain bamboos and forest trees also practice vivipary.

This mechanism of rearing the young one by the parent is termed vivipary. It is a specialized characteristic evolved by mangrove plants as a strategy for survival.

SAQ 5

Fill in the blanks in the statements given below with appropriate words.

a) Dispersal of seeds by means of wind is termed ..... .

b) ..... involves built-in mechanism in the fruit for expulsion of seed.

c) Seeds of the banyan tree are mostly dispersed by ..... .

d) Edible banana is a ..... fruit.

e) ..... and ..... are plant growth regulators employed for induction of parthenocarpy.

f) Vivipary is observed in ..... plants.

6.9 SUMMARY

In this unit we have studied the development of fruits and seeds. You have seen that the fruit and seed structure display immense diversity. We have examined the nature of reserve materials and the sites of their storage in the seed. The interesting aspects of the range of seed dispersed agencies have been discussed. The formation of seedless fruits (parthenocarpy) and their importance in horticulture have been explained. The rather rare phenomenon of vivipary in plants has been highlighted as an adaptive feature by the mangroves.

What we have learnt can be summarized as follows:

- A true seed is a fertilized ovule. It contains an embryo and generally the endosperm. These are enclosed by the seed coat derived from the integuments.
- Seed protects the young sporophyte and serves as an efficient propagule.
- One or both the integuments form the seed coat. A layer called palisade or sclerotic layer is usually differentiated which forms the protective shell.
- In some plants Perisperm (persistant nucellus) forms an additional nutritive tissue. In many seeds, principally leguminous seeds, the cotyledons perform the function of storing reserve food.
- Seeds may have appendages such as aril, caruncle, operculum or wings and hairs which help in their dispersal.

- Carbohydrates (in the form of starch and cell wall materials), lipids and proteins are the chief sources of nutrition for the germinating embryo and young seedling.
- A true fruit develops from the gynoecium as a result of stimulus provided by pollination and/or fertilization.
- Fruits are classified on the basis of their water content (fleshy or dry), number of carpels and locules, number of seeds and importantly on the criterion of their ability to dehisce or not.
- Fruits and seeds are variously adapted for self-dispersal (autochory), or dispersal by agencies such as wind (anemochory), water (hydrochory) and animals (zoochory).
- Parthenocarpy involves formation of fruits without fertilization so that no fertile seeds are produced. Varieties of horticultural plants can evolve by mutations or hybridization to bear seedless fruits. Parthenocarpy can also be induced by environmental factors and application of growth hormones regulators.
- Vivipary is a unique phenomenon observed chiefly in mangrove plants. It involves nurturing and germination of the young sporophyte while the fruit is still attached to the parent plant.

The study of fruits and seeds becomes fascinating by a visit to a garden, crop field, open land or a forest. You will need observant eyes, probably a knife and of necessity a magnifying glass. The types of fruits and seeds you have studied in this Unit can be found in your immediate surroundings, some even in a vegetable market or kitchen garden! The spectacle of seed dispersal can be observed in nature at all places and at all time.

6.10 TERMINAL QUESTIONS

1) What are the chief advantages of the seed habit?
2) Which are the food storage tissues in the seed? In what form is the food stored?
3) Name some seed appendages and describe how they help in dispersal or germination of seed.
4) What is a true fruit? Why are apple and Jack fruit considered false fruits?
5) What are the chief criteria for the classification of fruits?
6) What parts of the seed or fruit is edible/useful in the following plants:
   i) banana
   ii) tomato
   iii) coconut
   iv) groundnut
   v) apple
   vi) litchi
   vii) cotton
   viii) castor
   ix) pineapple
   x) strawberry
   xi) pomegranate
   xii) mustard
7) How are the fruits/seeds of [illegible]ing plants dispersed? What type of adaptations have been developed by these [illegible]

i) coconut
ii) mistletoe
iii) banyan
iv) balsam
v) milkweed

8) What is the commercial importance of parthenocarpic fruits? How can seedless fruits be induced artificially?

9) What is vivipary? How does it help the mangrove species to survive in their saline/ estuarine habitat?

6.11 ANSWERS

Self Assessment Questions

1. a) True
   b) False
   c) True
   d) False
   e) True
   f) True
   g) False
2. a) aril
   b) ants
   c) third integument
   d) three
   e) inner integument or nucellus
   f) Apocyanaeae and Asclepiadaceae
   g) wing
   h) Euphorbiaceae
3. a) True
   b) False
   c) False
   d) False
   e) False
   f) True
   g) True
4. a) True
   b) False
   c) False
   d) True
   e) True
   f) False
5. a) anemochory
   b) autochory
   c) birds
   d) parthenocarpic
   e) auxins and gibberellins
   f) mangrove

Terminal Questions

1. The seed not only protects and nurtures the young sporophyte, but also serves as a propagule for wider distribution. It may remain viable for long periods, or stay dormant till conditions are suitable for germination and growth of the seedling.

2. Endosperm, cotyledons, perisperm and rarely the chalazosperm are the food storage tissues of the seed. Carbohydrates (in the form of starch and wall materials), lipids and proteins are the chief stored food reserves.

3. Aril is consumed by birds and the animals and the discarded seed gets dispersed widely. The caruncle is sought after by ants, which carry the seeds far from where they have fallen after dehiscence of the fruit. In some aquatic plants the spongy aril provides buoyancy to the seed to stay afloat and travel long distances. During seed germination, the operculum (a lid-like structure at the micropylar part of seed) gets detached and facilitates the emergence of embryo to form the seedling. Wings and hairs also help in dispersal of seed by wind.

4. A true fruit develops from a carpel. Apple is regarded a false fruit because the floral tube and the receptacle around the inferior ovary also contribute to the fruit wall. In the fruit of the jack tree the perianth of the flowers in the inflorescence also proliferate and contribute to the edible portion.

5. The chief criteria for classification of fruit are: degree of hardness, number of carpels and locules; number of seeds per locule; ability of the fruit to dehisce or stay intact.

6. i) Mesocarp, endocarp and placentae

   ii) The whole fruit (sometimes including seed) minus the persistant calyx.

   iii) The whole seed.

   iv) Whole seed (sometimes without seed coat, and sometimes only cotyledons).

   v) Hypanthium (basal portions of perianth and stamens) and the fleshy receptacle

   vi) Aril

   vii) Lint hairs yield textile fibre fuzz hair which are used for high class cellulose acetate/nitrate and the cotyledons give oil. The oil cake is used as manure or a medium for fungi/bacteria in industry or as source of gossypol.

   viii) Endosperm

   ix) Bracts

   x) Receptacle of the fruit (flower) plus individual fruits developed from all the carpels (including seeds)

   xi) Testa

   xii) The whole seed is crushed for oil.

7. i) by sea water; impervious exocarp, buoyant mesocarp and hard endocarp

   ii) by birds; seeds are sticky

   iii) mostly by birds; the figs (*Syconia*) consumed by birds, and the tiny fruits bearing hard seed (one each) emerge ready for germination

   iv) autochory; high turgor pressure in the mesocarp

   v) by wind; seed hairs

8. Seedless fruits are more convenient to consume. These are particularly suitable for fruit preservation industries.

Seedless fruits can be evolved by mutations or hybridization and environmental conditions such as low temperature. Application of growth regulators can induce parthenocarpy in some plants.

9) Vivipary involves germination of seed and nurturing of the young sporophyte in the fruit while it is still attached to the parent plant. It is an adaptation to the peculiar environmental conditions that prevail in mangroves ecosystems. If a seed of mangrove is liberated, it is likely to be carried away by the tidal waters. Moreover, germination of seed and growth of young seedling may be seriously impaired by the high salt content. As an adaptation the seedling in mangrove plants separate from the parent only when it is sufficiently large and capable of establishment in saline water as an autotroph.

GLOSSARY

androecium : male reproductive organs of a plant; stamens taken collectively

archesporium : a cell or mass of cells, dividing to form microspore mother cells.

anticlinal : line of division of cells at right angles to the surface of apex of a growing point.

apomixis : a reproductive process without fertilization in plants, akin to parthenogenesis but including development from cells other than ovules, as apogamy and apospory.

cleistogamy : the condition of having flowers which never open, and are self pollinated.

dehiscence : the spontaneous opening of an organ or structure along certain lines or in a definite direction.

embryogeny : the process by which the embryo is formed.

embryo sac : the megaspore in angiosperms, containing the female gemetophyte.

endosperm : the nutritive tissue of most seeds.

endothecium : one of the wall layers of anther that helps in the dehiscence of the anther.

endothelium : synonymous-integumentary tapetum; specialized cells having nutritive role, these develop from the inner-most layer of the integument and closely surround the nucellus.

generative cell : the smaller of the two cells of the pollen grain, divides and forms the sperms.

haustorium : an outgrowth of embryo sac which extends to the nutritive tissue to draw nutrition.

indehiscent : fruits which do not open to release seeds, but the whole fruit is shed from the plant.

massula : a group of pollen grains that occur in a mass.

megaspore : it develops to form the female gametophyte, or the embryo sac.

microsporangium : pollen sac; a sporangium containing a large number of microspores.

microspore : the cell from which pollen grain — the male gametophyte, develops.

parthenogenesis : reproduction without fertilization by male gamete.

periclinal : division parallel to the surface of the cell, or the apex of the growing point.

pollen tube : a tubular structure developed from pollen grain after pollination, it grows towards the ovule, carrying male gametes to the embryo sac.

polyembryony : formation of several embryos in one ovule.

proembryo : an embryonic structure preceding true embryo.

suspensor : a chain of cells developed from hypobasal segment of angiosperm zygote; it attaches embryo to the embryo sac.

tapetum : nutritive layer investing the sporogenous tissue in a sporangium; it supplies nutrition to the developing microspores.

tetrad : 4 spores formed after the Ist and 2nd meiotic division of the microspore mother cell.

FURTHER READING

1. Bhojwani, S.S. & Bhatnagar, S.P. 1993. *The Embryology of Angiosperms.* Vikas Publishing House Pvt. Ltd., New Delhi.

2. Maheshwari, P. 1950. *An Introduction to the Embryology of Angiosperms.* Tata-McGraw Hill Publishing Company Ltd., New Delhi.

3. Shivanna, K.R. & Johri, B.M. 1985. *The Angiosperm Pollen: Structure and Function.* Wiley, New Delhi.,

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-06
Block-1

Enrolment No.

1) How many hours did you need for studying the units?

Unit Number	1	2	3	4	5	6
No. of hours						

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

Assignment Number	1	2
No. of hours		

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

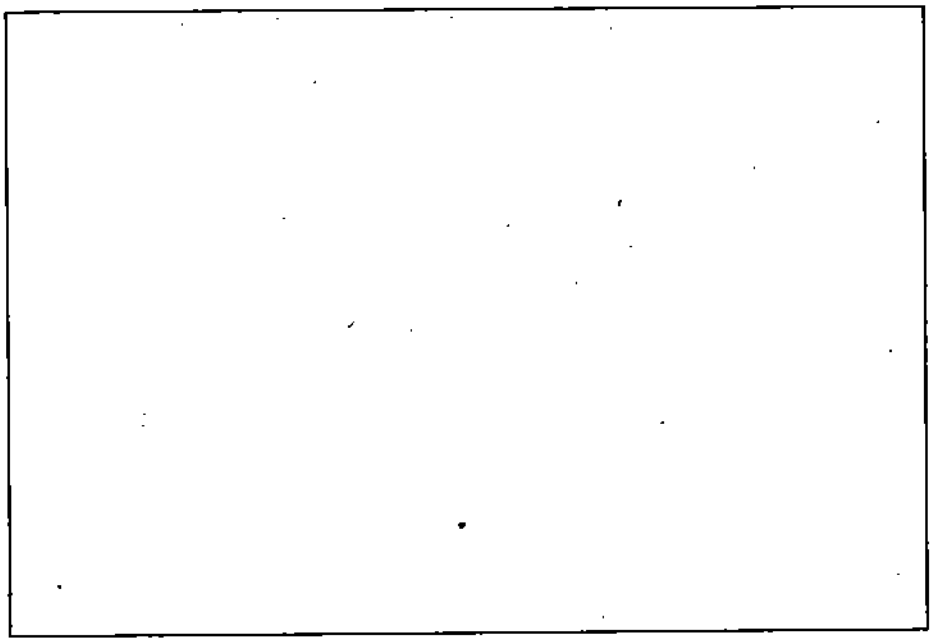
Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

4) It is possible that you could not attempt some SAQs and TQs. In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Type of difficulty			
			Not clearly posed	Cannot answer on basis of information given	Answer given (at end of unit) not clear	Answer given is not sufficient

5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

6) Any other suggestion(s)



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To

The Course Coordinator (LSE-06, Developmental Biology)
School of Sciences
Indira Gandhi National Open University
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NOTES

NOTES

UTTAR PRADESH
RAJARSHII TANDON OPEN UNIVERSITY

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Developmental Biology

Block

2

PLANT DEVELOPMENT-II

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UNIT 10
Secondary Growth 52

UNIT 11
Plant Tissue and Organ Culture 85

UNIT 12
Current Trends in Developmental Studies 111

BLOCK 2 PLANT DEVELOPMENT-II

The entire developmental span of flowering plants can be categorised either into vegetative or reproductive phase. Several processes become operative during these two phases. Now that you have a fairly good background about the structures and the events associated with sexual reproduction, i.e., upto the formation of embryo, let us know more about the post-embryonic developmental processes in flowering plants.

Unit 7–Root And Shoot Morphogenesis. The vegetative growth of plants is an extremely complicated but well coordinated series of events that begins with the growth of the embryo. Even before the embryo begins to grow, the zygote formed by fusion of male and female gametes, marks the begining of polarity that sets the stage for the formation of the mature plant. The initial stages of growth begin by cell division and subsequent cell elongation, followed by differentiation. As cell division, elongation and differentiation proceed, morphogenesis of the principal plant parts, that is, root and shoot occurs. In this unit you will study about the root apex, shoot apex, formation of leaf primordia and floral induction. Also you would learn about the polarity, tropic movements and regeneration in plants.

Unit 8–Effect Of Plant Growth Regulators On Development. During the natural course of development, several kinds of organic molecules come into play. These substances commonly known as hormones are required in a very low concentrations and their action may be involved in sites far removed from their place of origin. Presently, the term growth regulator instead of hormones is widely used. It includes all the naturally occurring and synthetically created substances that affect growth and development in plants. In this unit we have discussed physiological effects of naturally occurring plant growth regulators and their role in organogenesis. We have discussed also the orderly cycle of development that the whole plant undergoes namely dormancy, senescence and abscission.

Unit 9–Apical Dominance. The phenomenon of apical dominance includes the influence of the shoot apex on the development and positioning of leaves, axillary shoots, stolons, tubers, rhizomes and roots. The correlative inhibition of lateral buds by the apex has been investigated experimentally for over a century and an awareness of the dominating role of the apex in gardening, horticultural and pruning practices has been well known and it is discussed in this unit. We have also discussed the role of chemical factors namely auxins, cytokinins and ethylene in controlling apical dominance.

Unit 10–Secondary Growth. The activity of the root and shoot meristems results in the production of the primary body of the plant. In many instances, the primary body constitutes the entire plant, but many times it undergoes further development and increases in girth to form the secondary body. This additional component of growth is designated as secondary growth. It is brought about by the activity of the vascular cambium, which normally cuts off xylem on inner side and phloem on outer side. Besides the vascular cambium, another cambium known as cork cambium is also found which forms layers of protective tissue in the plant body. In this unit you will learn about the secondary growth in plants as well as some cambial variants.

Unit 11–Plant Tissue and Organ Culture. In this unit we have discussed aseptic growth of cells, tissues and organs in artificial media. You will come to know that all living cells in a plant body irrespective of their ploidy level and the form of specialization can give rise to whole plants – cellular totipotency. Tissue culture has become an invaluable aid in the field of experimental botany and has acquired many practical applications in agriculture and horticulture.

Unit 12–Current Trends In Developmental Studies. People all over the world utilise the crop plants as their staple food. What we consume as food is derived mostly

from the reproductive structures. A good harvest depends largely on the success of plant reproduction. In the recent years there has been a tremendous impetus in research concerning sexual reproduction. The last unit of this block apprises you of the important breakthroughs in this area. You would study about some of the recent findings in the field of pollen biology, incompatibility phenomenon, female gametophyte, embryo, suspensor and endosperm. The information provided in this unit pertaining to some of the frontier areas of research in plant developmental biology, when read in conjunction with the relevant aspects of plant reproduction discussed in Block-1, will enable you to obtain a comprehensive account of reproduction and development in plants.

Objectives

After studying this block you should be able to:

- record the major events associated with root and shoot morphogenesis;
- discuss the role of plant growth regulators in the various developmental processes in plants ;
- describe the phenomenon of apical dominance and discuss its importance in horticulture;
- explain the structure and function of vascular and cork cambium and some cambial variants;
- explain the role of plant growth regulators in growth and differentiation of plant tissues in *in vitro* conditions;
- enlist the applications of tissue culture techniques;
- describe the recent advances made in the field of reproductive biology of higher plants.

Acknowledgement: Ms Usha Chawla and Ms Kiran for word processing.

UNIT 7 ROOT AND SHOOT MORPHOGENESIS

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7.1 INTRODUCTION

Plants, like animals can be divided into two groups on the basis of cellular organisation. 1) Prokaryotic: composed of one or many cells (organised into a colony), but cells are simple in structure and without an organised nucleus.2) Eukaryotic: composed of one or many cells organised into a colony or thallus or with a complete body structure like shoot and root with all the cells having an organised nucleus.

When a plant body consists of one cell (unicellular) or a colony of cells (multicellular) each cell carries on the functions, both vegetative as well as reproductive. But as the plant body acquires greater complexity greater is the need for specialised tissues, and organs. each meant for a special function. Leaves perform photosynthesis and transpiration. Roots anchor the plant and absorb water and minerals from soil. Shoot supports branches that bear leaves and flowers.

Flowers on fertilisation produce fruits. Fruits contain seeds which protect an embryo in side. On germination seeds(embryo) give rise to a new–offspring–that continues the material existence of the parent plant.

You know by now that embryo arises from the zygote essentially a diploid cell (2n)–which receives one set of chromosomes from female parent and one set of chromosomes from male parents. The whole plant is born out of mitotic divisions and differentiations of the zygote and its products. When a seedling grows up into a full plant, the capability to divide and to differentiate is restricted to a few regions like shoot apex, root apex,intercalary meristem, cambium, buds etc., despite the fact that everywhere we find living cells in all organs.

On the one hand the process of differentiation itself is quite a perplexing phenomenon and we really do not know satisfactory answer to all the questions that come to our mind. Also, a fundamental question that requires answer is why a cell irreversibly looses its ability to divide and differentiate on maturation.

At the same time 'in vitro' studies (plant cell and tissue culture), have amply proved by now that a living parenchyma cell from a carrot freed from its confinement within the plant body can divide and differentiate to produce a whole carrot plant when supplied with 'right type of mixture' of nutrients, hormones and physical factors at the right time. F.C. Steward's research has vindicated the dream of Haberlandt about totipotency of cells. We will read more about cellular totipotency in Unit-11 (Plant Tissue and Organ Culture) of this course (LSE-06).

The study of molecular , biochemical, physiological/cellular mechanism involved in this process of growth and differentiation of an embryo into a morphological form true to its genetic characteristics that can conduct all the functions (both vegetative and reproductive) is called morphogenesis.

Objectives

After studying this unit, you should be able to

- define the basic meaning of differentiation of root, shoot and leaf,
- explain the morphogenetic phenomena of root, shoot and leaf,
- appreciate the intricacies of mechanisms involved in these two processes,
- correlate the role of physical factors like light, gravity, temperature, touch etc. in affecting differentiation and morphogenesis and,
- list complexities involved in the process of emergence of a floral apex from a vegetative apex.

7.2 ROOT APEX

The mature embryo of higher plant is uniquely different from an embryo of an animal like mammal. For example, in case of mammalian embryo, by the time embryo growth is complete all the organs of mature individual are present in complete or at least in rudimentary form. On the contrary, in plants, relatively 'autonomous' growth centers are located in root, shoot, cambium and intercellary meristems. A fully grown embryo or an animal (foetus) like a cow or an elephant, when delivered into the outside world, not only does it resemble the fully grown mother but can just stand up on its four legs in a few hours. There is a definite size relationship between the adult and the young one. A seedling of banyan (***Ficus benghalensis***) tree hardly looks like its fully grown up counter part. Another striking difference is that the "young one" of a mammal is delivered after a predictable period by the mother. The plant embryo enclosed in a mature seed is in a state of inactivity. Capable of lying dormant and germinating after prolonged periods extending over several years.

A mature plant embryo is a product of genetic recombination of both parents. It is also an 'ecologically sound package' in the sense that there are inbuilt sensing devices to judge the external conditions.

Before really examining the structural intricacies of root apex and dynamics of its morphogenesis, let us look at certain bare facts. The area under the ground covered by root system of a plant is far more than that covered by over ground shoot system (i.e. axis, branches and leaves). As plants do not 'move', both water and minerals need to be fetched from greater depths and from greater distances from the site of the plant.

The apex of root is simple in structure. In general, the root apices are protected by a multilayered parenchymatous covering called the root cap. This apical meristem remains deep seated. In most monocots and dicots, the root meristem is divisible in three zones when seen in a longitudinal section (Fig. 7.1).

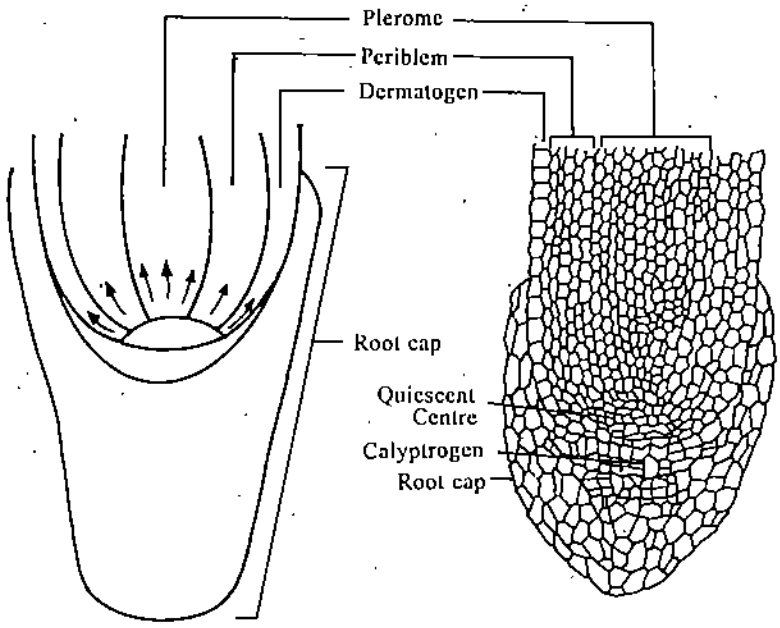

Fig. 7.1: Longitudinal section of maize root showing Four meristematic Zones and quiescent centre.

These include the plerome that gives rise to a vascular cylinder and pith; the dermatogen which produces the epidermis and root cap and the periblem which gives rise to the tissues between the epidermis and the vascular cylinder. In some monocots such as maize and garlic, there is found a fourth zone a meristematic cells which gives rise to root cap alone and is called calyptrogen. As the root grows downwards the layers and new cells are added by the activity of calyptrogen. A root apex does not form primordium like a shoot apex. Experiments in which root tips are exposed to x-rays have suggested that the ensuring chromosomal anomalies in the top cells can be passed on to all the new cells in the root. This limits the size of apex to 3 cells).

But when tritiated (3_H) thymidine (specific for its location in DNA) is applied to roots and autoradiographic tests were carried out, labelling could be noticed in many cells. These studies also point out to the presence of a 'quiescent center'. The quiescent center located just behind the tip of root and Comprising cells that do not divide actively is surrounded by a layer of actively dividing cells.

The cells in quiescent center are considered as a 'standby zone'. When the root apex is 'injured', the cells in quiescent center become meristematic. This is a quite an advantage when you realise that growing roots have to penetrate through hard terrains. It may also be a zone of hormone synthesis (cf: Unit 11).

7.2.1 Root Growth

In vitro studies by P.R. White have established that roots can be cultured in a medium containing sugars, salts and brewers yeast extract. Cytokinins seem to be needed for root growth.

7.2.2 Differentiation of Tissues

New cells generated from the divisions of meristematic cells start expanding and differentiating further. Epiderms, cortex and stele are formed. Stele is organised into xylem forming a star shaped central axis with columns of phloem between the points of the star, encircled by the pericycle. But what are the processes that control differentiation?

Certain experimental studies involving surgical removal of small portion of root tips and observing their differentiation in culture has thrown much light on root development. Smaller pieces while differentiating produced monoarch and diarch type vascular organisation even though originally the plant root showed triarch pattern. This experiment indicates that a definite size of root tip is needed for realization of its full morphogenetic potential. But on further growth even these small pieces revert back to normal pattern of organisation of vasculature. If auxin at 10^{-5}M concentrations was provided in the culture exarch vascular tissue was formed.

The ontogenic development of the primary vascular system of the root is simple. The differentiation of the root tissues behind the apical meristem is summarized as follows: Periclinal divisions in the cortex cease near the level where the sieve elements mature: beyond this region the root undergoes rapid elongation and the maturation of the protoxylem usually takes place when the process of elongation is almost completed: Casparian strips develop in the endodermal cells before the maturation of the protoxylem elements and also before the appearance of root hairs.

Phytohormones influence the development of secondary vascular tissue. When the roots are cultured on a nutrient medium containing Indole Acetic Acid (10^{-5}m) along with sucrose can induce secondary vascular tissue. Cytokinins and hexitols in small amounts promote secondary vascular tissue. Roots also depend upon vitamin 'B' for their proper growth.

7.2.3 Lateral Roots

Lateral roots normally arise at a definite distance behind the tip from areas close to or opposite the points of xylem star . So, a triarch root can have 3 rows of lateral roots and a tetrach root four rows of lateral roots. It is interesting to look at the growth of lateral root primordia through the tissue of parent root. In gymnosperms and angiosperms the lateral roots are generally initiated in the pericycle. Primordia are formed by the periclinal and anticlinal divisions of groups of pericycle cells. According to one view growing primordia partially 'digest' the tissues of cortex through which they pass. Another view point is that this process of penetration is essentially a mechanical one i.e. growing lateral roots essentially push through the tissues of the cortex. Relatively low concentrations of auxins and high concentrations of cytokinins near the tip inhibit lateral root formation (auxins are produced in shoot apex and are transported basipetally and cytokinins are synthesized in the root tip also). As the auxin concentration rises (as we go up) lateral root formation is induced.

7.3 SHOOT GROWTH

The apical meristem produces stem, branches, leaves appendages like stipules. In response to signals which may be genetic, age and maturation, or external stimuli like light or temperature, the apex becomes transformed into a reproductive apex. The branches and appendages arise as bumps on the apical dome.

The shoot apical meristem is difficult to visualize as it is deeply embedded in the leaf bases. One of the earliest methods of studying shoot apices is by serial sectioning. By dissecting out the leaves it is now possible to obtain a three dimensional view of the shoot tip with the help of scanning electron microscopy. (Fig. 7.2).

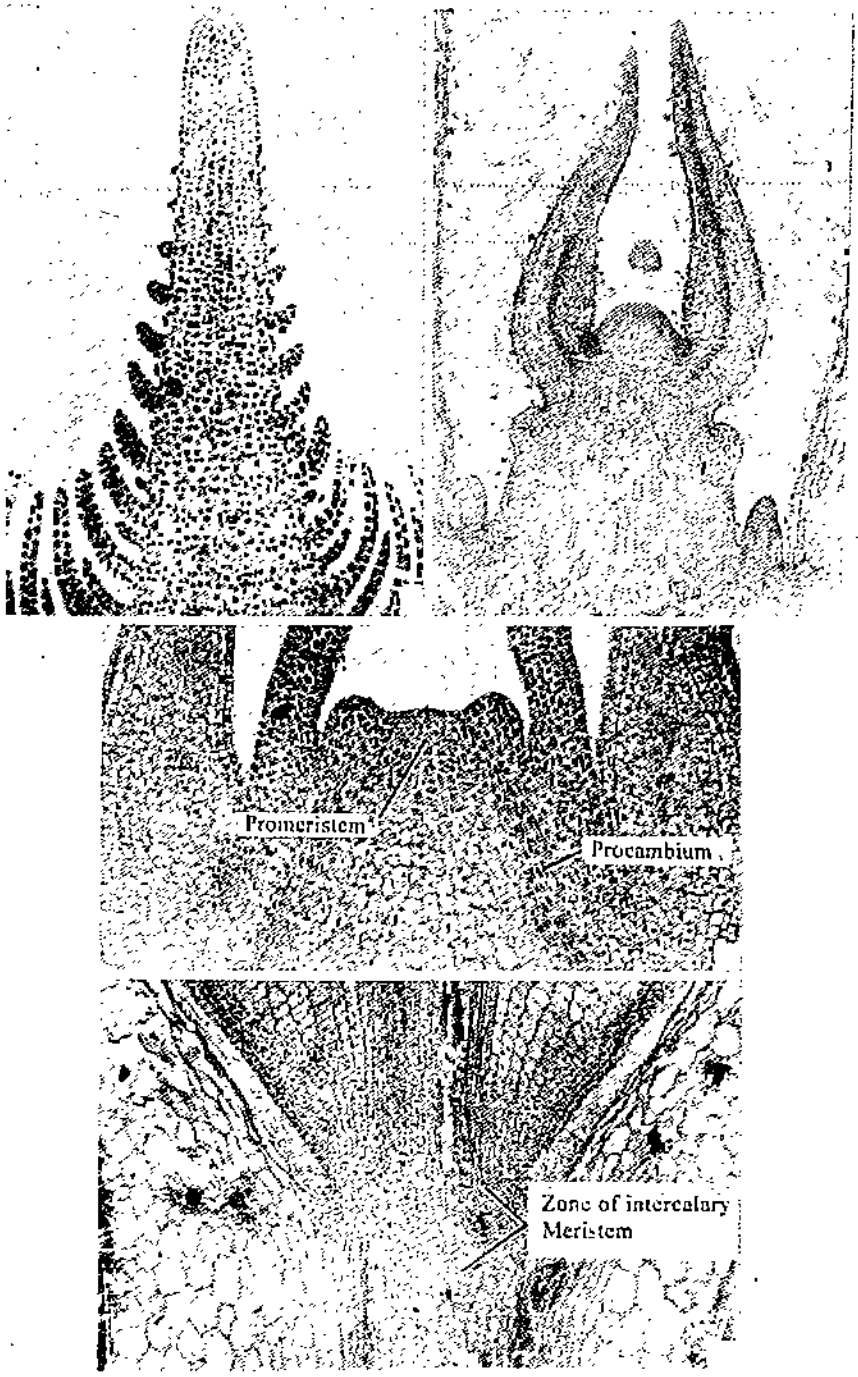

Fig. 7.2: Micrographs of longitudinal section of vegetative shoot apex 1,2,3,4

There are several concepts put forward to explain the organisation and development of the shoot apical meristem. According to Tunica–Corpus theory, apical meristem consists of an outer layer tunica undergoing chiefly anticlinal divisions (at L^0 to surfaced) and inner corpus undergoing mainly periclinal divisions. Tunica contributes to the development of epidermis and corpus to the all other tissues inside the plant body like cortex, endodermis, xylem, phloem and various associated tissues. (Fig. 7.3).

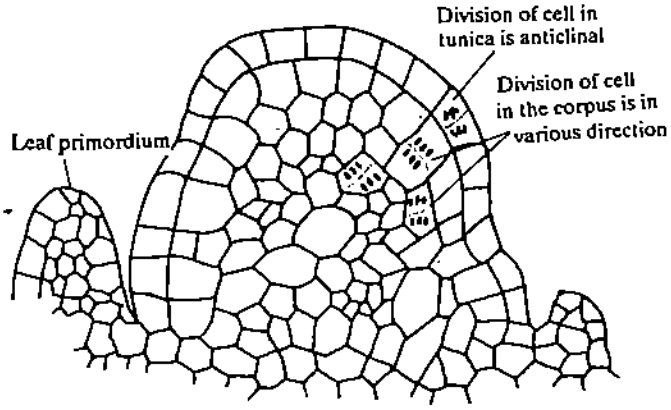

Fig.7.3: Longitudinal section of shoot apex showing one layered tunica and corpus with its cells dividing in various plains.

Experimental anatomy involving surgical removal of a small, terminal portion of the shoot apex by Ernest Ball in **Cuscuta** indicate that even a portion of apex consisting of 25,000 cells are sufficient enough for complete regeneration and differentiation.

Auxins promote cell elongation. Cytokinins are needed to promote cell division. They might be translocated all the way from roots or from young leaves. Gibberellin also promotes cell elongation particularly in case of rossette plants. Branches develop from axillary buds and have vascular connections with the main axis. These vascular connections are termed branch traces. At the node, the branch traces are situated very close to the leaf traces that enter the leaf in whose axil the branch develops. The branch gap is situated above the leaf gap and together these two gaps appear as one. In the dicotyledons and gymnosperms the vascular supply to the axillary branches usually consists of two traces. However, in some plants only one trace is present and in others more than two. In the branches themselves, the stele is similar to that of the main axis.

SAQ 1

a) Describe the following:

   i) Root apex

   ii) Shoot apex

   iii) Shoot growth.

b) Explain the role of hormones on shoot and root growth.

c) Discuss the origin of a lateral root.

7.4 LEAF PRIMORDIA

Buds or elevations (leaf primodia) appear on the periphery of apical meristem, which gradually mature into leaves. The arrangement of leaves on stem is called phyllotaxy. Although it is easy to recognise alternate, spiral, opposite and whorled

arrangement, it is possible to mathematically calculate the spiral arrangement of leaves and express them in definite terms. Normally phyllotaxic series of 1/2, 1/3, 2/5, 3/8, 5/13, 8/21 (Fibonacci series) are found in different species. The interval of time between the appearance of a given leaf and the next is called plastochron.

Light affects leaf development through phytochrome in the sense that chlorophyll development does not occur without light except in some gymnosperms. The concentrations of CO_2 controls the length of petioles. In heterophyllous plants even water has a role in determining the number and shape of leaves. For example in **Limnophila** sp. leaves in the submerged shoot are finely divided. Five to nine leaves appear at a node. Aerial shoots bear opposite and decussate leaves with entire broad Lamina. IAA can affect growth of veins. Cytokinins are known to regulate the rate of cell divisions in leaf primordia and leaf expansion.

How to find out the phyllotaxy number in a plant. Select an old leaf at the top. Carefully examine its position. Now go down slowly, carefully counting the number of leaves observing their positions till you come across another leaf which is exactly below (in vertical alignment) the leaf with which you started. Now the ratio.

$$\frac{\text{No. of turns you have taken around stem}}{\text{No. of leaves.}} = \text{is the phyllotaxy of this particular plant.}$$

You will find a good deal of variation in the arrangement of leaves on stem. In the large majority of stems leaves arise alternately and spirally. In the grasses they are alternate but arranged into two rows.

Be it a branch or leaf–when plant body is diverging from the main vertical axis connection with vascular tissue of main axis needs to be maintained. Just recollect (junctions) in pipe lines. These 'junctions' in plant are called as leaf traces or branch traces.

7.5 FLORAL INDUCTION

The transformation of vegetative apex into a floral apex is a multifactor and multistep phenomenon. Despite researches on flowering carried out during the last seventy and odd years what we know is far less than to what we do not know.

Light

The effect of day length on flowering of some of the temperate plants was first recorded by Garner and Allard. Subsequent work has established a classification of plants on the basis of their day length requirement to flower.

Day Neutral Plants: Flower irrespective of the length of the daily light (cucumber, sunflower, maize)

Long-day plants: Require a light period longer than a "critical period"(spinnach lettuce herbane)

Short-day Plants: Flower when exposed to a light period less than a "critical period". The classical example is cocklebur *Xanthium strumarium*. Others are chrysanthemum, strawberry etc.

Apart from this some plants are obligatively long day and some are obligatively short day plants. In some plants, they flower more in inductive conditions but induction of flowering may not be affected by light period as such. The affect of

light on flowering which is different from the role of light in photosynthesis is called as **Photoperiodism.**

7.5.1 Perception of Light Stimulus

Leaves are found to be organs that perceive light 'stimulus'. K.C. Hamner and J. Bonner showed in 1938 that even if all the leaves are stripped in a cocklebur plant and only as little as one eighth of a leaf is allowed to stay on, the plant would flower in response to one short day exposure. The 'stimulus' continues to stay even when the plants are brought under non-inductive conditions. Moreover, this stimulus can be 'transferred' to another plant through grafting. Chailakhyan's classical grafting experiment using short day *Nicotiana* species and long day *Nicotiana* species clearly established this point. He suggested for the existence of 'Florigen'–a flowering hormone. So far it has not been possible to isolate the flowering hormone, although there is evidence to suggest that it consist of (1) anthesin and (2) gibberellin. For the present flowering may be considered to be controlled by the balance between GA, cytokinin and ABA.

7.5.2 Nature of Light

Red light promotes flowering in short day plants. If the dark regime of a short day plant is interrupted by red light, flowering is inhibited, indicating the importance of the uninterrupted dark period. If a brief exposure to far-red light follows the exposure to red light it can over the inhibitory effect of red light. All these observations suggest the involvement of a pigment. Chlorophyll can be ruled out as chlorophyll never shows any such far–red light reversal effects.

7.5.3 Phytochrome

Phytochrome a pigment that exists in two different interconvertible forms was discovered by Hendricks and Borthwrick et. al. and was chemically extracted and purified. Since then–exact chemical nature is now established. Phytochrome has two parts the light absorbing portion (chromophore) and the large protein. Action spectra of photoperiodism, germination, breaking of dormancy etc.–clearly establish phytochrome as the 'photomorphogenetic' pigment as its absorption spectra genuinely overlaps the action spectra of the above phenomenon.

Phytochrome was found located mostly in the cell membranes. It exists in red-light absorbing form and far-red light absorbing forms. Phytochrome (R) absorbs red light and is converted into phytochrome (FR), a far-red light absorbing form. Phytochrome Fr on exposure to far red light is reconverted to phytochromoe R. Phytochrome (Fr) can also be converted to phytochrome (R) in prolonged darkness.

The action of phytochrome through gene repression and depression production of flowering inducing hormones enzyme activation leading to 'floral apex' from a vegetative apex is roughly understood. But all the steps are not clear yet. Phytochrome regulated morphogenesis results for change in gene 13 transcription. Several phytochrome activated genes have been identifed and isolated. One of them is PAL gene. (Fig. 7.4).

7.5.4 Floral Apex

Each plant must pass through a minimal 'ripeness to flower' stage. That is, even to perceive and respond to a specific photoperiodic regime, a plant must be ready. This may be age, size or stage by which a specific number of leaves must have been formed. In perennials this is called a 'Juvenile phase'. A phase shift from Juvenile to adult stage must be crossed before flowering ensures.

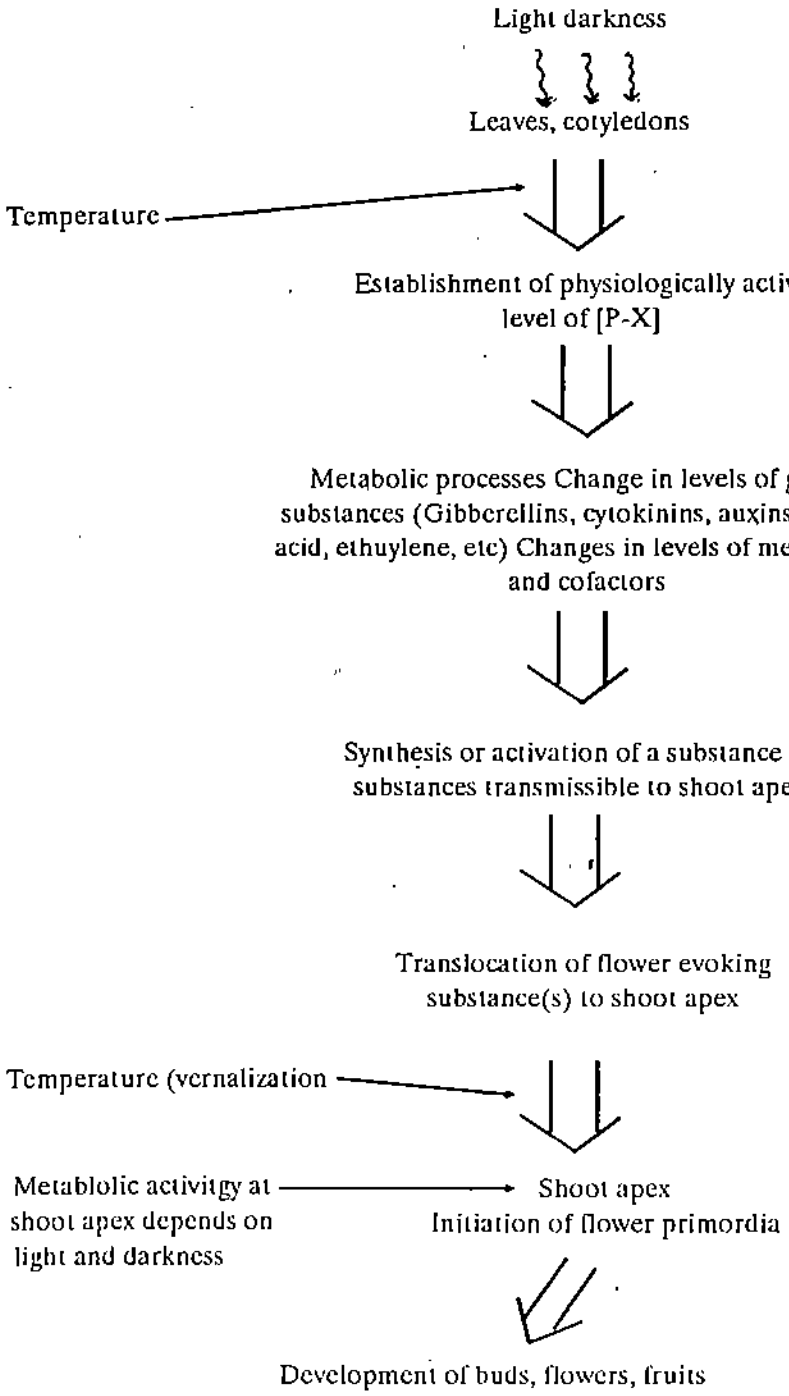

Fig. 7.4: Summary of processes participating in the initiation of flower primordia

There are speculations of the existence of a quiescent center in shoot apices but it has not been upheld. A floral apex do not produce branches but a 'thalamus' that carries floral parts. A 'Thalamus'does not bear leaves but floral parts like sepals, petals, stamens and carpels-which are modified leaf like structures. This fact was well established by the carpel theory of origin of floral parts. Indian scientists like Prof. V. Puri have more contributions in this field. (Fig. 7.5)

Floral Initiation

Activation is generally most marked in the central zone of the meristem. Most of the responses following inductive treatment have been recorded in different meristem components. For example in the pheripheral zone in *Sinapis alba*, central corpus in *Xanthium* and sunflower, central tunica in tobacco, leaf primordia in some cases–are some of the zones where greater activity in terms of cell division and differentiation are noted in the shoot apex after exposure to inductive stimuli.

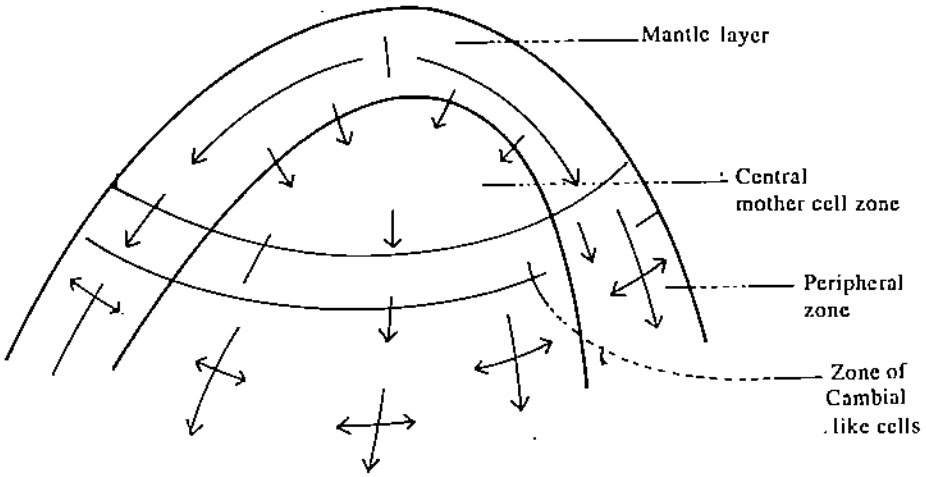

Fig. 7.5: Zonation in the shoot apex of chrysanthemum.

Rise in RNA levels, increase in synthesis of new proteins, increase in general in translation, increase in ATP levels are some of the major molecular events that follow floral induction.

Some of the morphological changes that accompany floral induction are:

- change in the shape of meristem,
- increase in the rate of growth of apendages like plastochromes,
- precocious initiation of axillary meristems,
- increased growth of internodes.

The transformation of the vegetative apex into reproductive apex is manifested in different ways in different plants. These examples such as sunflower, chrysanthemum etc. in which the shoot apex itself transforms into an inflorescence bearing florets. There is a tremendous increase in the radial growth (as opposed to vertical growth) occasionaly reaching an area of 1000 fold. This flowering and fruiting generally result in the cessation of growth of the mother plant, senescence and death. There are also instances in which the axillary buds give rise to inflorescences or single flowers. This you find in perennial plants with seasonal flowering and fruiting. Ofcourse we have annuals like pea *Pisum* or Sarason (*Brassica*) in which a large number of axillary buds metamorphose into reproductive apices. In pea the vegetative apex ends up in a leaf, but in all cases flowering and fruiting affect vegetative growth negatively.

Let us keep in mind that floral induction is a genetically programmed process. Wheat flowers within four months. Even if exposed to conducive photoperiodic conditions–immature plant can not flower. A mango tree does not flower in just two years of planting. Agave tree can flower in 7–10 years.

7.5.5 Effect of Temperature

Certain plants such as the winter rye (*Secale cereale*) and the biennial strain of henbane (*Hyocyamus niger*) require exposure to low temperature conditions before they start flowering. A phenomenon known as 'vernalization' or 'Yaronization' was noted by the Russian Plant physiologist Gustav Gussner in 1915. Here also embryo or shoot apex seems to be the site of perception and the role of a hormone "Vernalin" is suggested. Unfortunately like florigen, vernalin has been still elusive.'

Gibberellin treatment is known to substitute for cold treatment.

7.5.6 Role of Hormones

The role of hormones has already been discussed as part of photoperiodism and vernalization . But remember that the general nutritional status of a plant also controls the extent of flowering. Poor nutritional status of the plant may produce few flowers, irrespective of inducive light and temperature conditions.

7.5.7 Flowering and Endogenous Rhythms

The last four decades of studies in animals and plant physiology have clearly established the existence of a biological clock mechanism to measure time in all living organisms. Circadian rhythms are one such type, that require 24 hours (diurnal) to complete one full cycle of the rhythm. Leaf movements, opening and closure of flowers, photosynthesis, auxins production and rate of cell division follow this pattern. A **rhythm** controlled from within is independent of artificial changes in environment once initiated. Only a prolonged change in light and dark conditions can dampen the rhythm.

Bunning did pioneering work on the role of rhythms in flowering. We know that interruption of dark period by red light leads to inhibition of flowering. But at what point it does during the dark period is also important. These studies lead to the concept of photophil and skotophil which means there are light loving phases in dark period (exposure to red light is not inhibitory) and dark loving phases (where exposure to red light is inhibitory).

Morphogenesis and Totipotency

A chlamydomonas mother cell gives rise to two daughter cells by a simple division. But a leaf cell can not give rise to a new plant except in cases like Bryophyllum or Kalanchoe. What makes a daughter cell of a zygote to loose the 'potential' to develop into a whole plant? Or if all the daughter cells possess this potential– i.e. if they are totipotent–why it does not express?

These questions troubled Haberlandt–hundred years ago. He postulated that any living plant cell should be able to grow into a full plant. He experimented with mesophyll cells at a time when tissue culture had not been unheard for. His experiments failed because we now know that isolated mesophyll cells are not really easy to grow to unleash their morphogenetic potential. F.C. Steward-who took up this work sixty years later-successfully cultured whole carrot plants from carrot phloem parenchyma cell.

He published the papers in the American Journal of Botany in the late 1950's. Steward and his coworkers took 2 mg. tissues of secondary phloem of carrot roots and grew them in special flasks with nipples or tubes called tumble tubes. The medium was whites' medium with coconut water. These tubes/flasks were mounted on a wheel that was rotated on a shaft at the rate of 1 r.p.m. So that the pieces were alternately aerated and bathed in the liquid medium. There was an enormous increase in size of the explant. A callus ensued. A few peripheral cells slaughtered off into the medium and started dividing and gave rise to clumps,–occasionally with roots these could be transferred to semi-solid medium in (still) tubes. Shoots arose opposite the roots to yield full plants. Subsequently other parts were also used to demonstrate cellular totipotency (Fig. 7.6).

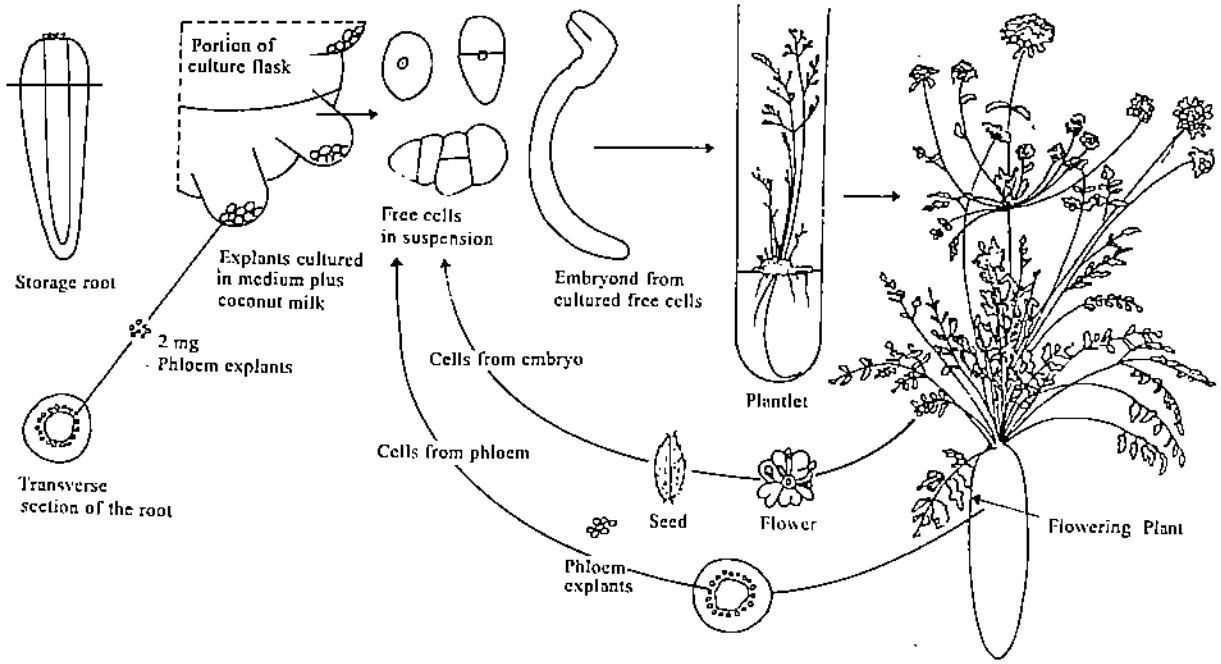

Fig. 7.6: Diagrammatic representation of the cycle of growth of the carrot plant; Successive cycles of growth are linked through free cells derived from phloem of the embryo.

His meticulous methodology well illustrated in his cronnian lecture while receiving FRS in London outlines his scheme.

Concepts of Totipotency and its experimental realisation opened up new vistas in tissue culture, cell biology, molecular biology and biotechnology.

7.6 POLARITY

Polarity is defined as having a difference (morphological, physiological or both) between the two ends of an axis or cells. This is largely determined by the position. In an embryo sac located in an ovule, egg cells and synergids are located at one end and antipodals at the opposite end. This is a kind of polarity. In an eucaryotic unicellular organism like chlamydomonas–flagellae are situated at anterior end. In higher plants, right after division of the zygote, the radicle end and plumule end are well defined. The location of cotyledons is also established very soon. The auxin transport is polar basipetal. Cytokinins move from roots to shoot apex.

Polarity is a common phenomenon observed in plant body right from embryo upto fully grown plant. You should know that Genotype contains information to control polarity. Genes control this process through hormones i.e. gradient of all the growth regulating hormones control and regulate phenotypic expression of polarity.

7.7 THERMODYNAMICS AND CELL SHAPES

Why are protoplasts (cells devoid of cell wall) spherical? Why are most of the unicellular organisms (prokaryotes and eukaryotes) spherical? A sphere is a form which provides maximum surface area for absorption and interaction. In this form a cell remains at the lowest possible energy. In tissues, cells acquire different forms like polygonal, rectangular, irregular etc. Here the concern is to accommodate maximum number of cells in minimum area, so that complete functions are being taken care of.

SAQ 2

a) Define polarity

b) Explain the terms–Short-day plant
Long-day plant
day neutral plant.

c) What are the morphological changes that accompany floral induction.

d) Discuss the role of chemical factors and physical factors in flowering.

7.8 TOPOLOGY AND CELL ORGAN SHAPES

Topology is an abstract mathematical exercise that deals with the possible shapes and surfaces of an endlessly/infinitely elastic body. Most cells are plastic and elastic to a great extent. An abstract activity like topology finds its application in understanding the shapes and forms cells acquire. The probable shapes and forms under different circumstances are some of the questions this interaction of topology and biology can answer. Also, what are the probabilities in shapes if certain circumstances are changed in a certain direction, is another exercise in abstract speculation–having bearing on space biology. Why is a root tip tapering in shape? Why a shoot apex is like the way it is? Or what are the possible laminal shapes? In this unit we will not discuss more about the interaction of Topology and Biology.

7.9 PHOTOTROPISM

Paratonic growth movement of part of a plant in response to light, e.g. bending of stems of indoor plants towards a window, brought about by increased elongation of cells in growth region at tip of stem on shaded side. Coleoptiles of dark grown Avena seedlings bend towards light, when we provide unilateral light. The tip is found to be playing a role. Actually the tip is site of auxin production. If the tip is removed there is no response to unilateral light. But an agar block containing auxins can substitute for the tip and coleoptile would bend towards the light as if it had not been decapitated.

The coleoptile bends towards light because of unequal elongation of cells on two sides. The cells on the darker side elongate faster than those cells on two sides exposed to light. Generally accepted that effect is due to greater concentration of auxin on dark side than on side facing light but some recent evidence suggests asymmetric distribution of some co-factor that influences auxin effect. (Fig. 7.7).

Shoots always bend and grow towards light. It is an adaptive mechanism, no doubt. Here, also the auxin are agents that mediate light control just as in coleoptile.

One question that needs to be asked is whether the extent of phototropism is proportional to the duration of exposure to light. Secondly, whether the magnitude of curvature is proportional to the energy content of the inducing light.

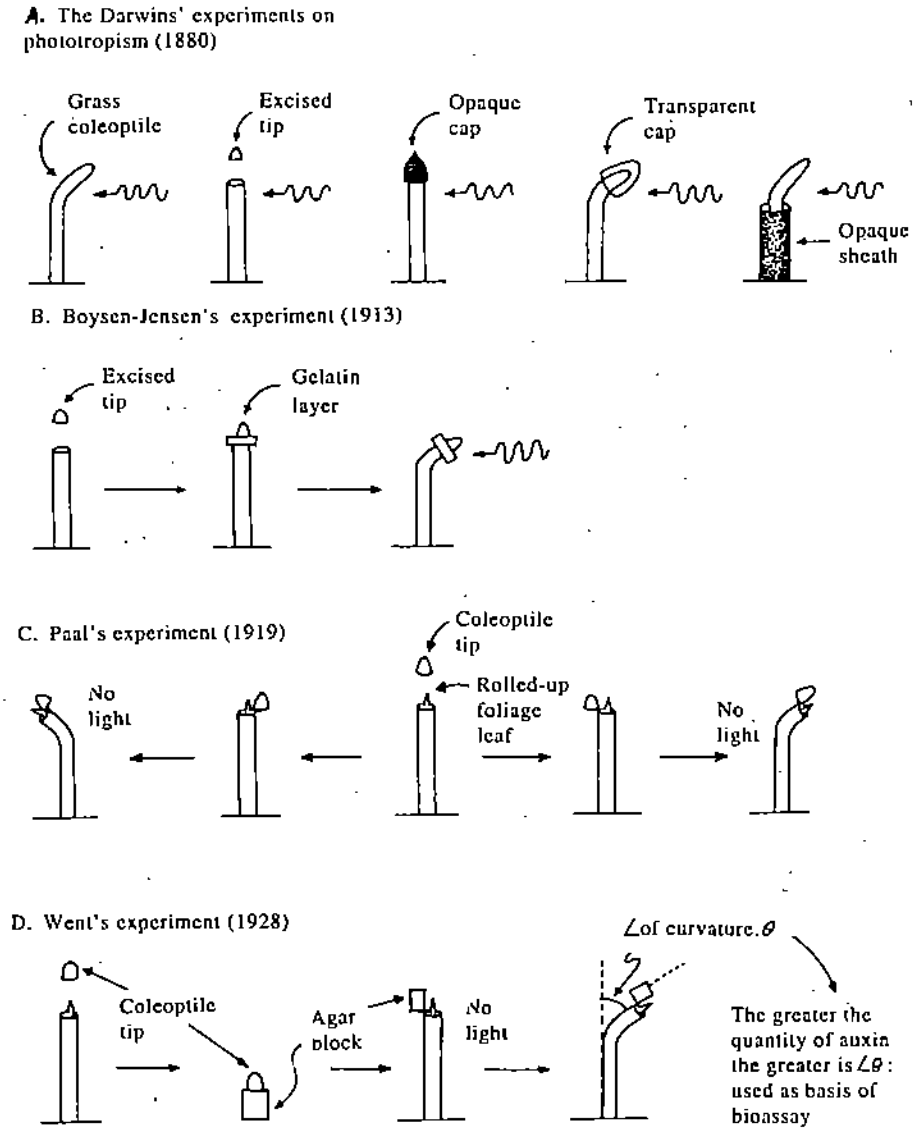

Fig.7.7: Some of the key experiments in the discovery of an auxin (light)

Photoreceptor

The molecular nature of the photoreceptor is unsolved. Initially in the 1930s, some form of carotenoid was thought to be the phototropic receptor. General opinion at present, however, favours some kind of flavin as phototropic receptor, largely on the basis of the spectral match between phototropic action and flavin absorption in the ultra violet region.

The action spectra for phototropism compare with the absorption spectrum of riboflavin and carotene, Clearly favours riboflavin as the absorbing pigment. In some cases it can also be a flavoprotein.

7.10 GEOTROPISM

Roots always grow towards gravity. The lot threshold of geotropic response requires as little time as one minute. Gravity pulls auxin to lower side, causing promotion of growth of root tip and elongation. Ethylene induced by auxins also play a role in geotropism. Immediate transient release of ethylene is often part of geotropic response.

There are special crystals in plant cells which are heavy enough to perceive gravity. These crystals move towards the direction of gravity and exert a pressure on those of sides of cells, perhaps stimulating a series of changes including auxin production influencing the growth of cells in the direction of gravitation field.

i) **Perception:** How does a plant part detect direction of the environmental stimulus that causes the tropism? Where in the plant is located the perception mechanism? It has been difficult to answer these questions for plants, because, in contrast to animals, they do not have specific organs for each function (Fig. 7.8).

ii) **Transduction:** Whatever be the mechanism of perception. How does it convert or transduce it into a message of stimulus direction to the cells in the organ where tropistic movement occurs? What metabolic or growth regulator changes occur in response to the environmental stimulus? This has been an especially active area of research in biology.

iii) **Response:** What actually happens during tropistic bending or other responses? Any hypothesis put forth to explain the mechanism of perception and transduction must account for the observed response. Yet the details of each response have been rather neglected for the past few decades–until quite recently. Early researches in the late 1800s and early 1900s made many careful studies of tropistic responses, discovering that cells on one side of the organ grow more than those on the other side, accounting for bending. The result of early workers were either overlooked or completely forgotten, and are beginning to be appreciated for their significance.

Two generalizations have come from work on tropism during the past two or three

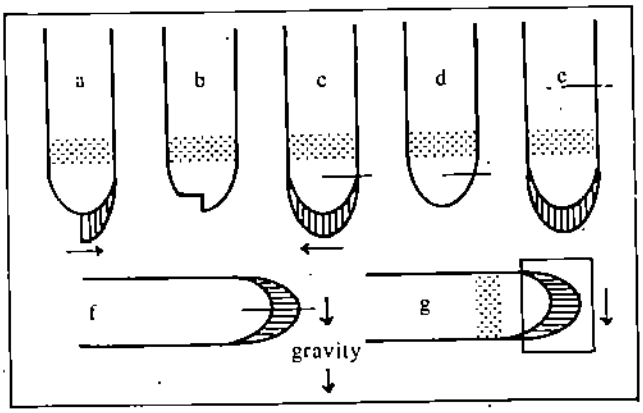

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Fig. 7.8 : Diagramatic representation of various treatments applied to the roots of maize (*Zea mays*) and peas (*pisum sativum*). The experiments suggest that the lower side of the root cap produces an inhibition of root growth that is transmitted to the growing cells (shaded). Arrows show the direction of curvature of the root after treatment (a) The vertical tip bends towards the remaining portion of a root cap. but (b) removal of cap and a portion of the meristem has no effect (c) insertion of a horizontal barrier between the cap and the growing zone causes bending away from the side where the barrier was inserted, but (d) such a barrier has no effect in the absence of the root cap or (e) when the barrier is above the growing zone. Insertion of a horizontal barrier in a horizontal root. (f) nearly prevents bending (suggested by the short arrow), but a vertical barrier (g) has little effect on bending.

decades. Because we must necessarily limit our subject matter in the following pages, these generalizations will not always be apparent. They are–first, similar plant mechanism often cause different responses. For example, K^+ movement in and out of cells causes such diverse responses as stomatal action and thigmonastic leaf folding in the sensitive plant. K^+ may also be important in some of the tropisms. Second, different mechanisms may produce similar responses in different or even the same organisms. For example, different pigment systems are apparently responsible for phototropic bending in different organisms, although the exceptional ones, which respond to red (instead of blue) light, are rare and will be discussed.

7.11 NASTIC AND EPINASTIC RESPONSES

Paratonic variation movements of plant parts in which direction of movement is independent of the direction of stimulus. Nastic responses are like closing of leaflets by touch (Eg. ***Mimosa pudica)*** and episnastic responses are like bending downward of the petiole bringing the leaf to a hanging position.

Ethylene causes epinasty of leaves by promoting elongation of cells on the upper side. If one half of a leaf of plant like xanthium is shaded, then the corresponding site of the petiole elongates so that the petiole bends displacing the leaf blade towards irradiated side. Secondly upward bending of the shaded side of the leaf (hyponasty) this mechanism helps the plant in avoiding overlapping of leaves and maximum exposure to light. The canoppy forms leaf mosaics.

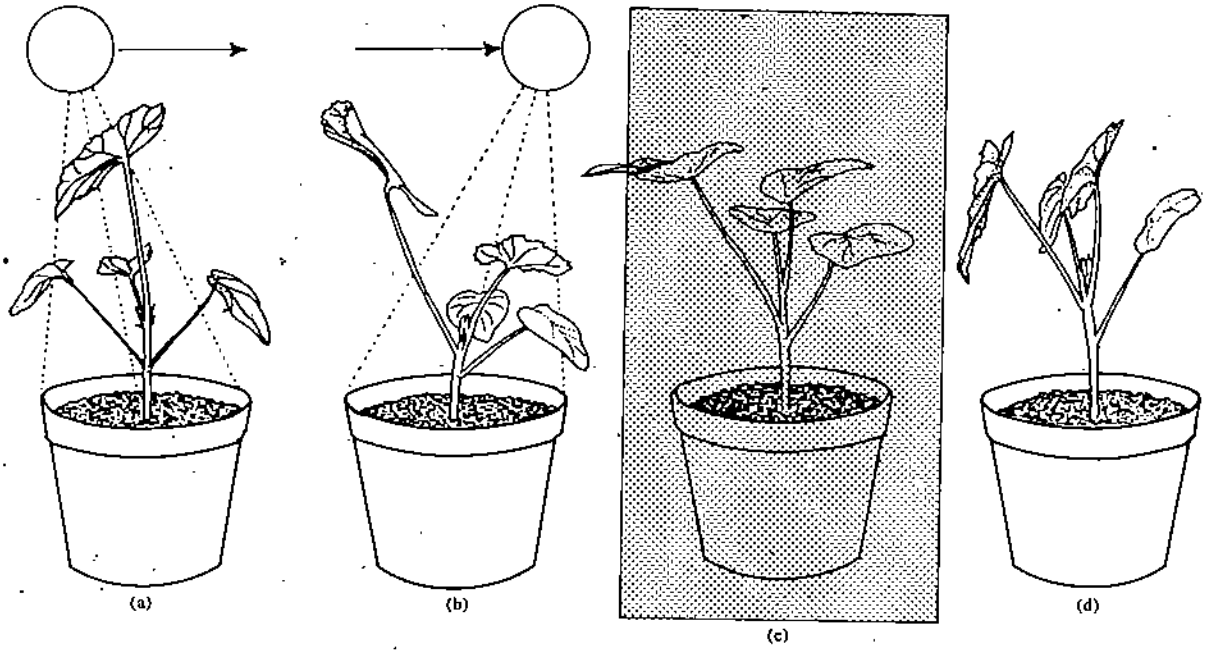

Fig. 7.9 : Solar tracking in a plant with cup-shapped leaves such as some members of the Malvaceae family (e.g., Malva or Lavatera). The leaves receive directional signals from the sun and swivel around to face it as pulvinus cells at the base of the blade gain or lose water. (a and b) The leaves track the sun during the day, much as a radio telescope tracks a satellite (c) An hour or two after sunset, the blades are nearly horizontal in the "relaxed" position they maintain during most of the night (d) About an hour before sunrise, the blades face the point on the eastern horizon where the sun came up the day before.

Solar Tracking: Many plants such as sun flowers are capable of solar tracking in which the flat blade of leaves or inflorescence will remain at right angles to the sun throughout the day, maximising the light harvested by the leaf. Darwin studied the phenomenon first.

This movement is presently thought to be controlled through a mechanism of maintenance of turgidity levels in petiole (pulvinus). Later studies also indicate a role for active pumping of K^+ ions across the membrane. (Fig 7.9)

7.12 THIGMOTROPISM

A tropism in which the stimulus is touch. Many plant organs like tendrils respond to touch. Tendrils bend towards the point of contact, thus helping a climbing plant to climb round a twig or a support. It has also been shown that if you take two potted plants (young seedlings) of **Arabidopsis thaliana** and keep one untouched and the other is touched twice a day, the latter will be shorter (as a result of inhibition in elongation) than the control.

7.13 REGENERATION

Higher plants have three kinds of regenerative activities:,

Reconstitution (as in animals)	Restoration (formation of – missing parts)	Reproductive regeneration (Vegetative , reproduction)

All plants possess inherent ability to regenerate lost parts. For example when a part of mature tissues are removed from stem, the exposed mature cells undergo differentiation, divison and redifferentiation to restore the normal pattern. When a branch is cut and wound is made–damaged tissues are cast off and new cells take this place. A wound also results in the formation of a zone of cells that form a thick protective layer. Induction of hormones like auxins and cytokinins that promote cell divison is also a reaction to wound and part and parcel of regeneration.

Mobilization of nutrients towards the site of injury, synthesis of new hormones, increased cell division and thickening of cell walls etc. are some of the responses and contribute to regeneration.

7.14 REACTION WOOD

As a plagiotropic branch elongates, it might be expected to bend ,downwards because of its increased weight and distance from the trunk. It is resisted by formation of **reaction wood.** Reaction wood is the increased xylem produced on either the upper or lower side of a branch by more rapid divison of the vascular cambium on that side. In conifers compression wood reaction wood forms on the lower side and by expansion.

7.15 SUMMARY

What we have discussed in this unit could be summarised as follows:

- The origin and development of form is genetically determined and environmentally modified.
- Morphogenesis is the result of cell division, cell elongation, and cell differentiation.
- Phytohormones influence the development of Root and Shoot.
- Lateral roots develop endogenously from inner cell layers.
- Shoot apex has an essential role to play in the orderly development of the plant.
- Apical meristem consists of outer layer "Tunica" and inner layer "Corpus". Tunica forms epidermis, and corpus forms cortex, endodermis, xylem and phloem.
- The growth and development of the leaf is complex function of hormonal balance, genetic predetermination, and environment.
- Floral organs of plants are homologous to vegetative shoots. Sepals, petals, stamens and carpels are modified leaves.
- On the basis of their day length requirement to flower, plants have been named as long day plants, short day plants and day neutral plants.
- During phylogenetic development of the flower, processes of cohesion, adnation and organ abortion have taken place.

7.16 TERMINAL QUESTIONS

1. Discuss the role of Phytohormones in flowering and sex expression
2. Write short notes on:
   i) Tunica corpus theory
   ii) Abscission
3. Explain various developmental changes that floral organs undergo before anthesis
4. Define Fibonacci sequence
5. The transition from vegetative to reproductive growth occurs in response to environmental signals, discuss.
6. What is Phytochrome? What developmental phenomena are mediated by phytochrome

7.17 ANSWERS

Self assessment questions

1. (a) i) *Root Apex* : Root apex is simple in structure. The size of root apex is not clear. At the very apex there are a few initial cells that give rise to the cells of the root and root cap. Just behind the tip of the root is "Quiescent Center" comprising of cells that do not divide actively. This center is surrounded by a layer of actively dividing cells.

   ii) *Shoot Apex* : shoot apex has an essential role to play in the orderly development of the plant. Shoot apex is composed of aggregations of cells enged in the organization and initiation of tissues and organs. The meristematic cells of the distal region are responsible for maintaining the continuity and integrity of the growing shoot.

   iii) *Shoot growth* : The apical meristem produces stem, branches, leaves appendages like stipules. In response to signals which may be genetic, age and maturation, or external stimuli like light or temperature, the apex becomes transformed into a reproductive apex. The branches and appendages arise as bumps on the apical dome.

b) Phytothormones influence the development of secondary vasular tissue in roots. When the roots are cultured on a nutrient medium containg indole acetic Acid (10-5m) along with sucrose can induce secondary vascular tissue. Cytokinins promote cell division. Gibberellin also promotes cell elongation particularly in case of rossette plants.

C *Origin of a lateral root* : Lateral roots normally arise at a definite distance behind the tip from areas close to or opposite the points of xylem star. Lateral roots arise endogenously from inner cell layers in region of relatively mature tissues. The initiation of lateral roots usually commences immediately behind the region of root hairs. In gymnosperms and angiosperms the lateral roots are commonly initiated in the pericycle but in pteridophytes lateral roots are initiated in the endodermis.

2a) *Polarity* : Polarity is defined as having a difference between the two ends of an axis or cells. This is largely determined by the position. For instance in an ovule, egg cells and synergids are located at one end and antipodals at the opposite end. This is a kind of polarity.

b) *Short-day Plant* Flower When exposed to a light period less than a critical period.

Long-day plant : require a light period longer than a critical period.

Day neutral plant : Flower irrespective of day length.

c) Some of the morphological changes that accompany floral induction:

1. Change in the shape of meristem
2. Increase in the rate of growth of appendages like plastochromes
3. Precocious initiation of axillary meristerms.
4. Increased growth of internodes.

Terminal Questions

1. Phytohormones play an essential role in flowering and sex expression. In monoecious cucumbers during ordinary development the male flowers are produced before the female flowers. If the plant is treated with auxin the female flowers appear sooner. Gibberellin treatment causes an increase in the number of male flowers, in dioecious hemp plants having male flowers. Thus there is much evidence that hormones are related to sex expression and that a balance of hormones is probably necessary for normal development.

2. i) *Tunica corpus theory* Apical meristem consists of an outer layer "Tunica" and inner layer "Corpus". Tunica undergoes periclinal divisions and corpus undergoes anticlinal divisions. Tunica forms epidermis and corpus forms all other tissues like cortex, endodermis, xylem, phloem and various associated tissues.

   ii) *Abscission* Abscission is a physiological process occurring in a specialised region of the leaf petiole base. It is marked by an increase in physiological activity including protein synthesis and increased respiration. The leaf produces auxin which is transported from lamina to the petiole. Continuous production of auxin by the leaf during development and maturation apparently prevents abscission. When auxin production slows during aging the abscission zone becomes sensitive to ethylene and abscisic acid.

3. Before mature flowers open (anthesis) the floral organs undergo a series of development changes that prepare the flower for pollination and fertilization. Stamen development includes elongation of the filament and pollen grain formation. In the pistil the style and stigma develop where as the basal section enlarges as the ovary containing one or more ovules is formed.

4. The Fibonacci sequence is one in which each number is sum of the two numbers preceeding it, with the exception of the first two like 1,1,2,3,5,8,13,21,

5. The transition from vegetative to reproductive growth occurs in response to environmental signals. Day length, temperature or water regime can act as an environmental stimulus. The effect of day length on flowering of plants has established a classification of plants on the basis of their day length requirement to flower.

6. Phytochrome is a pigment that exists in two different interconvertible forms (i) red-light absorbing form and (2) far-red light absorbing form. Phytochrome (R) absorbs red light and is converted into phytochrome (FR) a far red light absorbing form. Phytochrome FR on exposure to far–red light is reconverted to phytochrome R. Phytochrome (FR) can also be converted to phytochrome (R) in prolonged darkness.

UNIT 8 EFFECTS OF PLANT GROWTH REGULATORS ON DEVELOPMENT

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8.1 INTRODUCTION

In developmental biology so far you have studied the structure and development of anther, ovule and endosperm. You have learnt about gamete formation, pollination and fertilization, and embryo development. You have become aware of the processes involved in the development of seed and fruit. Subsequently we have discussed certain important physiological phenomena like apical dominance. You have learnt about the structure, organization and functions of roots and shoots, leaf production and transformation of a vegetative shoot apex into a floral apex. Growth of secondary tissues and meristematic tissues responsible for secondary growth have also been discussed. Methods used in the culture of tissues and organs and their applications in agriculture and crop improvement were also covered.

All the above mentioned processes are part and parcel of our knowledge of developmental biology i.e. growth, differentiation, development and morphogenesis. Like animals plants require input of energy from the outside

environment to sustain growth and development. But unlike animals, plants can draw energy directly from sunlight and by using water and a few essential mineral nutrients from soil, can synthesize the molecules that constitute their structure and function (autotrophic). There are parasites, semi–parasites, saprophytes and insectivorous plants which are exceptional to this generation.

There are certain physical factors like light, temperature, gravity and touch which trigger one or other physiological processes like flowering, germination, chlorophyll development etc. or modify the pattern and direction of growth e.g. phototropism, geotropism and hydrotropism responses.

There are also certain chemical substances endogenously present in plants which at a very low concentration initiate a particular physiological process like enhancement of production of α–amylase in barley seed endosperm or acceleration of a specific m–RNA or initiation of a major physiological process involving number of specific steps like germination, flowering, senescence, etc. There are just five plant growth regulators in plants. They are auxins, gibberellins, abscissic acid, cytokinins and ethylene. They are also called as plant hormones. But, the word hormone is actually borrowed from animal, as hormones were first discovered and well characterized in animal systems. Unlike in the case of animals, each one of these five groups of 'plant hormones' can influence/ initiate a number of physiological processes. The same hormone can promote growth at one concentration and retard growth at some other concentration in the case of same organ like a shoot, bud, root etc. Same physiological process like germination can be influenced positively by two different types of hormones (gibberellins and cytokinins). Plant physiologists have recommended the use of the phrase "plant growth regulators" (PGRs) to these compounds. These are naturally occurring as well as synthetic compounds which can be included under PGRs. Some scientists call the former as hormones. In recent times there is much discussion whether the term hormone should be used for plants as plant and animal hormones are markedly different in their origin, structure and mode of action.

Nevertheless let us ask ourselves the question. What is a plant hormone? A plant hormone is an organic compound which plays a major role in regulating growth. Some hormones are synthesized in one part of a plant and translocated to another part where they provide specific physiological responses. Some other function in

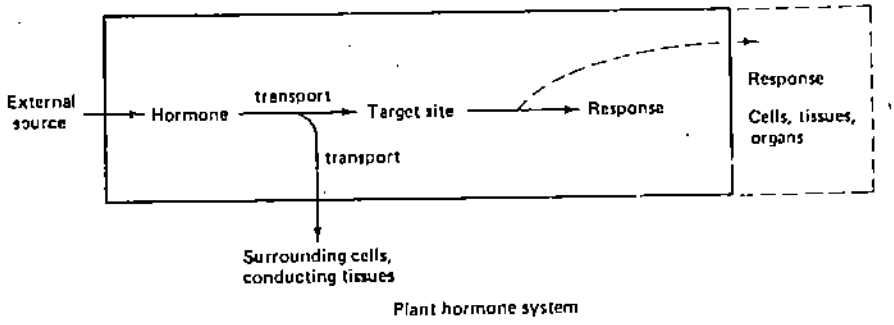

Fig. 8.1: Model of plant hormone system.
In plants all elements–synthesis, transport, and response may occur within a cell

the same tissues where they are formed in very low concentrations. This response may be promotive or inhibitory to growth, development and differentiation.

Objectives

After studying this unit, you should be able to:

- determine the biochemical and physiological events these growth substances can effect at cellular level,
- describe the growth and differentiation of different plant organs modified/ mediated by these substances.
- correlate physiological phenomenon like dormancy, polarity, senescence, abscission, flowering and root formation to these growth substances.
- list "combined action" of these growth substances, and
- apply the 'fundamental' knowledge of plant growth substances in horticulture, in agriculture, in forestry etc.

8.2 HISTORICAL BACKGROUND OF PLANT GROWTH SUBSTANCES

To understand the effects of plant growth substances on development of plants, following questions need to be asked.

1. Where is a particular growth substance produced in plants?
2. Is it really 'extractable' from plants?
3. What are the 'effects' of removal of sites of 'production' of a particular growth substance on plants?
4. What are the effects if we 'apply' isolated/extracted plant growth substances on plants?
5. What are the probable 'Mechanisms of Action' of these growth substances?

A brief and quick review of early history of plant growth substances will give us an idea about how these questions were raised and attempts were and are being made to answer those questions.

Auxins: Julius Sachs (1882) first suggested on the basis of his observations that there are special substances for forming roots and some special substances for forming leaves etc. in plants (quite a far sighted hypothesis on the basis of strength of his scientific foresight). A decade earlier, Charles Darwin (known to all of us for his famous "Theory of Evolution") and his son Francis studied the effect of gravity and unilateral light on the movement of plants. They demonstrated that the effects of light and gravity on the bending of both roots and shoots are mediated by the tip and that this influence can be transmitted to other parts of the plant.

Darwin was primarily interested in the coleoptile, which is a specialized leaf in the form of a hollow cylinder that encloses the epicotyl and is attached to the first node. It affords protection to the delicate growing tip of a grass seedling until, eventually, the more rapidly growing first leaf emerges above ground (Fig. 8.2 a,b,c)

Paal and Hans Soding first approached this problem through experimental methods. They decapitated the coleoptile (removed the tip) and observed that this resulted in failure of coleoptiles to respond to light.

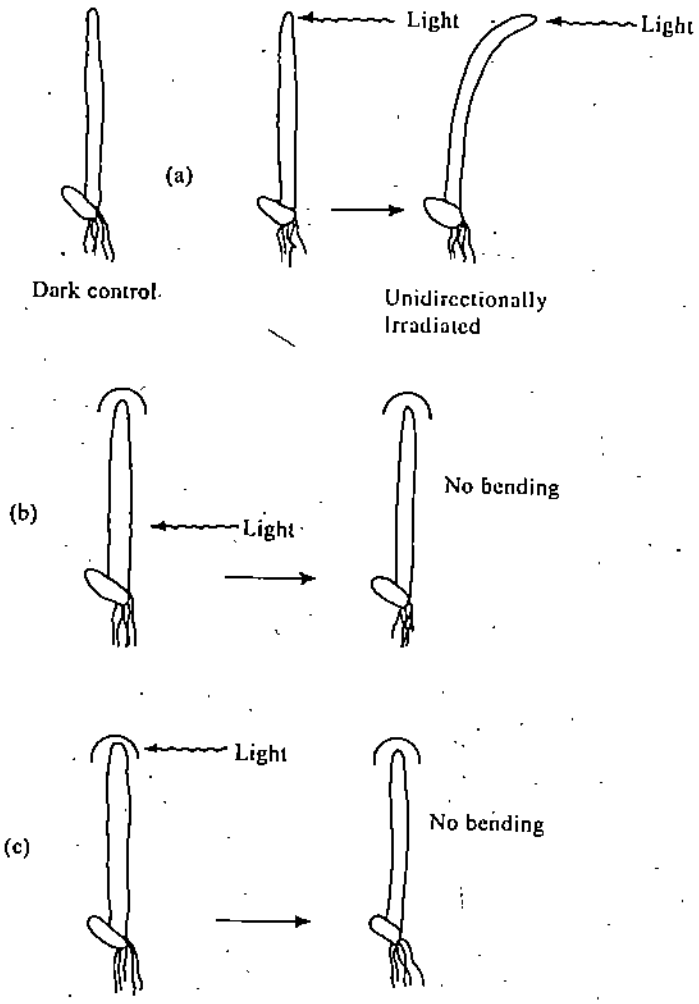

Fig. 8.2: a,b,c Some of Darwin's Experiments on phototropism in grass coleoptiles.

Frits Went conducted his classical experiments designed in a most ingenious manner (Fig. 8.3). These experiments firmly established the existence of a 'growth substance' in plants.

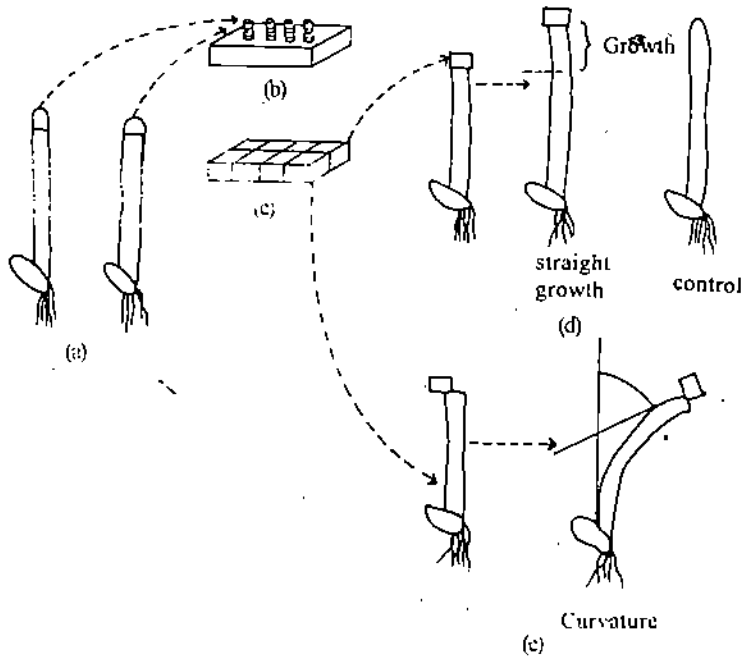

Fig. 8.3 : a,b,c,d Avena coleoptile growth experiment of F.W Went. All manipulations were carried out in darkness.

True auxin indole–3 acetic acid was isolated from urine, from yeast cells, from the fungus **Rhizopus** sp. and from higher plants as chemical extraction methods of organic compounds have become more and more refined.

From this period onwards extensive work was done and is still being done on the physiological effects of auxins, mechanism of action etc.

Gibberellins

In late 1920's Kurosowa observed that the medium in which the fungus **Gibberella** had grown could cause the abnormal tallness in rice seedlings. This work was later taken up by Yabuta and Sumiki (1938) who isolated a crystalline product from **Gibberella** and named it Gibberellin.

Later, Gibberellins were isolated from bean plants by MacMillan, and from citrus plants by Sumiki, from Bamboo shoots by Takahashi and Tamura. This established the wide occurrence of Gibberellins in plants. Today, we know that more than 70 different Gibberellins exist. All have a common skelton but variations are caused by substitutions at several positions in the molecule (Fig. 8.4).

Fig. 8.4: Effects of varying concentrations of gibberellic acid in nutrient solutions on corn (zea mays) plants. Concentrations of GA3 from left to right are:(i) control (on phytohormone), (2) 0.005 ppm, (3) 0.05 ppm (4)0.5 ppm and (5) 5.0 ppm.

Cytokinins

Folke Skoog observed that the cell division or differentiation is affected by AMP and other purines. A nucleic acid from yeast was also found to be useful or effective. Isolation efforts from yeast t-RNA using the differentiation of tobacco tumor cultures as a bioassay has lead to the discovery of kinetin or 6-furfury laminopurine. Lethan and others isolated another cytokinin zeatin from corn plants.

Today, we know quite a few types of natural and synthetic cytokinins. We call them cytokinins because they essentially promote cell divison. Other effects of cytokinins are retardation of senescence, development of buds in moss protonema, release of inhibiters from buds.

Ethylene

This hydrocarbon $C_2 H_2$ is a gas and is unbelievably a small and simple molecule to be accepted as a hormone. Ethylene is not produced by animals. The role of ethylene in the inhibition of growth and in geotropism was observed in 1901. Denny observed that ethylene can promote fruit ripening. Wallace observed the role of ethylene in leaf abscision. Gane noted that ethylene was actually produced by ripening fruit in 1934.

Improvement in techniques like gas chromatography had immensely helped us in estimating the minute quantities of ethylene produced in plants.

Abscisic Acid (AbA)

In 1961 Lice and Carns isolated a substance in crystalline form from mature cotton fruit. This substance stimulated the abscission of debladed cotton petioles. It also promotes flowering in some short day plants. The dormancy response may be through an effect of RNA and protein synthesis. Some effects of Abscisic Acid seems to be reversed by gibberellins (seed dormancy) or by cytokinins (stomatal closure).

8.3 SUMMARY OF PHYSIOLOGICAL EFFECTS OF NATURALLY OCCURRING PGRs

- **Auxins**

Organ formation (interacts with cytokinins)

Tissue organisation (interacts with other factors)

Stimulation of cell division (interacts with cytokinins) through stimulation of proton secretion.

Cell wall relaxation

RNA and protein synthesis

Direction of translocation

Enzyme effects

Ethylene production

Tropic and nastic responses (perhaps sometimes due to ethylene)

Apical dominance

Prevents abscission.

- **Gibberellins**

Cell elongation in intact plants, notably genetic dwarfs

Cell division

Enzyme induction

Flowering (long day plants),

Overcome dormancy (antagonize ABA)

Precocious flowering of trees.

- **Cytokinins**

Cell division especially soyabean cotyledons, tissue cultures etc. (induction as well as promotion interact with auxins).

Cell enlargement

Organ formation (interact with auxins)

Overcome dormancy

Release apical dominance

Prevent senescence

Mobilize nutrients

Regulate polyribosomes

- **Abscisic Acid**

Imposition of dormancy

Gibberellin antagonist

Flowering (short day plants)

Abscission

Stomatal closure

Control of embryo development (with cytokinins and GA)

- **Ethylene**

(Auxin causes its production)

Epinasty–promotes, causes, retards

Geotropism

Causes ripening of fruits

Hastens senescence

Causes abscission.

8.4 PLANT GROWTH SUBSTANCES AND ORGANOGENESIS

Plant growth substances play an important role in the initiation of organs like roots, shoot, leaves and flowers. Not only initiation but differentiation of all tissues in all these organs is also mediated by plant growth substances singly and through interaction of hormones. Both in-vivo and in-vitro studies on organ growth and differentiation have immensely helped us in understanding the role of plant growth substances in organ initiation and differentiation. In the following subsections we will discuss in detail the induction of root, stem, flower and fruit.

8.4.1 Root

Induction of roots by auxins in stem cuttings is a well known phenomenon. The concentrations needed are much lower as compared to that which promote shoot growth.

Auxins also induce lateral root growth at lower concentrations. High concentrations of auxins are inhibitory to root growth. The auxin transport is basipetal in nature. Produced in shoot apex and lateral active shoot buds-auxins percolate towards roots. Obviously, the concentrations that reach roots are low. Effects of auxins in mediating the differentiation of root tissues is already discussed in Unit 7.

The presence of cytokinins is a must for promotion of cell division in root tips. Cytokinins also interact with auxins in the cellular differentiation to produce roots and shoots. Gibberellins do not seem to be having much role in root growth.

Ethylene plays vital role in affecting the nastic responses and geotropism. It also promotes dormancy in buds.

8.4.2 Stem

The role of auxins, cytokinins, ethylene and abscisic acid in controlling the apical bud dominance and lateral bud dormancy has been discussed in detail in Unit 9 (Apical Dominance).

Auxins also play a role in phototropism, geotropism and other responses of stems. Auxin-cytokinin interaction regulates the differentiation and growth of shoots from callus. Gibberellins stimulate cell division and cell elongation in shoot apices. Gibberellins abolish the dwarfism (rossette conditions) in plants and promote stem elongation.

8.4.3 Leaf

F Went has shown that IAA affects linear growth in leaves particularly the length of veins, the leaf area and shape. Now it is well known that the expansion of leaf discs and of isolated cotyledons is stimulated by kinetin even in dark.

8.4.4 Flower

Floral initiation is a dramatic event involving a total change over of the character and developmental pattern of the meristem. The stimulus can be internal i.e, age of the plant, endogenous hormones etc. and external factors like light (photoperiod) and temperature (vernalisation) as well. External stimuli are perceived by a pigment called phytochrome which in turn triggers a series of steps including gene activation, new enzyme synthesis and rise of endogenous hormone levels.

It is also possible that certain quiescent cells in the meristem may be carried along at the apex and become activated during floral initiation. However, it is also possible that genes which control floral initiation are different from those responsible for leaf development.

The chemical nature of any physical stimulus like light (photoperiodism) or temperature (vernalisation) has always been thought to be "hormone like" in plants. This was inferred on the basis of studies that concluded that:

a) The 'stimulus' caused by physical factors can 'stay on' even after plants are brought to normal/uninductive conditions.

b) Can be transferable through 'grafting' of leaves or a stem cutting with leaves from plant exposed to inducive conditions onto a plant kept under uninductive conditions and

c) The fact that stimulus 'moves' from sites of 'perception' to sites of 'action' e.g. from leaves to shoot apex.

Gibberellins are known to promote flowering apart from shoot elongation. Gibberellin treatment can replace long day treatment in plants and also can promote germination without light in photoblastic seeds. Gibberellin treatment to seeds can also substitute for their 'chilling' requirement. The role of a hypothetical hormone called 'anthesins' is much discussed in flowering of long day plants. Unfortunately this hormone could not be isolated so far. Perhaps the balance between the levels of cytokinins, GAs and ABA might be the principle mechanism of hormonal control of flowering.

8.4.5 Fruit

Auxins promote development of fruits without fertilisation (parthenocarpy). Embryological studies revealed that auxins are involved in pre-fertilisation activities like pollen tube growth and ovary development. Auxins directly or indirectly through ethylene production also promote fruit ripening.

SAQ 1

a. Name various plant growth regulators in plants.

b. What is a plant hormone? Discuss in 50 words only where are plant hormones synthesised.

c. Write five physiological effects of auxins, Gibberellins and ethylene.

8.5 DORMANCY

Dormancy can be defined as a state of suspended growth and metabolism. When most plants are exposed to seasonal periods of very inclement whether during which they would be damaged or killed if some protective mechanism did not exist. The most common safeguard against freezing cold or extreme dry heat is dormancy.

Dormancy takes many forms like seed dormancy and bud dormancy. Dormancy as a state is marked by very low levels of water content; low metabolic rates, tolerance to low temperatures and dry conditions, etc. Dormancy is 'innate' (because of certain inbuilt causes and controlled genetically) in some cases or 'induced' by external factors in other cases. Induced dormancy is avoidable if dormancy inducible external factors are avoided.

Dormancy is a defence mechanism against winter frost or summer drought and is a necessary part of the lives of many plants. Dormancy must occur at the right time. It must last for a sufficient time, and it must be relieved or broken when the conditions are right for resumption of growth. There are, four basic questions that must be asked about dormancy of a particular plant or organ. What are the environmental signals that start the process, and how are they perceived? What is the duration of dormancy? A timing mechanism appears to be necessary to ensure that the plant does not accidentally reawaken during unfavourable conditions. What is the nature of dormancy and what are the mechanisms for bringing about the dormant condition? Dormancy is not just an inactivation of metabolism, but frequently involves the development of specialised organs (for example, bud scales) or substances (for example, gummy waterproofing materials). Dormancy is evidently a programmed developmental event that requires specialised synthetic metabolism in addition to turning of metabolic activities.,

8.5.1. Bud Dormancy

Environmental Factors: The most important factor inducing dormancy appears to be photoperiod. Short days induce dormancy in many woody plants. The photoperiod is perceived by the leaves, but the apex or buds are the main initial responding parts of the plant.

Cold itself does not appear to be necessary in the induction of dormancy. Infact, cold appears to be the most important prerequisite for breaking dormancy.

Moisture, or a lack of it, appears to be important in initiating dormancy in some plants, particularly those that rely on dormancy to survive hot dry spells. A shortage of nutrients, particularly nitrogen, also appear to trigger dormancy in some plants. However, dormancy apparently does not result from a slowing down of metabolism resulting from nutrient deficiency. On the contrary, slowed metabolism is a result, not cause of dormancy.

8.5.2 Seed Dormancy

Seed dormancy is critically important for the survival of plants. Factors involved in seed dormancy are:

1. **Environmental Factors**
   a) Light requirement for germination-positive or negative
   b) High or low temperatures
   c) Absence of water.
2. **Internal Factors**
   a) Seed coat-prevention of gas exchange
   b) Seed coat-mechanical effects
   c) Embryo immaturity
   d) Low ethylene concentration
   e) Presence of inhibitors
   f) Absence of growth promotors.
3. **Timing Mechanism**
   a) After-ripening
   b) Disappearance of inhibitors
   c) Synthesis of growth promotors.

Light Requirement: The light requirement for germination of many seeds is presumably a mechanism that prevents the germination of small seeds burried deep under ground, where they would exhaust their reserves before reaching the surface and becoming autotrophic. However, after a fire many seeds germinate at once, and forest regeneration begins. This mechanism prevents overcrowding in a mature forest and ensures rapid regrowth after a disaster.

Not all seeds require light for germination: some are unaffected and a few are inhibited by light. Blue light at quite high intensity has some effect on the germ ination of certain seeds, but it is not clear whether this is mediated through the absorpton of blue light by phytochrome or by some other pigment.

Temperature: Low temperature treatment is an essential prelude to germination in many seeds, and high temperature may be inhibitory at the time of germination. The low tempeature chilling equirement is frequently met artificially by the process of stratification. The seeds are layered in trays in cold moist air for a period of several weeks or months. Temperatures between 0 and 10°C are most effective. The chilling requirement is variously located in the embryo or the seed coat, sometimes in both. The chilling requirement of apple seeds, for example, is much longer for intact seeds than for seeds with the coats removed, or for isolated embryos.

Red light and GA have a synergistic effect, that is, the combination of both factors stimulates germination more than the sum of the two separately.

8.5.3 Seed Coat Effects

In some seeds dormancy is imposed by the presence of the seed coat; if this is removed, the seed germinates. Two possible types of mechanisms could be involved, one biochemical or physiological and the other purely mechanical.

Scarification

The seed coat is nearly impervious to the diffusion of gases. Wareing's group found that seeds of Birch (*Betula pubescens*), which would not germinate intact, would do so if the seed coat were scratched or broken. Moreover, added oxygen greatly stimulated germination of such damaged seeds. Evidently the embryos themselves were not dormant; they would germinate if isolated from the seed. An alternative

possibility is that the seed coat might prevent the leaching out of a diffusible inhibitor.

The second, or mechanical alternative has been investigated by Y. Esashi and A.C. Leopold using seeds of *Xanthium pennsylvanicum* (also called *Xanthium strumarium*), the cockleburr. This plant produces two kinds of seeds in each fruit large, nondormant ones and small, dormant ones. The investigators used the specially designed apparatus to show that neither type of seed generates enough force to rupture the testa during imbibition. During growth, however, the large, nondormant seeds generate sufficient force to break it whereas the smaller dormant seeds do not. This shows, for *Xanthium* at least, that the longheld opinion that the embryo must generate sufficient force during germination to rupture the seed coat is correct. Moreover, it is clear that the forces generated by imbibition alone are not sufficient. Active growth is also needed.

8.5.4 Other Factors

Numerous seeds become capable of germination if they are extensively leached or washed in running water (castor bean seeds). Another substance that may be important in the germination of some seeds is ethylene. A number of workers have shown that ethylene is produced by seeds during germination and that seed dormancy may be broken by ethylene treatment.

IAA Stimulates Cell Enlargement

Cell wall contains layer of cellulose fibrils and are normally quite rigid. Thus for a cell to grow, there must be a mechanism for relaxing the cellulose fibrils. IAA releases the crosslinking bands which hold the microfibrils together. Auxin is thought to cause the walls to become plastic and them osmotic water intake causes the cell to swell like a balloon. American physiologist P.M. Ray has observed that IAA effect on elongation begins after a lag period of only 8–12 minutes, in tissues as coleoptiles. Recently it has been found that with certain auxins or even with IAA itself when used under correct conditions, the lag period can be reduced to 1 minute or less. These effects are now considered to be as a result of activisation of proton pumps in membrane, that pump protons in cell wall and cause a rise in acidity. Acidification loosens bonds in cell wall and cell wall plasticity increases.

This rapid response is followed by activation of m-RNA translated into enzymes that catalyze synthesis of cellulose and other cell wall materials which cause the strengthening of elongated cell wall completing the cell elongation.

α – amylase activity by GA_3

Of the enzymes required for the digestion of starch α-amylase appears immediately after the start of germination. It was found that if the embryo was removed no α-amylase appeared, however, if very low concentations of GA_3 were added to the seed the production of digestive enzyme proceeds. The work over the last 30 years proved the fact that GA_3 initiates action at transcriptional and translational levels. Not only activation of pre-existing m-RNAs coding for α-amylase but synthesis of m-RNA also takes place after the exposure of seeds to GA_3.

Cytokinins and Cell Division

Cell division involves synthesis of new DNA, RNA and a host of cell proteins in interphase (cell cycle –S_1 and S_2). All these events are promoted by cytokinins. Cell division also means synthesis of a number of enzymes that are needed for the synthesis of cell wall and middle lamella.

Ethylene

Ripening of fruits involves a chain of cellular events like:

a) rise in the rate of respiration,

b) breakdown of higher carbohydrates into carboxylic acids, chlolrophyll breaks down, other pigments become prominent (Carotene, Xanthophyll, Lycopene),

c) break down of proteins into amino acids,

d) formation of sugars from polysaccharides like starch that is why ripe fruits are sweeet in mango, banana and sour in citrus (contains carboxylic acids),

e) decay of cell wall and organellis. All these events are mediated through a number of enzymes which means activation or denovo synthesis of enzymes is an integral part of Hormone action,

f) causes sex change in flowers.

ABA

Abscisic acid functions as an antogonist of all three promoters like IAA, GAs and Cytokinins. ABA nullifies the activity of GA, IAA and cytokinin right by a) repressing the genes b) by inhibiting the synthesis of enzymes at translation level (of those enzymes to be promoted by IAA, GA, Cytokinins); c) by promoting enzymes involved in abscission, dormancy etc. Some seeds are shed before the embryo has developed sufficient size or maturity for it to start growing. In this case, the seed may be dormant for a period because of the immaturity of the embryo.

All these dormant systems seem to share essentially the same mechanism of dormancy–short days somehow promote the synthesis of ABA, through the agency of a phytochrome–mediated system and long days prevent the onset of dormancy.

In certain cases, it also seems likely that shortage of nutrients or changes in the balance of hormonal constituents can bring about dormancy.

Leopold's group has pointed out that the dormant condition is considered to be one in which the metabolic machinery of the cells lies idle because of a repressed condition of the nucleic acid system.

SAQ 2

a. Define dormancy. Differentiate between innate Dormancy and induced dormancy. Write upto 30 words only

b. Name three major events of senescence in plants

c. Why do older leaves on an intact plant senesce, whereas younger leaves do not? Write in 50 words only.

8.6 SENESCENCE

All five major growth regulator groups: auxins, gibberellins cytokinins, abscisic acid and ethylene can influence senescence in a wide range of species and this may indicate that all these compounds participate in natural senescence.

In many organisms ageing and loss of viability occur as a result of accummulated metabolic errors and cell damage. On the other hand, senescence of leaves and homologus organs (cotyledons, petals, etc.) is believed to be a programmed process. In the following subsections we will discuss various aspects of senescence.,

8.6.1 Senescence of Chloroplasts

Dark stress induces senescence causes a red shift in the red absorption band of the absorption spectrum of chloroplasts isolated from senescing barley leaves, which indicates that dark senescence brings about some structural alterations of chloroplasts which affect their function.

When the relationship between the structure and the function of chloroplasts in ageing tobaco leaves was studied under electron microscope, earlier degradation of stroma lamellae in comparison with grana were observed.

During the dark stress induced, ageing of leaves of barley–the ability of different exogenous electron donars like manganese chloride ($mnCl_2$) and diphenyl carbazide (DPC) to feed electron to PSII was found to be different. $MnCl_2$ supported DCPIP reduction only up to 4th day where as DPC sustained till day 7 of incubation. These results suggest a sequential alteration of the sites in ETP (Electron transport chain) between H_2O and PSII reaction of chloroplasts during dark induced senescence.

If cytokinins are supplied, this dark induced senescence of leaves and chloroplasts can be delayed. In certain other plant species auxins and gibberellins are also found to be effective. On the contrary ABA and ethylene can promote senescence of leaves and chloroplasts.

8.6.2 Patterns of Ageing and Death

Plants and their parts develop continuously from germination until death. The latter part of the developmental process, which leads from maturity to the ultimate complete loss of organisation and function, is termed senescence. It is a characteristic of plant behaviour that senescence is not simply a running down of the life processes but is a highly ordered and programmed process or series of processes.

According to their habit of growth, plants senesce in many different ways. We will briefly discuss major events of senescence in plants:

- **Whole Plant Senescence:** The whole plant may senesce and die at one time after flowering and fruit formation (all annual like wheat, rice, mustard belong to this category). Delay in flowering postpones senescence phase by keeping the plants alive in unfavourable conditions for flowering.
- **Organ Senescence:** Parts of the plant like leaves undergo senescence after maturation. Rest of the plant remains alive. New leaves replace senescing leaves.
- **Tissue Senescence:** Some tissues like sclerenchyma, tracheids and xylem vessels may senesce and die although the plant as a whole is in a state of good growth.

8.6.3 Variation in t' Range of Senescence in Plants

We have a range in plant that complete their life cycle in a *season* upto a species like *sequoia* that can live upto 5,000 years.

Metabolic Aspects of Senescence: At the cellular level, senescence appears to be tightly controlled at genetic level.

Nutritional Competition in Senescence: The German physiologist H. Molisch suggested in the 1920s that senescence might be caused by nutritional deficiencies Rapidly growing parts act as 'sinks' to draw nutrients from mature organs like old leaves, making them to senesce.

Effects of Growth Factors: A clue to the causes of senescence came from the observation that if a detached, senescing leaf begins to form roots, then senescence is reversed. This suggests that roots produce something that is translocated to leaves and prevents or reverses senescence. Scientists working in both America and Germany discovered that cytokinins applied to leaves would reverse senescence in the area of the leaf to which they were applied. This is called the Richmond Lang effect. Later work also showed that roots do indeed produce cytokinins.

The mechanisms of action of cytokinin is not wholly clear. Cytokinin might promote translocation of labelled amino acids to senescing leaves and thus delay breakdown of protein and senescence.

The British physiologist A.C. Chibnall earlier found that detached leaves without roots invariably became senescent, even if cultured on a complete nutrient solution; therefore, it would appear that rejuvenation is not merely the result of cytokinin-induced mobilisation of nutrients. However, cytokinins are known to cause cell division and to enhance many metabolic processes, including protein, DNA and RNA synthesis.

The question might be asked: Why do certain (that is, older) leaves on an intact plant senesce, whereas younger leaves do not? Both apparently have the same access to the root system. The answer may lie in the fact that nutritional traffic in the plant tends to be strongly directed toward younger and more actively growing parts of the plant. This direction of translocation is possibly the result of more vigourous auxin production in rapidly growing tissue; auxins have been shown to increase translocation toward the site of their application or production. The movement of cytokinins from the roots may be affected in the same way; thus, senescence may be the result of the starvation of older leaves.

As usual, there are complications. Not all plants respond to the same hormones. Cytokinins appear to be more effective in many herbaceous plants. Gibberellins are effective in retarding senescence of dandelion (*Taraxacum officinale*) and the ash (*Fraxinus*), and endogenous gibberellin levels fall progressively during leaf senescence. Auxins (IAA and 2,4-D) have been found to retard senescence in certain trees, although they cannot always be shown to have this effect in all plants. Ethylene strongly promotes senescence in many tissues, it appears to be physiologically involved in ripening fruits, in which its concentration may build up to effective physiological levels.

There is a strong evidence to suggest that ethylene is intimately connected with ageing. It has a strong "Phytogerantological" effect if applied externally.

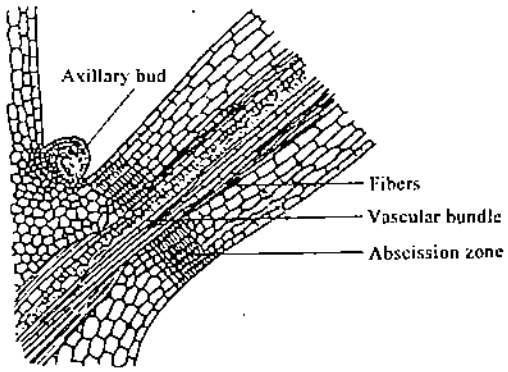

Fig. 8.5: The abscission layer

8.7 ABSCISSION

Abscission of leaves and fruit is one of the more obvious characteristics of senescence. Leaves do not fall simply because they are dead. A zone of cell division, the abscission zone, develops near the base of the leaf so that numerous crosswalls form at right angles to the long axis of the petiole. Then pectinases and cellulases are induced in these cells of the abscission zone. These dissolve the lamellae of the crosswalls of these cells, so that the petiole breaks off. The vascular connections break and usually become plugged by the formation of tyloses (deposits of gummy substances) and layers of corky cells. Thus, at least two important events are involved in abscission: cell division and the induction of hydrolases. Both of these are processes of active metabolism and must therefore, be a programmed part of the development of the plant(Fig 8.5).

Causes of abscission involve several interlocking events. It seems possible that certain growth–inhibiting substances may be involved. ABA clearly stimulates abscission in cotton petiole explants, as does GA. Addition of auxin to the petiole or blade of a senescing leaf prevents the formation of the abscission layer, and thus inhibits abscission.

Ethylene appears to play a role in leaf abscission. When petioles are excised, an abscission layer forms within about 3 days and the force required to break the petiole decreases abruptly. The addition of ethylene greatly accelerates this process.

SAQ 3

a. Explain the following : i. Receptors ii. Tyloses iii. Synergistic action iv. Sequential action v. Totipotency

b. Discuss briefly the role of the following:

i. Cytokinins in senescence ii. Ethylene in senescence

iii. Gibberellins in senscence

c. Differentiate between abscission and bioassay

.....

.....

It has been suggested that ethylene has a two fold effect: a gerantological action, which causes or accelerates senescence in the leaf, and a stimulation of induction of cell wall degrading enzymes in the abscission zone. Although IAA treatment has been found to prevent abscission, it actually stimulates the rate of abscission when it is applied late (after senescence has begun). This may relate to an IAA stimulated formation of ethylene. There is also evidence that abscission is influenced by the direction of application of auxins to a leaf.

8.8 SYNERGISTIC AND SEQUENTIAL EFFECTS OF HORMONES

In vitro studies on callus and suspension cultures have brought out two interesting findings. The nature and direction of differentiation is guided by the fact that whether two hormones are supplied together (synergistic effect) or in a sequence (sequential effect). Different effects result depending on whether auxins supply is followed by cytokinins and vice versa. It also matters whether only auxins and cytokinins are supplied together or whether auxins and gibberellins or similar combinations of all the five growth hormones.

The latent built in capacity to grow which resides in the mature cells of carrot or of many other angiosperms may be released by appropriate exogenous stimuli. When growth is so induced the cells respond to normal range of organic and inorganic nutrients and of vitamins if these are furnished under aseptic conditions, F.C. Steward, through his studious efforts spread over years, cultured full grown plants from free cells of carrots in suspension.

It is these studies that demonstrated the synergistic and sequential effects of hormones. More of cytokinins and less of auxins stimulate shoot growth and less of cytokinins and more of auxins induce roots. Light is also needed for greening of leaves.

8.9 TOOLS AND TECHNIQUES

In the preceding units we have discussed about the "effect of auxins". In this section we shall discuss the methodology being followed in hormone studies:

a) **Observations:** The knowledge about role of light or temperature in flowering and germination of avena coleoptile curvature or apical dominance or other phenomenon like senescence resulted out of emperical studies during the late 19th and early parts of the 20th centuries.

b) The fact that a hormone like substance is involved was realised through simple experimental studies like removal of supposed sources of hormone production like

apices, leaves etc. and observing this effect on growth and development of plants.

c) The chemical nature of hormones was then elucidated through biochemical techniques of isolation, purification and structural studies using techniques like i) chromotography, ii) chemical purification, iii) studies of chemical properties etc.

d) In vitro studies of cells, tissues and organs over the last 60 years contributed to our knowledge the effects of hormones in more detail.

e) Studies with transcription and translation inhibitors yielded and still are throwing new light on hormone action at the molecular level.

f) Isolation of proteins that bind to hormones called 'receptors' is throwing focus on 'perception of signals' at cell level.

g) Studies on linkages between respiration and hormone action are increasingly stressing the role of phosphorylation, energy requirement and the role of other membrane bound proteins.

8.10 MOLECULAR GENETICS AND PLANT GROWTH HORMONE STUDIES

In the preceding section, we discussed the tools and techniques useful in understanding plant growth hormones and their role in controlling plant growth and development. Molecular genetics has advanced over the last three decades by leaps and bounds, providing knowledge regarding the control of developmental events nucleic acids and proteins. Further, scientists have perfected techniques in identification, isolation, sequencing of genes, their transplantation into new hosts, production of transgenic crops, so on so forth. It is no wonder that there is a temptation to make use of all this in plant hormone studies also.
Following are the various aspects of these studies:

a) Mutant varieties in various plant species which are identified for over production or under production of a given plant hormone are being isolated from nature or some times these mutatns are produced through mutation breeding techniques. Studies on phenotypic nature of these mutants would throw new light on the fact how far our earlier assumptions on the physiological responses of various plant hormones are correct or not.

   Mutants which are deficient in the production of particular hormone step in biosynthetic pathway are also found. Studies of these mutants are helping us in understanding the intricacies of biosynthetic pathways of hormones. Mutants which fail to show a particular physiological response to a given hormone (Eg. cytokinin retards leaf senescence, promotes leaf expansion, promotes chlorophyll biosynthesis etc., in wheat). There can be one wheat mutant that shows all other responses except leaf senescence.

   Identification of genes that produce these hormones, cloning them and transplanting them in some other plants also helps us a good deal in knowing the interaction of hormones.

   Changes in the levels of molecules like m-RNA i.e in terms of total quantity or quality i.e. how many new species of m-RNA etc. are being followed in cells exposed to hormones over the last two decades. These studies have given new information about hormonal effects at transcriptional and post-transcriptional levels.

   Observations have shown that polyribosomes formation is higher in cytokinin treated cells. Auxins stimulate both production of ribosomal RNA and m-RNA meant for ribosomal proteins. ABA has shown to cause deactivation of ribosomes. GA_3 treated cells show enhancement of ribosomal activity.

   Above are just few of many areas of molecular genetics under study to develop a better picture of hormonal role in plants.

8.11 SUMMARY

In this unit you have learnt that ,

1. Basically there are five classes of Plant Growth Regulators. Auxins, Gibberellines, Cytokinins, Abscisic Acid and ethylene.
2. There is no phenomenon in plant growth and development and differentiation that is not mediated by Plant Growth Regulators.
3. All physical factors like light, temperature gravity etc. act through controlling the endogenous levels of Plant Growth Regulators.
4. Plant Growth Regulators function at two levels:
   a. Rapid responses-mediated through membrane leval changes.
   b. Long term responses-mediated through gene expression, transcriptional and translational control.
5. Recent advances in techniques of biochemistry, biophysics and molecular biology has immensely helped in elucidating the structure, biosynthetic path ways, mechanism of action etc. of different Plant Growth Regulators.
6. All the knowledge gathered from the study of Plant Growth Regulators finds its application in agriculture.
7. The whole plant may senesce grow old and die at one time after flowering and fruit formation.
8. Abscission of leaves and fruit is one of the more obvious characteristics of senescence.
9. Plants and their parts develop continuously from germination until death.
10. Dormancy is a state of suspended growth and metabolism, that takes many forms like seed dormancy and bud dormancy.

8.12 TERMINAL QUESTIONS

1. Discuss the role of hormones in:
   i) Growth of shoot
   ii) Development of leaves
   iii) Fruit ripening
2. Define dormancy: What is the importance of seed coat in contracting dormancy.
3. Discuss the biochemical changes in chloroplast senescence.
4. Discuss briefly the recent advances in plant molecular biology in relation to growth hormones.
5. What are the factors involved in seed dormancy.

8.13 ANSWERS

Self assessment questions

1. a. There are just five Plant Growth Regulators in Plants viz auxins, gibberellins, abscisic acid, Cytokinins and ethylene.
   b. A Plant hormone is an organic compound which plays a major role in regulating growth. Some hormones are synthesized in one part of a plant and translocated to another part where they provide specific physiological responses. Some others function in the same tissues where they are formed in very low concentration.

c. Physiology effects of:

Auxins	*Gibberellins*	*Ethylene*
i. Organs formation	i. Cell division	i. Promotes : Causes, Retards Epinasty
ii. Cell wall relaxation	ii. Cell elongation	ii. Causes ripening of fruits
iii. Enzyme effects	iii. Flowering	iii. It is a $\overset{0}{+}$ sex promoter
iv. Apical dominance	iv. Overcome dormancy	iv. Hastens senescence
v Prevents abscission	v Precocious flowering of trees	v. Causes abscission

2. a. Dormancy can be defined as a state of suspended growth and metabolism. *Innate-dormancy:* dormancy is innate because of certain inbuilt causes and controlled genetically.

Induced dormancy: dormancy is induced because of external factors and is avoidable if dormancy inducible external factors are avoided.

b. Major events of senescence in plants are:

- Whole plant senescence
- Organ senescence
- Tissue senescence

C. Older leaves senesce whereas younger leaves do not because nutritional traffic in the plant tend to be strongly directed towards younger and more actively growing parts of the plant. This direction of translocation is the result of more vigourous auxin production in rapidly growing tissue. This senescence may be result of the starvation of older leaves.

3. a. i. Isolated proteins that bind to hormones

ii. Deposits of gummy substances

iii. Action of two harmones supplied together

iv. Action of one hormones followed by other hormone

v. The ability of individual living cell to form an organism when freed from the body provided it is given suitable nutrients, hormones, etc.

b. i. Cytokinins delay senescence

ii. Ethylene strongly promotes senescence

iii. Gibberellins are effective in retarding senescence.

c. *Abscission:* The controlled separation of leaves, twigs, flowers and fruits form the plant body as a result of formation of abscission zone in their stalks. The abscission zone consists of a layer of delicate, thin walled cells extending across the stalks.

Bioassay: Quantitative estimation of biologically active substances by the amount of their actions in standarized conditions on living organisms or parts of organisms, e.g. Avena Curvature test for auxin.

Terminal Questions

1. i) *Growth of shoot :* Auxins play a role in phototropism, geotropism and apical dominance. Gibberellins stimulate cell division and cell elongation in shoot apices. Gibberellins abolish the dwarfism (rosette conditions) in plants and promote stem elongation. Cytokinins release apical dominance.

ii) *Development of leaves:* Indole acetic acid affects linear growth in leaves particularly the length of veins, the leaf area and shape. The expansion of leaf discs and of isolated cotyledons is stimulated by kinetin even in dark

cytokinins promote translocation of labelled amino acids to senescing leaves and thus delay break down of protein and senescence.

iii) *Fruit repeinig* Auxins promote development of fruits without fertilisation (Parthenocarpy). Auxins directly or indirectly through ethyelene production also promote fruit repening.

2. *Dormancy*: Dormancy can be defined as a state of suspended growth and metabolism. When most plants are exposed to seasonal periods of very unfavourable weather during which they would be damaged or killed if some protective mechanism did not exist. Dormancy is a defence machanism against winter frost or summer dorught and is a necessary part of the lives of many plants. In some seeds dormancy is imposed by the presence of the seed coat. The seed coat is hard and nearly imperious to the diffusion of gasses. If seed coat is removed the seed germinates. Two possible types of mechanisms could be involved, one biochemical or physiological and the other purely machanical.

3. Dark stress induced senescence brings about some structural alterations of chloroplasts which affect their function.During the dark stress induced ageing of leaves of barlay the ability of different exogenous electron donars like manganese chloride ($MnCl_2$) and diphenyl carbazide (DPC) to feed electrons to Ps 11 was found to be different. $MnCl_2$ supported reduction only upto 4th day whereas DPC sustained till day 7 of incubation. These results suggest a sequential alteration of the sites in ETP (Electron Transport Chain) between H_2O and Ds·II reaction of chloroplasts during dark induced senescence. When the relationship between the structure and the function of chloroplasts in ageing tobacco leaves was studied under electron microscope, earlier degradation of stroma lamellae in comparison with grana were observed .

4. Recent advances in plant molecular biology in relation to growth hormones are:

i) Mutant varieties in various plant species which are identified for over production or under production of a given plant hormone are being isolated from nature or some times these mutants are produced through mutation breeding techniques.

ii) Mutants which are defecient in the production of particular hormone step in biosynthetic pathways of hormones. Mutants which fail to a given hormone (e.g. Cytokinin retards leaf senescence, promotes leaf expansion, promotes chlorophyll biosynthesis etc. in wheat) there can be one wheat mutant that shows all other responses except leaf senescence.

iii) Identification of genes that produce these hormones cloning them and transplanting them in some other plants also helps as a good deal in knowing the interaction of hormones.

5. Factors involved in seed dormancy are:

Environmental factors :	***Internal factors :***	***Timing Mechanisms:***
a. Light-Positive or negative	a. Seed coat-Prevention of gas exchange	a. After-ripening
b. Temperature-high or low	b. Seed coat-mechanical effects	b. Disappearance of inhibitors
c. Absence of water	c. Embryo immaturity	c. Synthesis of growth promoters
	d. Low ethylene concentration	
	e. Presence of inhibitors	
	f. Absence of growth promoters	

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9.1 INTRODUCTION

In the previous units of this course you have studied certain basic developmental processes like development of anther, ovule, pollination, fertilisation, endosperm, embryo, seed and fruit. These are developmental processes which are fundamental and basic for plant life. Now, we shall move forward to certain finer aspects of developmental biology (Plants). Apical dominance is a correlative phenomenon in the developmental biology of plants.

Branching of the main shoot into lateral branches is one of the main characteristics of the growth of most of the plants. More branches mean more leaves and more of photosynthesis. Branching also helps a tree to avoid crowding of leaves. Branches provide an opportunity for exposure of maximum number of leaves to sunlight.

Branching has a distinct evolutionary advantage, as it helps a plant to survive in a variety of habitats. A plant that does not branch finds it difficult to survive in crowded forests or in areas where the light intensity is low.

If you go around your place of stay or your place of work and start observing the big trees (flowering plants), you will come across two different types of tree growth. One, "monopodial" where the main trunk grows tall bearing a crown of leaves at the top without much of lateral branches such as the large majority of palms and the other- "sympodial" where lateral branches start spreading out leaving a gap from the apex. Plants which exhibit the second type of growth pattern are quite useful as they provide lot of shade from hot sunlight apart from providing economic benefits like timber and fruits.

Plants with a monopodial growth habit find it difficult to survive in temperate forests where light availability is poor. Palms mostly grow in tropical regions. Interestingly, bamboos have underground stems (rhizomes) like grasses which show both monopodial and sympodial branching pattern.

In those plants which exhibits sympodial growth, lateral branches always sprout a little distance away from the shoot apex and in the case of some plants this distance

is quite significant. Some of the questions that emerge in mind are: Why do not the lateral branches sprout immediately below the shoot apex? What is the advantage of this process to plants in terms of growth and viability? What are the physical and chemical factors involved in controlling this process apart from biological reasons at cellular level?

Apical dominance is well-known to plant physiologists for over two hundred years even though the mechanism that controls this process has become a matter of investigation more recently particularly after the discovery of phytohormones and their role in growth and development.

Objectives

After studying this unit you should be able to:

- define the terms related to the apical dominance,
- explain the role of chemical factors like phytohormones in controlling this process,
- correlate the role of factors like nutritional in the expression of apical dominance,
- determine the significance of this process in optimizing development and
- list the applications of modification of apical dominance in agriculture and horticulture.

9.2 SOME RELEVANT TERMS

Here are a few terms that will be used in discussing apical dominance. A clear understanding of these terms is needed for understanding the intricate mechanisms of physiological control of branching.

Apical Dominance

The inhibition of growth of lateral buds by the presence of active shoot apex of either the main stem or branches is called apical dominance.

Lateral Buds

Buds which are present in the axils of leaf primodia which are similar to main shoot apex in structure but generally remain dormant.

Dormancy

Arrested condition of growth.

Phytohormones

Specific chemical substances that are produced at one site in plants and travel to other areas (targets). At the target they regulate physiological responses.

Shoot Apex

The terminal tissue of main stem or of a branch. Very simple in structure consisting of just two different types of cells which are actively dividing to produce multiple number of cells which differentiate into various structures and tissues of the stem.

Root Apex

The terminal tissue of root consisting of actively dividing cells that differentiate into various tissues of root as the elongation of root takes place. A root apex does not generally give rise to other organs or structures.

Apical dominance can be recognized if we carefully observe the growth of plants. That the shoot apex is responsible for the inhibition of the growth of lateral buds can also be demonstrated "experimentally". You choose any actively growing plants in your courtyard and count the number of branches present immediately below the shoot apex. Take a fine blade and severe the shoot apex. Observe the growth pattern for a period of 10 or 15 days. Count the number of lateral branches sprouting below the cut.

SAQ 1

a) Complete the following sentences:

i) Apical dominance means. . . . . .
. . . . . .

ii) Apical dominance is regulated by. . . . . , ... and. . . . . plant hormones.

b) Differentiate between the following terms: Answer in about 50 words

i) Apical bud and lateral buds

ii) Monopodial and sympodial types of branching

iii) Shoot apex and root apex

9.3 ROLE OF CHEMICAL FACTORS IN CONTROLLING APICAL DOMINANCE

There were indications about the existence of plant hormones in the last part of 19th century. The plant hormones had been isolated and characterized in the third decade of this century. By the middle of this century we became aware of different types of plant hormones like auxins, gibberellins, cytokinins, abscisic acid and ethylene. The first four are chemical substances that influence growth in solutions and interestingly ethylene is a gas and perhaps the only hormone of its type. The first four plant hormones are isolated in crystal form from plant sources and their chemical structure is well characterised. Unlike the case of animal hormones, plant hormones exihibit a broad spectrum of effects on growth and development. Quite often same growth process may be regulated by more than one plant hormone. This applies to apical dominance also.

Prior to the discovery of plant hormones and their role in apical dominance it was thought that apical dominance is a kind of **struggle for existence and competition** between apical bud and lateral buds for nutrients from the root and leaves.

9.3.1 Auxins

The role of auxins in controlling the apical dominance was shown by the experiments done by Skoog and Thimann with the broad bean plant (see Fig. 9.1). They removed the terminal bud. This resulted in the development of lateral buds and branching occurred. If an agar block containing auxins was placed after the removal of apical bud, no growth of lateral buds occurred. This meant that the role of terminal bud in supressing the growth of lateral buds can be substituted by auxin. The agar blocks containing auxins is playing the same role as the apical bud. It was inferred that auxins present in the shoot apex might be playing a role in apical dominance.

As auxins could be isolated from the terminal bud, it was believed that shoot apex is also the site of synthesis of auxins. Experiments have also showed that terminal bud (shoot apex) contains a higher amount of auxins than lateral buds. A logical question that may arise in your minds is: If the auxins present in the main shoot

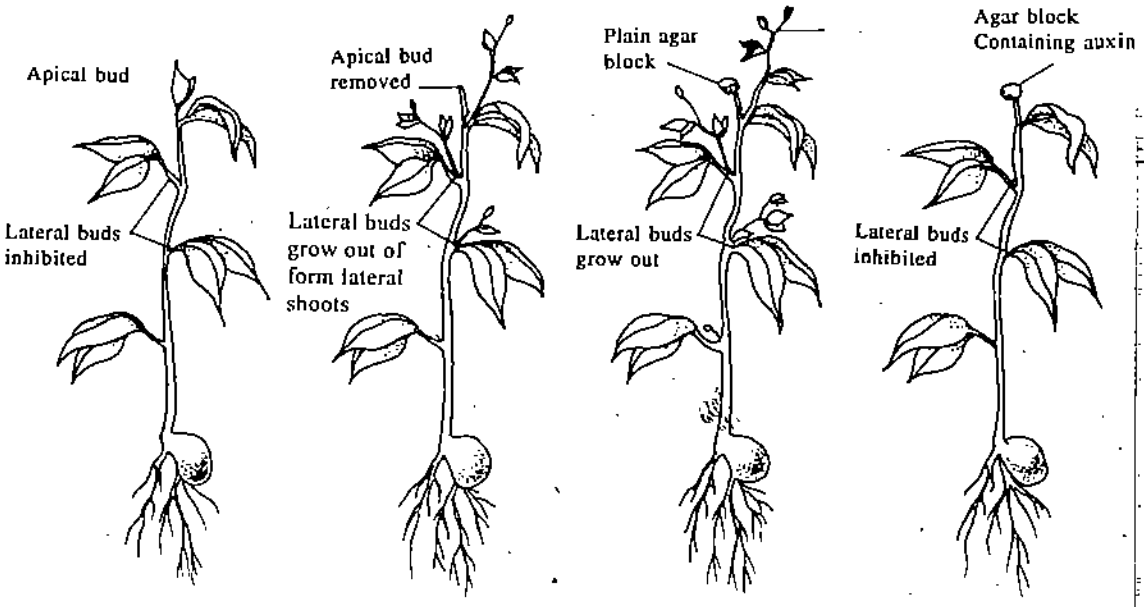

Fig. 9.1: Effect of apical bud removal and auxin on lateral bud growth in a leguminous plant.

apex promote its growth, why should the growth of lateral buds which contain lower amount of auxins be inhibited?

Thimann (1937) suggested that lateral buds respond to auxins in much the same manner as roots and stems but the optimum concentration for the promotion of shoot apex is much higher than that for lateral buds. The lateral buds need very low concentration of auxins for growth promotion. Hence the amount of auxin present

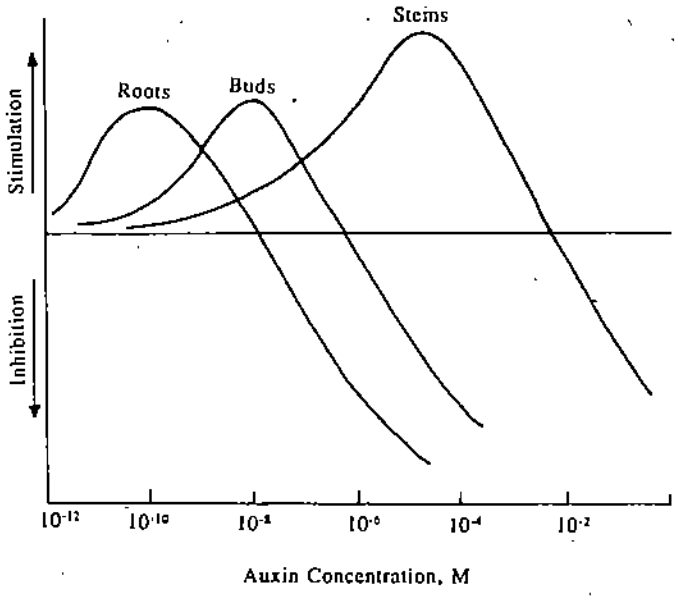

Fig. 9.2: Differential action of IAA on roots, buds, and stems as envisioned by K.V. Thimann.

in lateral buds and auxins transported from the apex, added together are sufficient enough to prevent the growth of lateral buds.

The transport of auxins is basipetal i.e., from top to bottom. Auxins synthesized in the shoot apex are transported downwards. In addition to this young leaves also produce auxins. The auxins transported from the shoort apex and from the young

leaves accumulate in excessive amounts to cause inhibition of the growth of lateral buds. There is a progressive decline in the concentration of auxin when you go down from the apex (see. Fig.9.3). This facilitates inhibition of growth of lateral buds immediately below the shoot apex (high concentrations of auxin inhibit growth of lateral buds). But at a distance from the apex growth is stimulated (low concentrations promotegrowth).

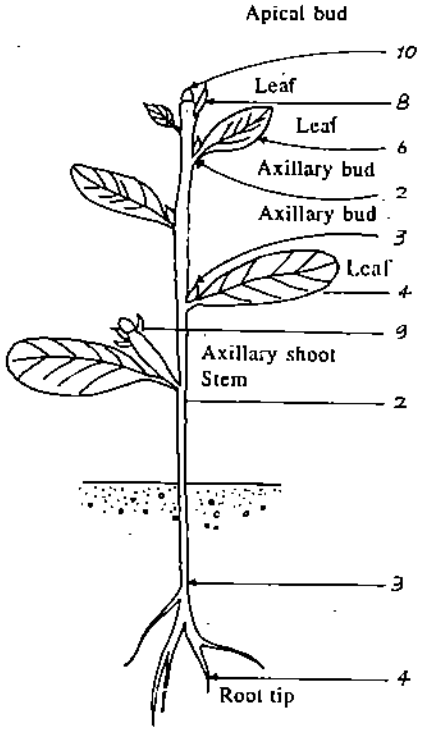

Fig. 9.3: The relative concentrations of auxin in different parts of seedling. Relative units of auxin: 10 = high, 1 = low.

9.3.2. Cytokinins

Cytokinins are also involved in the regulation of apical dominance. Wickson and Thimann studied the interaction of cytokinins and auxins in controlling the apical dominance. They isolated and cultured pea the stem sections in nutrient media containing auxins and no auxins. In the absence of auxins growth of lateral buds was not prevented. However, if cytokinins were added along with auxins, the inhibition of lateral buds was released.

Wickson and Thimann also demonstrated the effect of cytokinins on entire shoots. When the intact shoot was soaked in a solution of kinetin, growth of lateral buds occurred even in the presence of shoot apical buds. From this observation it was inferred that apical dominance exhibited by the shoot apex was overcome by the application of cytokinins.

The present concept is that apical dominance is controlled by a balance between cytokinin and auxin concentrations. Some investigators suggest the possible inhibitory influence of cytokinins on auxin production. The cytokinin application to the lateral buds may inhibit the synthesis of certain forms of IAA oxidase, normally induced by IAA translocated from the terminal bud. With the repression of IAA oxidase, the spared auxin may stimulate lateral bud growth and shoot development. In addition to the possible inhibition of IAA degradation, cytokinins may initiate a sink effect at the site of lateral buds to attract diversion of nutrients from other regions (Fig. 9.4).

9.3.3 Ethylene

Ethylene also plays a role in the inhibition of lateral bud growth. It is present in those tissues like shoot apex where auxins are also present. In normal mature

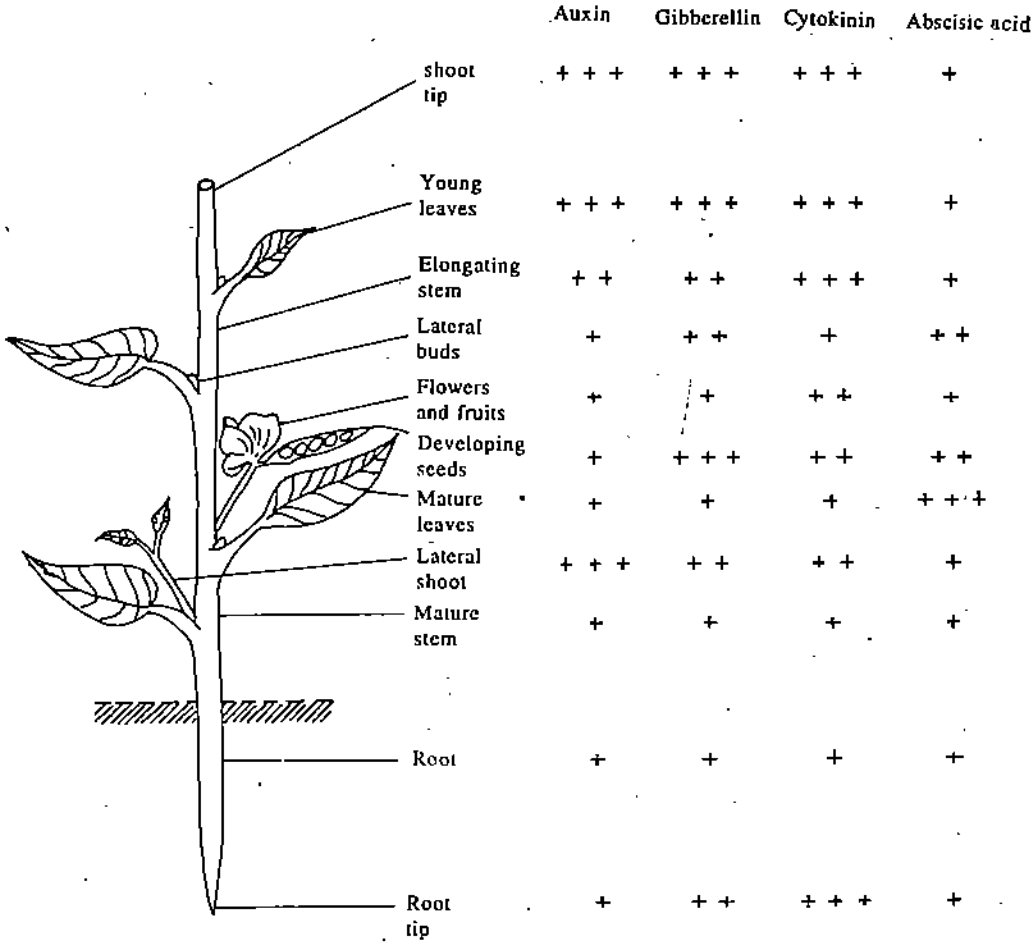

Fig. 9.4: Relative concentrations of some plant growth substances in various parts of the plant. +++ high concentration, ++ medium concentration, + low concentration.

light-grown plants the inhibition of lateral buds seems to result from the action of ethylene stimulated by auxins translocated there from the apical buds and leaves. This inhibitory effect of ethylene on lateral buds is released by the application of cytokinins.

9.3.4 How do Hormones Regulate Apical Dominance?

Auxins promote cell expansion and differentiation. Cytokinins accelerate cell division. A proper balance of these two hormones stimulates the production and differentiation of a variety of tissues in the stem. This rapid growth turns the shoot apex into a sink to draw nutrients from roots and adjacent leaves. As the main shoot apex generally draws most of the nutrients, lateral buds are starved resulting in the inhibition of their growth.

9.4 NUTRIENT DIVERSION THEORY

It has been repeatedly observed that if labelled nutrients like C^{14} sugars, S^{35} amino acids are applied leaves lowerdown the shoot are translocated towards the shoot apex. Application of cytokinins that break lateral bud dormancy reverts this process It is suggested that growth promoting hormones can enhance the transport of nutrients by increasing the rate of metabolism at the site of their application.

9.5 APICAL DOMINANCE AND PHYTOCHROME

We do not know the exact role of phytochromes in apical dominance. However short day induce lateral bud dormancy, suggesting the involvement of phytochromes. In those plant varieties in which phytochrome is genetically over expressed, apical dominance is poorly expressed.

9.6 APICAL DOMINANCE AND GENETIC STUDIES

In plants genetically programmed to over produce IAA, extreme cases of apical dominance is observed. If a genotype with a characteristic phenotype of over production of IAA is crossed with a genotype causing over production of cytokinins, apical dominance decreases phenotypically. This suggests that the balance between levels of cytokinins may be genetically regulated.

9.7 APPLICATIONS IN HORTICULTURE AND AGRICULTURE

While going round in public parks, you might have observed plants trimmed in such a way giving appearances of animals like camel, elephant, horse, and lion. This art traditionally used by gardeners, called topiary involves cutting of shoot apical buds, and force lateral branches such that they take on the shapes of animals.

Coniferous trees such as *Thuja* and *Biota* are also trimmed to produce globuler shapes. Another interesting case is that of *Picea (abies)* which grows straight, producing numerous lateral branches at equal intervals. It is commonly used as Christmas tree.In India *Araucaria* is commercially grown to serve as the Christmas tree. In *Araucaria* if you cut the terminal portion of a horizontally growing branch and plant it in a pot, it keeps growing horizontally after rooting. The plant appears to have "forgotten" to grow vertically (topophysis).

Another plant in which apical bud removal is being practised since times immemorial is the tea. Here, the commercial interest is in the production of new twigs bearing fewer leaves. Apical buds of the main axis and the branches are

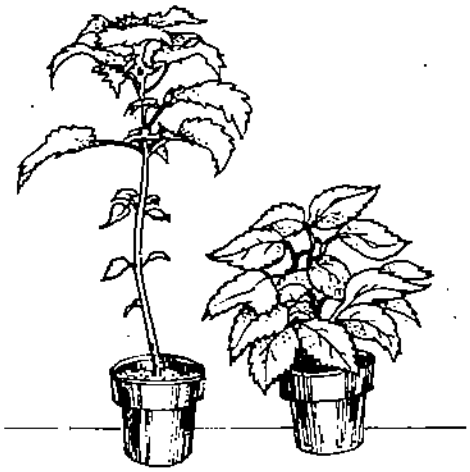

Fig. 7.5: The strong influence of the apical bud on the growth of lateral buds is easily demonstrated by removing it from the plant. In the absence of the apical bud, active growth begins in the lateral buds. However, in a short time the lateral bud nearest the apex will establish dominance over the other lateral bud and causes them to become inactive again. Of course, the response to removal of apical bud on the growth of lateral buds follows different patterns in different plants. In Bryophyllum and Kalanchoe, for example, the lateral bud closest to the apical bud grows out first; not the buds far-

removed seasonally so that the tea bush table is maintained at a convenient height to low picking of two leaves and a bud periodically.

9.8 SUMMARY

What we have studied in this unit could be summarised as follows:

1. Apical dominance has a great eco-Physiological significance and adds to survival value of plants.
2. Auxins play a role in association with cytokinins and ethylene in controlling the nature and extent of apical dominance.
3. Competition for nutrients between apical and lateral buds also decides the influence of apical bud.
4. Hormones activate synthetic machinery and create as 'Sink' to draw nutrients from other parts of the plant resulting in stimulated growth.
5. Recent Genetic studies indicate a role of phytochrome and confirm the earlier findings of the role of hormones in apical dominance.

9.9 TERMINAL QUESTIONS

1 Name some plants that exhibit monopodial branching and sympodial branching.

2. Mention the contributions of the following scientists in our understanding of apical dominance:
   i) Wickson & Thimann
   ii) Skoog
3. Does branching provide any extra survival value to plants? If so, explain
4. What is the nature of competition in the apical dominance?
5. What is the nutrient diversion theory? Explain with reference to phytohormones.
6. What is the effect of red light on apical dominance?
7. What is the contribution of recent genetic studies to our knowledge on apical dominance?
8. Describe briefly the application of techniques based on apical dominance in horticulture and agriculture.

9.10 ANSWERS

Self Assesment Questions:

a. i) Suppression of growth of lateral buds by the presence of shoot apex (apical bud).
   ii) Auxins, cytokinins and ABA.

b. i) The apical bud is the topmost meristematic tissue of main shoot or branch that contributes to the growth of main shoot/axis. Lateral buds are meristematic tissues that arise in the axils of leaves and that give rise to lateral branches.
   ii) Monopodial is a type of branching where the main axis grows continuously with less and little of lateral branching, bearing a crown of

leaves. (E.g. Palms). Sympodial type of branching is marked by a number of lateral branches, giving a bushy appearance to the tree (mango, lemon etc.).

iii) Shoot apex consists of meristematic tissue whose division and differentiation lead to the formation of all tissues and most organs of shoot. Slightly large in size and prominant, it is often protected by scaly leaves.

Root apex consists of actively dividing cells leading to the differentiation of tissues in root. It is protected by a layer of cells called as root cap. Root apex is relatively small in size.

Terminal Questions

1. Monopodialgrowth: Palms such as coconut, *Bonassus*,
   Sympodial growth: Neem (*Azadirachta indica*)
   Mango (*Mangifera indica*)
   Sisham (*Dalbergia sissoo*)
2. Wickson and Thimann Confirmed the role of auxins in apical dominance, through surgical removal of apex. Demonstrated the role of cytokinins in apical dominance.
3. Yes, because plants with more branches bear more leaves and can conduct more photosynthesis: spreading of branches helps in optimum utilisation of solar radiation.
4. The nature of "Struggle for existence" competition is as follows: The growing shoot apex functions as a sink in drawing nutrients from leaves and hormones from roots while lateral buds fail to get enough of nutrients.
5. Nutrients are diverted towards growing shoot apex from leaves thus depriving lateral buds from the supply of nutrients. Phytohormones are also produced in leaves and roots (like cytokinins, Gibberellins etc.) so along with nutrients, phytohormones supply is also mainly towards shoot apex. Cytokinins can break bud dormancy. Lack of supply of cytokinins to lateral buds induces bud dormancy.
6. Red light breaks apical dormancy. This effect is mediated through phytochrome that perceives red light and further mediates this process through production of phytohormones, like cytokinins that are needed to break dormancy.
7. The earlier observations on phytohormone interactions have now been confirmed by studying mutants that produce relatively high amounts of auxins and low amounts of cytokinins or relatively more cytokinins and less auxins.

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10.1 INTRODUCTION

You have learnt that among seed bearing plants, herbaceous annuals attain a limited height and do not need to increase in girth. Primary growth is often sufficient to meet their structure needs. However, in woody perennials that reach enormous height and produce large canopies, increase in girth is necessary to support the weight of the shoot. Secondary growth, derived from secondary or lateral meristems results in increase in diameter of stems and roots.

Objectives:

After reading this unit you will be able to:

- explain the phenomenon of secondary growth in woody plants,
- describe the structure and give function of each of the following
  - * vascular cambia,
  - * cork cambium,
  - * lenticels,
- identify the secondary growth of monocotyledonous and dicotyledonous stems
- distinguish between types of wood, its annual rings, sapwood, heartwood and bark,
- explain the commercial uses of cork and different wood,
- list various types of unusual secondary growth in stems and roots.

10.2 SECONDARY GROWTH IN A TYPICAL DICOTYLEDONOUS STEM

You have already read in unit 8 that the primary plant body is in itself structurally and functionally complete; for example the majority of monocotyledons and pteridophytes. In gymnosperms and most dicotyledons primary growth is followed by secondary growth.

In stem, the secondary growth in thickness in diameter is confined both intrastelar, i.e, within the stele and extrastelar regions.

The cells that form secondary tissues are produced by **lateral meristems.** The lateral meristems grow and join to make a circular ring known as the **vascular cambium** which lays down cells that become the **secondary vascular tissues.** In the stem, cells which are situated between the primary xylem and primary phloem in the vascular bundles become meristematic and form part of the vascular cambium. Additional cells between the vascular bundles also become meristmatic. Hence the vascular cambium can be seen in a cross section of the stem as a continuous ring of tissue, with the xylem and pith on the inside and phloem, cortex, and epidermis on the outside (Fig. 10.1).

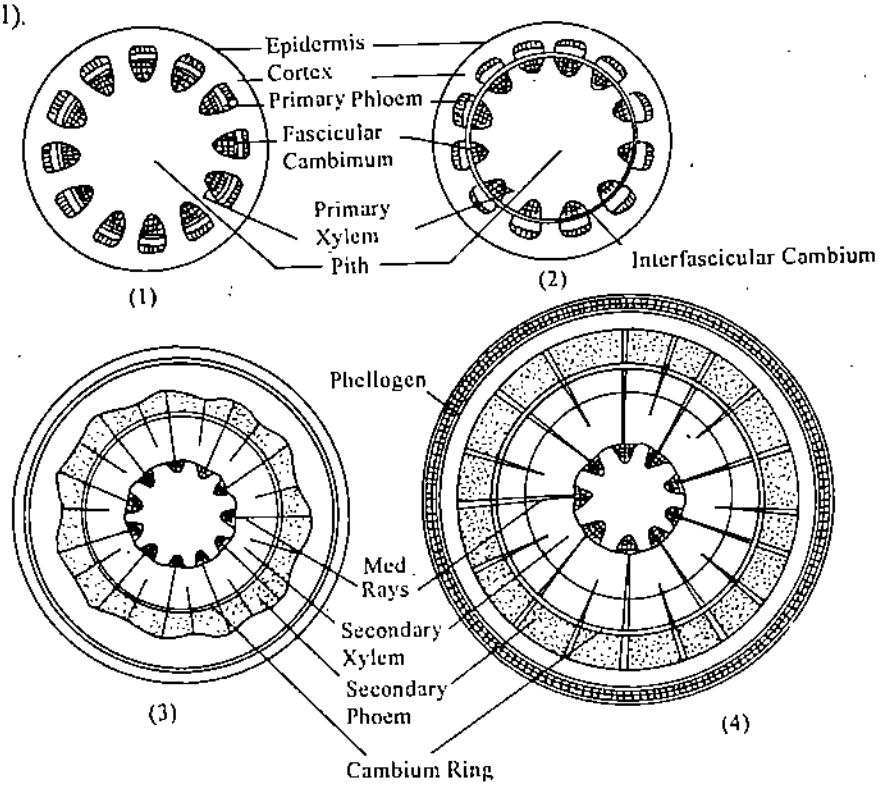

Fig. 10.1: Diagrammatic representation of secondary growth in a dicot stem upto two years (stages in T.S.)

The vascular cambium usually, if not always, has a dual origin within the primary tissues; from provascular strands, and from the "ground" meristem tissues between those strands. These two modes of origin are termed **intrafascicular** (within fascicles) and **interfascicular** (between fascicles). The term "provascular tissue" will be used in the text. We should know what this means. Provascular tissue is the precursor of all vascular tissues and "Procambium" is that part of the provascular tissue that is the precursor of the vascular cambium (which may also produce some metaxylem). The transitional stages between procambium and cambium are denoted as **metacambium.**

Both procambium and then metacambium differentiate acropetally within the provascular bundles. If we see the transections of *Populus*, metacambium can be first detected by a series of radial, anticlinal divisions in laterally extended sets of tangentially aligned cells. Most of the divisions in this layer are periclinal, producing metaxylem and metaphloem. The cells between the metaxylem and metaphloem eventually begin to function as cambial initials.

Cambial initials consists of two morphological types of cells–axially short, blocky, ray cells. Procambium at first consists of short cells from which longer cells may arise in two ways (Soh 1990): (1) Different cell lengths result from locally different rates of transverse and/or pseudotransverse cell divisions during growth. Thus the shorter cells become ray initials and the longer become fusiform initials. (2)All procambial cells first become quite elongated. Then some of them by nonrandom transverse and/or pseudotransverse divisions are secondarily transformed into sets of axially short ray initials.

As soon as a circle of vascular cambium is completed, its cells divide to produce

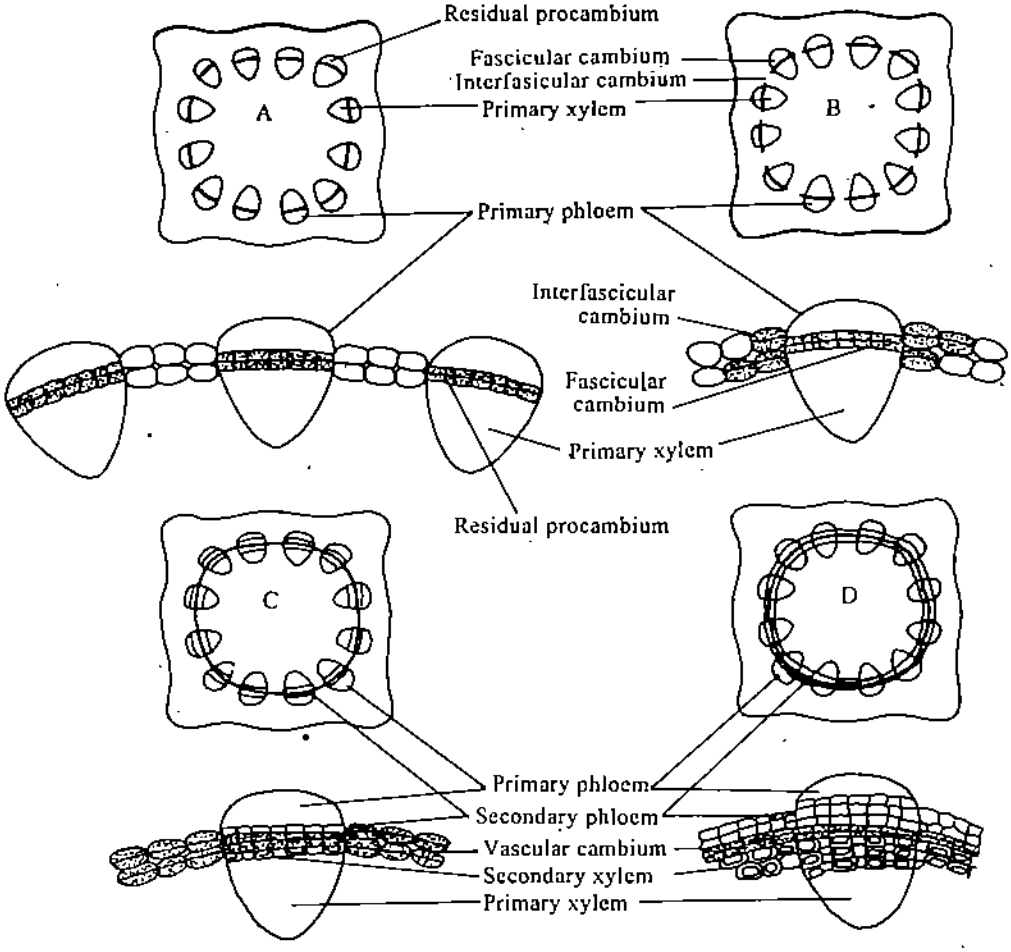

Fig. 10.2: Formation of complete vascular cambium. A. After completion of primary growth some meristematic cells remain between primary xylem and primary phloem. B. The residual procambium become reactivated to form fascicular cambium and some parenchyma cells of pith become meristematic to form the interfascicular cambium. C. Formation of complete cylinder of vascular cambium. D. Cylinders of secondary phloem and secondary xylem have been formed by vascular cambium.

new cells. Those formed inside the ring of cambium differentiate into secondary xylem or wood, about which you would study in the later part of this unit. Most of the cells of secondary xylem have very thick walls. As the cambium produces new wood, the stem increases in diameter, the phloem peripheral to the vascular cambium becomes stretched. In the mean time cells produced just outside the vascular cambium becomes differentiated into secondary phloem, and participate in the transport of organic substances.As more secondary xylem is formed, the first formed secondary phloem is destroyed and replaced by the newly formed secondary phloem. (Fig.10.2)

With the addition of secondary tissues, the stem grows thicker with secondary xylem and phloem, the peripheral primary tissues, cortex and epidermis become compressed and destroyed. The epidermis is replaced by a new protective layer of secondary tissue. Simultaneously another lateral meristem called phellogen (formerly termed **cork cambium**), is differentiated. It divides and produces new cell towards the outside. These cells become suberised i.e., impregnated with a waterproof waxy material and die, giving rise to a protective layer of cork.

Secondary growth in roots is similar to that in the stem. The main roots of a tree are large and woody and provide anchorage to the plant.The tasks of absorbing water and minerals are performed by younger roots at the far ends of the root system. A detailed account of the structure and description of secondary tissues and secondary growth will be given later in the unit.

SAQ 1:

a. Define secondary growth.

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b. Name the region in a dicot stem where secondary growth occurs.

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10.3 VASCULAR CAMBIUM-GENERAL DEVELOPMENT AND STRUCTURE

In certain plants, including monocotyledons, all the cells of the procambium undergo differentiation into primary vascular tissues. In almost all the dicotyledons and gymnosperms, a portion of the procambium remains meristematic even after the completion of primary growth and develops into the cambium of the secondary body. The cambium that arises within the bundles of primary vascular tissue of the stem is called **fascicular** cambium, because it originates within the bundles or larger segments of the primary vascular system. Commonly the bands of fascicular cambium become interconnected by additional bands of meristem, the interfascicular cambium, which originates from interfascicular parenchyma. A completely formed cambium of the stem has the shape of hollow cylinder, extending through the nodes and internodes. If the axis is branched, the main axis of the cambium is continuous with that of the branches, and it may extend some distance into the leaves.

The procambium and cambium may be looked upon as two developmental stages of the same meristem; they intergrade with regard to their morphological and physiological characteristics. The typical features of cambium of a woody dicotyledons and gymnosperms is segregation of its initials into fusiform and ray initials, the occurrence of apical growth, and the precise method of division in a tangential plane during the formation of xylem and phloem. The origin of the

interfascicular cambium in the more or less vacuolated interfascicular parenchyma results from a resumption of meristematic activity by a potentially meristematic tissue. Usually, no cytological changes are noticeable in connection with the return to meristematic activity. In most of the dicotyledons and gymnosperms the cambial cylinder develops between the primary xylem and phloem, a position that is retained throughout the life of the plant. It is from this point that the cambium produces the secondary xylem centripetally and secondary phloem centrifugally. (Fig.10.3). Now we will study about the strucuture of vascular cambium.

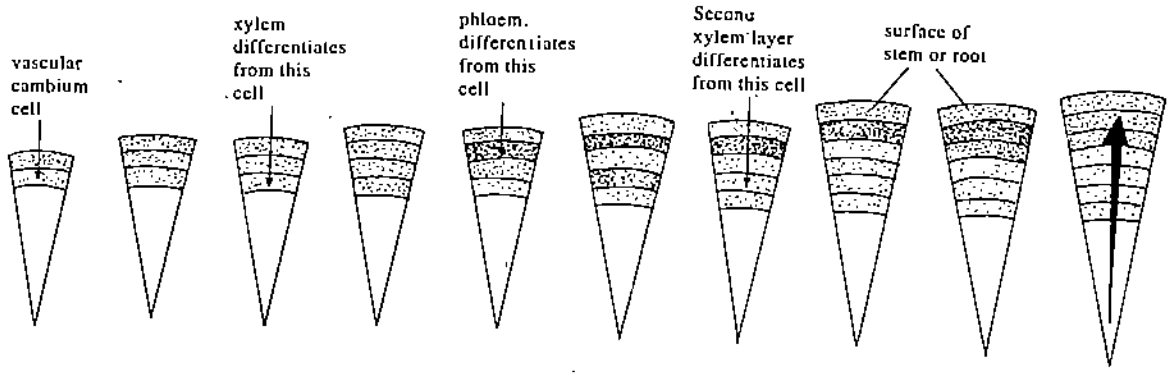

Fig. 10.3: Production of new xylem and new phloem each year. Vascular cambium is shifted away from the centre of the plant.

10.3.1 Structure of the Vascular Cambium:

In gymnosperm initials 1000-8700µm long were reported.

This meristem, in the anatomical sense of the term usually includes two histologically distinct kinds of cells: i) Fusiform Initials, ii) Ray Initials

i) Fusiform Initials:

Fusiform initials sometimes become very long in old trunks of *Sequoia sempervirens* e.g. they reach a maximum length of 8700µ m. (Bailey, 1923).

These are typically axially elongated cells with tapered ends. The mean length of fusiform cells varies, among taxa and within an individual plant. It tends to increase withthe age of the plant. These cells are shaped somewhat like flat shoe laces. It would be easy to assume that they have about 8 faces but a study of *Pinus sylvestris* has shown that 8 sides are minimum; they can often have up to 32 faces with an average of18; they have 14 contact faces with other such cells.

ii) Ray Initials:

They are smaller than fusiform initials and are nearly isodiametric (equal dimensions, small slides) or only about two or three times as tall as wide!

10.3.2 Types of Cambium:

On the basis of the arrangement of the fusiform cells as seen in tangential section, cambium is divided into:

i) Storied or Stratified Cambium:

The groups of ray initials may become taller either by the loss of fusiform initials located between two groups of ray initials, allowing them to fuse; or a fusiform initials can by transverse division, convert itself into a row of ray initials. All the structural elements which extend radially are produced by the ray initials.(Fig.10.4 A).

In this type the fusiform cells are arranged in tiers, or stories. That is, the ends of large tangential groups of cells are aligned at the same levels of axis. If you view them tangentially the ends of cells in axially adjacent stories generally overlap only slightly making a zigzag pattern.

Storied cambia occur in about 50 families of dicots, but commonly not in all genera of a family. Storied cambia do not occur in gymnosperms.

ii). Non-storied or Nonstratified Cambium :

In this type of cambium the ends of cambial fusiform cells typically overlap much more extensively and in a seemingly random manner. In nonstoried cambia there is no lateral alignment. In vesselless dicotledons the fusiform initials may reach a maximum length of 6200 µm . Thus nonstoried initials are longer. They are also of more common occurance. (Fig.10.4B)

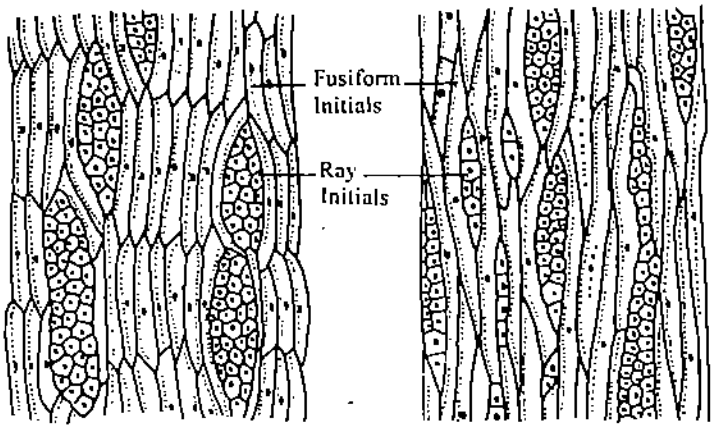

Fig. 10.4: L.S. views of fusiform initials and ray initials. A. Storied cambium B. Non-storied cambium.

10.3.3 Structure of Protoplast and Cell Growth:

Active cambial cells are richly cytoplasmic and are not wholly undifferentiated. They are obviously different in shape from embryonic ground meristem and apical meristem cells. They also differ cytologically from the other meristematic cells. They are more highly vacuolated, have large mitochondria and often have more highly differentiated plastids (Catesson 1990). In a cambial fusiform cell, the nucleus is quite elongated. Whereas in a ray cell it is usually more nearly spherically.

Active fusiform cells commonly have one or two large vacuoles transversed by many slender cytoplamic strands, and small vacuoles in the peripheral cytoplasm.

The cambium initials form pholem and xylem by tangential division. These vascular tissues are laid down in two opposite directions, the xylem cells towards the interior of axis and the phloem cells towards its periphery. The consistent tangential orientation of the planes of division during the formation of vascular tissues determines the arrangement of cambial derivatives in radial rows. Such radial seriation may persist in the developing xylem and phloem or it may be disturbed through various kinds of growth readjustments during the differentiation of these tissues.

The thickness of xylem cylinder increases by secondary growth, and the cambial cylinder also enlarges in circumference. Although active cambial cells undergo repeated periclinal divisions and radial growth, the width of cambial zone does not increase indefinitely. Conversely, although differentiation of cambial derivatives

into xylem and phloem continually removes cells from the cambial zone, the zone itself does not disappear. If the rates of radial growth and periclinal division are just balanced by the rate of cell loss through differentiation, the cambial zone thickness remains constant. However, the balance is often imprecise, and cambial zone thickness tends to vary during the active season. The rate of production of cambial derivatives depends on the number of cells in the cambial zone and on the duration of the cell cycle.

Addition of new fusiform initials is brought about by longitudinal anticlinal divisions of the existing initials in the storied cambium while in nonstoried cambium, the fusiform initials undergo oblique, pseudotransverse anticlinal divisions, followed by intrusive growth, and each of the new cells becomes as long as or even longer than the cell from which it was derived. (Fig. 10.5).

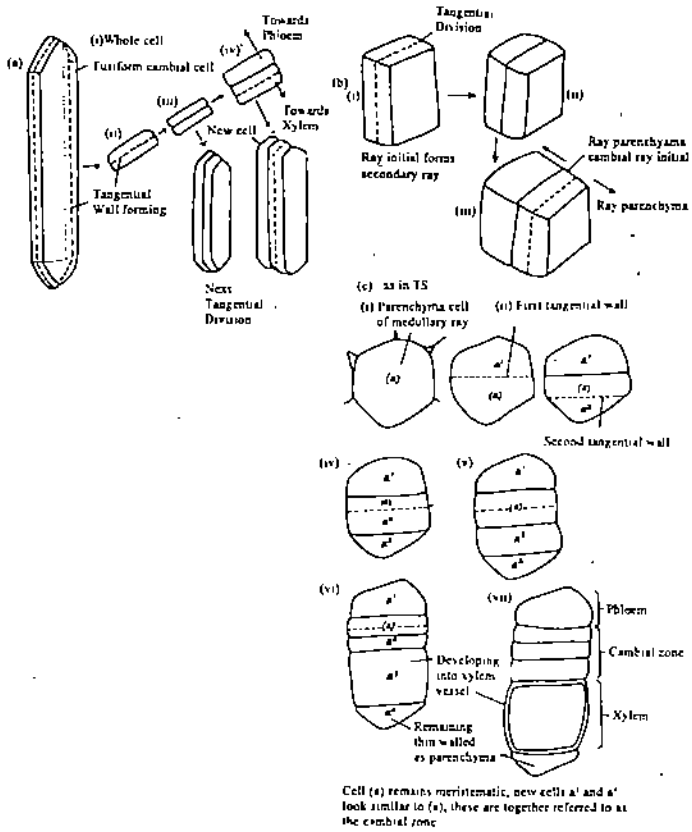

Fig. 10.5: Cambial development.
A. Fusiform cambial initial
B. Ray initial.

Because of the excessive length of the fusiform initials, the formation of the cell plate during the process of longitudinal division is peculiar to these cells. The cell plate begins to form between the two nuclei and it spreads slowly. The plate takes a long time to reach the end walls.

10.3.4 Ray Initiation:

With the enlargement of the cambial cylinder new ray intials develop and single fusiform initials are continuously lost from the cambium and are replaced by new ones (Fig 10.6). Let us now study how the ray initials are formed in the cambium, i) A single cell may be cut off along the side of a fusiform initial (lateral division). ii) A single cell may be cut off the end of a fusiform initials. iii) A declining fusiform initial may be reduced to a single ray initial. iv) In the last whole or part of a fusiform initial may be segmented by transverse divisions to form a tier of ray initiale. But various degrees of transverse divisions may occur between these types.

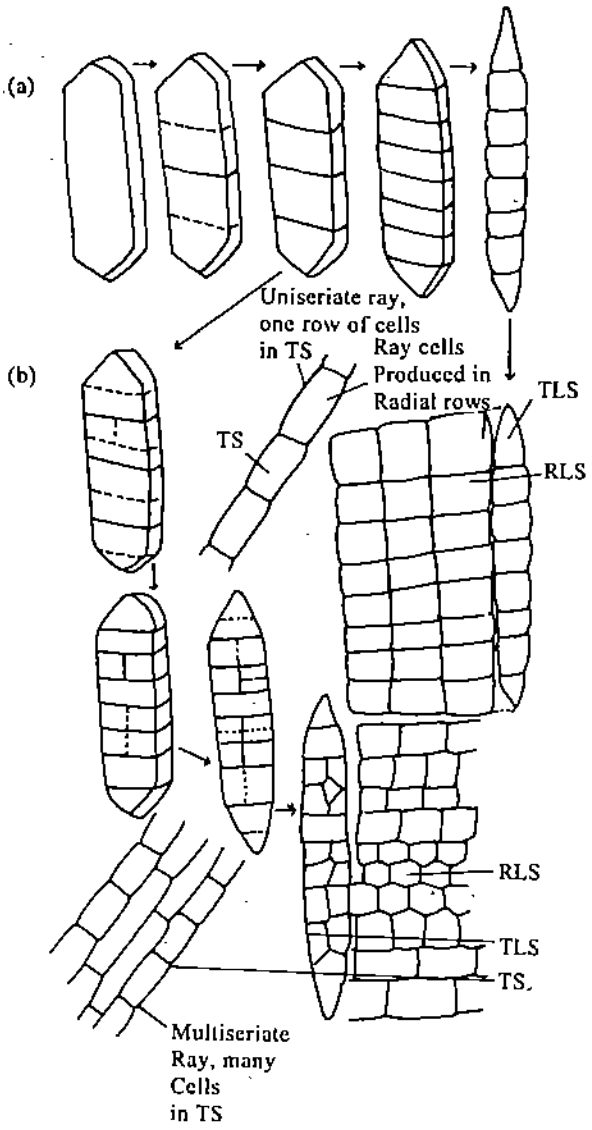

Fig. 10.6: Origin of a secondary ray.
A–Division of fusiform cambial initial to uniseriate.
B–Multiseriate ray.

SAQ2:

a. Name the two cambia which join and form a cylinder of of cambium.

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b. Describe the types of cells found in the vascular cambium.

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c. Differentiate between Storied or stratified cambium and Non storied or nonstratified cambium.

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10.4 CAMBIAL ACTIVITY

The radial growth is directly correlated with the rate of cambial activity. The variation in the number of cell across the cambial zone seems to express the balance between the rate of cell division and the rate of differentiation of the derivatives. However, at the time of cambial activity cell divisions are faster than cell differentiation. This results in a wide cambial zone. But as soon as differentiation begins, a balance is established and the width of the zone remains more or less constant. When the rate of division becomes less and the rate of differentiation faster the cambial zone becomes narrower.

The vascular cambium shows variation in the period and intensity of activity. These variations result from internal and external factors.

10.4.1 Formation of Annual Rings:

The secondary xylem in perennial axis commonly consists of concentric layers, each one of which represents a seasonal growth. If you see a cross section of the axis, these layers appear as rings, and the terms annual ring and growth ring or growth layer are applied to each layer.

An annual ring or growth ring of xylem is a layer of secondary xylem formed in one growing season over the entire plant and is, therefore, an extensive tubular structure having the general form of the axis of the plant. It is open at ends where meristems occur. There are some plants in which cambium is active during the entire life. These plants commonly occur in the tropical regions where seasons are mot markedly different. However, not all tropical trees exhibit a continuous cambial activity. In warm temperates climates, the percentage of ringless trees is still lower. In geographical regions where there are clear cut seasons, cambium shows decreased activity with the onset of autumn, and it enters a dormant state during winter. This may last till the beginning of the following spring. In spring the cambial activity is resumed and becomes maximum during summer. Thus periodical activity of cambium results in the formation of ring wood. The wood thus formed in spring is called springsummer wood or earlywood and that formed in autumn is called autumn wood or latewood.

It is noted that each annual ring corresponds to one year's growth hence the age of plant can be determined roughly by counting the total number of annual rings in a log (trunk) as seen in a transverse section (Fig. 10.7). Tree ring analysis is also known as **Dendrochronology**. By analysing the tree rings, a great deal can be learned about past climatic conditions. This study is known as **Dendroclimatology** (See box 10.1).

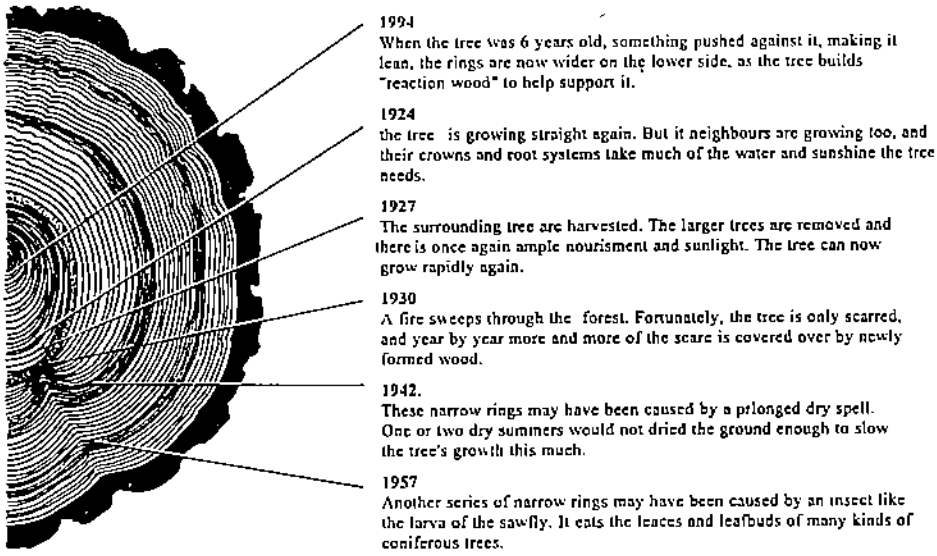

Fig. 10.7: T.S. of dicotyledonous stem showing annual rings.

Box 10.1: TREE RING ANALYSIS –A WAY TO TELL THE LIFE HISTORY OF A TREE

Every year a new growth ring is added to trunk of the tree. In spring growth is fast and wood is light in colour. In summer the wood is darker. By counting the dark rings you can tell the age of tree. In addition other useful information can be obtained by analysing tree rings as well. For example, the size of each ring varies depending on environmental conditions, including precipitation and temperature. Some times the variation in tree rings can be due to a single environmental factor. Then similar patterns appear in the rings of many tree species in a large geographical area. For example, if a certain year is drought year then in that particular year much smaller wood layer's will be produced. Some times locusts may have eaten the leaves just after they have appeared. This will result in a marked decrease in photosynthesis, causing low wood production. This will also result in two annual rings being very close each other because very little growth will take place.

To study the sequence of rings, in trees that have lived for several thousand years first a master chronology of complete records of sample of rings dating back as far as possible is developed. Then by matching the rings one can know the exact age of the living tree or trees (Fig. 10.8).

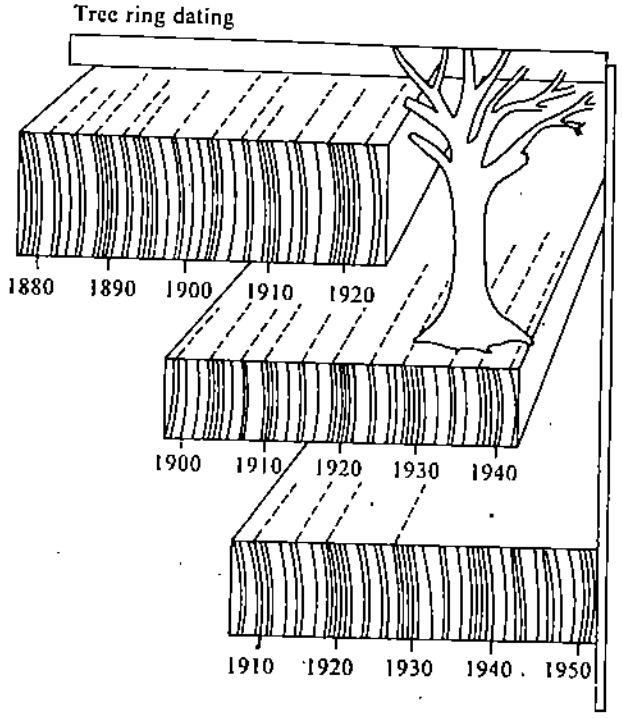

Fig. 10.8: Tree ring dating: A master chronology is developed using progressively older pieces of wood from the same geogrophical area. The age of the sample can be accurately determined by matching the rings of a wood sample of unknown age to the master chronology.

A great deal can also be studied from tree rings about the past climatic conditions. For several years A.E. Douglass, Harlod C. Fritts and others associated with the Laboratory of Tree Ring Research of the University of Arizona Phoenix, USA studied ring widths in trees from various sites. They found that a very significant statistical relationship exists between the growth of trees and climatic condition. With the help of computers and statistical analysis they developed techniques that takes into account of climate and other environmental variables. They were able to reconstruct relatively precise histories of climatic changes and fluctuations dating back thousand of years. Presently by ring analysis data are gathered to determine climates of prehistoric times.

To determine the age of living old tree you do not have to cut it down, to examine the rings. A simple instrument known as increment borer is used. The increment borer is primarily made up of a rigid metal cylinder. It is driven in the stem of a tree and a core of wood is removed. The hole is then treated with a disinfectant and covered up without harm to the tree. The rings are examined counted from the core and then analysed.

SAQ 3:

Which of the following statements are true. Write T for true and F for false in the given boxes.

a) All tropical trees exhibit a continuous cambial activity. ☐

b) Regions with warm climate have a low per cent of ringless trees. ☐

b) It is possible to calculate the approximate age of a tree by counting the total number of rings in one log of wood. ☐

10.5 SECONDARY XYLEM

The products of the cambium formed towards the centre of the stem and root constitute secondary xylem. Secondary xylem is composed of tracheids, vessel members, different types of fibres, parenchyma cells, xylem ray cells and sometimes secretory cells. The occurrence and the arrangement of these elements vary in different group of plants. The quantitative differences in the number of cells and the size of the elements that exist between the species of a single genus, make it possible to identify individual species on the basis of secondary xylem alone.

10.5.1 Basic Structure of Secondary Xylem:

Secondary xylem is characterised by the existence of two systems of elements which differ in the orientation of their longitudinal axis. One system is horizontal and other vertical. The horizontal system is made up of xylem rays (Fig. 10.9) and the vertical or axial system consists tracheary elements, fibres and wood parenchyma. The living cells of the rays and of the vertical system are usually interconnected and a continuous system of living cells is formed, which in turn is connected with the living cells of the pith, phloem and cortex.

10.5.2 Wood Parenchyma:

Two types of parenchyma are found in secondary xylem : The axial parenchyma and the ray parenchyma. The relatively short, special cambial initials give rise to ray parenchyma, whereas fusiform initials from axial parenchyma. The axial

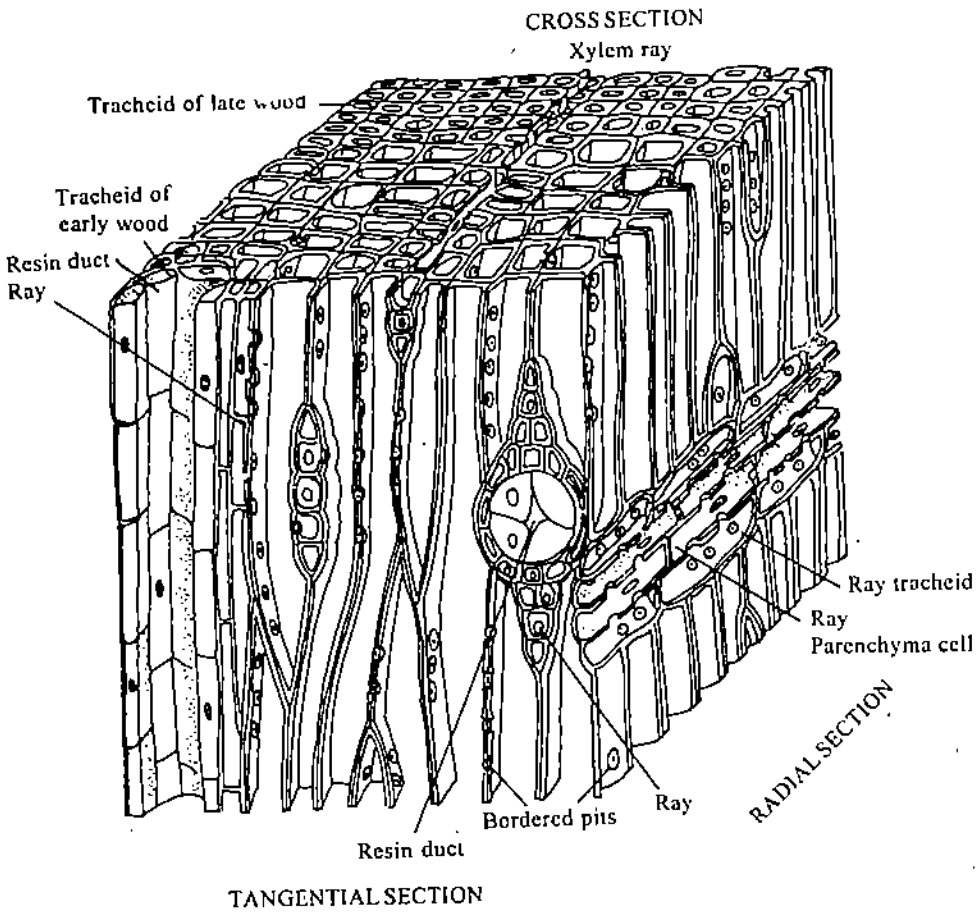

Fig. 10.9: Structure of secondary xylem in three dimensional diagram of a cube of *Pinus*.

parenchyma cells may be as long as the fusiform initials or much shorter. It is more common to find shorter parenchyma cells (Fig. 10.10).

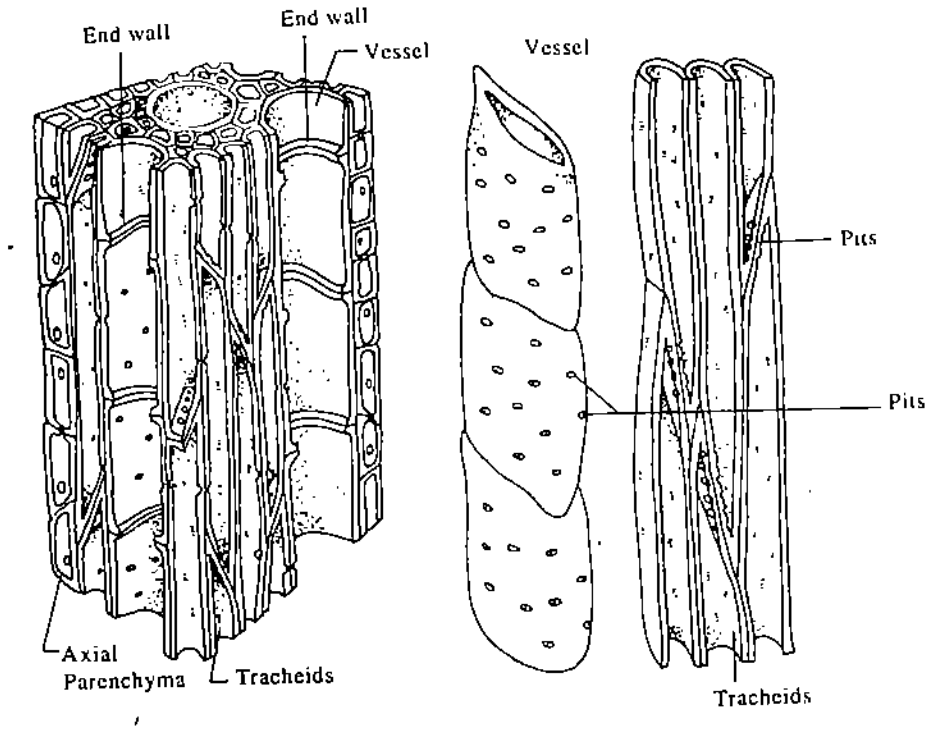

Fig. 10.10: Basic structure of xylem.

The ray parenchyma may be of different kinds, the two most common forms are : i) in those which longest axis of the cell is radial, ii) in which it is vertical.

The number of xylem rays increases with the expansion of stem girth.The length, width and height of each ray can be measured by cross sections and tangential sections respectively,. When the ray is one cell wide it is called **uniseriate ray;** when two cell wide it is **biseriate** and when more than two cells wide, it is **multiseriate.**

All the cells of ray parenchyma may have primary wall or only secondary walls are found. The secondary walls may develop pitpairs that are to be simple, halfbordered, and sometimes even bordered. These secondary cell walls are commonly characterized by the presence of depressions or cavities varying in size, depth and structure. Such cavities are termed pits (Fig. 10.11 A). The parenchyma cells of the xylem serve to store reserve food materials such as starch and fats. Tannin, crystals, silica bodies and other substances are also deposited in these cells. The ray parenchyma is the main route of radial symplasmic transport between xylem and phloem.

In several plants wood parenchyma cells form protuberances which penetrate into the vessels through the pits after they become inactive, or later injured. These outgrowths are termed tyloses (singular : tylose) (Fig. 10.11 B). The nucleus and part of cytoplasm of the parenchyma cells may enter the tyloses. Tyloses may also divide. Although the formation of tyloses is considered a natural phenomenon, in many species it has been reported to result from mechanical injury or diseases.

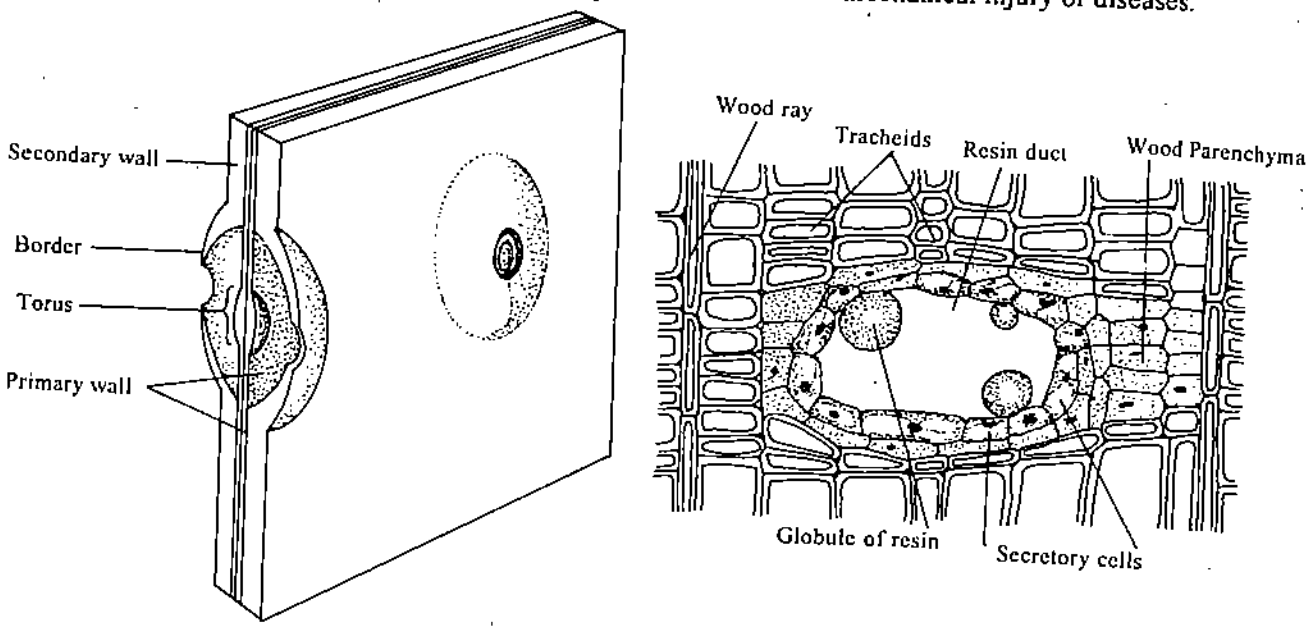

Fig. 10.11: A. Boarded pit.
B. Resin duct in *Pinus* wood.

10.5.3. Heartwood and Sapwood:

The outer part of the secondary xylem contains living cells and at least one or two outermost rings participate in the conduction of water. The outer part of secondary xylem with living parenchyma is named as Sapwood or **alburnum** (Fig. 10.12). In nearly all the trees the central portion of the xylem consists of dead parenchyma which ceases to conduct water. This is called heartwood or **duramen.** The events that occur in the formation of heart wood include disintegration of protoplast, loss of cell sap and hydrolysis of reserve material stored and formation of tyloses. In such species in which the tyloses are formed the inner portion of the cell is totally blocked by the tyloses. Oil, gums, resins, tanins, aromatic compounds and coloured substances which develop in the cells are accumulated in the heartwood.

Heartwood may sometimes develop as the result of pathological conditions.

The amount of heartwood and sapwood varies in different speices. These differences are influenced by genetic and environmental conditions.

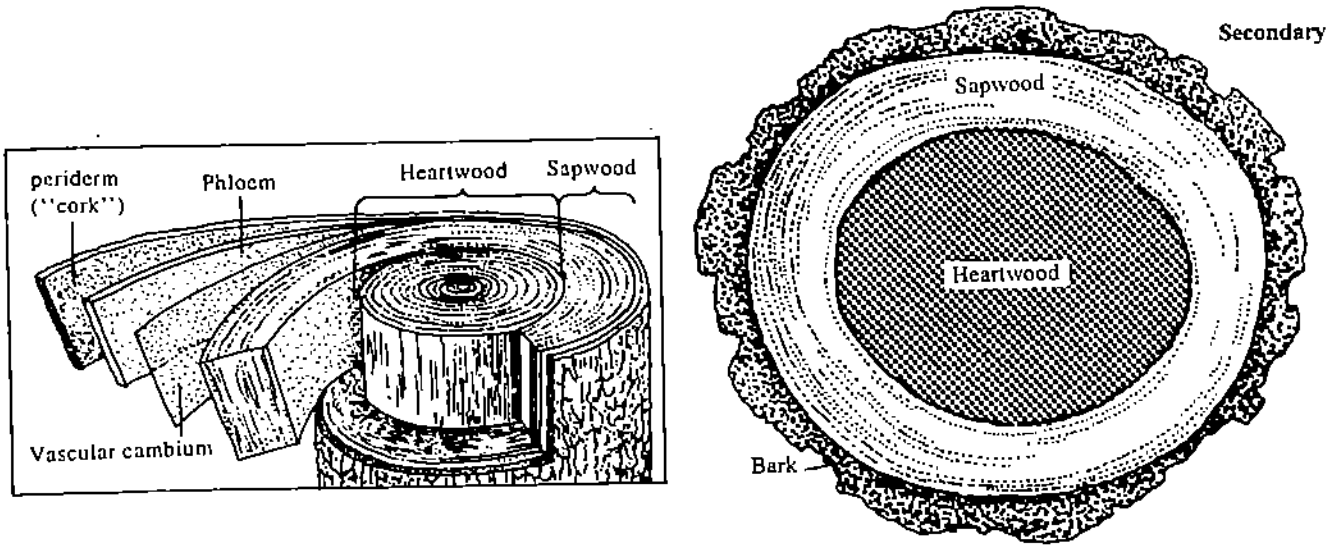

Fig. 10.12: Heartwood and sapwood in C.S. of mulberry trees.

In the timber trade wood of dicotyledons is known as hardwood and that of gymnosperm as softwood. These terms do not accurately express the degree of hardness, as in both groups wood with both hard and soft structure can be noted. However, there is an important difference in the wood of dictoyledon and gymnosperm. The former have vessels and the later lack them. If we see the histological structure of the wood of dicotyledon and that of gymnosperm there are fundamental differences.

10.5.4 Economic Importance of Woods and its Characteristics.

Wood has much importance in day to day life in form of furniture, paper gum, resin and several other industrial purposes. We will discuss some of the properties of wood by which wood quality is judged.

i) **Weight:** The wood may be either light or heavy. Differences in weight are due to variations in the proportions of wall substance and of lumen space , when the lumen is small the wood is dense and heavy. The abundance of slender, thick walled fibres make wood heavy. Extremely light woods such as *Ochroma* is also found. The majority of well known commercially important wood range from 0.35 to 0.65 in specific gravity. Balsa wood (*Ochroma lagopus*) and sola (*Acshynomene*) have abundant of parenchyma and very few fibres.

Light woods have limited economic importance. Balsa (*Ochroma*) with a specific gravity of 0.12 to 0.35, is used extensively in insulation, as material for modelling (by hitects) and for life rafts. The heavy woods are used in construction, wagons, carts, railway sleepers and furniture etc.

ii) If a large proportion of the wood is made up of fibers or fiber tracheids it tends to be strong. Thus the dense and heavy woods are of greater strength. Strong woods are used for building, structural works, furniture etc.

iii) **Durability:** The ability of wood to withstand decay by the action of fungi and bacteria is largely dependent upon the chemical nature of the wood and is referred as its durability. The presence of tyloses and other natural constituents of wood such as tannin, resin and oils largely determine the durability of wood. Both light and heavy woods can be durable.

The durable woods are used for ship building, boats, masts, ships, carts, bodies of campartments, railway wagons, construction, bridges, railway compartments, railway sleepers.

Some other important uses of Wood:

Teak, sisham and rose woodware decorative timbers for panelling furniture,

cabinet, boxes, for carving idols, inlay works, aspects of art etc.

iv) **Pines:** They are chiefly used for doors, windows, pattern making in cabinet, boxes and matches. In India *P. roxburghii* is used for house building, packing cases, matches, music instruments, railway sleepers etc.

v) **Sandal Wood:** Sandal wood is smooth and tends itself to exquisite used for carving as well as for extraction of the oil. The sandalwood oil is fragrant and finds use in perfumes, cosmetics, soaps, incense sticks etc.

SAQ 4:

a. List the two types of systems and various cells found in secondary xylem.

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b. How do uniseriate, biseriate and multiseriate rays differ from one another.

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c. Distinguish between hardwood and softwood.

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d. What structural features gives strength to wood?

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e. List as many independent uses of wood as you can think.

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10.6 SECONDARY PHLOEM

In the secondary phloem also there are two systems the vertical and horizontal-products from those cambial intials as in the case of xylem. Though these two tissues secondary xylem and secondary phloem differ in ontogeny and structure at maturity.

The important components of the vertical system are sieve elements, phloem parenchyma and phloem fibers (Fig.10.13). The horizontal system consists axial and ray phloem which is made up of parenchymal cells. As in the xylem, the arrangement of the tissue in phloem is primarily determined by the nature of cambium i.e. whether it is storied or not. Secondly it depends upon the extent of elongation of various elements of vertical system during the differentiation of the cells.

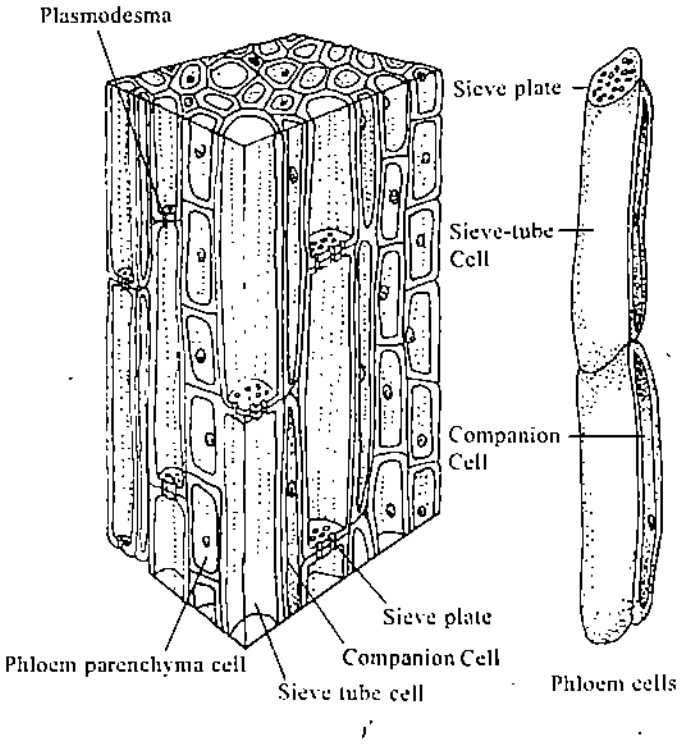

Fig 10.13: Basic structure of phloem

In several species of dicotyledonous trees, growth rings can be observed in the secondary phloem but they are less distinct than in secondary xylem. This is because cells which are produced at the beginning of the season are extended radially while in the end of the season they are flattened. After some growth the arrangement of growth ring becomes obscured due to the obliteration of the sieve elements. The primary reason is also because of absence of lignin that is why they can not be used as an indication of the age of secondary phloem.

As you have already studied, that the ray initials in the cambium produce cells towards both the xylem and phloem. Thus xylem and phloem rays are continuous. In the vicinity of the cambium the xylem and phloem rays are equal in size but in many plants the mature outer portions of phloem rays are wider. The widening of the phloem ray may be accomplished solely by the lateral expansion of the existing cells or as is more common by an increase in the number of cells on the periphery by radial divisions.

In the dicotyledons the functional secondary phloem is restricted generally to that produced in the last growing season. In some cases when the cambium starts producing new phloem, almost all the previously produced sieve tubes cease to function. However, in *Tilia* the sieve tubes are active for several years including winter.

10.6.1 Economic Value of Secondary Phloem:

The secondary phloem may be quite rich in secretory tissues. This is possibly related to its role in protecting the plant. The phloem may contain well developed

duct systems in some species. It is the system of lactifers in the bark of *Hevea* that is tapped to obtain rubber and the resin canals of the bark of conifers that is are harvested for pine resin which is further distilled to make turpentine and resins.

Bast Fibers: These are sclerenchyma fibers associated with the phloem of certain stems of plants. They are rather easy to separate from underlying woody tissues. They arise with primary tissues from the apical meristem or with secondary tissues produced by the lateral meristem, the cambium. Important sources of bast fibres are flax, jute, sunnhemp etc.

SAQ 5:

Write T in front of true statement and F for false statement in the bracket provided.

a. In phloem there are two systems the vertical and horizontal. ( )

b. We can judge the precise age of tree by counting the number of rings of phloem. ( )

c. In several dicotyledonous species we can see more distinct ring in secondary phloem than in secondary xylem. ( )

10.7 SECONDARY GROWTH IN MONOCOT STEM

Normally in a monocot stem, no secondary growth takes place, as the vascular bundles are closed i.e. cambium is absent. But in some herbaceous and treelike woody monocotyledons plants belonging to families Liliaceae, Agavaceae etc., the increase in thickness is through secondary cambium (which is entirely a secondary meristem). At the time of secondary growth some of the innermost parenchymatous cell become meristematic, the cells divide tangentially forming a band of secondary cambium, a few layers in thickness. The secondary cambium is made up of rectangular fusiform cells. They do not produce secondary phloem or xylem outside and inside respectively as in dicotyledonous stems and roots. Instead the secondary cambium cuts of secondary tissues on the inner side first and then a small amount of new tissues on the outerside. These newly formed inner secondary tissues directly differentiate into oval shaped collateral vascular bundles and radially arranged parenchyma cells called conjunctive tissue. Thus the vascular bundles remains embedded in the conjunctive tissue.

In a monocot stem a periderm is absent but some storied cork cells, forming a protective tissue with suberisation (*Dracaena*) are present.

10.8 PERIDERM

Before dealing with **periderm** formation we must know what periderm is? The term periderm is collectively given to the protective tissues **phellem** and **phelloderm** and the meristem that lies between them and gives rise to them. This meristem, the **phellogen,** similar to vascular cambium, is a uniseriate layer of initial cells that by periclinal divisions gives off derivatives from their adaxial and abaxial faces. A phellogen produces phelloderm adaxially and phellem abaxially (Fig. 10.14). Phellem, a major constituent of periderm, is generally suberized and inhibits translocation of water and solutes to tissue abaxial to it. As a result, these tissues senescence and die. The cork tissue forms a protective layer of the tree after the epidermis dies and is shed. Cork is generally formed in the stem and root of dicotyledons which have a continuous and pronounced secondary thickening cork is not formed in leaves with the exception scales of winter buds of certain plants.

Cork is an important part of secondary tissue which is termed periderm (Fig. 10.14). Peridem is usually divided into three parts:

i) The phellogen–cork cambium

ii) The phellem–cork which is produced centrifugally by the phellogen.

iii) The phelloderm–a parenchymatous tissue in some species, and produced centripetally by the phellogen.

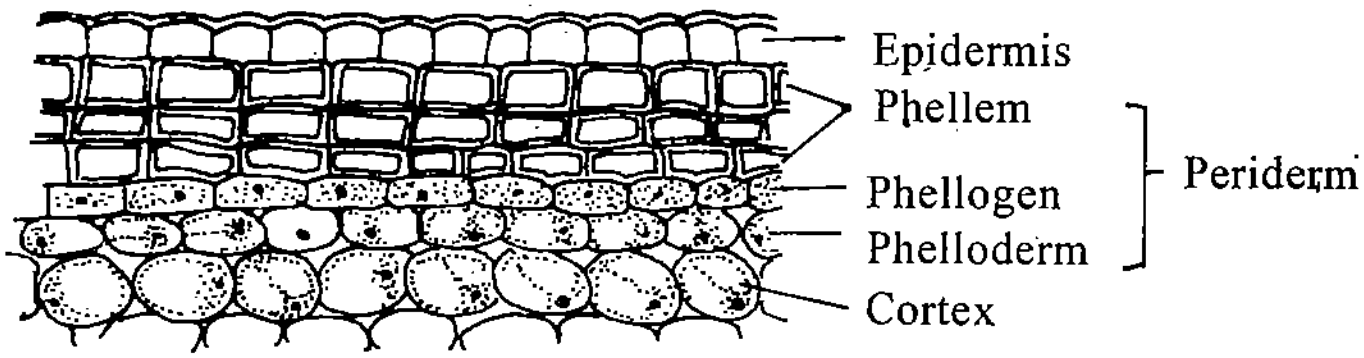

Fig. 10.14: Periderm formation.

10.8.1 Structure

Phellogen is a lateral meristem consisting of a single layer of initial cells. The cells are uniform, rectangular in cross section with their shorter axis in the radial direction. In a T.L.S they appear as regular polygons. The protoplasts of phellogen cells contain vacoules of various sizes and may contain chloroplasts and tannins. Phellogen has no intercellular spaces except in the regions of lenticels.

The phellogen has distinct periods of activity and nonactivity. The activity period may or may not concide with that of the cambium. However, in some plants two periods of phellogen activity have been noticed in a single annual period of cambial activity.

The two types of cell of cork may found in one species e.g. *Arbutus* and *Betula*, where they occur in alternating layers in *Betula* this feature causes an interesting feature and cork is peeled off like sheets of papers.

10.8.2 Phellem

Phellem or cork arises from the abaxial derivatives of phellogen. The cells of phellem or cork are usually polygonal, and radially flattened and divide tangentially.In a cross section cells are devoid of intercellular spaces except in lenticular region. The cells of phellem divide tangentially. Cork cells are dead. They may contain crystal containing cell, they may be sclerieds, or even nonsuberised. In certain species the cork cells's primary walls are suberized and contain a thick suberin layer interior to the primary wall called the suberin lamella. This substance suberin is highly impervious to gases, water and resist the action of acid. This phenomenon of impregnation of walls with suberin is referred as suberization.

10.8.3 Phelloderm:

The phelloderm cells are living cells with nonsuberised walls. They are similar to the parenchyma cells of the cortex but, if the phelloderm is multiseriate, they are usually arranged in radial rows. In some plants cells of the phelloderm have chloroplasts and are photosynthetic.

10.8.4 Origin and Development of Periderm

The phellogen may differentiate in a living epidermal (Fig.10.15), collenchyma or a parenchyma cell. Just before the onset of meristmatic activity the cell loses the central vacuoles, the volume of cytoplasm increases and it undergoes a periclinal division. Following the first periclinal division, two similar cells are formed, of which the inner ceases to divide further. The outer divides periclinally. The outer cells differentiates into the cork cell and the inner constitutes the phellogen initial and continues to divide.

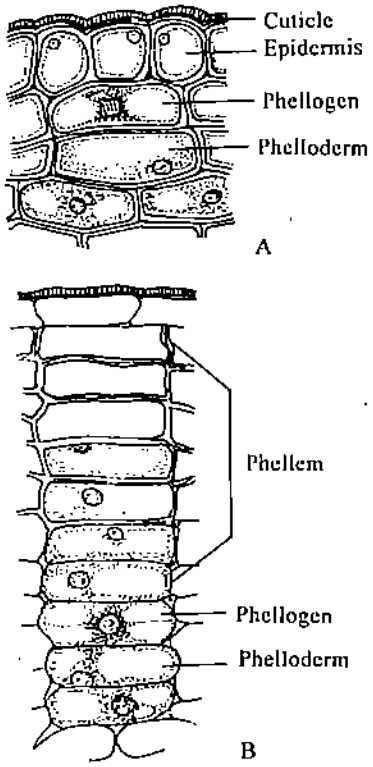

Fig. 10.15: Formation of periderm stages.

The initials of phellogen cells occasionally undergo anticlinal divisions, to keep pace with the increase in the circumference of the cork cylinder. The number of phellem layers is usually greater than the number of phelloderm layers.

If the firstformed periderm remains on the axial organ for many years, the other layers of cork show cracks and are shed off. Thus the thickness of cork on a plant remains constant.

10.8.5 Commercial Cork

The source of commercial cork is. *Quercus suber* which occur naturally in the countries boarding the medeterranean sea. In this species phellogen arises in the epidermis. It may remain on the plant for an indefinite period of time. For commercial purpose the first formed periderm is removed when the tree is about 20 years old and about 40 cm in diameter. The exposed cells of the phelloderm and cortex dry out and die, and a new phellogen is formed a few millimeters within the cortex. The subsequent phellogen produces cork more rapidly and in about 10 years a sufficient thickness to be of commercial value. The stripped cork shows a rough outer surface and a smooth inner surface.

The cork is valuable because it is impervious to gases and liquid, nonreactive and has strength, elasticity and lightness. It is used for insulation, sound proofing and in the manufacture of sportsgoods. Cork is unparallel as a material for making stoppers for wine and champange bottles.

You may like to know why commercial cork is to be cut in a particular plane. Cork is several centimeters thick and the lenticels remain active for a long time and result in the formation of cylinders of complementary tissue which extend from the phellogen to the surface of the phellem. This complementary tissue forms the patches of dark brown crumbling tissue found in commercial cork. Because of the radial orientation of the tissues, bottle corks to be in a direction parellel to the surface of the trunk..This way the cylinderical lenticels extend transversely through them. Sheets of cork from the tree are rarely more than 3 cm. thick. Cork with a diameter greater than that can not be obtained by cutting in the usual manner. Large corks are usually cut from sheets of ground and compressed cork or from "multiple sheets" composed of layers cemented together. These kinds of cork are of low quality.

You must also know about lenticels. These are restricted area of relatively loosely arranged cells, in the periderm. lenticel protrude above the periderm because of their larger size and loose arrangement of numerous cells.There is continuity of intercellular spaces of lenticels with tissue in the axial organs. Therefore, lenticels are believed to participate in gas exchange.

SAQ 6:

Complete the sentences from section A with those given in section B:

	Section A		Section B
a.	**Periderm is usually divided into**	i)	**consists only one type of initial cells.**
b.	**In some plants phellogen has**		**ii) three parts** **a) Phellogen** **b) Phellem** **c) Phelloderm**
c.	**Histologically phellogen**		**iii) is impermeable to water gases and can withstand the action of acid.**
d.	**Cork**		**iv) alternating periods of activity and inactivity.**

10.9 DISTRIBUTION OF LENTICELS

Lenticels are highly differentiated lens shaped areas of periderm. They are usually found on stem and roots and appear on young branches or other organs as rough dark patches. Due to many intercellular spaces, lenticels have a loose structure. Mostly scattered over the entire surface of stem (Fig. 10.16).

In the roots of *Phoenix dactylifera* lenticel like structure occur which take part in aeration of the root but which differ from the described ordinary lenticels. Here the lenticels form collar like structure around the thinner roots.

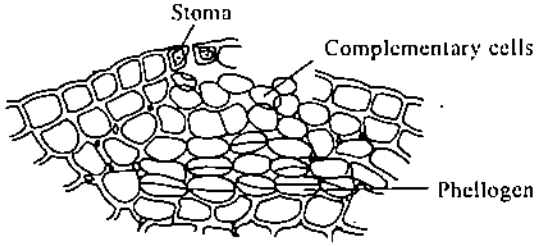

Fig. 10.16: Lenticel in L.S. early stage in formation.

If we examine lenticel it usually looks like a convex lens both internally and externally.

10.9.1 Development and Structure of Lenticels

Lenticels originate form localized regions in the phellogen that become continuous with the nonlenticular phellogen. Lenticular phellogen has more intercellular spaces and produces derivatives at a higher state than do nonlenticular phellogen. The first formed lenticels generally appear below a stoma or group of stomata. Cells below the stomata begin to divide in different directions and the chlorophyll in them disappear so that a loose colourless tissue is formed. The cells that are derived from the divisions become more and more periclinal until the phellogen of the lenticels is formed (Fig. 10.17). The cells which are derived from the divisions of the substomatal cells, as well as those produced towards the exterior by the phellogen of the lenticels are termed complementary cells. After division the number of cells increases and masses of complementary cells are pushed out and rise above the surface of the organ.

Only a few plants e.g. *Philadephus Anabasis, Haloxylon, Campsis radicans Vitis* and some other species, many of which are climbers do not possess lenticels.

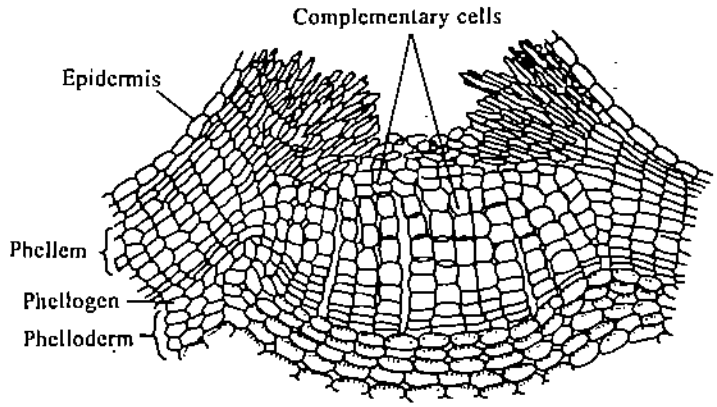

Fig. 10.17: Lenticel (in L.S.) – well formed

In the temperate regions lenticels become closed by the end of autumn season by a closing layer. Whereas in some plants lenticels are formed relatively early in the life of the plant and are shed together with the bark, in others they may remain active for several years.

SAQ 7:

Define the following in two or three sentences:

Lenticels and Complementary cells.

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10.10 CAMBIAL VARIANTS

In this unit you have studied the function of vascular cambium and cork cambium in dicotyledonous and monocotyledonous stems accounting for development of secondary tissues. Cambium shows variation in its activity giving rise to conditions which are rather typical. Various terms such as cambial variants, anomalous or

aberrant secondary growth have been used to describe these instances (Fig. 10.18). As the variants are of quite regular occurence in certain plants, anatomists have discouraged the use of the term anomalous secondary growth. Instead cambial

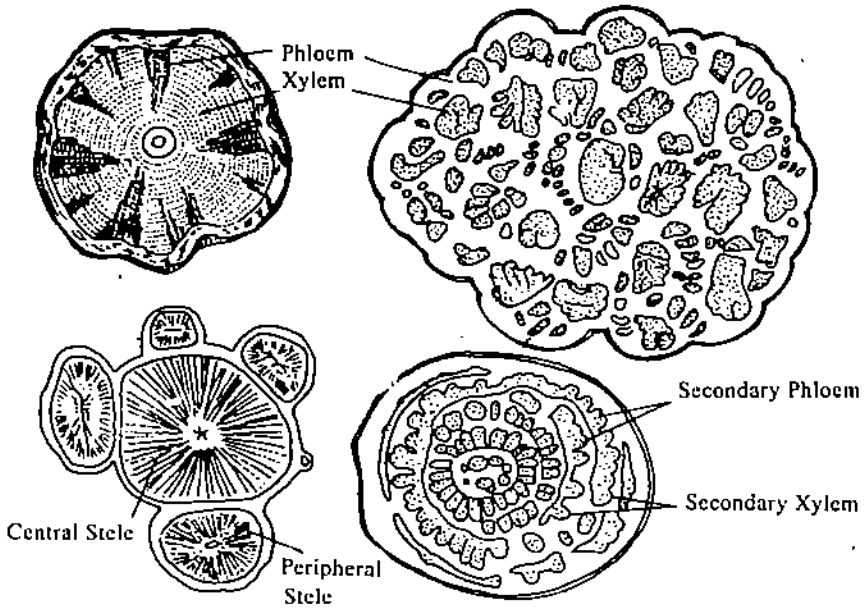

Fig. 10.18: Cambial Variants (Anomalous stem structures. A. In *Bignonia*. B. In *Serjania*. C. *In Bauhinia*. D. *In Boerhaavia*.)

variant has been recommended for usage.

10.10.1 In Stems

I. The cambium is persistent and normal in position. Its products show unusual arrangement and proportion.

In *Bignonia* (Fig. 10.19) and some other members of the Bignoniaceae family. The cambium produces secondary xylem and secondary phloem in dififerent amounts. Thus in some part of the plant the amount of xylem is much greater than phloem while in the other, phloem is much more abundant than xylem. This feature results a characteristically ridged and furrowed xylem cylinder. Phloem can be identified by

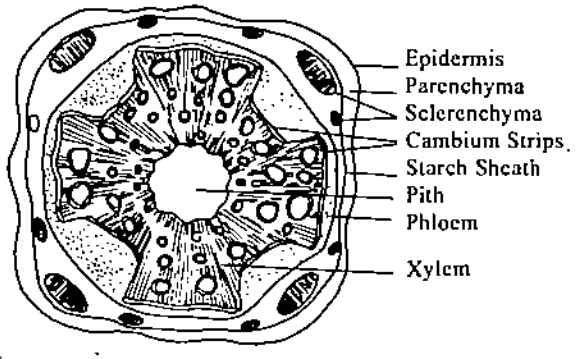

Fig. 10.19: T.S. stem *Bignonia* sp. showing cambial variant.

the presence of wedges. There, as usually, four such wedges symmetrically arranged and corresponding in position to the larger primary vascular bundles.

b) In some climbing species of genus *Vitis* (graphs) *Clematis, Aristolochia* (Fig. 10.20). *Tinospora* etc. acomplete ring of cambium is formed. The fascicular cambium functions normally but the interfascicular cambium

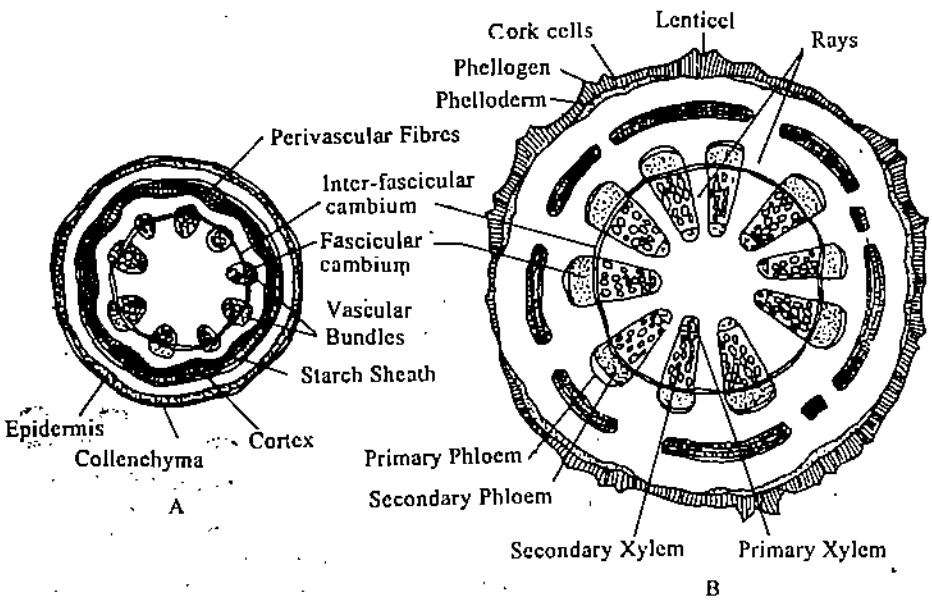

Fig.10.20: Aristolochia in T.S. A. One year old
B. Two years old

produces only raylike parenchyma cells. As a result, broad and long medullary rays and fluted vascular cylinders are formed.

II. The Cambium is abnormal in position but normal in activities:

This situation is noted in some climbing species of *Serjania* (Fig. 10.21). During development the cylinder of primary vascular bundles become notched at certain points, so that groups of bundles are formed. Thus bundles become contricted from the cylinder. They may be cut off even at the procambial stage. These groups of bundles function as independent cylinders and give rise to separate cambia, each of

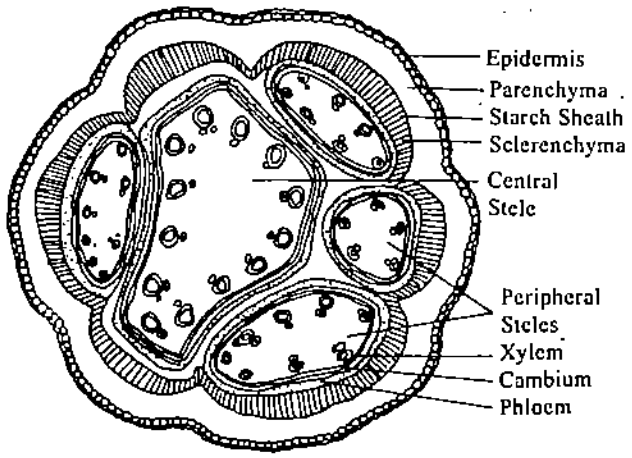

Fig. 10.21: T.S. stem *Serjania*.

which functions normally and independently. Thus the stem appears as if it is made up of several discrete woody cylinders, each of which has its own periderm. Sometimes the woody cylinder is only lobed.

III. Anomaly due to the formation of accessory cambium and its activity:

a) In some species of Chenopodium and members of the Amaranthaceae, the anomalous secondary growth results from accessory cambia. A hollow cylinder of vascular tissue or a ring of irregularly arranged bundles. The bundles are of secondary nature but their cambial activity soon ceases. Just outside the bundles a new secondary cambium arises in the pericycle. In some species the cambium forms tissues centripetally, consisting of bundles embedded in nonvascular tissue. The bundles formed in this way may be arranged irregularly or in defenite concentric rings

c) In *Tecoma* sp. secondary xylem and phloem are produced in the beginning by the activity of a normal cambium ring. At later stage an accessory secondary cambium arises in two arcs on the inner side of the normal wood or towards the pith. This accessory cambium cuts off xylem and phloem in an inverse order i.e. xylem towards the periphery and phloem on the inside. This newly formed phloem is intraxylary phloem and is secondary in origin. And the secondary xylem merges gradually with the previously formed secondary xylem (Fig. 10.22).

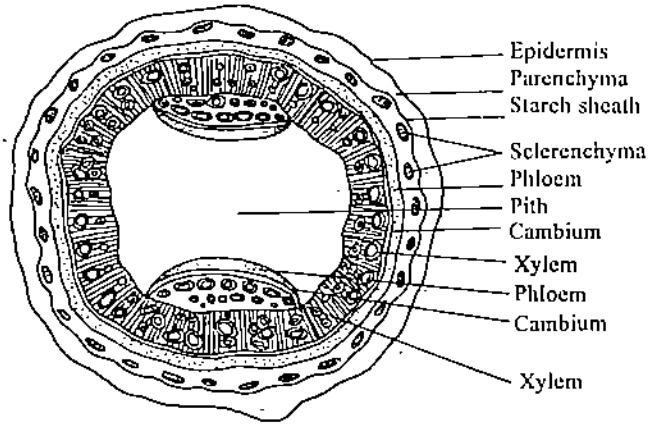

Fig. 10.22: T.S. of stem *Tecoma* showing accessory cambia.

IV. Anomaly due to the formation of interxylary (included) phloem as a result of aberrant activity and position of cambium.

Secondary phloem patches are sometimes embedded in secondary xylem in the form of strands. Those extra patches of secondary phloem present inside the secondary xylem are known as **interxylary** or included phloem.

In certain plants there are strands of secondary phloem within the secondary xylem, e.g., in *Avicennia, Thunbergia, Bougainvillea. Salvadora* and the families Amaranthaceae and Chenopodiaceae. In these plants cambium differentiates outside the primary vascular bundles, in the pericycle or in the inner cortical layers. Later a series of vascular cambia arise successively outward, each of which cambium produces xylem toward the inside and phloem towards the outside until a new cambium develops from parenchyma cells on the outside of the phloem.

b) In Chenopodiaceae the successive cambia are seen in the form of long or short arches. They produce irregularly or spirally arranged phloem strands (Fig. 10.23). Frequently the additional cambia in this family form more or less entire rings.

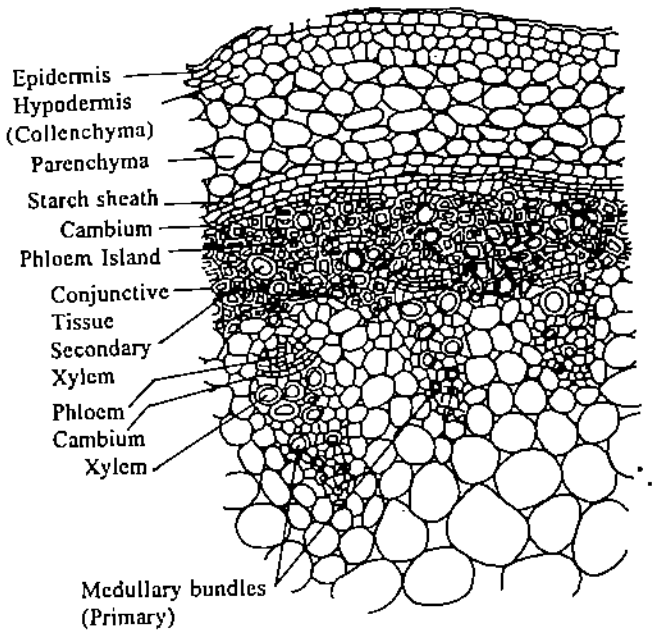

Fig. 10.23: T.S. *Chenopodium* stem.

SAQ 8:

i). List various types of cambial variants.

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10.10.2 In Roots

Cambial variants are also found in the roots of some plants, especially those that serve a storage function.

a). In the beet roots *Beta vulgaris* first the cambium ring develops near the primary xylem patches which in turn produces secondary xylem towards inside and secondary phloem toward the outside. Soon its activity ceases. Then from the cells of pericycle and phloem a secondary cambium ring is formed. This is followed by the formation of several concentric cambia. All the cambial layers continue to function and produce a large amount of storage parenchyma and strands of xylem and phloem. Although cambia are in continuous rings they produce separate bundles which are surrounded by conjuctive tissue. The bundles are separated from one another by wide radial panels of parenchyma formed due to the activity of newly formed cambium. Thus alternate bands of proliferated pericycle and vascular bundles are formed which can be seen as dark coloured and light coloured rings respectively. The bundles are themselves largely parenchymatous with a few lignified elements in xylem. (Fig.10.24).

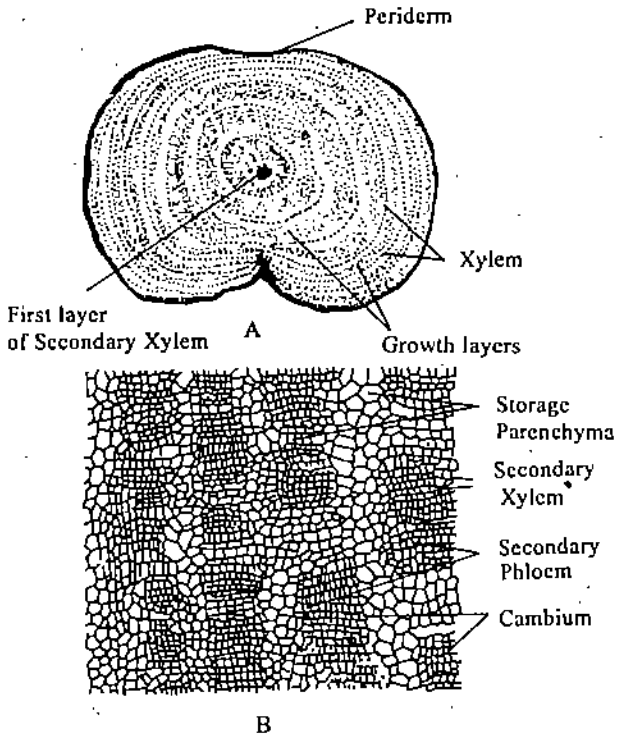

Fig.10.24: A. T.S. of root of sugar beet. B. Magnified part of the root .

b). In the sweet potato root of Ipomoea batatas (Fig. 10.25).Convolvulaceae) secondary growth is unique. The xylem, has an adundant amount of parenchyma Secondary cambia develop in the parenchyma around the individual vessels or vessel groups. The cambia produce tracheary elements towards the vessels and sieve tubes away from the vessels; A considerable amount of storage parenchyma is produced in both the directions. Thus the phloem appears to be a portion of root that originally differentiated as xylem.

SAQ 9:

Explain how dark cololured and light coloured rings are formed in the beet roots.

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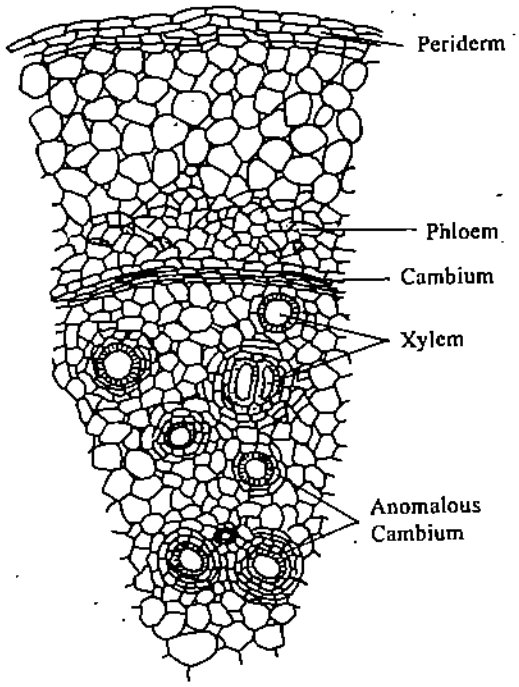

Fig. 10.25: T.S. of root of sweet potato.

10.11 SUMMARY

- In general the plants having only primary growth are limited in size and longevity. secondary growth helps the perennial gymnosperms and dicotyledons to increase the diameter and support the height and growth size.
- After the procambium strand become differentiated into primary vascular tissue, active meristematic regions lie between primary xylem and primary phloem from which continued, addition of fresh tissues is possible. These meristematic cells constitute the fascicular cambium.
- An interfascicular cambium arises from ray parenchyma cells between vascular cambia.
- The fascicular and interfascicular cambium joins together and form a complete cylinder of vascular cambium. The divisions in cambium are longitudinal, so that the stem now increases only in girth.
- In many plants, phellogen differentiates near the surface of the stem. This gives rise to phellem and phelloderm. Cork cells have suberin in their wall, which makes cell impreviousto gas and liquids.Lenticels in the bark facilitate gas exchange.
- Cork cambium may originate in successively deeper tissues: epidermis, cortex and phloem.
- Woody dicotyledons have most of their secondary tissues arranged in concentric layers: The most conspicious tissue is wood (secondary xylem). The urly wood

usually has relatively large vessel elements, while late wood has smaller vessels and/or a predominance of tracheids.

- Generally an annual ring comprises one year's growth of xylem. The age of atree and other aspects of its ecological history can be determined by studying histological details of the annual rings. Older wood ceases to function and in gradually accumulated in the centre to form the heartwood or deadcore which becomes plugged with tyloses. The younger, more peripherally located or living wood is called sap wood. The scientist have shown that only one or two recently formed annual rings or wood are actually involved in the ascent of sap.
- Some monocots such as *Agave* have true secondary growth, with a type of cambium that produces secondary vascular bundles and parenchyma.
- Certain dicotyledonous stem have cambial variants that contribute to unusual secondary growth. Cambial variants may arise from the following situations/conditions: i) The cambium is persistent and normal in position. Its products showunusual arrangement and proportions. ii) The cambium is abnormal in position but normal in activities. iii) Formation of acessory cambium and its activity. iv) The formation of interxylary phloem because of abnormal activity and position of cambium.
- Cambial variants are also noted in some roots such as *Ipomoea batatas, Beta vulgaris* letc.

10.12 TERMINAL QUESTIONS

1. If a nail is driven into the trunk of a tree say at breast height, will it remain at the same distance from the ground and more up or down in the course of the next 10 years. Explain why?

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2. What is secondary thickening (growth)? Explain the mode of secondary growth in dicotyledons and a monocotyledons. List the similarities and differences in their secondary structures.

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3. What are tyloses? How are they formed?

   .....
   .....
   .....
   .....
   .....
   .....
   .....
   .....

4. Suggest some reasons for preferring to heartwood to sapwood as material furniture.

5. Describe some important features of secondary phloem.

6. What is periderm? Explain how it divides and the tissue it produces.

7. How cork is formed? Explain the structure, properties and uses of commercial cork. Why is orientation of the elements of cork is important in making bottle stoppers?

8. What are lenticels? How they are formed? What are their functions?

9. Describe the structural anomaly arising as a result of formation of accessory cambium and its activity provide suitable diagram?

10. Write the names of plants in which interxylary (included) phloem is formed.

11. Explain briefly the main features of unusual secondary growth in roots.

10.13 ANSWERS

Answers

SAQ 1:

a) Secondary growth is growth in diameter due to addition of new tissues due to activities of lateral meristems.

b) Secondary growth occurs both in intrastelar amd extrastelar regions.

SAQ 2:

a) Fascicular cambium and interfascicular cambium.

b) Vascular cambium consist two types of cell: Fusiform initials and ray Initials

The fusiform Initials: are elongated cell with tapering ends. They are found in tracheary elements, fibres, xylem and phloem parenchyma and sieve elements. **Ray Initials:** are much smaller than fusiform initials and are almost isodiametric. They have intense vacuolation. the cell wall possess primary pit fields with plasmodesmata. The radial walls are thicker than the tangential wall.

c) See section 10.3.2.

SAQ 3:

a) False (b). True (c). True.

SAQ 4:

a) Secondary xylem can be classified in two systems:

a) One system is horizontal and

b) other is vertical.

The horizontal system is made up of xylem rays and the vertical consists of tracheary elements, fibres and wood parenchyma. The living cells of rays are usually interconnected and a continuous system of living cells is formed.

b) Uniseriate Ray; when the ray is one cell wide it is called uniseriate ray.

Biseriate Ray: When two cell wide the ray is called biseriate rays. On stem they are longitudinally or horizontally arranged but most of the time they are scattered all over the entire surface. Usually itlooks like a convex lens both internally and externally.

Multi seriate: When it is more than two cell wide.

c) In timber trade wood of dicotyledonous is known as hardwood and the wood of gymnosperms as soft wood. But these words do not express the degree of hardness. But histologically the dicotyledonous woods has vessels while gymnospermous wood lack then.

d) The large position of the wood is made up of fibers or fiber tracheids and they give strength to wood. These woods are dense and heavy.

e) Woods have several uses which are listed below:

i) building material for houses in windows, doors, cabinets, boxes, furniture.

ii) building boats, ships, masts.

iii) bodies of automobiles, railway wagons, railway sleepers, bridges.

iv) electric poles.

v) various aspects of art are also made up of wood.

vi) wood is a good material for carving idols, inlay work.

vii) packing cases, matches.

viii) several music instruments.

ix) cosmetics, soaps, perfumes and incense sticks are made from oil derived from woods.

SAQ 5:

a True b False c False.

SAQ 6:

a (ii) b (iv) c(i) d(iii).

SAQ 7:

Lenticels: Restricted areas of relatively loosely arranged cells, in the periderm. They protude above the periderm because of the loose arrangement, larger size of the numerous component cells.

Lenticels usually found on young branches of stem and roots. They are involved in exchange of gases between internal tissues and the atmosphere through periderm.

Complementary Cells: The cells which are derived from the divisions of the substomatal cells as well as those produced towards the exterior by the phellogen of the lenticels. As the division progresses, masses of complementary cells are pushed out and rise above the surface of an organ.

SAQ 8:

Cambial variants are of following types:

i) The cambial is persistent and normal in position but its products show unusual arrangement and proportion.

ii) The cambium is abnormal in position but normal in activity.

iii) Abnormaly due to the formation of accessory cambium and its activity.

iv) Anomally due to the formation of interxylary phloem as a result of aberrant activity and position of cambium.

SAQ 9:

In the beet roots first formed cambium activity ceases very soon. Then there is formation of several concentric cambia. All the cambial layers continue to function and produce a large amount of storage parenchyma and strands of xylem and phloem. Though the cambia are continuous they produce separate bundles which are surrounded by conjuctive tissue. Bundles are also separated from one another by radial panels of parenchyma. Thus alternate bands of proliferated pericycle and vascular bundles are formed which is seen as dark coloured and light coloured rings respectively.

Answers to TQs:

1. Stem increases in height due to the activity of shoot apical meristem.So if a nail is driven in trunk. It will always remain at the same height above the ground.The nail may eventually become embedded as the stem increases in girth. and you can see the differences between the activities of the apical meristem and of the vascular cambium.

2. In the dicotyledons the stem generally increases in girth due to the activity of the vascular cambium. The growth is known as secondary growth or secondary thickening.

	Dicot Stem		Monocot Stem
1.	Lateral meristem joins and makes vascular cambium which in turn cuts off secondary tissues.	1.	In some monocotyledons seems it is secondary meristem
2.	The cambium cuts secondary xylem inside and secondary phloem outside.	2.	Vascular cambium produces only parenchyma. Some of these parenchyma elements differentiates into vascular, bundles and remain embedded in the parenchyma or conjuctive tissues.
3.	Examples: Most of the dicoty yledonous woods plants such as *Helianthus, Bougainvillea,*	3.	Examples: Families. Dracaena, Agave etc

3. See Section 10.5.2.

4. The outer part of the secondary xylem with living parenchyma is named sapwood. In nearly all the trees the central portion consists of dead parenchyma which ceases to conduct water.This is known as heartwood. Heartwood is formed after disintegration of the protoplast, loss of cell sap, hydrolysis of stored reserve materials and formation of tyloses. Tyloses totally block the cell. Oils, gum, resins, tanins are accumulate in the heartwood making it extremely durable. Thus heartwood is preferred to sapwood for making furniture.

5. See section 10.6.

6. See section 10.8.

7. Cork is an important part of secondary tissue which is termed as periderm. It is divided into three parts.

   i) The phellogen cork cambium

   ii) The phelloderm-parenchymatous tissues in some species, produced by the phellogen.

   iii) The phelloderm-parenchymatous tissues in some species, produced by the phellogen.

   Phellogen abaxially cuts off derivatives as phellem. The phellem or cork cells are usually polygonal, and radially flattened. The cells are arranged in compact radial rows which are devoid of intercellular spaces. Cork cells are dead. Some cells are hollow and thinwalled, and somewhat radially rounded, Some others are thick walled and radially flattened. The later type of cells may be often filled with dark resiniferous or tanninferous substance.

   Most of the commercial cork comes from *Quercus suber*. In this species phellogen arises in the epidermis. The first formed periderm is removed when tree is about 20 years old and 40 cm in diameter. A new phellogen differentiates a few millimeters within the cortex. This cambium divides rapidly and in about 10 years it forms sufficiently thick cork for commercial uses.

   Cork is of commercial value because it is impervious to gases and liquids and has strength, elasticity and lightness and see section 10.8.5.

8. See section 10.9.

9. Some time unusual secondary growth occurs due to the formation of accessory cambia and their activity. There are two types of vascular tissues (i) a hollow cylinder or (ii) irregularly arranged bundles. Their cambial activity ceases and outside a bundle in the pericycle a new secondary cambium arises. The cambium forms tissues centripetally, consisting of bundles embedded in nonvascular tissue. The bundles may be arranged in concentric rings or irregularly.

10 Interxylary phloem is found in following plants.

   Avicennia

   Thunbergia

   Bougainvillea

   Salvadora.

11. See section 10.10.2.

UNIT 11 PLANT TISSUE AND ORGAN CULTURE

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11.1 INTRODUCTION

In the preceeding units you have studied that cells grow and divide in a selfregulating manner, and differentiate into diverse tissues and organs. The concept that the individual cells of an organism are totipotent is implicit in the cell theory put forth bySchleiden and Schwann. A totipotent cell is capable of regenerating into a whole plant. In this unit you will learn how the fundamental tenets of the cell theory have been experimentally verified by the techniques of plant tissue culture.

In plant tissue culture isolated protoplasts (i.e., cells without cell walls), cells, tissues or organs are grown aseptically in an artificial nutrient medium, under controlled temperature and light. Gottlieb Haberlandt, a German plant anatomist and physiologist, was the first to attempt the cultivation of isolated mesophyll cells of several flowering plants. The cells increased in size, synthesised starch and survived for a month but failed to divide. His failure could be attributed to two main factors: (i) selection of highly differentiated cells as the experimental material and (ii) the lack of knowledge of plant growth substances, that promote cell division. The progress of plant tissue culture has since been fast, aided by some important discoveries, notably: (i) recognition of the importance of B–Vitamins in plant growth, (ii) identification of auxin as a natural growth regulator and (iii) discovery of cytokinins. White was the first scientist to succeed in establishing continuously growing cultures of tomato roots by the addition of B–Vitamins in the culture medium.

Initially the main concern in the plant tissue culture studies was to induce division in cultured cells and organs and to optimise the nutritional and hormonal requirements to establish continuously growing tissue cultures. Later, plant tissue culture was employed as a research tool to study basic problems of physiology and biochemistry and the complex process of differentiation. However, the discovery that whole plants can be regenerated from any living cell raised the technique of plant tissue culture to the status 'a major technology' in making important contributions to agriculture and plant biotechnology.

Objectives:

After studying this unit you should be able to:

- describe the methods employed in aseptic manipulation of plant cell and tissue culture,
- apply the methods of plant tissue and organ culture to culture tissues and single cells,
- justify the role of plant growth regulators in the growth and differentiation of plant tissues,
- differentiate between in-Vitro embryogenesis and organogenesis, and
- discuss the applications of plant tissue culture in agriculture, horticulture and industry.

11.2 TECHNIQUES OF PLANT TISSUE CULTURE

A standard tissue culture laboratory should provide facilities for washing and storage of glass ware, preparation and storage of nutrient media, aseptic manipulation of plant material, maintenance of cultures under controlled temperature, light and humidity. At least two separate laboratories or rooms should be available one for glassware washing storage and media prepration and another to store cultures. Also it is very essential to maintain a sterile environment during plant tissue culture. By following a few simple precautions against microbial contamination you will save valuable laboratory time in repeating experiments. In the following subsections we will discuss (i) Initiation of aseptic techniques (ii) Culture media and (iii) Culture conditions.

11.2.1 Initiation of Aseptic Cultures

The nutrient medium (basal medium) used for plant tissue culture would promote luxuriant growth of micro-organisms such as bacteria and fungi. Widely used basal medium for tissue and culture studies is that of Murashige and Skoog (1962). Reaching the medium, these microbes grow much faster than the plant cells and cover the tissue surfaces impeding its growth and finally killing it. The microbes may be present in the medium right from the beginning. To destroy them the culture vessels containing the medium are properly plugged and autoclaved (steam heating under pressure) at 121° C for 15-20 minutes. Also you can use pressure cooker for sterilising small volumes of media. If you use presterilised, nonautoclavable culture vessels then the medium is autoclaved in 100-1000 ml. corning or pyrex flasks or bottles and the sterilised medium poured into the culture vessels under aseptic conditions. You should know that most of the media constituents are heat stable and can be added to the medium before autoclaving. Growth regulators such as Gibberellic acid, Abscisic acid, Zeatin and some vitamins are thermolabile i.e. they are rapidly degraded by elevated temperatures. The solutions of such compounds are sterilised by passing them through a bacteria proof filter membrane (Pore size 0.2 um-0.45 um) and then added to the autoclaved medium cooled to 60° C.

The pieces of tissue used to start a culture referred to as the 'Explant' are the principal source of contamination of the cultures. To eliminate the micro organisms

carried on the surface of the explant, the latter are properly surface sterilised before planting them on the medium (inoculation). The most commonly used surface sterilising agents are sodium hypochlorite and mercuric chloride. Sodium hypochlorite is generally used at a concentration of 2 per cent for 5 to 30 minutes. While hard tissues such as stem pieces and seeds are directly exposed to the sterilants, soft and delicate tissues such as embryos and shoot tips are dissected from surface sterilised plant parts. Addition of a few drops of a surfactant such as triton-X or tween-80, to the sterilant solution enhances the efficiency of the sterilising agent. After treating with a sterilant solution, the plant material is rinsed several times in sterile distilled water to remove all traces of the sterilant.

All manipulations after surface sterilisation of the tissue are ,carried out in aseptic conditions. Presently a laminar air flow cabinet is used for this purpose (Fig. 11.1). Air is forced into the cabinet through a bacterial filter. It flows outward and forward

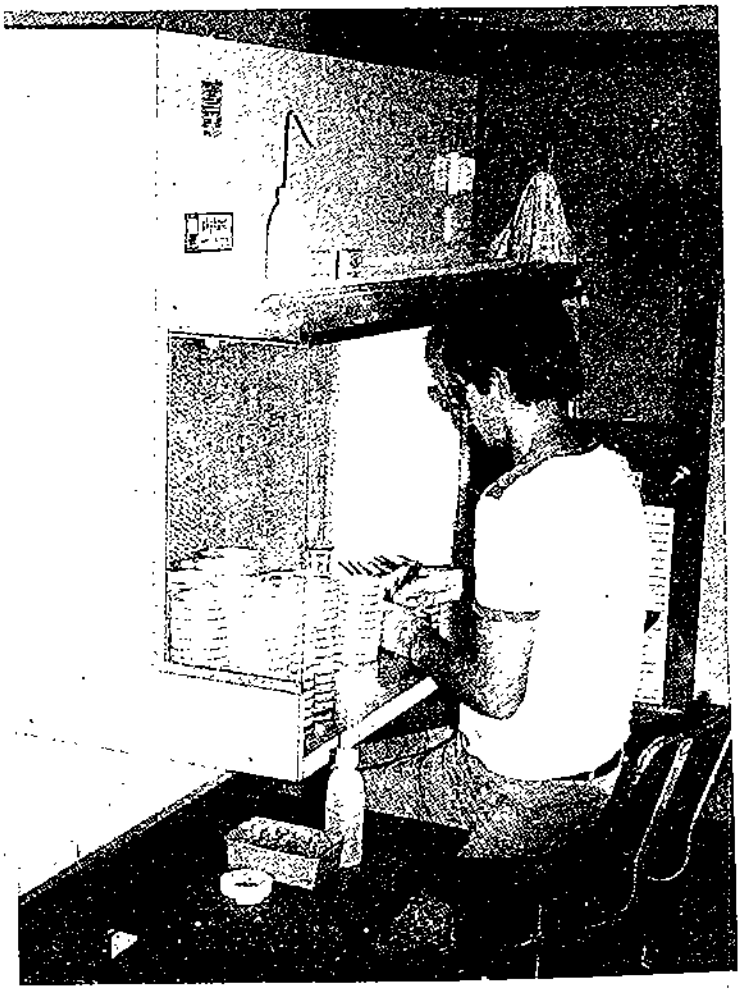

Fig. 11.1: Laminar airflow cabinet ready for use.

over the bench at a uniform speed. The work bench in the cabinet is cleaned in cotton soaked in ethanol (70-80 %) before starting the work. Instruments such as forceps, needles, and steel knives used for preparing materials for inoculation are sterilised by dipping in ethanol and flaming. This is done at the start of the work and several times during the operation. Even the hands of the operator are sterilised by dipping in alcohol and drying in air.

11.2.2 Culture Medium

In nature green plants are capable of synthesising organic compounds necessary for their growth and development from the mineral nutrients and they can absorb water from the soil and obtain CO_2 from the atmosphere. However, in tissue cultures the normal biosynthetic potentiality of the cells is weakened, therefore, it is necessary

to provide all the essential organic and inorganic nutrients (including sucrose) and growth regulators, particularly an auxin and a cytokinin.

Nutritional requirements for optimal growth of tissue cultures may vary with the source (plant). They are also affected by the age of the explant and the stage of development. For example very young embryos require a more elaborate component of the medium as compared to mature embryos. Similarly culture requirements of single cells are more complex than shoots.

Composition: A standard plant tissue culture medium (Basal medium) contains all the essential macroelements (Carbon,hydrogen, oxygen nitrogen, phosphorus, sulphar, calcium,potassium and magnesium) and iron and microelements(iron, manganese, copper, zinc, boron and molybdenum) but the concentration of their salts in different formulations vary. In addition some vitamins and sucrose (2-3%) are universal constituents of plant tissue culture media. The composition of Ms medium (Murashige and Skoog's) medium found satisfactory for a wide range of monocotyledonous and dicotyledons species is given in table 11.1.

Table 11.1: Composition of Murashige and Skoog's (MS) basal medium, widely used in plant tissue culture studies (1960)

Constituents	Concentration (mg/l)
Inorganic constituents	
A. Macronutrients	
NH_4NO_3	1,650.00
KNO_3	1,900.00
$CaCl_2 . 2H_2O$	440.00
$MgSo_4 .7H_2O$	370.00
KH_2PO_4	170.00
B. Iron	
$FeSO_4 .7H_2O$	27.80
Na_2 EDTA. $2H_2O$	37.30
C. Micronutrients	
$MnSO_4 .4H_2O$	22.30
$ZnSO_4 .7H_2O$	8.60
H_3BO_3	6.20
KI	0.83
$Na_2 MoO_4. 2H_2O$	0.25
$CuSO_4 .5H_2O$	0.025
$CoCl_2 .6H_2O$	0.025
Organic constituents	
Myo-inositol	100.00
Glycine	2.00
Nicotinic acid	0.50
Pyridoxine-HCL	0.50
Pyridoxine-HCL	0.10
Sucrose	30,000.00
Agar	8,000.00

Besides the nutrients, one or more plant growth regulators (PGR'S) are generally required for supporting good growth of the cultured material. The PGR'S most widely used in tissue culture media are auxins (2,4-D, IAA, NAA, IBA) and cytokinins (BAP, Kinetin). The PGR's are particularly important for the growth of Callus tissues and organogenic/ embryogenic differentiation. Usually the medium is gelled with 0.8% bacteriological agar.

Preparation: Now that you are familiar with the constituents the preparation of medium is quite simple. Several plant tissue culture media are now available commercially in the form of dry powders, containing all ingredients except growth regulators, sucrose and agar. They are very convenient to prepare media for routine maintenance of cultures. Generally concentrated stock solutions of major inorganic nutrients (200 times concentrated expressed as 20 x) micro inorganic nutrients (200 × concentrated) iron (200 × concentrated) and organic nutrients except sucrose are prepared and stored in a refrigerator at 4° c. Separate stock solutions are prepared and stored for each growth regulator by dissolving it in a minimal quantity of appropriate solvent and adjusting the final volume with distilled water.

A general protocol for media preparation is as follows:

1. Prepare stock solutions one day before the medium is to be made.
2. Weigh the required quantities of agar and sucrose and dissolove them in water (about 3/4th the final volume of the medium) by heating in a–waterbath or autoclaving at low pressure.
3. Pipette the required volumes of each of the stock solutions into the above solution kept on a stirrer.
4. Make up the final volume of the medium by addition of distilled water.
5. Adjust the pH to 5.8 with 0.1-0.5 N NaoH or Hcl.

11.2.3 Culture Conditions

The culture vessels placed in suitable trays or racks, are stored on the shelves in a hygenically maintained culture room, under controlled conditions of light and temperature. If possible the relative humidity should not fall below 50% to prevent rapid desiccation of the medium. To minimise the infection of cultures during incubation, the culture room should be dust free. It is often provided with double doors and entry of people is restricted.

The requirement for light and temperature varies considerably. The cultures of high altitude plants as well as those of desert species grow well at 25° c–28°c. However, induction of pollen embryogenesis require a high temperature treatment (35° C). As mentioned earlier, in cultures even green tissues and shoots do not exhibit active photosynthesis and are largely heterotrophics. The differentiation of shoots from callus or explants and axillary shoot proliferation are favoured by light. A callus is defined as an unrecognised mass of tissue varying widely in texture, appearance and growth rate. Such cultures should be maintained in continuous diffuse light (1-3K lux) provided by fluorescent tubes. You should know that unorganised callus and freshly isolated protoplast cultures are often sensitive to light and are incubated in darkness. Total darkness is also favourable for rooting and for the initial stages of pollen and somatic embryogenesis.

SAQ 1:

a) Why is it extremely important to sterilise the nutrient media used for plant tissue culture?Answer in about 40 words

b Choose the Correct statement from among the following:

i) Many undesirable microorganisms may find the culture media suitable for their growth and compete with plant tissue for various nutrients.

ii) The main aim of surface sterilisation is to remove all of the microorganisms with a minimum of damage to the plant system to be cultured.

iii) Plant tissues often require rich media for their growth, the presence of microorganisms as contaminants may hamper the interpretation of results.

iv) It is in fashion these days to autoclave the media before inoculation. Sterilised media, any way give a better look.

c) Some compounds are thermolabile and should be added separately to a sterilised medium, after passing themthrough a bacteria-proof filter. What does the term thermolabile mean?

d) State which of the following statements is true:

i) Thermolabile compounds are those compounds which retain their chemical structure and activity when exposed to heat.

ii) The compounds which can pass through a bacteria proof filter membrane are called thermolabile compounds.

iii) Some compounds become active only after passing through a bacteria proof filter membrane, such compounds are called thermolabile compounds.

iv) Compounds subject to destruction or loss of characteristic properties by the action of moderate heat.

e) You have used the term "Explant" in this section. Say which of the following statements best defines this term.

i) The plant which acts as a source of inoculum for tissue culture.

ii) The plants obtained from tissue culture grown on an artificial medium.

iii) The tissue pieces used to initiate cultures.

iv) The medium which provides nutrients to plant parts under culture.

e) Fill in the blank spaces with appropriate words from the text:

i) Besides the nutrients, one or more ..... are required for callus growth and organogenic/embryogenic differentiation.

ii) Culture requirements of callus tissue are..... elaborate than single cell.

iii) Media are sterilised by autoclaving at.....

iv) pH of the culture medium is adjusted at.....

v) Plant tissue cultures require light for.....

11.3 ESTABLISHMENT OF TISSUE CULTURES

By now you are familiar with the term "Explant". After a few days in culture the explant becomes slightly rough in texture and its surface glistens in reflected light. This is a sign of the beginning of callus formation. A "callus is defined as an irregular tissue mass varying considerably in texture, appearance and growth rate. In nature plants produce callus as a result of mechanical injury (wound tissue), invasion by microorganisms or by insect feeding. Callus formation has been observed in angiosperms, gymnosperms, ferns, mosses and liverworts. Infection by *Agrobacterium tumefaciens* causes the production of tumours or 'Crown gall' in dicotyledonous plants. The stimulus for cell proliferation in these cases is provided by endogenous hormones, auxins and cytokinins. Plant material typically cultured

included vascular cambia, storage parenchyma, pericycle of roots, cotyledons, leaf mesophyll and provascular tissue. You should know that all multicellular plants are potential sources of explants for callus initiation. In the laboratory you can easily establish callus cultures from the stem pith tissues of tobacco or slices of carrot root on MS medium supplemented with 2,4-D (Fig. 11.2). Other tissues may require a cytokinin for callus induction. In such cases a high 2,4-D. Cytokinin ratio favours callus formation.

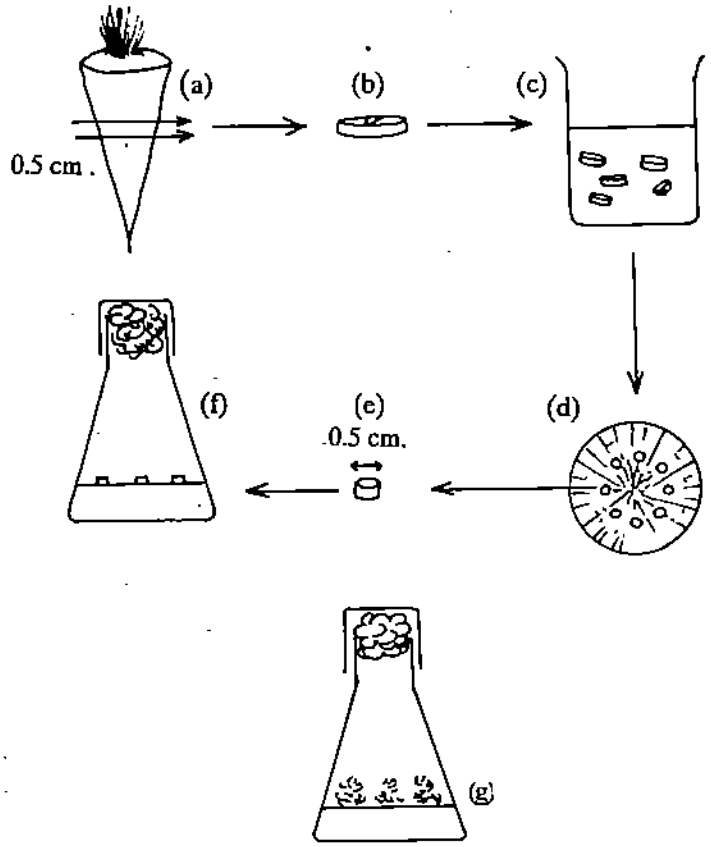

Fig. 11.2: **Fig. 11.2: Diagram showing the preparation of explants from the cambial region of carrot (*Daucus carota*) taproot. (a,b) A segment approximately 0.5 cm in thickness is removed from the taproot. (c) The segments are surface sterilized and subsequently rinsed repeatedly in sterile DDH_2O (not shown). (d,e) Short cylinders of tissue (0.5 cm O.D.) are removed with a sterile cork borer from the cambial zone of the taproot. (f) After trimming the ends of tissue that may have been injured by the sterilization, the explants are placed on the surface of the agar-solidified medium. (g) Following incubation at a suitable temperature, callus will arise from repeated divisions of the cultured cells.**

The most important characteristic of callus from a functional view point is that abnormal growth has the potential to develop normal roots, shoots and embryoids that can form plants. Callus cultures become brown and necrotic if allowed to grow on the same medium for an extended period because of the depletion of essential nutrients, gradual desiccation of the agar due to water loss and accumulation of toxic metabolites in the medium the tissue may eventually die. The callus is cut into two or more pieces and transferred to a fresh medium. Such cultures are referred to as "sub-cultures". After a few subcultures some tissue systems do not require exogenous auxin for callus proliferation. Such cultures are said to have become "habituated to auxin". However, this phenomenon is not a genetic change but a selective gene expression as habituated cultures are known to revert to auxin requirement with time.

In the following subsections we will discuss the establishment of suspension cultures and single cell cultures.

11.3.1 Establishment of Suspension Cultures

A callus crumbles into smaller clumps and single cells in liquid medium by gentle agitation (100-120rPM) on a shaker. Shaking the cultures also helps to aerate the

cells. Such suspension cultures however rarely comprise single cells alone because cells tend to aggregate in clusters of 2-100. Suspension cultures can be maintained indefinitely by inoculations of known aliquots of cells to a fresh medium. This process is termed as "batch cultures". Alternatively, the medium is replenished at regular intervals. This process is termed as "continuous culture". In the continuous culture process at the time of replenishing the medium, cells are also harvested (open continuous system) or the biomass is allowed to increase (close continuous system) . Suspension cultures are useful in studying problems related to cell biology including cell cycle and production of secondary metabolites like alkaloids, steroids, glycosides, napthaquinones, flavones etc. which find medicinal and industrial application. Pharmaceutical industries use large bioreactors for suspension cultures to obtain valuable bioorganic compounds. A bioreactor is a vessel of glass or steel in which cells are cultured aseptically and culture conditions are closely monitored. This results in higher yield of metabolites. In a bioreactor there is provision for adding fresh medium, for harvesting cells, for the aeration of products, for mixing and sampling, for controlling pH, O_2 content and temperature Plant cells are immobilised in alginate, agarose, polyacrylamide beads. Immobilisation of cells enables i) re-use of biomass by rotation of cells ii) separation of cells from the medium and iii) leaching of metabolites in it . Immobilised cells are cultured in column reactors. Column reactors are of different types with different agitation and flow systems. Such reactors may be i) stirred tank type ii) air lift type iii) bubble column type and iv) rotating drum type.

11.3.2 Single Cell Culture

This is an important invitro technique which enables the cloning of selected cells. Single cells can be obtained directly from plant organs by treatment with enzymes that dissolve middle lamellae. The separate cells can sieve into liquid medium to start a suspension culture. The most widely used technique for single cell culture is the Bergmann's method of Cell Plating and. Microchamber technique.

Bergmann's Method of Cell Plating:

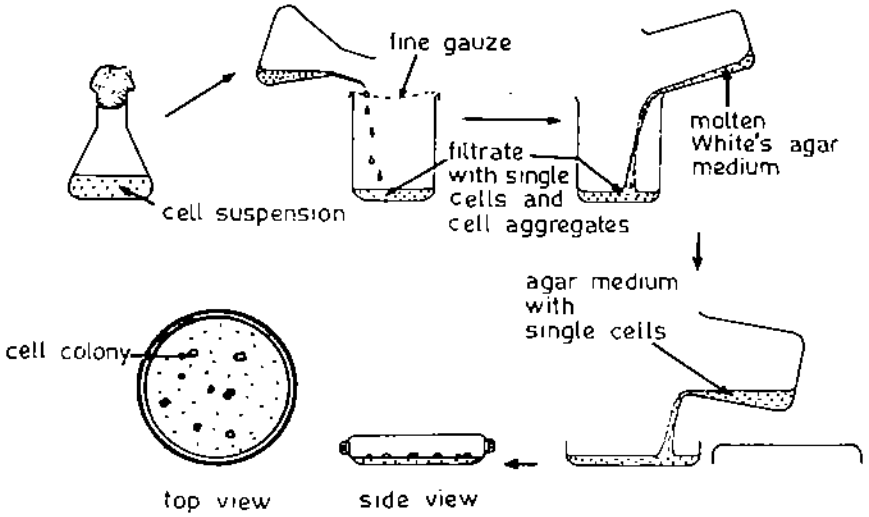

Figure. 11.3: A Summary diagram of Bergmann's method of cell Plating.

In this method free cells are suspended in a liquid medium at a density twice the finally desired plating density. Melted agarcontaining medium of otherwise the same composition as the liquid medium is maintained at 35°c in water-bath. Equal volumes of the two media are mixed and rapidly spread out in petri dishes in such a manner that the cells are evenly distributed and fixed in a thin layer (about 1 mm thick) of the medium after it has cooled and solidified. The dishes are sealed with parafilm. The cells to be followed are marked on the outside of the plate and before the colonies derived from individual cells grow large enough to merge with each other. They are transferred to separate plates. (Fig. 11.3).

Another popular method for single cell culture is the **microchamber technique,** developed by Jones et al. (1960). In this method mechanically isolated single cells are cultured in separate droplets of liquid medium. While Jones et al. used sterile microslides and three coverglasses to make microchamber, it is now possible to buy pre-sterilised plastic plates with several microwells (Cuprak dishes). Individual cells are cultured in separate wells each containing 0.25 ml of the liquid medium.

The culture requirement of single cells increases with decrease in the plating cell density, and the cell cultured in complete isolation require a very complex culture medium. A simple medium conditioned by growing cell suspension for some time also fulfils the requirements of single cell culture at low density.(Fig. 11.4)

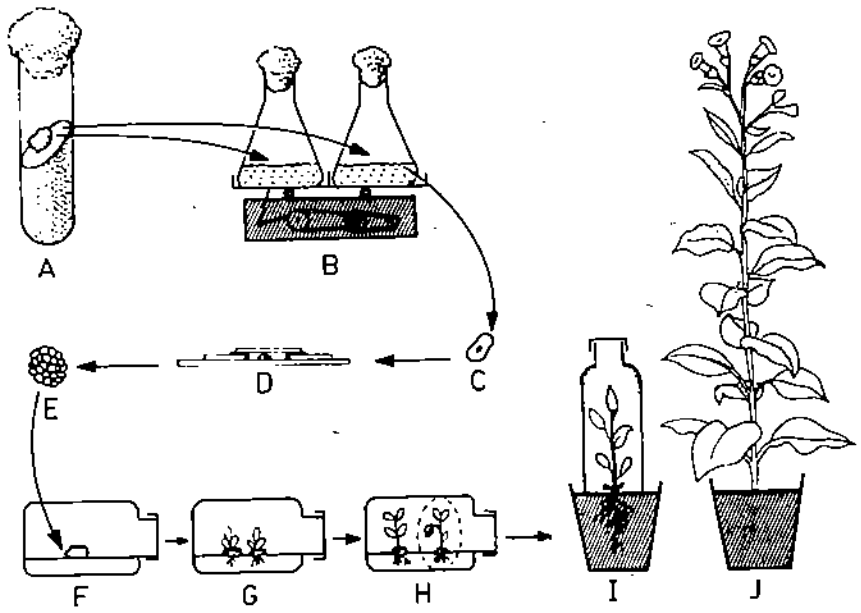

Fig. 11.4: Development of a tobacco plant from a single cell. A callus is raised from a small piece of tissue excised from the pith (A). By transferring it to a liquid medium and shaking the culture flasks (B) the callus is dissociated into single cells. A cell (C) is mechanically removed from the flask and placed in a drop of culture medium in a microchamber (D). A small tissue (E), derived from the cell through repeated divisions is then transferred to a semi-solid medium where it grows into a large callus (F), and eventually differentiates plants (G,H). When transferred to soil (I,J) these plants grow to maturity flower and set seeds.

SAQ 2:

Choose the correct word from the bracket and fill in the blanks:

i) An undifferentiated mass of cells, irregular in outline, produced in cultures, is known as.....(Callus/Crown gall).

ii) Callus formation can be induced in nature as a result of mechanical injury. Such a callus is called. . . . . (wound tissue or proliferated tissue).

iii) Callus formation is stimulated by a synthetic auxin, such as. . . . . , (2,4D/IAA)

iv) Periodic division of a callus tissue and transfer of each segment to fresh medium is called. . . . . (Inoculation/subculture).

b) Match the items given in column A with their respective explanation given in Column B and compare your answer with those at the end of this unit.

Column A	Column B
i) Single cell culture	i) A large vessel used for mass in-Vitro Propagation of cells in a liquid medium and monitoring their growth
ii) Batch culture	ii) Cells growing singly or in small clumps, in a liquid medium, gently agitated on a gyratory or rotatory shaker for better aeration and fast growth.
iii) Bioreactor	iii) An unorganised, undifferentiated mass of cells grown on an agar medium; usually having an irregular boundary
iv) Suspension culture	iv) Suspension cultures in which the medium is replenished periodically, the cells being simultaneously harvested or the biomass is allowed to accumulate.
v) Continuous culture	v) The practice of growing selected cells obtained either from suspension cultures or maceration of plant parts.
vi) Callus	vi) Suspension cultures in which aliquots of cells are periodically transferred to fresh medium.

C. Choose the false statement among the following:

i) Wound callus is initiated by auxin and cytokinin.

ii) Callus formation can be induced in numerous plant tissues and organs that do not usually develop callus in response to an injury.

iii) In the laboratory we can easily establish callus cultures from the stem pith tissues of tobacco or slices of carrot root.

iv) Initiation of callus is restricted to some specialised cells.

4. Callus cultures become brown and necrotic if they are left too long on the same medium why?

11.4 CELLULAR TOTIPOTENCY

In the preceding units of this course you have read that innumerable cells which constitute the body of a higher plant or animal and containing identical genetic material can be traced to a single cell–the zygote. During development cells undergo diverse structural and functional specialisation depending upon their position in the body. Leaf cells bear chloroplasts and act as the site of photosynthesis. The colourless root hairs perform the function of absorbing nutrients and water from the soil and some other cells become part of the colourful petals. Normally fully differentiated cells do not revert back to a meristematic state, which suggests that the cells have undergone a permanent change. In earlier sections of this unit you have read that the regenerative capacity is retained by all living cells of a plant. Several horticultural plants regenerate whole plant from root, leaf and stem cuttings. Highly differentiated and mature cells such as those of pith and cortex and highly specialised cells as those of microspores and endosperm,retain full potential to give rise to full plants under suitable culture conditions. G. Haberlandt was the first to test this idea experimentally. This endowment called "cellular totipotency" is unique to plants. Animal cells possibly because of their higher degree of specialisation do not exhibit totipotency. Whole plant regeneration from cultured cells may occur in one of the two pathways: i)shoot bud differentiation, (organogenesis) and ii) embryo formation (Embryogenesis). The Embryos are bipolar structures with no organic connection with the parent tissue and can germinate directly into a complete plant. On the other hand, shoots are monopolar. They need to be removed from the parent tissue and rooted to establish a plantlet. Often the same tissue can be induced to form shoots or embryos by manipulating the components of the culture conditions.

In the following sub sections we will discuss organogenesis and embryogenesis in detail.

11.4.1 Organogenesis

Organogenesis refers to the differentiation of organs such as roots, shoots or flowers. Shoot bud differentiation may occur directly from the explant or from the callus. The stimulus for organogenesis may come from the medium, from the endogenous compounds produced by the cultured tissue or substances carried over from the original explant. Organogenesis is chemically controlled by growth regulators. Skoog while working with tobacco pith callus, observed that the addition of an auxin Indole-Acetic Acid (IAA) enhanced formation of roots and suppressed shoot differentiation. He further observed that adenine sulphate, (Cytokinin) reversed the inhibition of auxin and promoted the formation of shoots. You should know that:

1) Organogenesis is contolled by a balance between cytokinin and auxin concentration i.e. it is their relative rather than the absolute concentration that determines the nature of differentiation.

2. A relatively high auxin: Cytokinin ration induces root formation, whereas a high cytokinin: auxin ratio favours shoot bud differentiation.

3. Differential response to exogenously applied growth regulators may be due to differences in the endogenous levels of the hormones within the tissue.

Organogenesis is a complex process. Whereas in the cultured tissues of many species organogenesis can be demonstrated in this pattern, some plants, notably the monocots, are exceptions. Plant tissues respond differently to

exogenously applied PGR's because of differences in the levels of endogenous Plant Growth Regulators (Fig. 11.5 A,B).

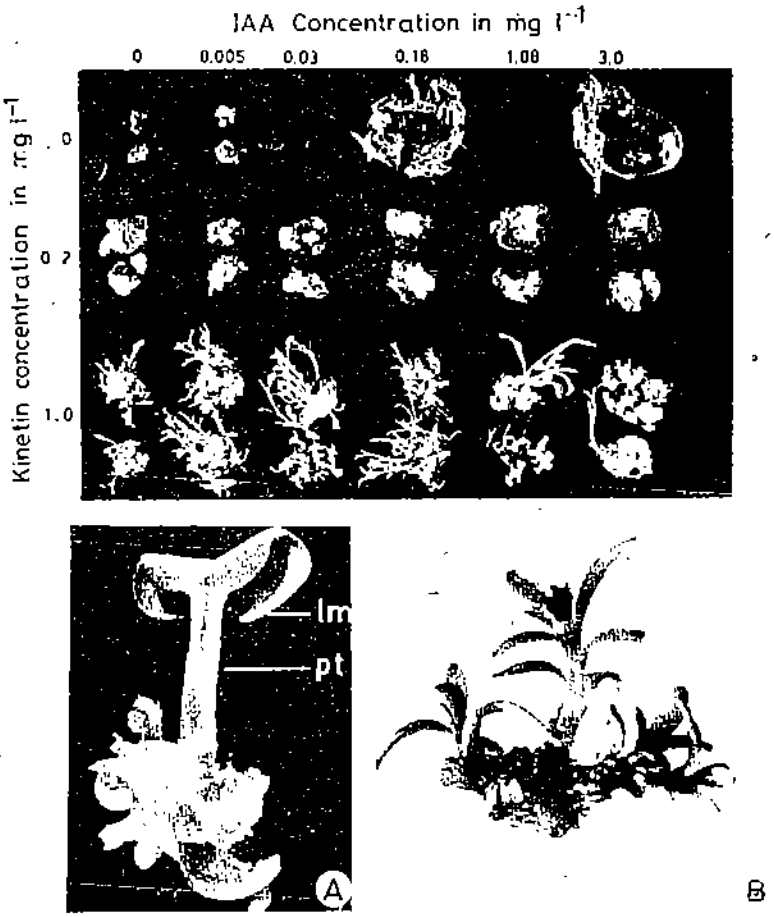

Fig. 11.5: Organogenesis in tobacco callus cultures as affected by IAA and kinetin at different concentrations, individually and in various combinations. Note that root formation has occurred only in the absence of kinetin (and in the presence of 0.18-3.0 mg/l of IAA) (A) and shoot differentiation in the presence of kinetin, particularly with 0.005-0.18 mg/l (B).

11.4.2: Somatic Embryogenesis

The process of embryo development is called embryogenesis. It is not the monopoly of the egg to form an embryo. Any cell of the female gametophyte (Embryo sac) or even of the sporophytic tissues around the embryo sac may give rise to an embryo. Thus we can say that "The phenomenon of embryogenesis is not necessarily confined to the reproductive cycle". In this subsection we will discuss some examples of "embryos formed in culture", also referred to as "somatic embryos".

The first observation of somatic embryos were made in *Dacus Carota*. Other plants in which the phenomenon has been studied in some detail are *Ranunculus scleratus, citrus and coffea spp.*

In *Ranunculus scleratus* somatic as well as various floral tissues, including anthers proliferated to form callus which, after limited unorganised growth differentiated several embryos. These embryos germinated in situ and a fresh crop of embryos appeared on the surface of the seedling. The embryos were derived from individual epidermal cells of the hypocotyl.(Fig. 11.6)

Citrus is commonly cited as an example of natural polyembryony.

In the preceding units of this course (Block 1) you have read about polyembryony and parthenocarpy. The nucellus cultures of monoembryonate as well as polyembryonate cultures of citrus could be promoted if malt is added to the basal

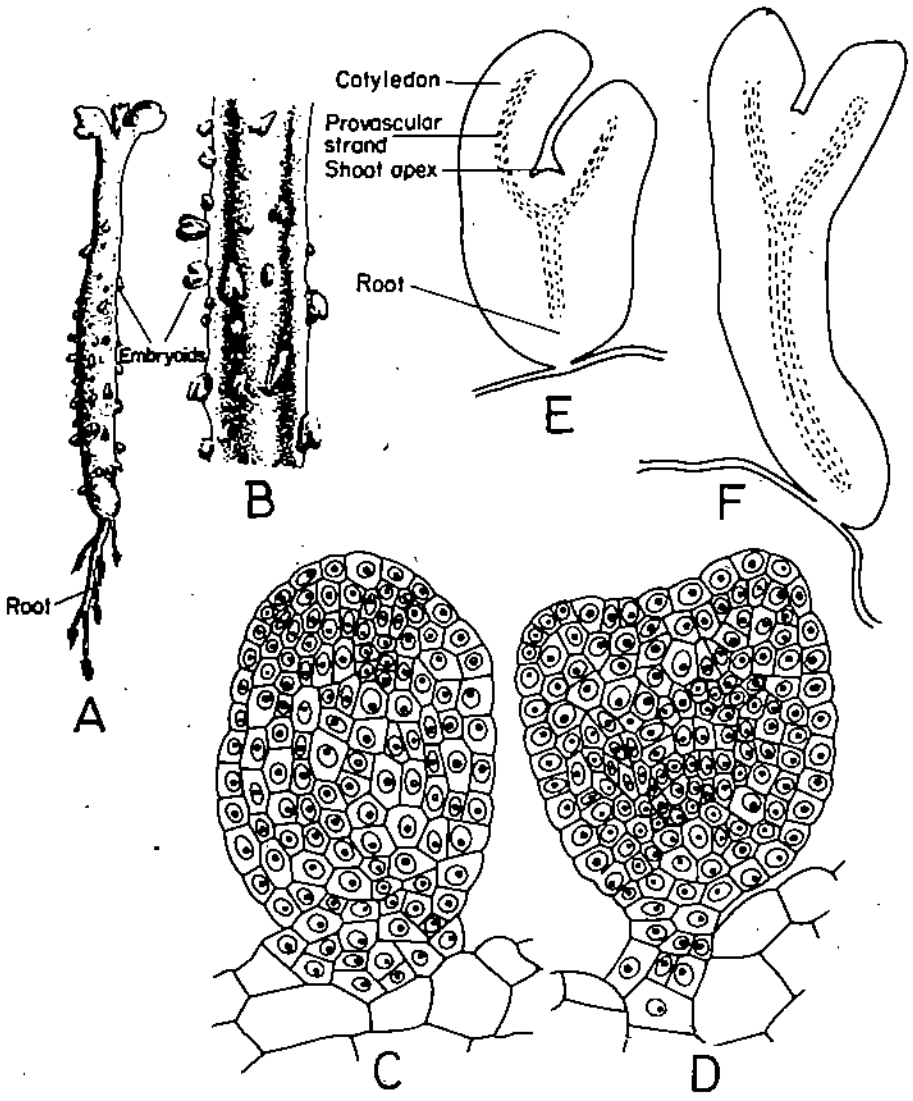

Fig. 11.6: Stages in the differentiation of somatic embryos from stem epidermal cells of Ranunculus. A One-month old seedling bearing embryos all over the stem. B. A portion of the seedling in A enlarged to show several embryos hanging from the surface of the stem. C-F. Sectional views of globular (C), heart shape (D), and cotyledonary (E,F) embryos differentiated from epidermal cells of the stem.

medium. You must be knowing that the seedless grapes are the result of parthenogenetic embryos (embryos formed by the unfertilized egg. In nucellus cultures of vitis embryo formation occurred in the presence of B. Naphthoxyacetic acid and BAP (Benzyl Amino Purine). However, minimal level of endogenous or exogenous auxin is necessary for in-vitro somatic embryogenesis. Supplementing the medium with activated charcoal facilitated embryogenesis in *Daucus carota* and *Hedera helix* (English ivy). Somatic embryos have been formed directly from leaf mesophyll cells of *Dactylis glomerata* L. (Orchard grass), without an intervening callus. Though regeneration of whole plants by embryogenesis has been relatively rare in Gramineae. (Fig. 11.7).

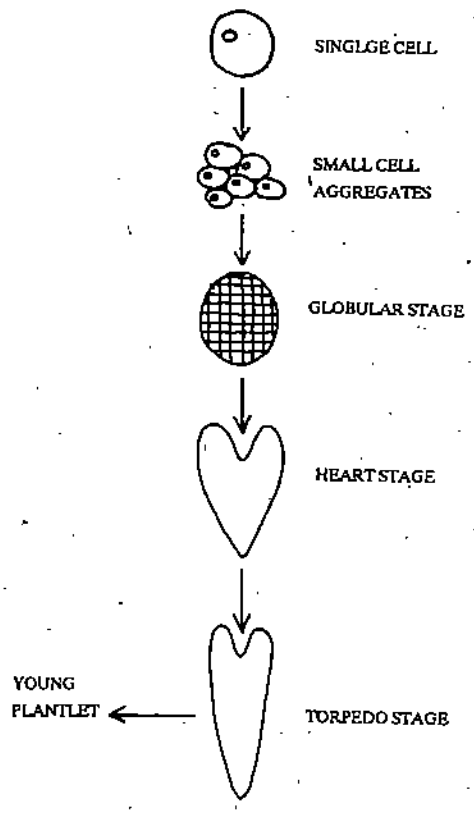

Fig. 11.7: Stages of somatic embryogenesis. Following repeated cell divisions, cell aggregates progressively develop and pass through globular, heart, and torpedo stages before ultimately forming plantlets.

11.5 APPLICATIONS

Plant tissue culture is an essential component of plant biotechnology. The possibility to regenerate whole plants from protoplasts, single cells, tissues and organs, in–vitro, has opened out entirely new approaches to plant improvement, and has considerably enhanced the efficiency of the conventional methods of plant breeding and plant propagation. This section highlights some of the applications of plant tissue culture in agriculture, horticulture and industry.

11.5.1: Production of Rare Hybrids

Hybridization is a well established plant breeding procedure to obtain superior plants by combining useful characters distributed in different plants. However, cross-pollination does not always result in the formation of viable seeds. The cross may fail due to a pre-zygotic barrier to crossability, which prevents fertilization, or the hybrid embryo may abort due to post-zygotic incompatibility. It is now possible to overcome both types of incompatibility barriers.

Hybrid embryo normally aborts on account of the failure of endosperm development or due to embryo endosperm incompatibility, In such instances it can be excised from the young seed and cultured in-vitro.

This technique was first used by Laibach to raise viable interspecific hybrids in Linum. Since then embryo culture also called embryo rescue has been successfully applied to several sexually incompatible crosses (e.g., *Hordeum vulgare x H. Bulbosum, Lycopersicon esculentum x L. Peruvianum*).

In several interspecific crosses of **Brassica** abortion of hybrid embryo occurs at such an early stage that it is not possible to excise and culture the embryo. A hybrid can be obtained by culturing the ovules **(Ovule culture)** or ovaries **(Ovary culture)** enclosing the hybrid embryo. Embryo culture has also been used to raise hyrbids between the sexually incompatible parents, *Gossypium hirsutum* and *G.arboreum*.

The pre-fertilization barriers to incompatibility include: i) failure of pollen to germinate on an alien stigma, ii) inability of pollen tube to reach the ovule due to its inherent short length or slow growth of pollen tube so that the ovary abscises before the pollen tube reaches the ovary, or iii) Pollen-pistil incompatibility. In such cases techniques of in vitro pollination or test-tube pollination (TTP), developed by Kanta et al. (1962) at the University of Delhi, hold promise. In TTP the ovules attached to a piece of placental tissue are excised one day before anthesis and planted on a suitable culture medium. The following day pollen grains are collected from the desired male parent and applied aseptically to the cultured ovules. Under suitable culture conditions the pollen grains germinate on the surface of the ovules, the pollen tubes find their way into the ovules and enable fertilization to take place. TTP has been successfully applied to obtain hybrids between sexually incompatible species of **Nicotiana** and to overcome self-incompatibility in **Petunia axillaris.**

Very recently, Kranz and Lorz (1990) have succeeded in fusing, in vitro, the excised male and female gametes of maize: the fusion product divided to form a small amount of callus. Regeneration of plants from the in vitro fertilized egg or the callus derived from it would open out an entirely new approach to overcome pre-fertilization barriers to incompatibility.

11.5.2 Somatic Hybridization and Cybridization

In the early 1970's an altogether new approach to raise hybrids which could not be produced through the conventional method of hybridization was proposed. It involves the fusion of somatic cells and regeneration of plants from the fusion products (somatic hybridization).

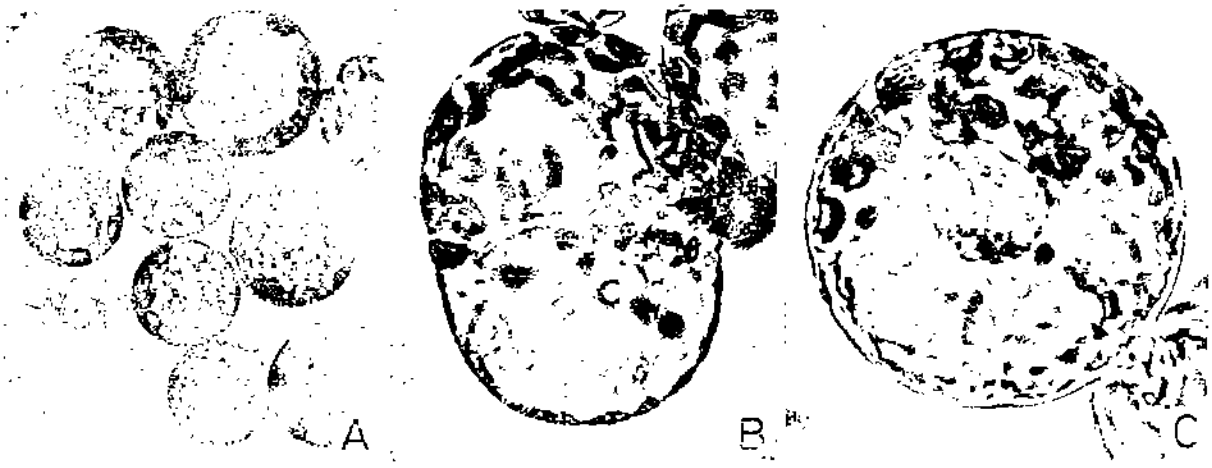

Fig. 11.8: A. Freshly isolated protoplasts of white clover. B,C. Stages in the fusion of a non-chlorophyllous protoplast from suspension culture of *Petunia hybrida* with a green mesophyll protoplast of *P. parodii*. First the fusion body apears dumbbell shaped which eventually becomes spherical. Even in C the chloroplasts are confined to one side of the heterkaryon. With the mixing of cytoplasm of the two protoplasts the chloroplasts would become evenly distributed.

Plant cells are bounded by a rigid cellulose wall and are cemented together by a pectin-rich matrix to form tissues. An essential step in fusion of plant cells is to bring together the plasma membrane by degrading the cellulosic wall. Thus, the first step in somatic hybridization is the isolation of plant protoplasts (spherical naked cells which have been stripped off their cell wall). (Fig. 11.8A)

Of the several kinds of materials tested for protoplast isolation mesophyll (leaf Parenchyma) and rapidly fast growing cell cultures have been most useful. Several potent and fairly purified enzymes (e.g., Pectolyase y-23, Onozuka R-10, Macerozyme R-10, Driesalase) obtained from fungi are available. These can convert plant tissues into a large number of protoplasts. In practice; small pieces of leaves or cells from actively growing cell cultures are incubated in a mixture of a cellulase and a macerozyme, at 30°c, in dark, for 3-12 hr (the duration of treatment varies with the tissue) to digest the cell wall and middle lamella respectively. The enzyme solution also contains a suitable osmoticum (Sucrose or mannitol) as the freshly isolated protoplasts may burst or shrink. After incubation the protoplast are cleaned by repeated washing in salt solution or culture medium. The protoplasts are directly cultured as single cells or used in fusion experiments. Freshly isolated protoplasts are also useful for genetic transformation as they behave like animal cells. They can readily take up macromolecules, such as purified DNA.

The protoplasts are cultured either in liquid medium or on agar plates. The protoplasts readily regenerate a cellulose cell wall, and under suitable culture conditions, the cells undergo divisions to form a totipotent callus. Complete plants have been regenerated from isolated protoplasts of several plant such as rice, cotton, potato, tomato and mustard.

The freshly isolated protoplasts readily fuse (Fig. 11.8 b,c) with each other when brought in intimate contact, irrespective of their taxonomic relationship. Several chemical substances that facilitate fusion of protoplasts (fusogens) have been used. Of these the high molecular weight (1,500-1,600) polyethylene glycol (PEG) applied in the presence of high pH (8-10) and high ca^{++} has been most effective. In recent years electrofusion of protoplasts has become popular because of the control, efficiency and versatility of this method.

A highly significant application of protoplast fusion is the production of asymmetric hybrids by partial genome transfer from an irradiated donor protoplast to an acceptor protoplast and the selective transfer of cytoplasmic genes. Many important agronomic traits, such as herbicide resistance and cytoplasmic male sterility, are often controlled by extra nuclear genes. Selective transfer of cytoplasmic traits is achieved by the fusion of normal protoplasts of the recipient parent with the donor's protoplasts in which the nucleus has been rendered inactive by irradiation or with its enucleated sub protoplasts or miniplasts. Such hybrids are called **Cybrids**. Medgyesy et al. (1980) transferred streptomycin resistance (controlled by chloroplast DNA) from *Nicotiana tabacum* to *N. sylvestris* by fusing iodoacetate treated, non-dividing protoplasts of streptomycin-resistant *N.tabacum* with normal protoplasts of streptomycin sensitive *N.sylvestris*.

Alloplasmic male sterile lines of *Brassica napus* and *Brassica oleracea* produced by substituting their cytoplasm by the ogura cytoplasm of male sterile *Raphanus sativus* could not be utilised for hybrid seed production because of their yellowing of leaves at low temperature (Jourdan et al., 1985). By fusing the protoplasts of these lines with those containing normal cytoplasm of respective species, researchers working with pelletier (1983),Robertson (1985) and Menzel (1987) replaced the sensitive chloroplasts by insensitive ones. The new alloplasmic lines retained the useful male sterility while acquiring functional chloroplasts.

Fusion of dissimilar protoplasts (from different parents) results in the formation of heterokaryons. After the fusion treatment the fusion mixture contains, besides heterokaryons, the unfused protoplasts and homokaryons (the fusion product of similar protoplasts from the parents). It has been possible in some cases to isolate

the heterokaryons mechanically, using micropipettes or by using a cell sorting machine. Generally, however, a suitable selection pressure is applied which permits the growth of only hybrid cells by selectively suppressing the division of the other types of cells. The nuclei in a heterokaryon fuse to form a hybrid cell. The latter may divide and produce a callus mass and eventually whole plants, thus may be differentated.

Several interspecific and intergeneric somatic hybrids have been produced. Fusion between potato and tomato created 'Pomato' (Melchers et al. 1978) and fusion between *Arabidopsis* and *Brassica* resulted in *Arabidobrassica*. However, such distant hybrids are generally sterile and do not produce viable seeds. Therefore, it has been now realised that somatic hybridization is likely to be successful only when closely related but sexually incompatible parents are involved. For example, *Solanum brevidense*, a wild species, is resistant to potato leaf roll virus (PLRV) and potato virus y (PVY) but it can not be directly crossed with *Solanum .tuberosum* (potato). Some of the somatic hybrids between these two species, showing resistance to PLRV and PVY, are cross compatible with S. *tuberosum* allowing introgression of virus resistance gene in potato cultivars.

11.5.3 Haploid Production

The higher plants are normally diploid, with two sets of chromosomes in their somatic cells. Their haploids (with one set of chromosomes) arise in nature by parthenogenesis due to malfunction in the normal sexual process. However, such events are extremely rare and unpredictable.

In 1964, two Indian scientists, Guha and Maheshwari, observed that in cultured anthers of *Datura innoxia* some of the microspores, instead of following the normal gametophytic mode of development, formed sporophytes **(Androgenic plants)**. As expected, those sporophytes were haploid (Guha and Maheshwari, 1966). This report caused much excitement because of the considerable importance of haploids in genetics and plant breeding. To-date androgenic haploids of over 200 species, including many major crop plants (Cereals, Brassica spp, tomato and potato), have been raised through anther and/or isolated pollen culture (Fig. 11.9).

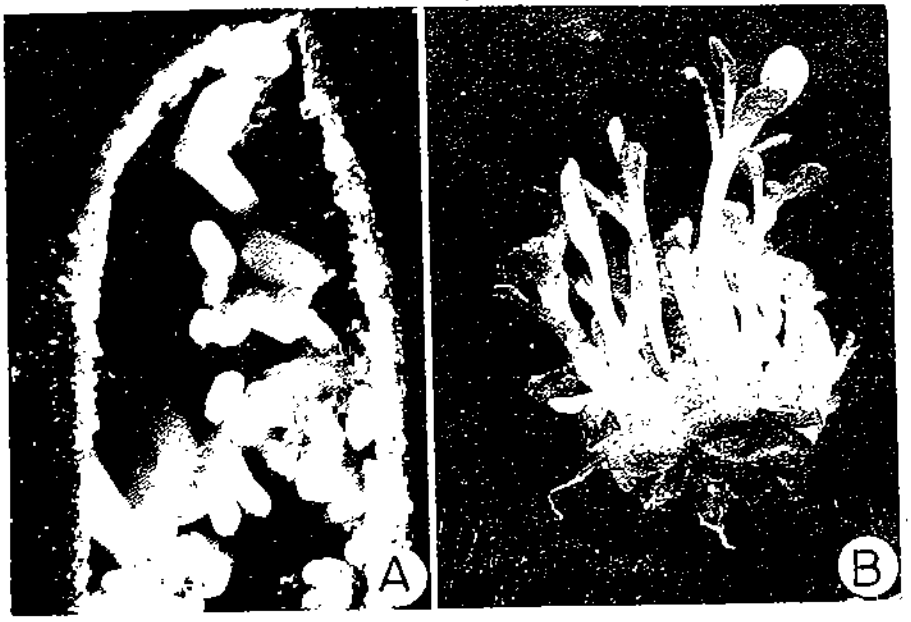

Fig. 11.9: Androgenesis in anther cultures of tobacco. A. A burst anther showing numerous pollen embryos. B. The pollen embryos have germinated.

In practice, anthers at the late uninucleate stage of microspore development are excised from surface-sterilised buds and cultured on a nutrient medium. Often a low

temperature (4-5°c) shock during initial 2-3 days of culture enhances the androgenic response. However, In *Brassica* species treatment with higher temperature (30-35° C) has proved beneficial. Under inductive conditions the

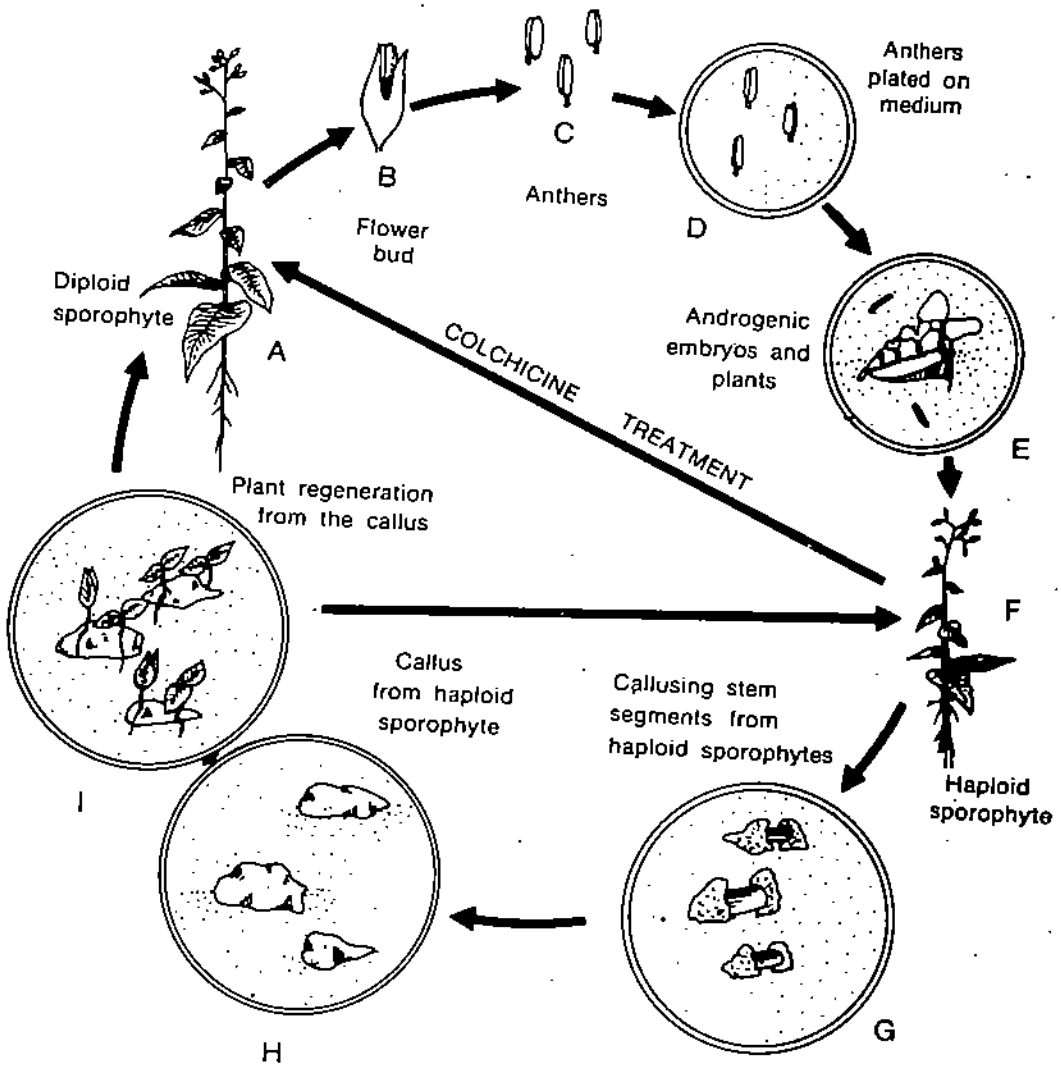

Fig. 11.10: A sumary diagram of haploid production by anther culture and their diploidization to raise homozygous diploid plants.

microspores undergo repeated divisions to form multicellular structures. Depending on the plant and the culture medium, such structures directly develop into an embryo or form a callus from which plants are regenerated via organogenesis or embryogenesis (Fig. 11.10).

Androgenic haploids of some species, such as wheat, mustard and tobacco, can also be raised through isolated microspore/pollen culture **(Pollen cultures)**. It must be realized that in spite of serious efforts androgenic haploids have not been possible in many other economically important plants.

In-vitro gynogenesis is another approach to produce haploids (Yang and Zhou, 1990). In this technique unfertilized ovules are cultured on media which stimulate the egg (parthenogenesis) or any other haploid cell of the embryo sac (apogamy) to undergo embryogenic development without fertilization. Invitro gynogenesis, first observed in **Hordeum vulgare** by San Noeum (1967), has now been reported in at least 16 species. This technique of haploid production is especially useful in plants in which the androgenic response is unsatisfactory, a large proportion of pollen plants are non-haploids or albinos, as in many cereals.

Haploids are extremely important in genetics and plant breeding. In haploids it is possible to detect recessive mutants which do not express themselves in diploid state due to the presence of the dominant allele. In cross pollinated plants and F_1

hybrids, with high degree of heterozygosity, the fixation of a particular trait through the conventional method of backcrossing takes 7-8 years. By anther or pollen culture this can be achieved in a single generation. Regeneration of plants from pollen grains also permits the screening of gametic variations at sporophytic level and selecting useful variants (gametoclonal variation). The Chinese scientists have developed and released about 20 new improved varieties of wheat and 61 varieties of rice through anther culture.

11.5.4 Clonal Propagation

Most cultivars of ornamental and fruit species and forest trees are highly heterozygous. Consequently, their seed progeny is not true-to-type. To preserve the unique characters of selected cultivars of horticultural plants nurserymen practise vegetative propagation, using stem, leaf or root cuttings or propagules such as tubers, corms, bulbs or bulbils. For plants which do not set seeds, such as edible bananas,grapes, citrus, petunia, rose and chrysanthemum, vegetative propagation is the only means of multiplication. A population of plants derived from a single individual by vegetative propagation is genetically uniform and is called a clone.

The conventional methods of clonal propagation are slow and often not applicable. For example, the only in-vivo method for clonal multiplication of cultivated orchids, which are complex hybrids,is "back-bulb" propagation. It involves separating the oldest pscudobulbil to force the development of dormant buds. This process allows, at best, doubling the plant number every year. Moreover,

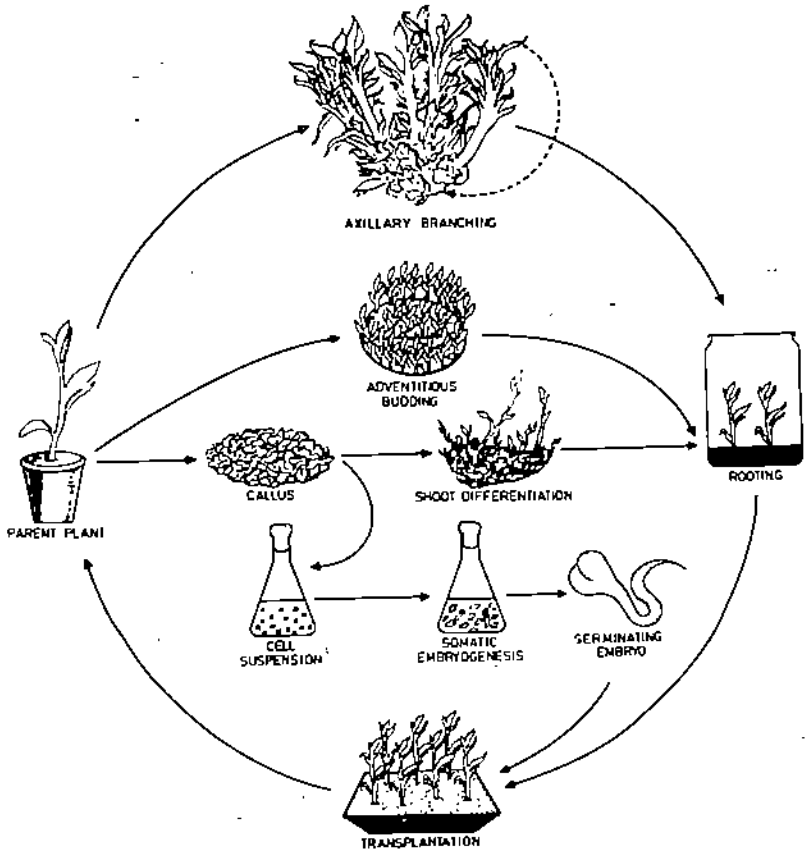

Fig.11.11: Diagrammatic summary of steps involved in aseptic multiplication of plants. Shoot multiplication is achieved through enhanced axillary branching adventitious budding from explants directly or after callusing. The shoots are rooted individually in a medium containing an auxin . The plantlets so obtained are transferred to well drained potting mix. After maintaining them under high humidity for3-4 weeks the plants are transferred to ordinary glasshouse or field conditions. Plant multiplication involving a callus phase may occur via shoot bud differentiation or somatic embryogenesis. In the latter case the rooting step is eliminated as the embryos possess a pre-formed root primordium.

monopodial orchids do not form pseudobulbils and, therefore, cannot be clonally multiplied. In 1960, a French scientist, G.Morel, described an in-vitro method for rapid clonal multiplication of orchids. This revolutionised the orchid industry, and today tissue culture is the only economically feasible method for clonal multiplication of orchids and is being widely used.

In-vitro clonal propagation, popularly called Micropropagation (Fig. 11.12) has been extended to a large number of species other than orchids and is being practised on commercial scale for numerous ornamental and fruit bearing plant and some forest trees. After the initiation of aseptic cultures micropropagation generally involves three steps: Shoot multiplication, rooting and transplantation (Fig. 11.11).

Shoot Multiplication:

This is the most important step with respect to the rate of propagation and genetic uniformity of the product. The most reliable and, therefore, themost popular method of shoot multiplication is forced proliferation of axillary shoots. For this, cultures are initiated from apical or nodal cuttings carrying one or more vegetative buds. In the presence of a cytokinin alone or in combination with a low concentration of an auxin, such as IAA or NAA, the pre-existing buds grow and produce 4-6 shoots (sometimes up to 30-40 shoots) within 3-4 weeks. By periodic removal of individual shoots and planting them on fresh medium of the original composition, the shoot multiplication cycle can be repeated almost indefinitely, and a stock of large number of shoots built up in a short period of time. Treatments with PGRs as described above can also help in a rapid build up of shoots by inducing adventitious buds by the explant directly or after callusing.

Somatic embryogenesis, which generally occurs after callusing of the explant, is another method of micro propagation. Somatic embryogenesis is not only fast, but may also allow partial automation of micropropagation and the propagules so produced (somatic embryos) bear both, shoot and root meristems. However, adventitive differentiation of shoots or somatic embryos, especially from callus tissue, has the risk of genetic variability in the progeny. Such variation, that develops in tissue culture called "somaclonal variation" is not desirable for micropropagation but is being exploited as a novel source of useful variations for crop improvement.

Rooting:

Shoots produced through axillary branching or adventitious differentiation are rooted in-vitro on a medium containing a suitable auxin, such as IAA, NAA or IBA. Alternatively, where possible, the shoots are treated with auxin and directly planted in potting mixture for in-vivo rooting.

Transplantation:

The shoots or plantlets multiplied on a medium containing organic nutrients, show poor photosynthetic capability. Moreover, in these plants mechanisms to prevent loss of water from leaves are poorly developed. Therefore, they require gradual acclimatization to the field conditions. In practice, the plants are maintained under high humidity (80-90%) for 10-15 days after they are removed from culture vessels. During the next few weeks the humidity around the plants is gradually lowered, before they are transferred to natural conditions.

The special merits of micropropagation are: i) it considerably increases the rate of multiplication 2) high rate of multiplication can be maintained throughout the year, 3) the multiplied plants are maintained in disease-free conditions 4) being free from microbes and insects valuable genotypes of exotic plants can be multiplied for export purpose, and 5) small size of the propagules and their ability to proliferate in a soil-less environment facilitates their convenient storage, handling and rapid transfer by air across international quarantine barriers.

11.5.4 Production of Disease-free Plants

Under normal conditions plants are infected by a wide range of pathogens such as bacteria, fungi, viruses, viroids, and insects like nematodes and insects. Many perennial plants and those propagated by vegetative means are systematically infected with one or more pathogens, which reduce yield, vigour and quality of the plant. If explants for micropropagation are derived from an infected plant, the pathogens can multiply and spread to a large number of plants. It is, therefore, essential to use disease free stock plants for micropropagation. Eradication of viruses and other pathogens is also desirable from the point of view of international exchange of plant materials.

Whereas bacteria and fungi present on the surface of the plant material can be easily eliminated by treatment with surface sterilizing agents, there is no dependable treatment against viruses. Viruses can multiply within the shoots in-vitro without symptoms. Traditionally, thermotherapy has been used for virus elimination but it is not effective against all viruses. Moreover, heat treatment may adversely affect the plant tissues.

For some reasons viruses are unable to enter or survive in the apical meristems. Therefore, even in infected plants the apical meristems are generally free of viruses. Taking advantage of this observation, Morel (1950) developed the technique of shoot tip culture to raise virus-free plants from infected individuals (Fig. 11.12). Since then it has become the most effective method of virus elimination. It involves excision of 0.5-1 mm long shoot tips, including apical meristem and one or two leaf primordia and their cultivation on a suitable medium to regenerate whole plants.

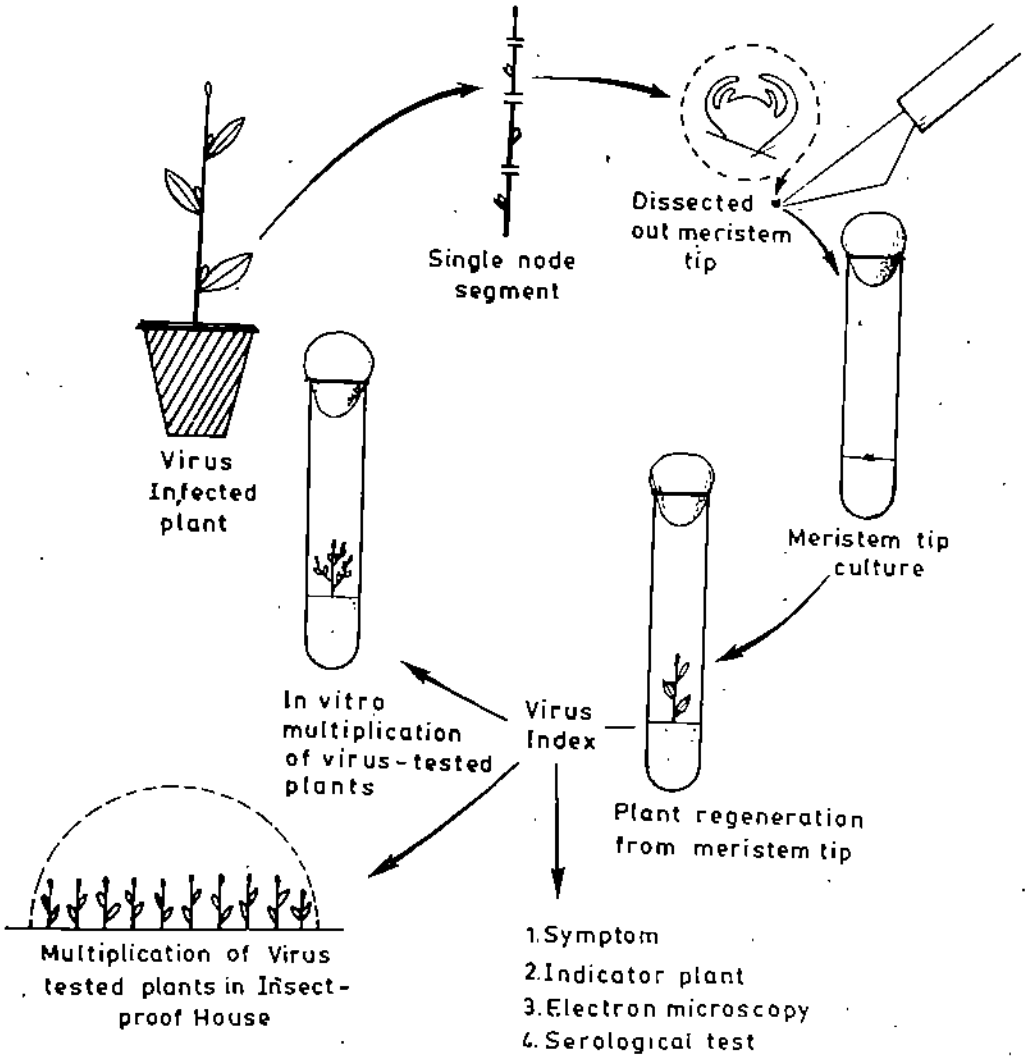

Fig. 11.12: Production of virus-free plants through shoot tip culture–A diagrammatic summary.

Some times a combination of chemotherapy or thermotherapy with shoot tip culture has enhanced the efficiency of the latter for virus eradication. However, the plants regenerated from shoot tips must be thoroughly screened for suspected viruses before declaring them virus-free.

11.5.5 Other Applications

Plants are source of a wide range of industrial products, including drugs. For considerable time the Ayurvedic system of medicine has been using dried plant parts or their crude extracts. However, the large scale, uncontrolled collection of plant materials from nature and habitat destruction by man have reduced the size of populations of certain species to such an extent that they are threatened to become extinct. In this context tissue culture provides two possibilities: (i) in-vitro conservation of endangered species and (ii) production of useful compounds by cultured cells.

Germplasm Conservation:

Totipotent plant cells and shoot tips can be freeze-preserved in liquid nitrogen (-196°c) for long periods, and when required they can be thawed and cultured to regenerate whole plants. Alternatively, for short term storage, the proliferating shoot cultures or roots can be incubated at growth-limiting conditions, such as decrease in temperature (4-12°c) or low nutrients. Such cultures have been shown to retain viability for 1-3 years, without a subculture. Root cultures have been kept for 25-30 years.

Production of Industrial Compounds:

Cultured plant cells retain their metabolic potential and synthesise secondary products of commerce. Cell cultures can also be used as factories for bioconversion of intermediate compounds into more valuable products. Shikonin, an expensive compound, obtained from the roots of *Lithospermum erythrorhizon*, has been used by the Japanese traditionally as a vegetable dye and in cosmetics and toiletries. However, due to over exploitation this plant has become almost extinct in Japan. To reduce dependence on import of this plant material, the Japanese scientists have developed a tissue culture method for the commercial production of Shikonin. Another example in which tissue culture production of an industrial compound has reached commercial level is Berberine from *Coptis Japonica*. In tissue cultures the yield of high value compounds can be enhanced by feeding the cells with precursors of their biosynthetic pathway (Biotransformation), manipulation of the culture conditions and selecting high yielding cell lines. The production of secondary plant products of commerce would considerably reduce the pressure on shrinking arable land. Besides its practical application, tissue culture systems have been found ideal to study basic aspects such as alternation of generation, morphogenesis, growth and differentiation and host-pathogen interaction. In fact, one important fundamental contribution of plant tissue culture is the discovery of cytokinins.

SAQ 3:

A) mark the correct statements:

a) Animal cells are totipotent

b) All living cells are totipotent

c) Plant cells are totipotent

d) Differentiation of shoot bud is referred to as cellular totipotency.

B) Select the right answer:

Cellular totipotency refers to the capacity of cells to produce:

a) Shoots

b) Embryos

c) Full plants

d) Roots

C) Fill in the blanks using appropriate words from the text:

a) Fusion of dissimilar protoplasts results in the formation of. . . . . .

b) A plant cell which has been stripped of its wall is called. . . . . .

c) . . . . . .is the most popular fusogen.

d) Hybrid embryos which abort at a very early stage can be rescued by. . . . . .

D) Give the technical term for the following statement:

a) In-vitro production of plant from pollen grains

b) In-vitro production of plants from unfertilized egg cell

c) In-vitro propagation of plants

d) Variation among the plants raised from pollen grains

E) Choose the false statement:

a) Microspores and unfertilized egg are known to form haploid plants in culture

b) Haploids are important in genetic studies as they help to detect recessive mutants.

c) Haploids production by tissue culture is of academic interest only as such plants are abnormal and can not be integrated into conventional breeding programmes.

d) Micropropagation allows the production of a large number of propagules in a relatively short time throughout the year under aseptic conditions.

11.6 SUMMARY

What we have learnt in this unit can be summarised as follows:

1. Plant tissue culture is the aseptic cultivation of isolated cells or protoplasts in standard plant tissue culture medium (Basal medium).
2. Tissue cultures can be raised from all living plant cells and multiplied indefinitely by periodic subculture on fresh medium.
3. All living plant cells are totipotent. By manipulating the composition of growth regulators in the medium it has been possible to regenerate whole plants from callus and suspension cultures via organogenesis or somatic embryogenesis.
4. Some chemicals used in Basal medium degrade on exposure to high temperature (Thermolabile) and some are stable (Thermostable).
5. Isolated protoplast released from the cell wall by either a mechanical or an enzymatic process is described as naked protoplast.
6. Tissue culture techniques, such as "hybrid embryo culture", "test-tube pollination or fertilization" and somatic hybridization are being used to

produce rare hybrids or cybrids which can not be produced by the conventional breeding methods.

7. Tissue culture has become an important horticulture technique to raise virus-free plants and for rapid clonal multiplication of selected genotypes.

11.7 TERMINAL QUESTIONS

1. Why is it essential to maintain a completely aseptic environment inside the culture vessels.
2. What are major categories of constituents of plant tissue culture media? Why is sucrose an essential constituent of all plant tissue culture media.
3. What is meant by cellular totipotency? Who was the first Scientist to test this concept experimentally?
4. Differentiate between inoculation and subculture.
5. Briefly discuss the role of plant tissue culture in plant breeding.
6. What are the advantages of micropropagation over the conventional methods of clonal propagation of plants?
7. Mention one major contribution of the following scientists to plant tissue culture:
   a) G.Morel
   b) F.Skoog
   c) P.R.White
   d) F.C.Steward
   e) F.Laibach
   f) S.Guha and S.C. Maheshwari
   g) L.H.San Noem.

11.8 ANSWERS

Self Assessment Questions:

1(a). It is extremely important to sterilise the nutrient media used for plant tissue culture because plant tissue culture media contain a high concentration of sucrose and support the growth of many microorganisms like bacteria and fungi.

(b). IV

(c) Compounds which can not stand heat are called "thermolabile compounds". They decompose and loose their activity on autoclaving.

(d). C.

(e). i) growth regulators

ii) less

iii) 120°c

iv) 5.8

v) Shoot bud differentiation and shoot multiplication.

SAQ 2:

2(a). i) Callus

ii) Wound tissue

iii) 2,4-D

iv) Subculture.

b) i) V

ii) VI

iii) I

iv) II

v) IV

vi) III

c) iii)

d) Callus culture becomes brown and necrotic if they are left too long on the same medium because of:

i) depletion of essential nutrients

ii) desiccation of the agar due to water loss and

iii) accumulation of toxic metabolites in the medium.

SAQ 3:

3) a) c

b) c

c) i) heterokaryon

ii) Protoplast

iii) Polyethylene glycol

iv) In ovule embryo culture

d) i) androgenesis

ii) parthenogenesis/gynogenesis

iii) micropropagation

iv) gametoclonal variation.

e) ii

TERMINAL QUESTIONS

1). The nutrient media used for plant tissue culture would support luxuriant growth of many microorganisms, such as bacteria and fungi. Reaching the medium these microorganisms grow much faster than the plant cells and cover the tissue surface, impeding its growth and finally killing it. It is therefore extremely important to maintain complete aseptic environment inside the culture vessels.

2) Any plant tissue culture medium should have the following categories of constituents: a) Sources of major and minor inorganic elements b) Organic nutrients, such as vitamins and amino acids c) Sucrose as a source of carbon and d) Plant growth regulators, such as auxin and cytokinin, use of agar is optional.

did not succeed because: a) he selected highly differentiated leaf cells as the experimental material and b) he did not use growth promotory substances as they were unknown at that time.

4) Inoculation is the process of planting fresh explants on culture medium at the time of initiation of cultures, whereas sub culture refers to dividing the cultured tissue into pieces and transferring them to fresh medium.

5) Application of plant tissue culture to plant breeding:

   a) Embryo culture for the production of rare hybrids.

   b) Anther Pollen and unfertilized ovule culture, to develop haploids for rapid production of homozygous diploids.

   c) In-vitro pollination and fertilization to overcome prezygotic barriers of sexual incompatibility

   d) Somatic hybridization and cybridization.

6) Micropropagation has many advantages over the conventional methods of clonal plant propagation:

   a) It is often faster than the conventional methods of vegetative propagation.

   b) Large number of plants can be multiplied in a short space.

   c) Multiplication occurs under disease free conditions.

   d) Under controlled conditions rate of multiplication is maintained throughout the year.

7) a) G.Morel: Shoot tip culture for virus elimination

   b) F.Skoog: Chemical control of organogenesis

   c) P.R.White: Continuous root culture

   d) F.C.Steward: Somatic embryogenesis in carrot

   e) F.Laiback: Hybrid embryo culture

   f) S.Guha and S.C. Maheshwari: Production of androgenic haploids ; anther culture

   g) L.H.San Noem: Production of gynogenic haploids by ovule culture.

UNIT 12 CURRENT TRENDS IN DEVELOPMENTAL STUDIES

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12.1 INTRODUCTION

In flowering plants, development commences with the growth of the zygote into embryo, that is enclosed in a seed. Intensive embryological investigations during the last four decades as presented in the Units 1-6 of Block-1, have provided some insight to understand and analyse various embryological processes and compare and correlate them. However, the factors and various metabolic changes that influence the specialization of embryological structures have mostly been unresolved.

With the advent of polarizing, phase contrast, fluorescence, electron (TEM, SEM) microscopy and interference (Nomarski) contrast optics, and cyto- and histochemistry, together with the application of complementing modern cell biology techniques such as: immunocytochemistry, immunofluorescence and video-image processing it has become possible to unravel and characterise some fine structural details of the components involved in embryology. These have added several new dimensions to investigations on the structure and function of reproductive organs and tissues.

During the last decade therefore there has been a surge of information on the complex array of interrelated processes constituting reproductive biology. The implications of these investigations are likely to enhance our understanding concerning sexual reproduction in flowering plants.

Some of the major achievements that have added to our knowledge in specific areas are described in this unit to familiarise you with the current trends in this field of study.

Before you begin your study of this unit, it would be useful to brush up your memory regarding what you have studied in Units 1 to 6. Combine your prior knowledge with the information provided here to get a clearer picture of sexual reproduction in higher plants.

Objective

After studying this unit you should be able to :

- understood the finer details of the embryological process in higher plants;
- interpret the use of modern techniques in solving the intricacies of reproductive structures of angiosperms;
- explain why it is essential to adopt other relevant techniques to unravel the finer details of the cellular components;
- integrate and assess the various steps involved in reproductive biology.

12.2 POLLEN BIOLOGY

As you know from your study of Unit-1 that during microsporogenesis deposition of callose around the microspore tetrads takes place. This phenomenon is reported in several taxa. The information regarding the mode and nature of deposition of callose has been made known to us largely by the use of fluorescence microscopy. Now it has been established that callose synthesis occurs in two phases between the persistent primary wall and the cytoplasm of the sporocyte. Beginning at the pachytene stage it continues in the form of two layers till prophase I. During telophase, callose develops afresh and forms layers 3 and 4. Finally, during cytokinesis 2, more layers develop of which layer 6 causes the separation of each microspore.

In addition, it has been found that in species with simultaneous cytokinesis four or six plates form a unit while in successive cytokinesis only three plates form the unit. The plates also show positive reaction to proteins, reducing substances, and polyphosphates, revealing the complexity of composition.

Fluorescence microscopy coupled with biochemical analysis has helped resolve the differential activity of the enzyme, β-1, 3- glucanase that catalyses the dissolution of callose. The enzyme activity is relatively low during the first meiotic division but reaches its peak at the time of spore release. This sequence of events controlling the enzyme activity is important, as an early dissolution of callose may lead to sterility. Use of autoradiographic and fluorochromatic techniques demonstrated the reduced permeability of the callose wall and the resultant chemical isolation of microspores. A combination of phase-contrast and fluorescence microscopy also revealed that the callose wall during exine formation serves as a mold or template.

Factors that trigger the onset and regulation of meiosis in anther and ovule can be worked out by employing labelled reagents. Biochemical studies through isoenzyme markers have already demonstrated specific stages of meiotic process. There is an increase in the activity of acid phosphatase at zygotene, followed by subsequent decline. At pachytene, marked activation of endonucleases is recorded. Isoperoxidases serve as markers in normal pollen development in *Mercurialis annua.*

Microcinematographic techniques clearly bring out the structure of thin-walled, transparent pollen grains, contents of pollen grains including their circulation and rotation, microstructure of pollen tubes, velocity and character of protoplasmic streaming, relative movement of nucleus of vegetative and generative cell, division of generative cell and movement of the two male gametes. The information would be interesting with respect to the changes accompanying the induction of androgenesis in vitro as that would indicate involvement of different components of pollen grain.

Additional evidences suggest that considerable cytoplasmic reprogramming takes place during generative cell ontogeny. In *Gasteria*, the amyloplasts become polarised and remain entirely within the vegetative cell during the first pollen mitosis so that the generative cell inherits only the mitochondria. There may be an

other polarisation of the organelles prior to second mitosis effecting unequal distribution, for example, generative cell of *Plumbago* carries both plastids and mitochondria.

Depending upon the number of cytoplasmic organelles (mitochondria and plastids) it is possible to distinguish the two male gametes in a pollen tube. This information, and by looking at the composition of components of cytoplasm of the post-fertilization products, it is possible to predict and demonstrate the male gametes involved in syngamy and triple fusion.

The phase-specific changes in gene expression become evident as the generative cell shows period-specific changes in shape, and interactions with the vegetative cell. It is important to note that the polarization of the organelles occurs prior to first and second pollen mitosis when generative, and sperm cells are formed respectively.

It has now become possible to isolate the generative cell in living condition and study the morphological changes. The change from natural spindle shape to spherical is dependent on osmolarity of the isolation medium.

EM studies suggest that wall formation around the generative cell is not a special one but corresponds to that in other cells. The presence of callose in generative cell is transitory.

The adaptation of recent techniques like ultra-thin serial sectioning, isolation of live sperms and computer-aided 3-D reconstruction has helped unravel the finer details of the 3- celled male germ unit (MGU). The studies have produced evidence that in the majority of angiosperms the MGU is organised in the pollen tube and it travels through the pollen tube as one unit. Upon reaching the ovule, the vegetative nucleus dissociates first and the sperms thereafter.

The presence of a cytoplasmic projection of one sperm cell (*Svn*) that is superficially associated with the vegetative nucleus in the pollen tube is also evidenced from three-dimensional reconstructions based on serial thin sections. It is linked to Svn by a common cell junction. Likewise, the three-dimensional reconstruction of sperm cells of *Rhododendron laetum* and T. *macgregoriae* shows that they are paired together and both have extensions that link with the tube nucleus, forming a male germ unit. Video-image processing has revealed the presence of an axial micro-tubic cage in generative cells. The sperm cells differentiate within the pollen tube about 24 h after germination in vitro. The relevance of such a complicated male germ unit in fertilization process is presently not understood.

In sperm cells the nucleus has a densely-stained granular chromatin packed around the periphery, usually with less dense regions as nuclear vacuoles. Relatively few pores are present in the nuclear envelope as compared to the vegetative nucleus.

Of the two sperm cells, one has a long extension and is associated with the vegetative nucleus (sperm *Svn*). It carries a significantly larger number of mitochondria, than the other sperm (*Sua*). The *Sua* sperms in *Brassica* have a lower content of spherical mitochondria; infact the lowest reported for any angiosperm cell. Where both mitochondria and plastids are present in the sperm cells, *Sua* has large number of plastids. These quantitative differences once again show varying degrees of differential gene activity. The evidence for such dimorphism in sperm cells is either lacking in other cases or needs to be worked out.

It has now become possible to study pollen tubes and isolate live sperm cells in barely. The isolated sperm cells examined under Nomarski interference optics show compact cytoplasm and a conspicuous nucleus with condensed chromatin. The male gametes remain in contact with each other without showing any directional movement. The mode of relative movement of the two sperm cells to their respective destinations, the egg cell and the central cell, is still a speculation. The sperm cells change from spindle to spherical and back to spindle shape. These changes are related to cytoplasmic microtubules present around the periphery of the sperm cells. It has been suggested that changes in shape occur even during the course of pollen tube growth through the micropyle and synergid. The cytoplasm of

the sperm cells is active with small external bulges appearing and disappearing rapidly. This may indicate an interactive role related to their mobility. Thus, research on such lines may ultimately help in our understanding towards the entire fertilisation process hitherto least understood. The cytoplasmic sheath of the sperm cell enters the egg apparatus at the time of pollen tube discharge. It is suggested that further information about the sperm cells may result if their activity is studied in the pollen tube 'sap' or embryo sac 'juice', or a combination of both.

Calcoflur is a fluorescence brightener used for detecting cellulose.

TEM studies show that the two sperm cells of *Plumbago* are connected by a calcoflur-positive cell wall transgressed by plasmodesmata. A microfibrillar ephemeral cell wall appears 15 min. after pollen germination in barely; absent in mature pollen and in pollen tubes. The sperm cells of *Brassica campestris* and *B. oleracea* are connected by interdigitating finger-like cytoplasmic evaginations. The importance of acquiring such a complex structure and arrangement needs to be worked out. Rapid-freezing and physical fixation procedures, such as, freeze-substitution will help determine the presence or absence of a periplasmic matrix component.

Recent researches in biochemistry reveal that during the development of pollen grains several haploid genome-specific genes are expressed that control pollen-specific functions, such as, pollen development, germination, sperm formation, and also stigma-style recognition. Further progress in understanding these processes can be achieved by isolating the genes that are expressed in pollen.

Recent studies on molecular genetics have thrown light on the role of microspores in controlling germination and regulating the metabolic processes. That the haploid genome is involved in transcription and translation during pollen development is evidenced from studies of several dimeric enzymes. The alcohol dehydrogenase gene (Adh 1) in maize specify a dimeric enzyme responsible for the activity of the pollen grain. Synthesis of the Adh enzyme depends solely on the genotype of the pollen nuclei and not influenced by the genotype of the diploid plant. The mRNA identified in mature pollen seem to be metabolic genes involved in tube growth. It would be challenging to determine the function of the pollen-specific genes that also control microsporogenesis.

The mature pollen grains of several species have mRNA synthesized prior to anthesis and in cell-free translation systems, code for similar polypeptides that are synthesised during germination and early tube growth. Biochemical experiments show that the mRNAs consist of three abundant classes: the first present in 26,000 (*Tradescantia paludosa*) and 32,000 (*Zea mays*) copies, the second are intermediate in number, and the third have 100 to 200 copies per pollen grain. In both the plants, the mRNAs in mature pollen grains are products of about 20,000 different genes. Based on colony hybridization, it is estimated that about 10% and 20% of the total genes expressed in maize and *Tradescantia* respectively might be specific to pollen. Zin 13 gene represents very few copies in maize genome but its specific mRNA has been demonstrated in the cytoplasm of the vegetative cell and throughout the pollen tube cytoplasm of the vegetative cell and also throughout the pollen tube after germination. Thus, it is a product of the vegetative cell nucleus. Such characterization of cell related mRNA and their product and finally their interaction with other components of the male sex structures will elucidate the nature and significance of male germ unit.

12.3 INCOMPATIBILITY

EM studies have established the concurrent development of Ü bisch bodies and sexine. The detection, identification and precise localization of exine and intine proteins became possible by employing fluorochrome-induced fluorescence and immunofluorescence. Now that so much information has become available, the two substances in both wall layers can be easily localized. The development of pollen

wall and simultaneous incorporation of proteins has also been worked out by using the same technique.

Specific regions in pollen wall have been identified which predominantly have esterases that are considered exine markers and acid phosphatases as intine markers. Hydration effect causes the release of esterases, amylases, galactosidases, glucosidases and phosphatases. These enzymes and other proteins account for pollen allergy.

It is already possible to use isoenzyme patterns of esterase and leucineaminopeptidase for the identification of maize inbred lines. The complex incompatible interactions require additional efforts to explore the possibilities of unraveling the mechanism involved. For that would help solve many unsuccessful breeding programmes. Already, enzyme and protein marking systems are being employed to find out the interrelationships between male and female reproductive structures. It has been reported that the glycoproteins related to S alleles 1,2,3,6 and 7 isolated from style extracts of *Nicotiana alata* are ribonucleasés. Ribonuclease activity has been implicated in the mechanism of gametophytic self incompatibility. It has been possible to generalize that stylar fluid in hollow-styled species show esterase and phosphatase and solid-styled taxa, in addition, show peroxidases and proteases.

Some recent biochemical genetic approaches have contributed significantly towards our understanding of the phenomenon of self-incompatibility, especially in *Brassica*. The accumulated data, however, shed little light on the relationships between sporophytic and gametophytic systems. It is interesting nevertheless, that the *Brassica* and *Nicotiana* genes appear to have similar patterns of expression in cells that occupy the path of growing pollen tubes.

Plant transformation experiments are now showing promise in the analysis of different aspects of the self incompatibility response.

A relationship has been worked out between the protein kinase of maize, to the S-locus glycoproteins. The enzymes are either serine/threonin-specific or tyrosine-specific. A complimentary DNA clone from *Zea mays* encoding a putative serine/threonin- specific protein kinase structurally related to the receptor tyrosin kinases have been identified. This protein kinase is linked through a trans-membrane domain to an extracellular domain similar to that of glycoproteins encoded in the self- incompatibility locus of *Brassica* involved in the self recognition system. The identification of gene sponsored compounds involved in the incompatibility mechanism may ultimately help in resolving the intricacies of self- incompatibility.

12.4 FEMALE GAMETOPHYTE

The deposition of callose in the nucellar cells became evident with the use of modern techniques. During megasporogenesis the walls of the nucellar cells adjacent to the chalazal megaspore between the embryo sac and hypostase show callose. The amount of callose deposition increases gradually. The functional significance is not well understood. Callose deposition has also been detected in the walls of the endothelial cells. The mode of callose appearance suggests that the cells might be involved in the translocation of metabolites. A similar role can also be assigned to the nucellar cells.

Antipodals, the cells at the chalazal region of the embryo sac have been speculated to have a nutritional role. Details of structural organization that establishes this fact have been worked out through ultrastructural and histochemical methods. The antipodals have abundant mitochondria, plastids, multicisternal dictyosomes and a large number of small vesicles derived from the ER or dictyosomes. The cells are reported to have papillate wall ingrowths that appear like filiform apparatus of synergids. The wall between the antipodals and with the central cell are interspersed

with plasmodesmata. The cells are rich in oxidases, ascorbic acid, sulfhydril compounds, starch, lipid and proteins.

The presence of projections in the synergids was long established. EM studies have now revealed that the filiform apparatus of synergids is not a simple finger-like projection of the wall into the cytoplasm. Each projection has a core of tightly packed microfibrils enclosed by a non-fibrillar sheath rich in polysaccharides. Infact, the filiform apparatus resembles the spongy wall of the 'transfer cells'.

Isoenzymatic studies indicate differences in specific female tissues. However they do not point to any conclusive explanation. It is interesting that in *Lilium regale*, higher levels of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) occur in ovary wall and inner integuments than in outer itegument. Synthesis of insoluble reserve polysaccharide takes place in the outer integument whereas, they are hydrolysed in inner integument. The metabolites diffuse from integuments through endothelium/nucellus in embryo sac through a gradient and enzymatic action.

Recent efforts demonstrate the possibility of enzymatic isolation of embryo sacs of *Plumbago, Lilium,* and *Zea mays*. Further experimentations should be possible to assess the respective participation of different components of the embryo sac. In *Lilium longiflorum*, all developmental stages of the female gametophyte can be isolated in living conditions. Recovery of viable eggs and central cell of *P. zeylanica* will make them amenable to physiological and biochemical studies, an advantage hitherto enjoyed by male gametophytic cells.

The contraction cycle of the nucleolus of the secondary nucleus is 7 min. to several hours in *Jasione montana*. In *Galanthus nivalis*, the sperm movement in central cell of the embryo sac is about 3μm per min. Such measurements further facilitate the understanding concerning the time required for the fusion of gametes with the egg, and with the secondary nucleus. The process requires about 150 min. at 20°C in a silicon fluid medium in *G. nivalis*. The nucleoli takes about 10-15 min.

12.5 ENDOSPERM

The examination of live material of *J. montana* reveals that the division of the primary endosperm nucleus is transverse, followed by the laying down a wall. The chalazal chamber divides by a vertical wall. The elongated tubular zygote is 60-80 μm long, and the growth rate is 5-6 μm per hour. The mitotic cycle of the endosperm nuclei is short as compared to that in somatic cells. It is also possible to analyse the rhythm of development of endosperm and embryo during the initial few stages. The information would help in finding out ways to prevent embryo abortion in distant hybridization.

Enzymological studies on endosperm during development point out variable activities of different enzymes. Free-nucleate endosperm cells of *Lilium regale* show high activity of 6-PGDH and G6PDH until cellularisation process begins. The most active period of nitrogen accumulation in maize coincides with a rapid increase in glutamate synthase. Even differences between the normal and opaque-2 maize endosperm can be marked by analysing isoenzyme profiles. At day 15, the normal endosperm shows proteases I and II, whereas, opaque-2 exhibits only I at both the ages.

The localisation of three isoenzymes of G6PDH in the plastids and one in cytosol of developing castor endosperm reflects that young castor endosperm is the seat of both, glycolytic and pentose phosphate pathways. The embryo-endosperm relationship and the role of endosperm during seed development is an important aspect needing further attention.

12.6 EMBRYO

The suspensor cells of young embryos of *Tropaeolum majus* show that the activity of acid phosphatase increases from micropylar to chalazal pole. The concentration of 6-PGDH and G6PDH is high in very young embryos of *Lilium regale* By globular stage the activity decreases considerably. Implicit in these results is the fact that different regions of embryo would provide some explanation of different embryogenic processes. Also, a marker system can be established for identifying differentiating areas in the developing embryos. What is needed is the uniformity of the enzyme system being explored in different climatic conditions which may affect metabolic processes thus influencing the sexual cycle. This may lead to detection of variations in developmental sequences or abnormalities so often reported in the literature.

12.7 SUSPENSOR

The structure and function of suspensor have not been given adequate attention During the last decade, however, investigations have produced noteworthy results. These include ultrastructural, isoenzymatic, physiological, and in vitro experiments. A great deal of variation occurs in suspensor structure, probably modified to support the developing embryo. During 1950's and 1960's embryologists believed that it the suspensor is merely a morphological organ that pushes the embryo deeper into the more friendly environs of endosperm. This view is gaining reconsideration. The suspensor plays a more dynamic role than was hitherto assigned. The special kind of plastids present in legumes such as *Pisum* and *Phaseolus* and in *Ipomoea* and *Tropaeolum* show remarkable ultrastructural changes around late heart-shaped stage of embryo. The significance of these unusual plastids needs to be determined.

Another feature of interest is the presence of wall embayments lined by plasma membrane in the suspensor cells supposed to be involved in short distance translocation of metabolites similar to transfer cells. Some experiments demonstrate the significance of presence of suspensor for proper development of the embryo. During early stages, removal of suspensor reduces embryo development but, at later stages, it has no effect. However, it is possible to replace, at least partially, the effect of suspensor loss by providing gibberellins in the culture medium. The finding is further substantiated by quantitative analysis of GA present in the suspensor and embryo proper cells. This GA has been identified as gibberellin A1.

The relative concentrations of auxin in embryo proper and suspensor of *Tropaeolum majus* have been studied through single ion detection. The suspensor proper yields significantly higher concentrations of auxin. Likewise, in *Phaseolus*, the suspensor of heart-shaped embryo shows more cytokinin. However, at mid-cotyledonary stage, suspensor contains low cytokinin and the embryo seems to become autonomous for cytokinin.

These findings clearly indicate that suspensor is a reservoir of growth hormones meant for supporting embryo development. Whether these hormones are synthesized de novo in the suspensor cell or it acts merely as a conduit remains to be answered.

12.8 THE FUTURE

Fertilization in flowering plants is essential for sustaining life on earth. Production of most crops depends on the effectivity of the fertilisation process. The emergence

of new cultivars and plant improvement until recently depended exclusively on normal fertilisation. Therefore, the active involvement of a number of research techniques bears testimony to the fact that reproductive biology is emerging as one of the most exciting areas of plant biology.

During the past decade major emphasis has been to understand the male gametophyte and the mechanism of incompatibility as these are crucial for the success of breeding programmes. The last five years have witnessed a surge of information on identification and control of haploid genome regulated activities during initial pollen germination and fertilisation. Characterization, and structure of sperm cells and structure and composition of pollen tubes are also attracting considerable attention. Immunofluorescence techniques confirmed the presence of proteins in the pollen walls which enhanced our understanding of the acceptance and rejection (incompatibility) response. Immunological principles would help explain the nature of the product of the sporophyte cells involved in gametophytic and sporophytic incompatibility.

Isolation of entire embryo sacs and its individual cells opens up new possibilities for the study of fertilisation process and embryo-endosperm relationship. Some ingenious experimental studies with suspensor demonstrated its significance during early stages in providing growth support to the developing embryo. Labelling studies may finally reveal the site of biosynthesis of nutrients and growth hormones and the creditable role of the suspensor.

With the aid of computerised imaging, the structure of sperm cells would become more clear, as yet incompletely understood.

The immensely growing body of knowledge of various embryological structures mainly from biophysical and biochemical investigations have provided added impetus for probing these structures in living state. Identification and localization of various cellular constituents therefore acquire additional significance. A creditable method capable of analyzing biochemical events in single cells or small aggregates will provide ample details and thus answer to a myriad of questions.

In embryological studies, now the principal criteria should be an estimation of the rate of success of any individual plant in parenting viable embryos. Towards this goal, together with several new techniques, embryologists can contribute extensively in making quantitative estimates of integral components of sexual process. This can also be used for a functional analysis of reproductive behaviour in any flowering plant, including tests of the capacity of the male or female organs involved in reproduction process.

It is thus surmised that rapid progress in techniques and consequently in approach has yielded valuable information in the area of angiosperm embryology. Further efforts will benefit our knowledge and enhance our understanding of various aspects of plant embryology hitherto unexplained.

12.9 SUMMARY

The study of this unit has acquainted you with the recent findings in reproductive biology, particularly in the fields of pollen biology; incompatibility reactions during sexual reproduction; several functional aspects of female gametophyte, endosperm, embryo and suspensor. The deployment of various modern tools and techniques have answered several questions that have been arising in the minds of biologists for a long time. The coming time holds great promises and it is going to be very exciting one; as clearer picture of plant development is likely to emerge. his has enormous application in the production of novel plants and in the improvement of existing plants.

12.10 TERMINAL QUESTIONS

1. Fill in the blank spaces with appropriate word (s).
   1. The ..... wall surrounding the microspore serves as a mold or template during the formation of exine.
   2. In the pollen tube, one of the sperm cells showing a large cytoplasmic projection is associated with the. ..... .
   3. The change in shape of the sperm cells from spindle to spherical and back to spindle shape is controlled by the ..... present along the periphery of the sperm cells.
   4. For studying the pollen wall, ... are mostly used as markers for exine and ..... as markers for intine.
   5. The filiform apparatus of the synergids is similar to the spongy wall of the ..... cells.
   6. For the proper development of embryo, the presence of ..... is necessary.
2. State which of the following statements are not true?
   (i) The synthesis of callose around the microspore tetrads occurs continuously.
   (ii) The microcinematographic technique enables us to study the following: the structure of thin walled pollen grains; microstructure of pollen tubes; velocity and character of plasma streaming, division of generative cells.
   (iii) The two male gemetes in a pollen tube differ from each other with respect to some of their cytoplasmic organelles.
   (iv) Several haploid genome-specific genes are expressed during the development of pollen grains, that control functions such as pollen development, germination, sperm formation etc.
   (v) By the application of modern techniques it has been established that the antipodals prevent the downward growth of the embryo sac.
   (vi) The filiform apparatus present in the synergids is composed of tightly packed microfibrils that are enclosed by a polysaccharidic sheath.
   (vii) Like the male gametophyte, viable embryo sac and its components can be isolated.
   (viii) The suspenser is a reservoir of growth hormones, and it supports the proper development of embryo.
3. During which time of microspore development, is the activity of enzyme β-1, 3-glucanase at its peak?
4. What would be the consequences, if the enzyme β-1,3-glucanase shows its maximum activity, say during meiosis-1?
5. What is the fate of 3-celled MGU (male germ unit): (i)while in the pollen tube, and (ii) on reaching the ovule?
6. Give two features, on the basis of which we can differentiate between the two sperm cells (*Svn* and *Sua*)?
7. What components of pollen wall cause allergenic reactions in humans?
8. Which characteristics of antipodal cells indicate that they perform a nutritive role?
9. Comment on the following statement: 'the mitotic cycle of the endosperm nuclei is short as compared to the somatic cell'.

10. What facts have been brought to light by the modern investigations on the functional role of suspensor?

11. Combining your prior knowledge on reproproductive biology of angiosperms from your study of Block-1, and what you have learnt in this unit, prepare on update write up on the following aspects : (i) microspore development; (ii) : incompatibility; female gametophyte; the roles of endosperm and suspensor.

12. Prepare a list of various techniques mentioned in this unit?

12.11 ANSWERS

Terminal Questions

1. 1. callose
   2. vegetative nucleus
   3. microtubules
   4. esterases, acid phosphatases
   5. transfer
   6. suspensor
2. (i)
   (v)
3. At the time of spore release.
4. This may lead to sterility.
5. (i) It remains as one unit, as they are connected through their extensions.
   (ii) The vegetative nucleus dissociates and the sperms separate later on.
6. The *Sm* sperm cell has long extension and is associated with vegetative nucleus, and contains a large number of mitochondria than the other sperm.
7. Esterares, amylases, galactosidases, glucosidases, phosphatases and some other proteins.
8. Hint: they have abundant mitochondria, plastids, multicisternal dictyosomes, small vesicles derived from ER or the dictyosomes, wall ingrowhts like synergids, walls between the antipodals and synergids have plasmodesmata, are rich in oxidases, ascorbic acid, sulphydril compounds, starch, proteins and lipids.
9. Hint: The endosperm has to develop faster to provide nourishment to the developing embryo.
10. Hint:The old view has gained firm footing that the suspensor pushes embryo deep into the nutritive medium - the endosperm. The presence of growth hormones like cell auxins and cytokinins; and unusual plastids, presence of wall embayments lined by plasma membrane suggest their role in short distance translocation of metabolites like the transfer cells.
11. You may refer to the related portions in Units 1-6 and the present unit.
12. Some of the techniques mentioned in this unit are: Fluorescence microscopy, Biochemical analyses, Microcinematography, Ultra-thin section, EM studies, Video image proceeding, Nomarski interference optics, Freeze substitution, Immunofluorescence; and Histochemical techniques.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-06
Block-2

Enrolment No. | | | | | | | | |

1) How many hours did you need for studying the units?

Unit Number	7	8	9	10	11	12
No. of hours						

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

Assignment Number	1	2
No. of hours		

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

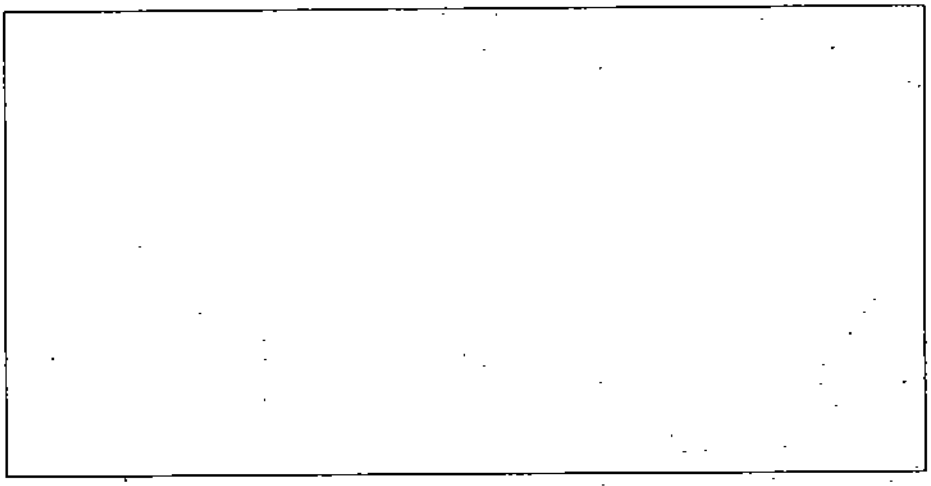
Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not explained	Terms are not explained

4) It is possible that you could not attempt some SAQs and TQs.
In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Types of difficulties			
			Not clearly posed	Cannot answer on basis of information given	Answer given (at end of Unit) not clear	Answer given is not sufficient

5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

6) Any Other Suggestion(s)



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To

The Course Coordinator (LSE-06; Developmental Biology–Block-2)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Delhi - 110 068

NOTES

NOTES

UTTAR PRADESH
RAJARSHI TANDON OPEN UNIVERSITY

Block

3

ANIMAL DEVELOPMENT 1

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UNIT 14
Cleavage and Gastrulation 32

UNIT 15
Morphogenesis and Tissue Organisation 75

UNIT 16
Mechanisms of Cell Interaction 98

UNIT 17
Organogenesis of Eye and Limb 123

BLOCK 3 ANIMAL DEVELOPMENT-I

You already know that animals reproduce in one or both of two ways, asexually or sexually (LSE-05; Physiology 2, Unit 8). All multicellular animals (Metazoa) reproduce sexually although some of them utilise asexual methods of reproduction. In most metazoans, sexual reproduction involves two parents, one of each sex, a male and a female, which generate the haploid sperms and eggs, respectively. Diploidy is restored in the zygote formed by fertilization of the egg by a sperm. The zygote develops into a new individual. Continuity of genotype from generation to generation in all organisms is ensured by reproduction; but multicellular animals are also characterized by diversity of cell phenotypes arising from the same genome present in all cells of individuals of each generation. Production of cell diversity or differentiation of various cell types within each generation is a function of development. It occurs not as an isolated phenomenon but during and as part of the elaborate process of the development of the new individual. This block deals with development of sexually reproducing metazoan animals from a single cell, the zygote, during the embryonic phase of their life history.

For a student like you, beginning the study of development, the first essential step is to acquire sufficient knowledge of the patterns and sequence of developmental events from egg onwards during embryogenesis of a variety of animal species. You should become familiar with the processes and mechanisms involved in emergence of structures and increasing organizational complexity in succession. You should gain an understanding of the casual relationships that have been discovered between various developmental events, how these are expressed and what factors are involved. This knowledge should enable you to appreciate the problems of development, the concepts and principles that are generally applicable to all developing systems in an animal and to the animal as a whole in all metazoans. These are the aims of this block.

Different animals have evolved a variety of strategies of development. The egg may develop in water, on land, in the womb of the mother, under the soil, in holes made in trees or in a parasitic manner in the body of another animal host etc. Small eggs such as those of mammals, dependent entirely for nourishment from the mother can give rise to large offsprings weighing from several to many kilograms at birth. Large eggs rich in yolk and self-sufficient such as those of birds and reptiles develop outside on land independently and directly produce miniature adults. The eggs of many others, e.g. many insects, sea urchins, frogs etc., having little or moderate amount of yolk hatch into small larvae which after a period of independent existence and growth transform into adults through a process called metamorphosis.

However, since all animals are related, the basic mechanisms of early development have been conserved in the course of evolution; and so there are some important similarities in early embryonic development of all metazoan animals. Formation of sperm and eggs (gametogenesis) occurs in almost identical ways in all animals. Development proceeds through a series of strictly coordinated, sequential changes that can be described in terms of stages common in all sexually reproducing metazoans; viz., fertilization-cleavage-morula-blastula-gastrula and formation of three germinal layers, establishment of basic body plan and rudiments of tissues and organs laying the foundations for organogenesis and elaboration of the form of the new individual in the later stages.

Much of what we know of development has been gained from descriptive, comparative and experimental studies of a wide variety of species of many invertebrates and chordates. However, as you study the units you will notice that while the text includes information about one or more aspects of development gained from a few invertebrate species, it is largely devoted to development of vertebrates exemplified by frog, chick and mammals. This is so because the similarities in developmental patterns and methods and also the variations owing to differences in egg size, structure and composition and strategies of development are rather more clearly seen in the embryonic development of vertebrates. Moreover, in addition to the fruit fly and sea urchin embryos the amphibian and chick embryos have been the most suitable material for experimental analysis of developmental events. Experimental manipulation of embryos of placental mammals including humans has become possible since only very recently with the help of very significant recent technological advances.

This block is divided into five units numbered as units 13 to 17.

Unit 13: After a brief review of forms of reproduction and the variety of sexual mechanisms in protozoans it deals with gametogenesis (spermatogenesis and oogenesis), types of eggs, process and mechanism of fertilization and its significance.

Unit 14: This includes description of various patterns and mechanisms of cleavage of eggs, emergence of multicellularity and formation of blastulae of different types. The section on gastrulation deals with fate maps, their utility in studying prospective fates of cells of early embryos and the rearrangement of embryonic cells into three germinal layers. Different methods of gastrulation as seen in sea urchins, frog, chick and placental mammals are described and compared.

Unit 15: This unit first describes the cellular mechanisms generally involved in morphogenesis. This is followed by accounts of and organization of some tissues derived from different germinal layers exemplified by neural tube (ectodermal), heart and red blood cells (mesodermal) and primoridal germ cells (endodermal).

Unit 16: This unit deals with concepts, principles and problems of development that have emerged from experimental causal analysis of developmental events during embryogenesis : viz (1) developmental potencies of early embryonic cells, (ii) genomic equivalence and potentialities of nuclei of embryonic and adult cells, (iii) problem of mosaic and regulatory eggs, (iv) role of cytoplasm in making the embryonic cells at an early stage to become committed to differentiate in specific direction and (v) the phenomenon of primary embryonic induction, concepts of instructive and permissive inductions, reciprocal cell interactions, competence and determination. The unit includes brief descriptions of the important experiments performed to elucidate these aspects.

Unit 17: This unit consists of detailed descriptions of the development of eyes and limbs of vertebrates highlighting the sequential interactions between cells and tissues derived from different sources in the embryo involved in the formation of these organs.

Study Guide:

i) The text is supported by many figures and illustrations. We advise you to study them carefully to understand and grasp the description in the text thoroughly.

ii) You must remember that embryonic development is a dynamic phenomenon involving continuous changes in processes, form and organization as time passes, in many cases from hour to hour or even sooner. This dimension, that is of time, must be kept in mind while observing and studying the process of development. It will demand much use of you power of imagination.

We hope you will enjoy beginning the study of Developmental Biology of Animals with this block.

Objectives:

After studying this block you should be able to:

- describe the processes of spermatogenesis and oogenosis, the structure of spermatozoa and the different types of eggs.
- explain the mechanism and process of fertilization of eggs by sperms.
- Describe the general patterns and sequential developmental stages during early embryonic period which lay the foundation for later elaboration of the structural organization and form of the new individual animal.
- Describe the general mechanisms and cell movements involved in morphogenesis.
- explain the origin of tissues and organs from different germ layers in the embryo and their development.
- explain the various concepts and principles of development and describe how different experiments have been performed on embryos for causal analysis of developmental events to understand the underlying mechanisms.
- Understand and explain how different cells and tissues interact in a coordinated sequential manner to construct complex organs.

UNIT 13 BEGINNING OF A NEW ORGANISM

Structure

13.1 Introduction
Objectives
13.2 Development in Eukaryotic Unicellular Animals
13.3 Spermatogenesis
Spermiogenesis
Structure of the Sperm
13.4 Oogenesis
Oogenesis in Amphibians
Oogenesis in Mammals
Egg Envelopes
13.5 Fertilisation
Events Prior to Sperm-Egg Fusion
Fusion of Sperm and Egg
Events after Sperm and Egg Fusion
Fusion of Sperm and Egg Pronuclei
Initiation of Development
13.6 Summary
13.7 Terminal Questions
13.8 Answers

13.1 INTRODUCTION

Developmental Biology deals with the progressive changes that a single fertilized cell undergoes to emerge as an adult organism. The science was earlier known as embryology. But the term embryology would mean the changes undergone from a fertilized egg to the emergence of the organism at birth. Since development does not cease either at birth or in the adulthood the term Developmental Biology has replaced the term embryology.

Development is concerned with two major functions; one it is ensures the continuity of life from one generation to the next and two it generates cellular diversity and order within the generation. The continued generation of new individuals of species is what is known as **reproduction.** The increase in size of tissues and an organ constitutes **growth.** The generation of cellular diversity is known as **differentiation.** The organisation of differentiated cells into tissues and organs is known as **morphogenesis.** Developmental Biology essentially deals with these various aspects namely reproduction, growth differentiation and morphogenesis.

We shall begin the study of Development Biology of animals with the description of the formation of **gametes,** the sperm and ovum. This will be followed by a study of structure of the gametes. We shall also discuss the union of male and female gametes, a process known as **fertilization.** Thus, in the formation of a new organism each sex of the species contributes a single cell from its own body. The fusion product of gametes, **the zygote,** undergoes divisions to produce cells that form the **embryo.** The process of division is known as **cleavage.** Cleavage is responsible for the origin of multicellularity. The cleavage, the formation of the embryo and the further development of the embryo will be discussed in the subsequent units of this course.

In multicellular animals the individual development may result from sexual or asexual reproduction. Asexual reproduction does not involve the gamete production and fertilization. Here a group of somatic cells of the parent body collectively form a bud or a rudiment which has the potential to form a complete new individual.

Sexual reproduction involves formation of generative cells or gametes each of which independently is incapable of developing into a new individual. Each gamete is haploid and the two gametes belonging to opposite sexes fuse to form a diploid cell or a zygote. Zygote is potentially capable of giving rise to a new individual. Sexual reproduction consists of many phases—gametogenesis, fertilization, cleavage, blastula formation, gastrulation, morphogenesis, histogenesis, cytodifferentiation and growth. These processes occur sequentially and in many cases the preparation for one phase is initiated with the onset of preceeding phase. As we mentioned earlier the reproduction process ensures fusion of gametes to generate the fertilized egg. In this unit the basic requisite for such a process, the production of the sex cells, the gametes—the egg and the sperm—will be discussed in detail. Also you will learn the various events associated with the fusion of sperm and egg, and subsequently the fusion of their pronuclei—the processes collectively known as fertilization.

Objectives

After studying this unit you will be able to:

- discuss the scope of Development Biology and explain the concept of development
- discuss the different types or forms of reproduction in animals
- describe the process and purpose of gametogenesis
- outline the mechanism of fertilization and explain that fertilization restores diploidy in most of the multicellular organisms
- discuss the significance of activation of egg in the event of fusion of egg and sperm
- list the events that prepare the fertilized egg for the next phase of development, namely the cleavage.

13.2 DEVELOPMENT IN EUKARYOTIC UNICELLULAR ANIMALS

Reproduction, transfer and exchange of genetic material in prokaryotes were discussed in detail in our LSE-03, Genetics Course. In this unit you will study the development in eukaryotes.

Unicellular eukaryotic animals resort mostly to asexual reproduction for the perpetuation of the progeny. But sexual reproduction does occur in many forms. Asexual reproduction is by **binary fission** as in *Amoeba, Euglena* and *Paramecium*, **multiple fission** as in *Amoeba, Elphidium* and many sporozoans, **budding** as in *Suctoria*, **plasmotomy** as in myxosporidians and mycetozoans, **schizogony** as in *Plasmodium* and *Monocystis*, and **endogamy** as in *Toxoplasma*. A detailed account of asexual reproduction in protozoans is provided in LSE-09, Animal Diversity-I course.

Sexual reproduction in protozoans is also of different types.

i) **Isogamy**—Gametes are formed, which are identical in shape and size but behave differently. It occurs in Sporozoa (*Monocystis*), Phytomonadina (*Chlamydomonas*) and Foraminifera (*Elphidium*). In Heliozoa (*Actinophrys* and *Actinosphaerium*) the two gametes are daughters of the same individual cell.

ii) **Anisogamy**—Two types of gametes are produced which differ morphologically as well as behaviourally. Usually male gametes are motile and small (microgametes) and female gametes are large, non-motile (macrogametes). It is found in Sporozoa (*Plasmodium*), Phytomonadina (*Volvox*).

iii) **Conjugation**—Two individual cells adhere together and exchange nuclei. It is the characteristic of ciliate protozoans (*Paramecium*).

iv) **Autogamy**—The two nuclei of the same individual, each representing a gamete fuse to form a zygotic nucleus. It is a form of nuclear reorganization and self fertilization (e.g. *Paramecium aurelia*).

v) Cytogamy—Somewhere in the middle of conjugation and autogamy. There is pairing between two individual cells but no exchange of nuclei between them. However, within each individual two nuclei (male and female) fuse with each other as in the autogamy.

One individual protozoan may adopt different means for reproduction depending upon the environmental conditions. In the life cycle of these individuals, the timing of meiosis (for gametogenesis) and fertilization (zygote formation) is variable. In many cases, zygote is the only diploid cell in the life-history while the other stages are haploid because the zygote divides meiotically as in *Volvox*. In other cases the zygote may divide mitotically so that subsequent stages are diploid.

SAQ 1

1) What is the study of Development Biology concerned with?

.....

.....

.....

.....

2) Describe the terms reproduction, growth, differentiation and morphogenesis.

.....

.....

.....

.....

3) List the methods of sexual reproduction in unicellular organisms.

.....

.....

.....

.....

13.3 SPERMATOGENESIS

In multicellular organisms the reproductive process commences with the production of gametes. The gametes are the sex cells that develop inside the gonads, the testes in males and ovary in females. In this section you will learn in detail the spermatogenesis or the formation of sperm, and in Section 13.4 oogenesis, the formation of the ovum is discussed.

The term gametogenesis includes both **spermatogenesis** and **oogenesis.**

In vertebrates as well as in higher invertebrates the testis, the site of production of sperm are composite organs, consisting of a number of seminiferous tubules. Sections of the testis would reveal the seminiferous tubules with sperm development in different stages. This means that the spermatogenesis is a continuous process and one can observe the various developmental stages of sperm in a single testis.

Spermatogenesis can be broadly divided into three phases(1) multiplication phase, (2) growth phase and (3) maturation phase (Fig. 13.1).

1) **Multiplication Phase**

The initial cells in the germ line are known as **primordial germ cells (PGC).** The PGC which arise at some distance from the prospective gonads migrate into them and are known as **stem cells.** The stem cells divide by mitosis and give rise to spermatogonial **cells.** The multiplication phase marks the production of a number of spermatogonia inside the testis.

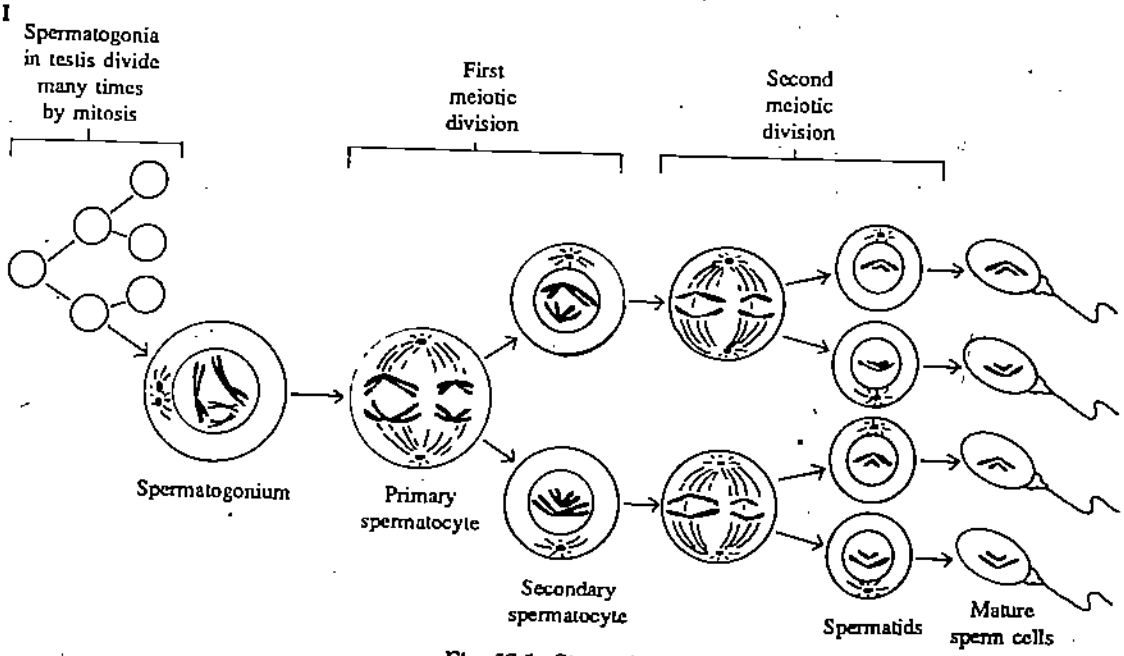

Fig. 13.1: Stages in spermatogenesis.

2) Growth Phase

Growth phase is characterised by the acquisition of the structural and functional characteristics of distinct sex cells. Also there is a pronounced increase in the size of spermatogonial cells which are now known as **primary spermatocytes.**

3) Maturation Phase

The maturation phase characterises the transformation of diploid primary spermatocytes into haploid **spermatids** (Fig. 13.2). The primary spermatocyte divides meiotically to produce two **secondary spermatocytes** each with a haploid nucleus. Each of the secondary spermatocytes undergoes second meiotic division to produce two haploid **spermatids.** Each spermatid undergoes an intracellular differentiation to produce a **spermatozoa.** The process of intracellular differentiation of a spermatid into a spermatozoa is known as *spermiogenesis* (Fig. 13.3). We shall now describe the **spermiogenesis** process in detail.

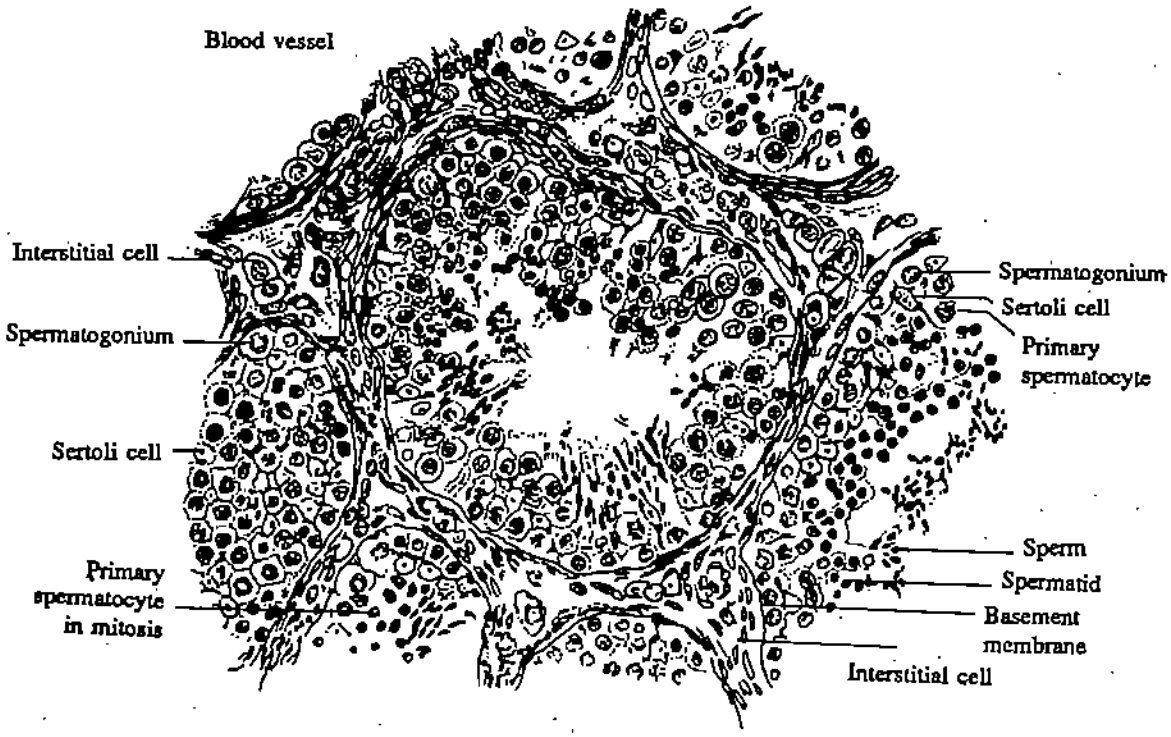

Fig. 13.2: Section of seminiferous tubules in a human testis showing different stages of spermatogenesis.

13.2.3 Spermiogenesis

At the end of the meiosis the spermatids appear as simple spherical cells with a centrally located nucleus. Their differentiation into sperm requires an extensive morphological transformation. The various cellular organelles like mitochondria, golgi body and centrosomes contribute to such a transformation. The first step involves the formation of an acrosomal structure from the golgi body. The acrosome lies proximal to the nucleus and forms a cap over the sperm nucleus. As the cap is formed the nucleus rotates and the acrosomal cap faces the basal membrane of the seminiferous tubule. This rotation is essential because the flagellum is to arise from the centriole on the other side of the nucleus. Subsequently the nucleus flattens and condenses and there is a loss of cytoplasm. The mitochondria tend to form a ring around the base of the flagellum and become the neck region of the sperm. The fully formed sperm enters the lumen of the seminiferous tubule.

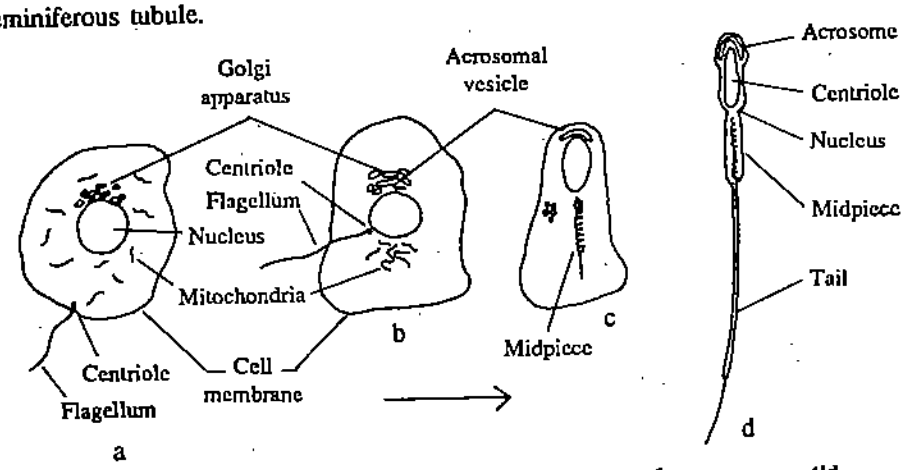

Fig. 13.3: Stages in the development of a sperm from a spermatid.

In mouse the entire process of spermatogenesis takes 34.5 days to complete and the spermiogenesis process alone takes up 13.5 days. In humans it takes 74 days to complete the process of spermatogenesis.

SAQ 2

Fill in the blanks:

i) ..... refers to the formation of sperm cells in male gonads.

ii) In ..... tubules different stages of sperm production can be observed.

iii) The three stages in sperm formation are (a) ..... phase,
(b) ..... phase and (c) ..... phase.

iv) At the end of maturation division during spermatogenesis diploid .....
are transformed into haploid ..... .

v) The process of formation of sperm from spermatids is known as .....

13.3.3 Structure of the Sperm

The spermatozoa in different animal groups display a wide variety of shapes (Fig. 13.4) and sizes but all share a basic morphological plan. The sperm in most animals is devoid of any stored nutrient materials within itself and is capable of performing locomotion in fluid and semifluid media. The size of sperm may be as small as 0.02 mm (crocodile) or as large as 2 mm (*Balanoglossus*). The shape of the sperm is species specific.

Structurally the sperm of different animals consist of a head, a middle piece and a tail. (Fig. 13.5) The account of the sperm structure presented here is based on the studies conducted on mammalian species, particularly, human beings.

Head

It exhibits a diversity of shapes in different animal groups (Figs. 13.4 and 13.6), e.g., spherical (teleost fishes), rod or lance shaped (amphibians), spirally twisted like a

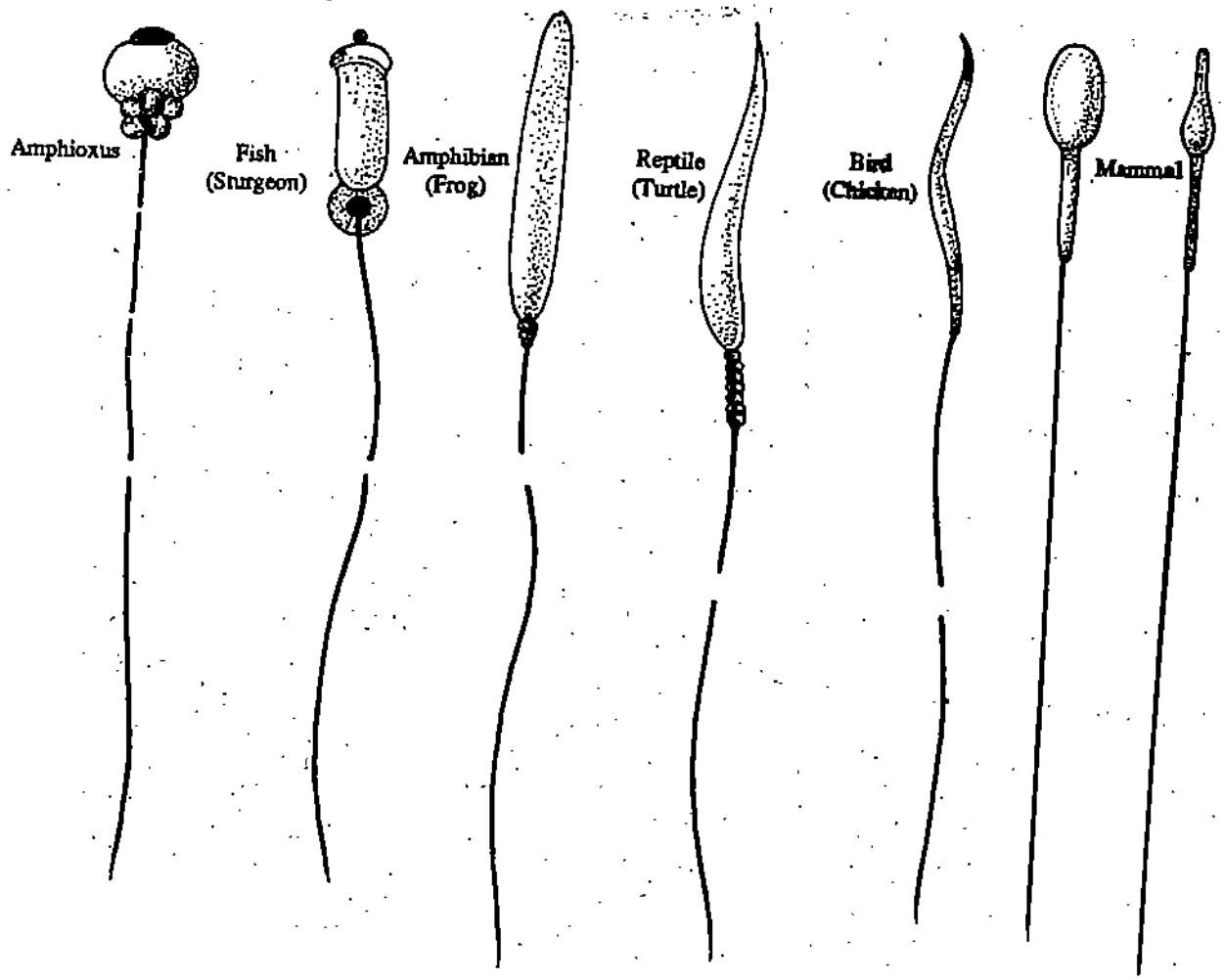

Fig. 13.4: Sperm of some chordates. The break in the flagellum of sperm indicates that a long portion is omitted.

corkscrew (birds), spoon shaped (man), flattened (buffalo) or hooked (mouse and rat) or occasionally round (bivalve molluscs). Some of these shapes may be interpreted as an adaptation to propulsion in fluid media. The genetic functions are embodied in the nucleus; activation of egg during fertilization is basically initiated by anterior cap like acrosome.

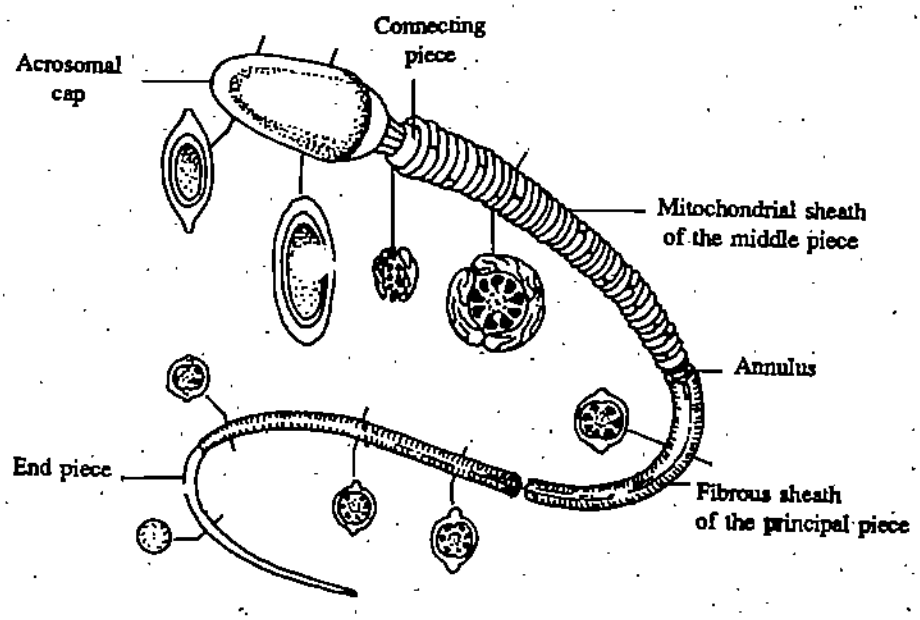

Fig. 13.5: Ultrastructure of a mammalian sperm. The cell membrane has been removed.

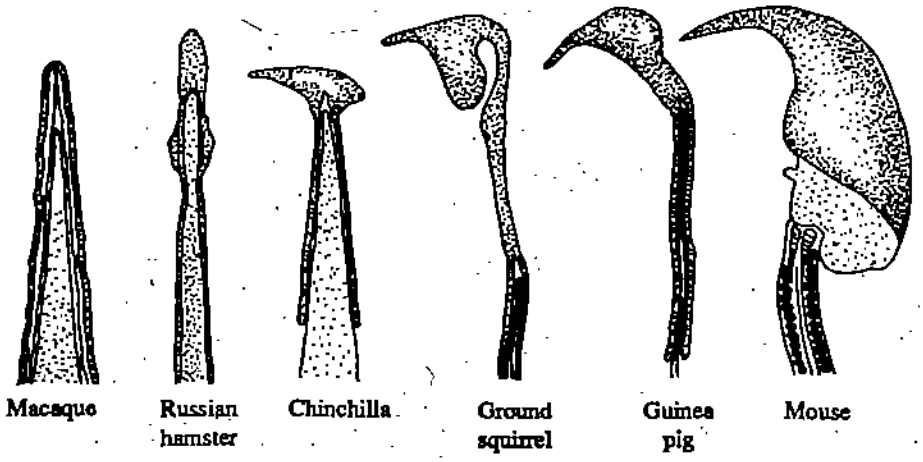

Fig. 13.6: Drawing of sagittal sections of head of sperm of different mammals to show the diversity in their structure.

The nucleus occupies the greater part of head (Fig. 13.7) and it also determines the shape of head. During the course of sperm differentiation (spermiogenesis), everything in the nucleus not directly concerned with the transmission of heredity characters is discarded leaving only the actual material of genes (DNA) which is tightly packed within the nucleus.

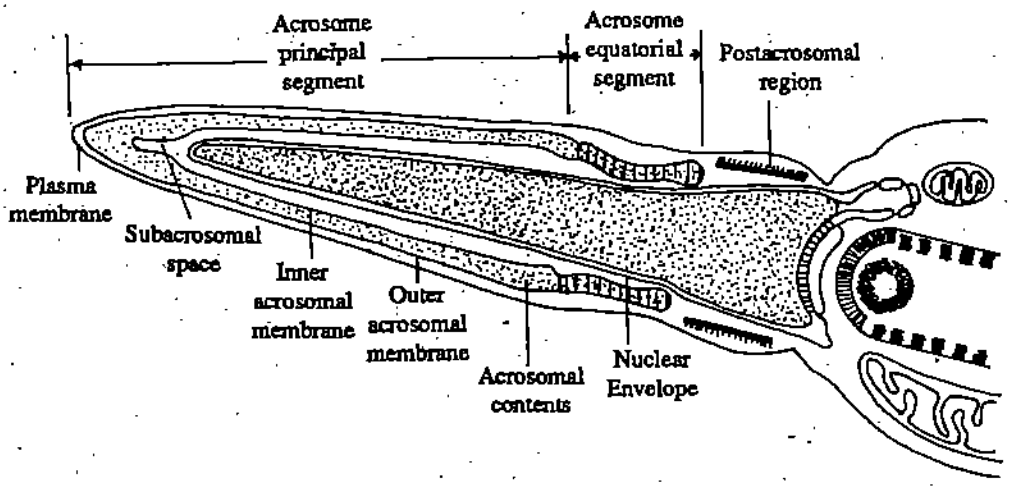

Fig. 13.7: Diagram of sagittal section of mammalian sperm head.

The anterior side of the nucleus (head) is made up of closely applied cap-like acrosome (Fig. 13.7). It enables the spermatozoan to penetrate through the egg evelopes and to establish connection with the egg. The interior of the acrosome (the acrosomal granule) contains the hydrolytic enzymes and certain polysaccharides. Periacrosomal cytoplasm is a thin layer of cytoplasm present between the outer membrane of acrosome and plasma membrane of sperm.

In certain species (e.g. teleost fishes), the acrosome has not been reported so far in the spermatozoa.

Middle Piece

Just behind the head, the small constriction (neck) carries a **distal** and a **proximal centriole**. The two lie at right angle to each other. The proximal centriole initiates the cleavage divisions of fertilized egg while the distal centriole provides attachment to **axial filament** of tail (Fig. 13.8).

The middle piece has mitochondria surrounding the base of the axial filament of the flagellum (tail). The mitochondria form compact and isolated clumps throughout the middle piece, or there may be a tightly coiled spiral of mitochondria around the proximal centriole of filament and distal centriole (Fig. 13.9). The mitochondria carry the oxidative enzymes responsible for oxidative phosphorylation. Therefore, it is the

'power plant' supplying the energy to the flagellum to be used for propulsion of the sperm through the fluid medium.

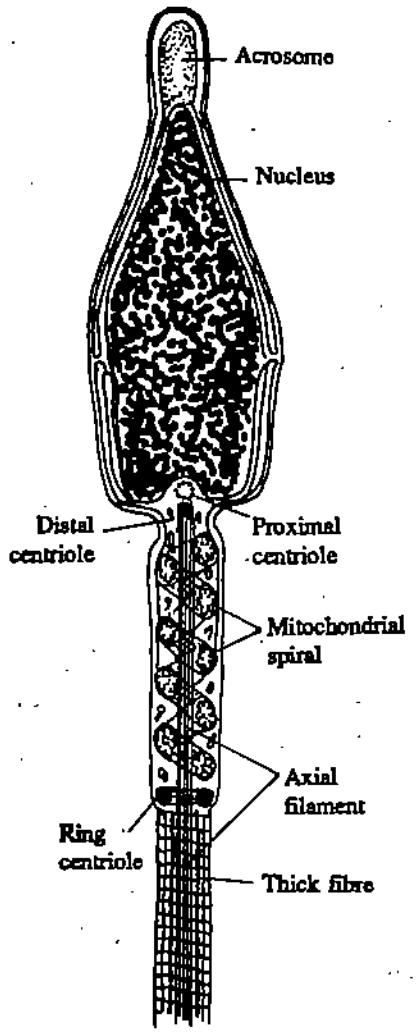

Fig. 13.8: Diagram to show the middle piece of sperm and location of proximal and distal centrioles.

A dark ring is sometimes seen at the posterior end of the middle piece as a boundary between middle piece and the tail. It is known as 'ring centriole', but electron microscopic study reveals that the ring centriole does not resemble a centriole in its structure. Its origin and function are unknown.

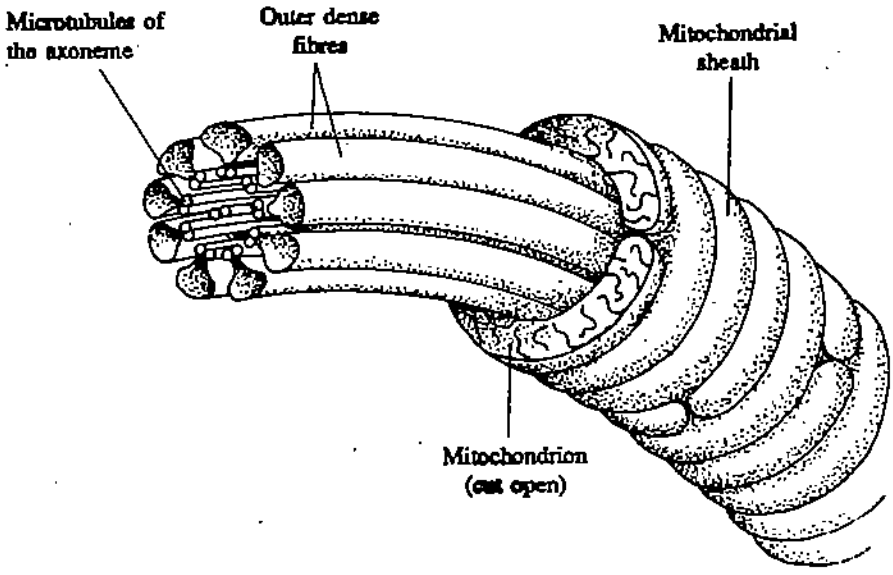

Fig. 13.9: Diagrammatic view of a segment of middle piece of a mammalian sperm showing the mitochondria wrapped helically around the axonema.

Tail

The tail or flagellum is the longest part of the sperm. By its movement it causes the sperm to swim with the head forward. It consists of a thin layer of cytoplasm surrounded by **plasmalemma**. The axial filament is the main part of the tail or **flagellum**. Its structure is essentially similar to a typical flagellum or cilium with the arrangement of 9 peripheral pairs and one central pair of longitudinal fibres (Fig. 13.10).

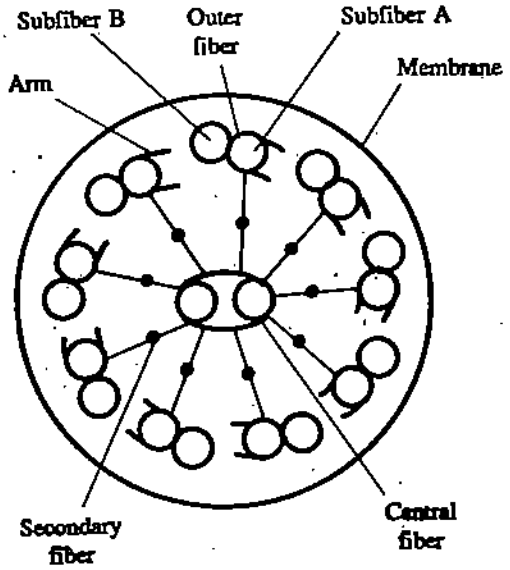

Fig. 13.10: Cross section of the flagellum of a mammalian sperm showing the central axoneme (9+2 arrangement) and the external fibres.

The major portion of the flagellum is called 'axoneme' (Fig. 13.11). An axoneme is formed by the microtubules emerging out of the centriole at the base of sperm nucleus. The core of the axoneme consists of two central microtubules surrounded by a row of nine doublet microtubules. In fact, only one microtuble is complete having thirteen protofilaments; the other is C-shaped having only eleven protofilments (Fig. 13.12). The protofilaments are exclusively made of the dimeric protein—**tubulin**. Another protein **dynein** is attached to microtubule. The dynein is capable of hydrolysing ATP molecule and can convert chemical energy into mechanical energy that propels the sperm.

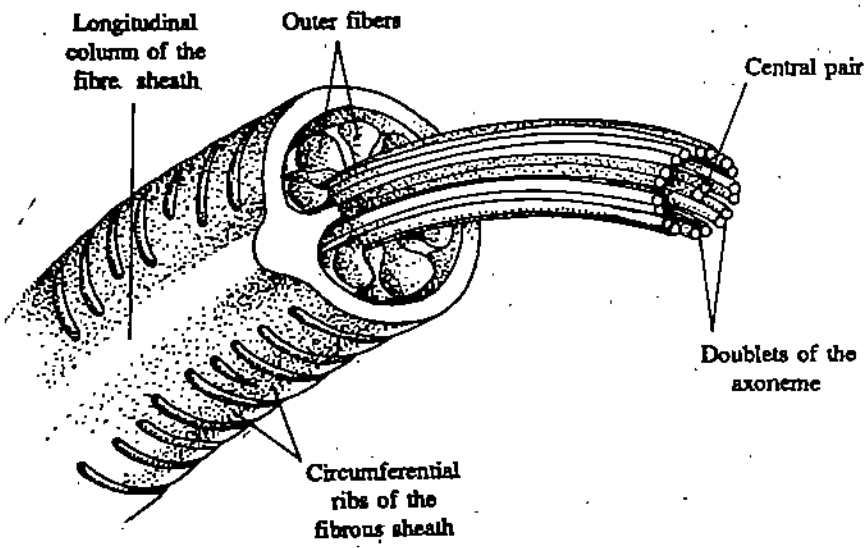

Fig. 13.11: Diagrammatic representation of the principal piece of mammalian sperm tail showing fibrous sheath and associated ribs.

The '9+2' microtubule arrangement with dynein arms has been conserved throughout the eukaryotic series. Therefore, it suggests that this arrangement is extremely suitable for transmitting energy for movement.

In the sperm of some fishes and amphibians there is an undulating membrane which stretches along most of the tail length and takes an active part in the locomotory activity of sperm.

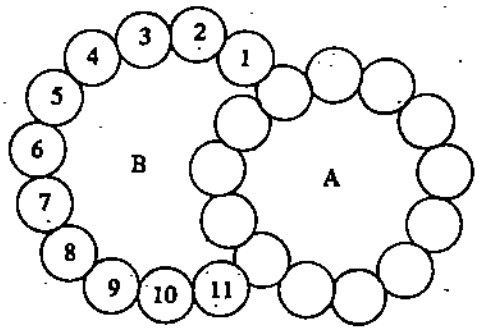

Fig. 13.12: Schematic diagram of the two microtubules one with 13 protofilaments and the other with 11 protofilaments.

Thus, sperm undergoes extensive modifications for transmission of its nucleus to the egg during its differentiation (spermiogenesis). The sperm of nematodes and decapod crustaceans are incapable of swimming because of the absence of flagella and mitochondria, and therefore exhibit amoeboid movement.

SAQ 3

a) What are the chief components of mature sperm?

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b) Trace the source of acrosomal contents.

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c) What is the purpose of motility of spermatozoa?

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d) What is the source of energy for the motility of spermatozoa?

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.....

13.4 OOGENESIS

Oogenesis is the formation of ovum from oogonial cells that are formed in the ovary from primordial germ cells. And as in spermatogenesis it involves meiosis to produce haploid ovum. You have learnt in the section on spermatogenesis that the differentiation of sperm occurs after the meiotic events. In oogenesis the process of first meiotic division is very prolonged; and it is during this process that growth and differentiation of oocyte take place. In many animals most events related to oocyte differentiation occur during prophase I of first meiotic division. In animals which produce yolk-laden eggs, this phase is divided into

1) Previtellogenesis (before yolk production)

2) Vitgellogenesis (yolk deposition)

3) Postvitellogenesis (after yolk deposition).

Vitellogenesis is the stage where much of the oocyte growth occurs. The completion of second maturation division (meiosis), which may occur after ovulation, results in ripe egg or ovum. In some species such as sea urchins and frogs, the female produces hundreds or thousands of eggs at a time where as in most mammals including humans only a few eggs are produced in the entire lifetime of the individual. In this section we shall discuss oogenesis in two groups of animals, (1) the amphibians as an example of organisms which produce yolky eggs and (2) mammals, as an example of organisms whose eggs do not contain any yolk.

The basic mechanism of maturation of ovum is more or less similar in all organisms (Fig. 13.13). The primordial germ cells that enter into the ovary divide mitotically and produce oogonial cells. The oogonial cells grow in size and become **primary oocytes**. The primary oocyte undergoes the first meiotic division and forms two haploids cells—a **secondary oocyte** and a polar body. The secondary oocyte undergoes the second meiotic division to produce an ovum and a polar body. In most vertebrates the second meiotic division is completed only outside the ovary after fertilisation. The polar body formed as a result of first meiotic division may divide again to produce two more polar bodies. All the polar bodies eventually degenerate. Thus, the meiotic process in oogenesis results in one ovum from a single oogonium unlike spermatogenesis where each spermatogonial cell gives rise to four sperm.

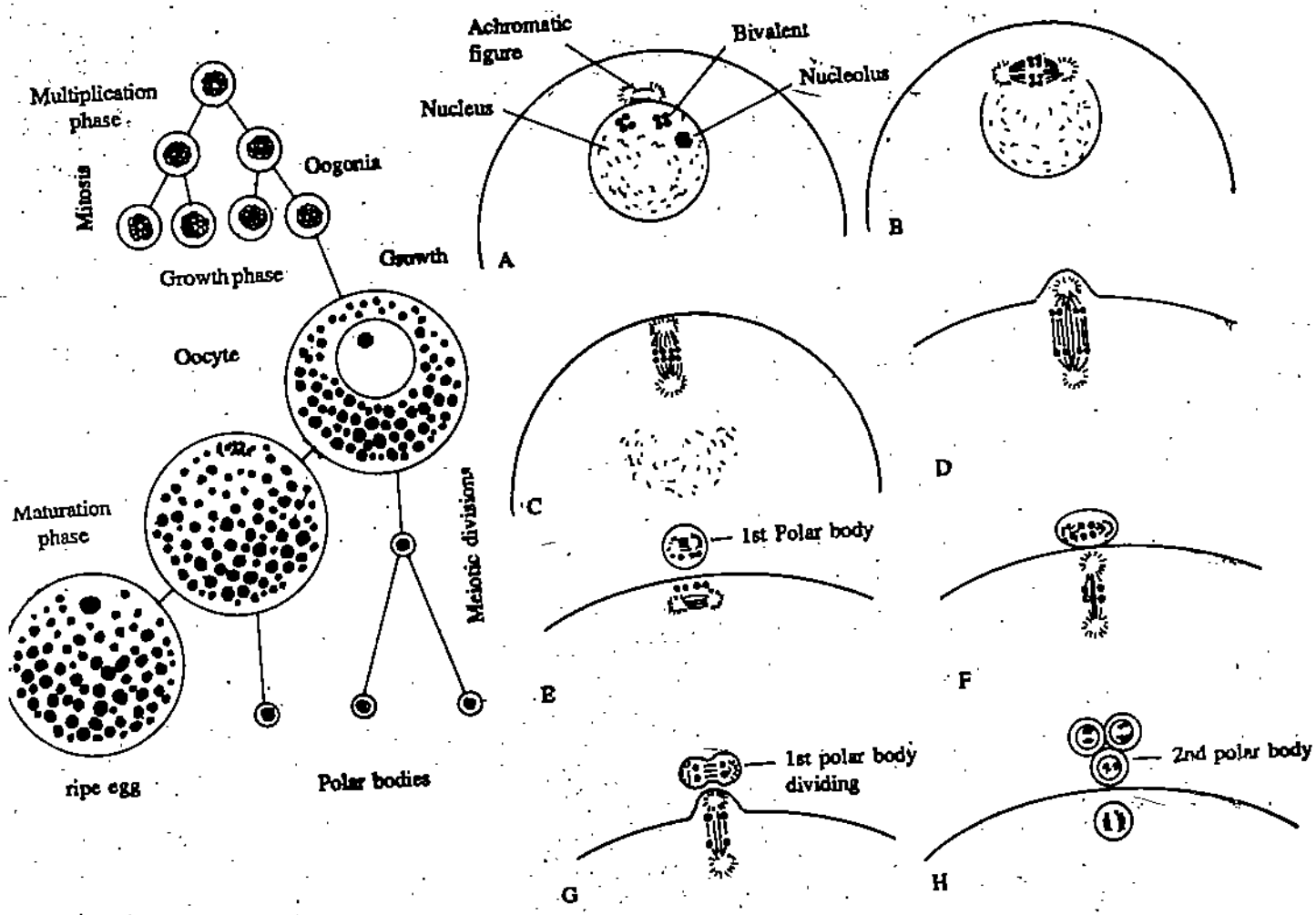

Fig. 13.13: Diagrammatic representation of (1) Stages in oogenesis, and (2) of first (A—E) and second (F—H) meiotic divisions in the oocyte.

13.4.1 Oogenesis in Amphibians

As we mentioned earlier, the eggs of amphibians contain an amount of yolk that is later utilized for the nutrition of growing embryo. During the oogenesis the oocyte cytoplasm accumulates material that is rich in energy sources. Further, the egg which is mainly responsible for initiating and directing the development, contains in its cytoplasm organelles such as mitochondria, enzymes, precursors for DNA, RNA and protein synthesis, stored mRNA, structural proteins and morphogenetic determinants (refer to Unit 16).

Eggs of amphibians like those of other animals are derived from stem cells which produce oogonial cells. The oogonial cells each year produce a fresh batch of oocytes. In the frog *Rana pipiens* oogenesis takes 3 years to complete (Fig. 13.14). The first two years mark the growth phase of oocyte when the size of the oocyte increases gradually. In the third year the oocyte rapidly accumulates the yolk material in its cytoplasm and there is further increase in its size. Oocytes undergo maturation in yearly batches, the first batch of eggs maturing about three years after metamorphosis and then every year a fresh batch matures at every succeeding breeding season.

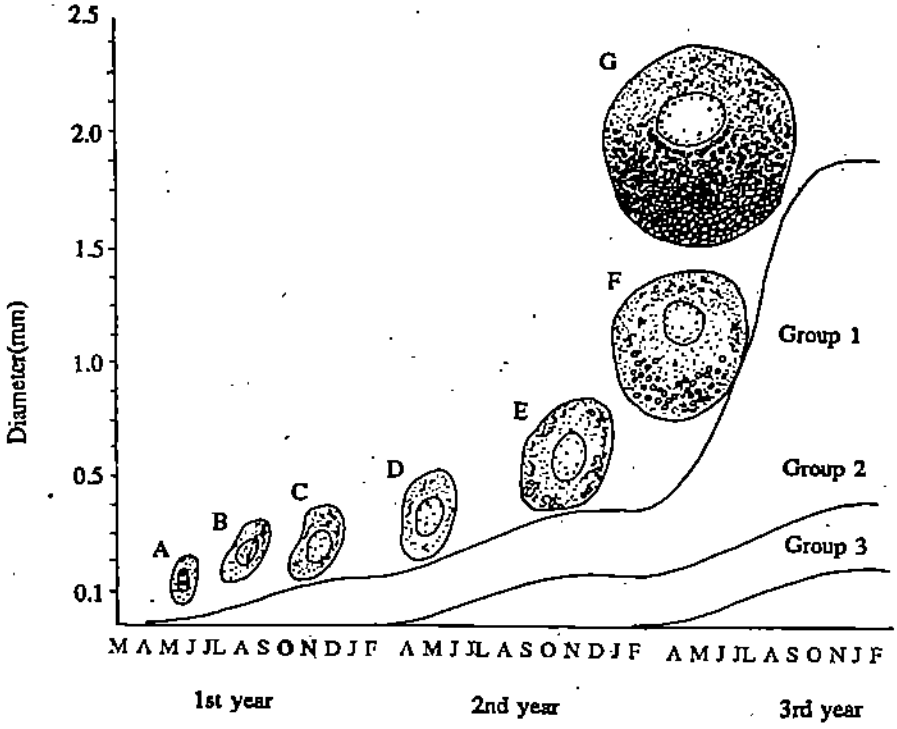

Fig. 13.14: Duration of oogenesis in *Rana pipiens*. Note that the first batch of eggs is ready for release after 3 years.

Lampbrush—chromosomes are non-polytene chromosome. The compacted amphibian chromosome during the diplotene stage will stretch out to form large loops of DNA and retreat them once the stage is completed.

Vitellogenesis, that is the formation and accumulation of yolk in the oocyte, occurs during the diplotene stage of the prophase of meiosis I. This stage is also characterised by active synthesis of RNA by lampbrush chromosome (Fig. 13.15) in the nucleus (see the marginal remark). Yolk is a composite substance consisting of many nutrients required for embryonic nutrition. The major yolk protein precursor **vitellogenin** is synthesised outside the ovary in the liver, transported by the blood and incorporated into developing oocyte by micropinocytosis. In mature oocytes the yolk contains two proteins which are modified from vitellogenins—a phosphorous containing protein, the **phosvitin** and a lipid containing protein called **lipovitellin**. These two yolk proteins together form membrane bound **yolk platelets**. Besides these proteins the yolk contains lipid and carbohydrates in the form of lipochondrial inclusions and glycogen granules respectively.

Amphibian eggs are highly asymmetric, that is, the distribution of yolk is not uniform throughout the egg. It is during oogenesis that the animal-vegetal axis of the egg is determined. The oocyte nucleus (the germinal vesicle) is confined to the upper half of the oocyte called **animal hemisphere**. The lower half of the egg is loaded with yolk and constitutes the vegetal **hemisphere** of the egg (Fig. 13.16).

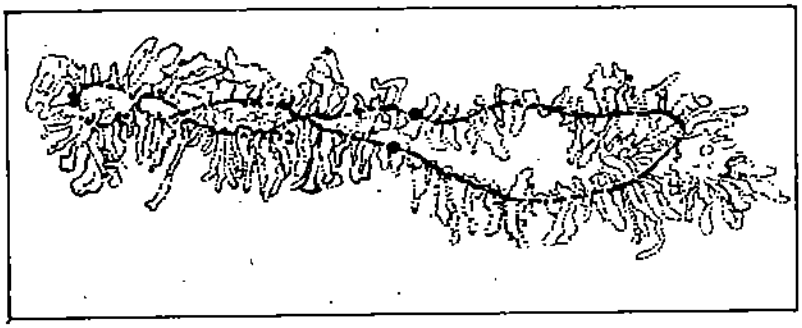

Fig. 13.15: Phase contrast diagram of the lampbrush chromosome of an urodele amphibian.

Towards the end of vitellogenesis the oocyte cytoplasm shows distinct regions. The golgi apparatus, mitochoadria and pigment granules are formed at the periphery of the cell forming the oocyte cortex. In the interior of the egg, yolk is largely accumulated in the vegetal hemisphere of the egg while the animal hemisphere is seen to contain largely clear cytoplasm including glycogen granules, ribosomes, lipochondria and endoplasmic reticulum.

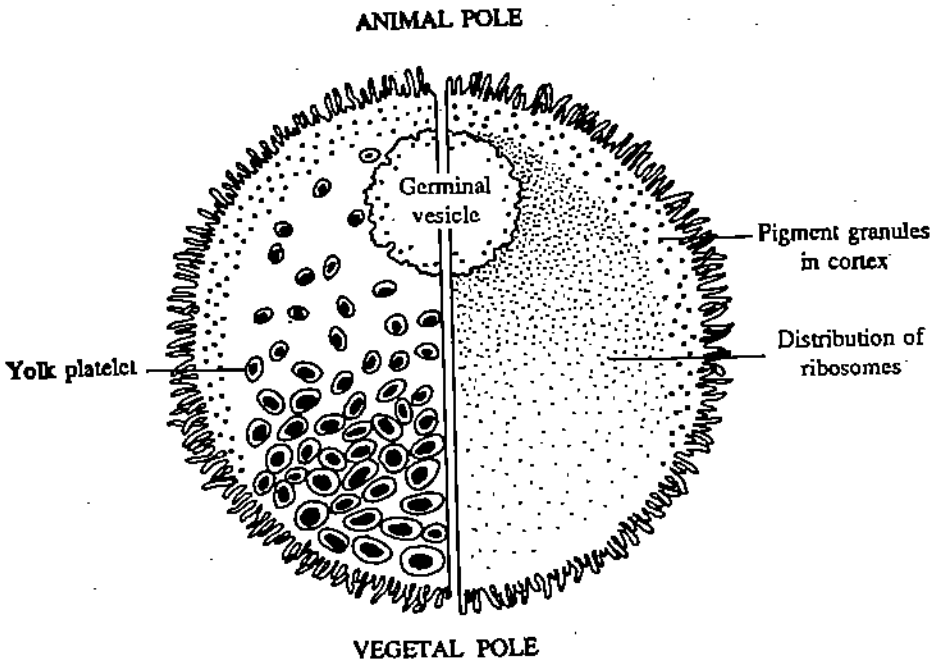

Fig. 13.16: A mature amphibian egg showing polarised distribution of oocyte components. Left side shows the distribution of yolk and the right side the ribosomes.

In *Xenopus*, the leptotene stage of meiosis I lasts 3 to 7 days, zygotene from 5 to 9 days and pachytene for 3 weeks. It is in the diplotene stage that the meiotic arrest occurs and the oocyte remains in this stage for 3 years. The interactions of hormones secreted by hypothalamus, pituitary and follicle cells of the ovary regulate further events of oogenesis. Once the mating season arrives the gonadotropin releasing hormone from hypothalmus causes pituitary to release gonadotropic hormones into the blood. These hormones stimulate follicle cells to secrete estrogen which in turn activates liver to synthesize the release vitellogenin which is taken up actively by the oocyte. Progesterone, another hormone secreted by follicle cells in response to gonadotropins of the pituitary, releases the primary oocyte from meiotic arrest. The hormone causes the breakdown of the germinal vesicle, prepares it for completion of the first meiotic division and also causes the release of the egg from the ovary—a process known as **ovulation** (Fig. 13.17).

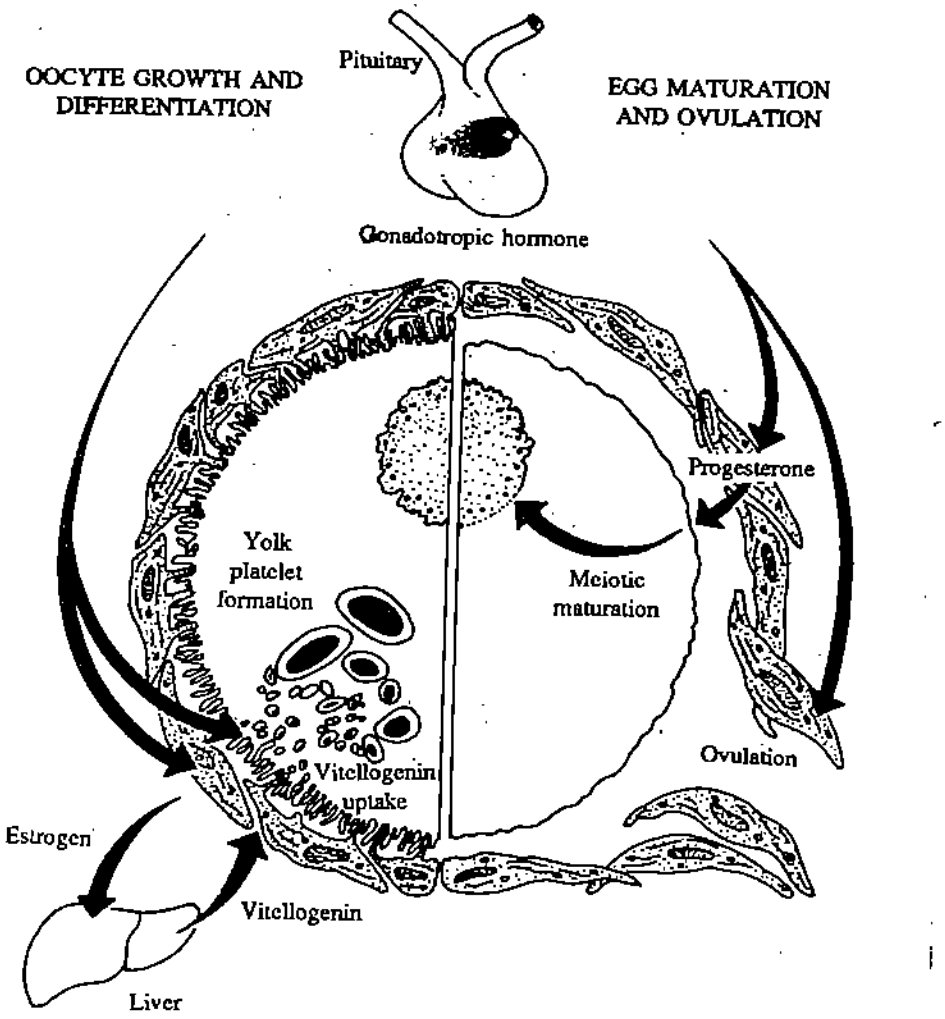

Fig. 13.17: Diagram showing the regulation of amphibian oocyte growth and differentiation (I) and egg maturation and ovulation (II).

13.4.2 Oogenesis in Mammals

In mammals also the oogonial cells are derived from primordial germ cells. The multiplication phase, that is all of the oogonial divisions and transformation of oogonia into oocytes are completed either before or shortly after birth. A number of oocytes are produced, all of which are held in meiotic arrest in prophase I.

Essentially in mammals the period of oogenesis covers the entire life of an individual from birth to ovulation. The meiotic arrest is released at the time of puberty after which a group of oocytes begin development during each cycle. A large percentage of oocytes fail to undergo maturation and therefore degenerate.

The oocytes in mammals are found in close association with non-germ cells in the ovary. The non-germ cells or the accessory cells produce steroid hormones, transport some of the essential cytoplasmic components into the oocyte and are also involved in the formation of cellular or noncellular layers that surround the fully differentiated egg. The accessory cells that surround the egg are of two types — (1) **follicle cells,** (2) **nurse cells.** The follicle cells are somatic cells which surround the oocyte as a single layer of the cells and are known as **follicular epithelial cells.** The nurse cells are derived from germ cell line and are connected with the oocyte by cytoplasmic bridges.

During the growth of the oocyte, the single layered follicular epithelium proliferates and becomes multilayered and the cells are called **granulosa cells.** The granulosa cells and the oocyte are separated by a space which is filled with sulphated glycoproteins. This layer becomes the **zona pellucida** of the oocyte. When proliferation of the granulosa cells is completed they secrete a fluid that accumulates in the intercellular spaces. The fluid filled spaces coalesce to form a cavity called **antrum.** Follicles with antra are

called **Graafian follicles.** With the formation of antrum the oocyte is displaced to one side of the follicle and is ready to be released (Fig. 13.19).

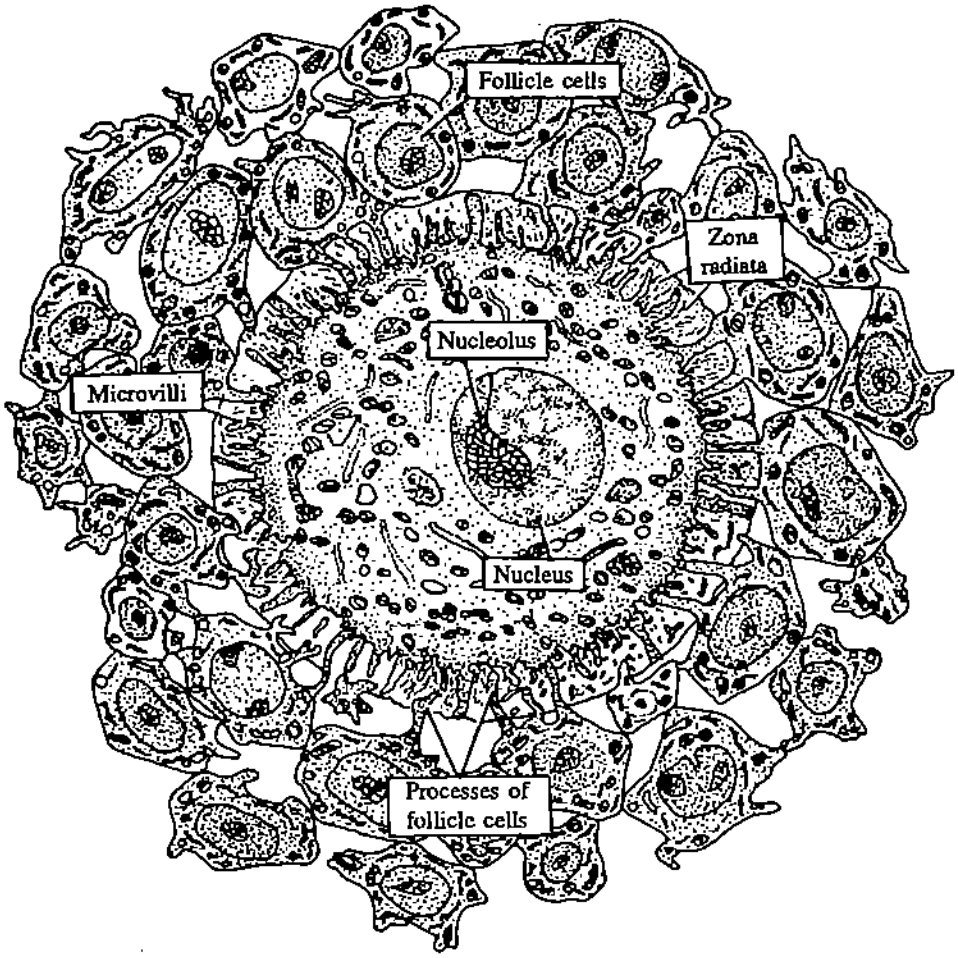

Fig. 13.18: Young oocyte of a mammal surrounded by follicle cells.

13.4.3 Egg Envelopes

Like any other cell, all the eggs are covered by the cell membrane or plasmalemma. It is with two layers of about 50 A° (unit membrane) separated by a gap of 60 A°.

In addition to plasmalemma, the eggs of practically all animals are surrounded by special egg envelopes (or egg membranes). Based on their origin, egg envelopes may be primary or secondary ones.

Primary Egg Envelopes

These are the egg envelopes which develop in the ovary between oocyte and follicle cells in the space occupied by the interdigitating microvilli. Such envelopes have been named variously in different animals.

a) It is named as **vitelline envelope** or **vitelline membrane** in insects, molluscs, amphibians and birds.

b) In tunicates and fishes, it is known as **chorion.** In many sharks and bony fishes the primary envelope has striated appearance and is referred to as **zona radiata** representing the degraded microvilli of the growing oocyte. The perforation in the zona radiata becomes the micropyle through which the spermatozoa can enter the egg.

c) In mammals, the unstriated and modified **zona pellucida** is formed as a result of joint efforts of egg and follicle cells (Fig. 13.18). While escaping the Graafian follicle mammalian oocyte carries on the surface of zona pellucida a layer of follicle cells known as **corona radiata.**

The primary envelopes usually stick closely to the egg surface. These later on participate in the formation of fertilization membrane.

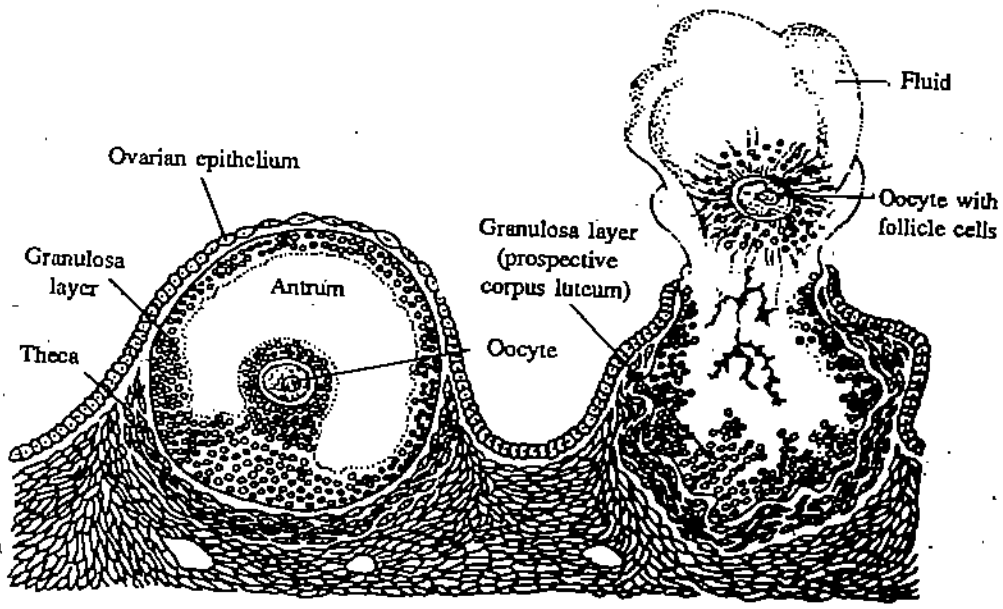

Fig. 13.19: A Graafian follicle and ovulation of egg in mammals.

Secondary Egg Envelopes

These are secreted by oviducts and other accessory parts of genital organs while the egg is passing through them from ovary to the exterior.

a) In ovoviviparous sharks, the egg is surrounded by a hard shell secreted by shell glands of the oviduct. The shell is drawn out as twisted horns which serve to entangle the egg among sea weeds.

b) In amphibians, the eggs are surrounded by a layer of jelly which protects the egg from adverse effects of sun rays, abrasion as well as from predators (because of its bad taste). The jelly absorbs water and swells (Fig. 13.20).

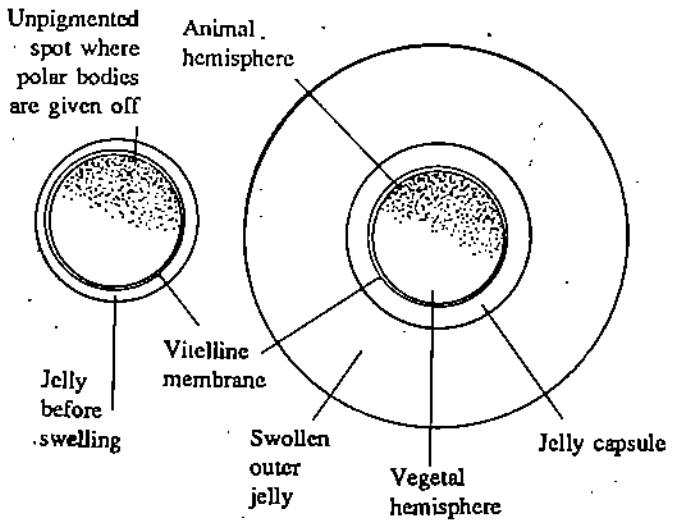

Fig. 13.20: An amphibian egg (a) as taken from oviduct and (b) sometime after in spawning in water with swollen jelly membrane.

c) In birds, reptiles and monotremes, the secondary envelopes include the white of egg surrounding the yolky ovum, followed by inner and outer shell membranes (which are in close contact with one another) and the outermost calcareous shell which is porous. The shell is elastic and compressible when the egg is laid but it hardens on exposure to atmosphere (Fig. 13.21).

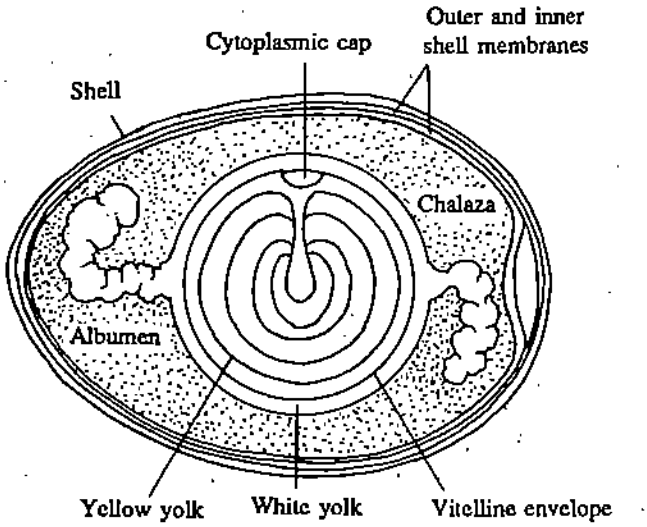

Fig. 13.21: A median longitudinal section of hen's egg to show the egg envelopes

SAQ 4

State whether the following statements are true (T) or false (F):

a) Vitellogenesis is the stage at which much of the oocyte growth occurs. (True/False)

b) Mammalian eggs are characterised by a high yolk content. (True/False)

c) Each polar body derived as a result of meiosis is a potential ovum. (True/False)

d) Prophase I of meiosis is divided into previtellogenic and vitellogenic phases. (True/False)

e) The first batch of eggs in *Rana pipiens* matures a year after metamorphosis and then a batch matures once in every three years. (True/False)

f) Amphibian eggs are highly symmetrical in the distribution of yolk in them. (True/False)

g) In *Xenopus* the meiotic arrest occurs in the diplotene stage. (True/False)

h) Follicle cells and nurse cells are two types of accessory cells in mammalian oocyte. (True/False)

i) Follicles with fluid filled space antra are called Graafian follicles. (True/False)

j) The shell of hen's egg is elastic and compressible at the rime of laying. (True/False)

13.5 FERTILIZATION

In the last two sections you have learnt the processes which lead to the differentiation of the male and female germ cells, the sperm and ova, respectively. In this section we shall describe the process by which the sperm and the egg unite to form a zygote. This process is known as fertilization. Fertilization results in (i) the restoration of the diploid chromosonal number in the nucleus, and (ii) the activation of the development. Activation would essentially mean that a sequence of metabolic and morphological changes are triggered that inturn cause the division of a unicellular zygote into a multicellular organism. Mostly we will describe the fertilization in mammals with occasional references to sea urchins. It is true that depending upon the habitat as well as the life styles there may be variations in the fertilization process in diverse organisms. But generally the events of fertilization are more or less similar in many different organisms. Prior to the actual fusion of the sperm and the egg nuclei, the germ cells prepare themselves for the fertilization process. In the first subsection we shall describe such preparatory events. Then you will learn about the fusion process and finally there will be a discussion about the various events that occur subsequent to the zygote nucleus formation.

13.5.1 Events prior to the Sperm-Egg Fusion

In a number of organisms that have been studied, the sperm remain immotile in the testis or in the semen. The sperm become active and motile when they are close to the eggs. Several factors have been attributed for the activation of the sperm which include pH, oxygen tension, presence of certain ions ect. Detailed studies in sea urchin have shown a change in the pH as the cause for the activation of the sperm (Fig. 13.22). In mammals the definite cause of activation is not known and it is presumed that sperm motility begins with the ejaculation of the semen into the female reproductive tract.

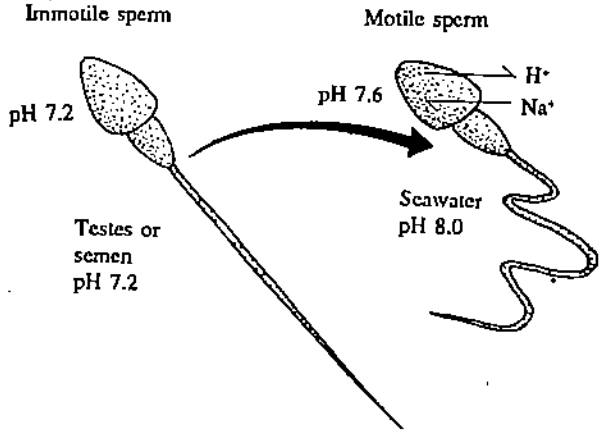

Fig. 13.22: The role of the pH in the activation of sperm motility in sea urchins.

Activation of the sperm does not ensure that the sperm will meet the egg. In instances where fertilization occurs outside the organism, i.e., external fertilization, chemotactic mechanisms have been evolved to attract the sperm towards the egg. In fish eggs substances found near the micropyle attract the sperm. The substances found near the micropyle make the sperm to move rapidly to the vicinity of the egg.

In jelly fishes the sperm attractant is found to be a protein molecule localised in a cupule (Fig. 13.23), a specialised extracellular structure found at the animal pole of the egg. In sea urchins, the sperm attractant is found in the egg jelly. It is an oligopeptide formed of 14 amino acids and named as resact - sperm respiratory activating peptide. In has been shown that injection of a small quantity of resact makes randomly distributed sperm change their swimming behaviour and accumulate near the site of injection within seconds.

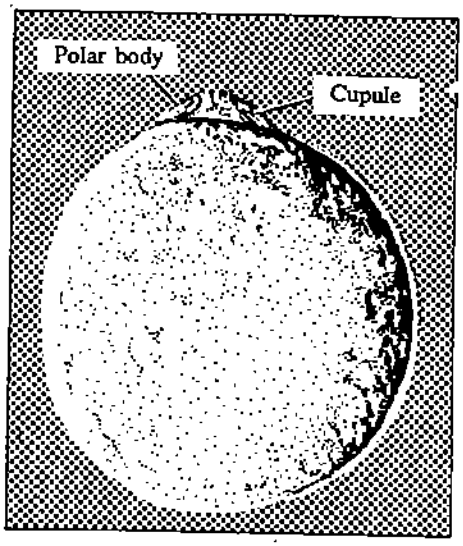

Fig. 13.23: Egg of the jelly fish showing the cupule which contains the sperm attractant protein.

In section 13.4.2 we described the structure of the egg as having the surface coats. These coats besides protecting the eggs are also responsible for specific sperm-egg interaction. The sperm need to cross the egg surface coats before they reach the plasma membrane. Mammalian sperm require a period of maturation in the female reproductive tract, a phenomenon known as capacitation. In mouse the capacitation period is one hour and in humans 5 to 6 hours. During capacitation the sperm undergo certain

changes such as removal of the surface components and rearrangement of the intramembranous particles. Capacitation mechanisms are generally poorly understood. Usually capacitated sperm consume more oxygen and this may be assisting their penetration through the surface coats.

The next stage in the process leading to fertilization is the acrosome reaction. In mammals the acrosome reaction commences when the sperm approach zona pellucida. This reaction is characterised by the fusion of the outer membrane of the acrosome with the plasma membrane of the egg. After the fusion, the acrosomal membrane vesiculates which results in the release of acrosomal contents. Subsequently the outer portion of the acrosomal membrane disappears and only the inner portion adjacent to the nucleus remains intact (Fig. 13.24). When the release of the acrosomal contents occurs, several enzymes including hyaluronidase are released.

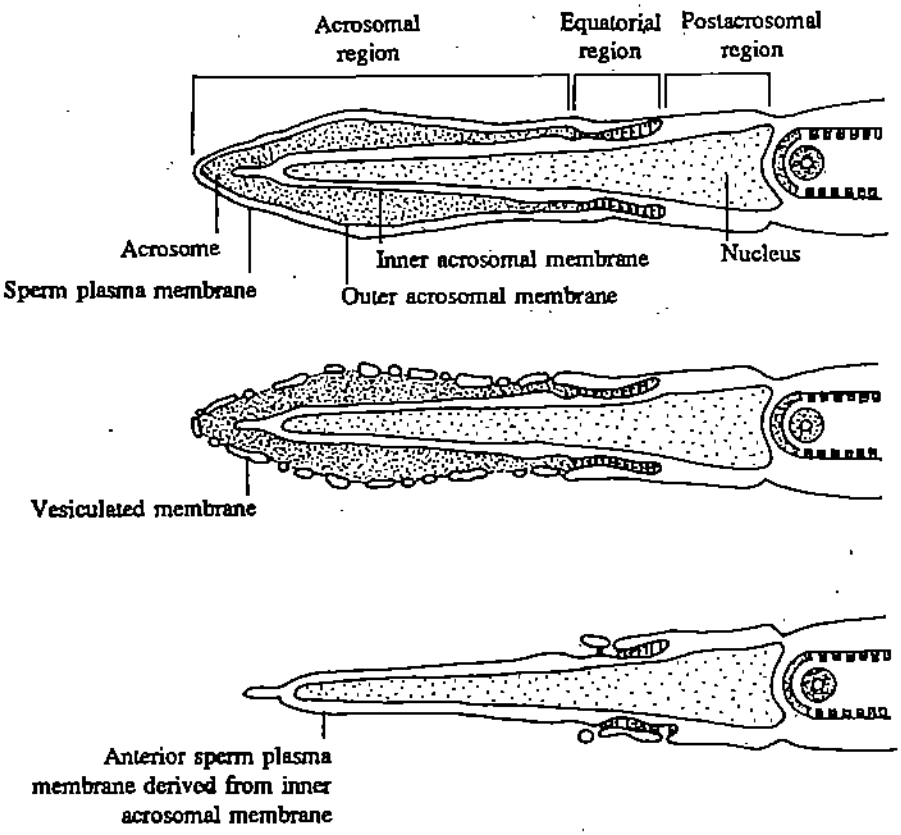

Fig. 13.24: Stages in the acrosome reaction in a mammalian sperm

a) Sperm head showing intact acrosomal membrane before the acrosome reaction.

b) Sperm head during acrosome reaction showing the vesiculation of the outer membrane.

c) Sperm head after acrosomal reaction showing the plasma membrane of the sperm derived from inner acrosomal membrane.

Hyaluronidase acts on the cell surface polysacharide hyaluronic acid. Also a corona penetrating enzyme is released. The function of these enzymes is to loosen the corona radiata cells and help the sperm to reach zona pellucida. It is believed that the sperm using a protease called **acrosin** present on the sperm head penetrate its way through the zona pellucida. Zona pellucida is believed to possess species specific sperm receptors that prevent interspecific fertilization in mammals. For example in mouse there are three glycoproteins in the zona pellucida termed ZP_1, ZP_2 and ZP_3, of which ZP_3 is the sperm receptor.

The acrosome reaction in mammals is initiated by an influx of Ca^{2+} into the sperm. In the glycoprotein ZP_3, the sugar moiety acts as a sperm recepter and the protein portion is believed to initiate the acrosome reaction.

In sea urchins the accumulation of Ca^{2+} ions in sperm appears to control the fusion of acrosomal and sperm membranes. Drugs which inhibit the Ca^{2+} ion movement into the cell prevent the occurance of acrosome-plasma membrane fusion. In sea urchins at the

end of the acrosome reaction (Fig. 13.25), the acrosomal process first attaches to the vitelline envelope and then the remainder of the sperm penetrates this layer. One of the proteins concerned in the acrosomal process is **bindin** which promotes species-specific attachment of acrosomal process to the vitelline envelope. From the acrosome a specific chymotrypsin helps sperm in the penetration of the vitelline envelope.

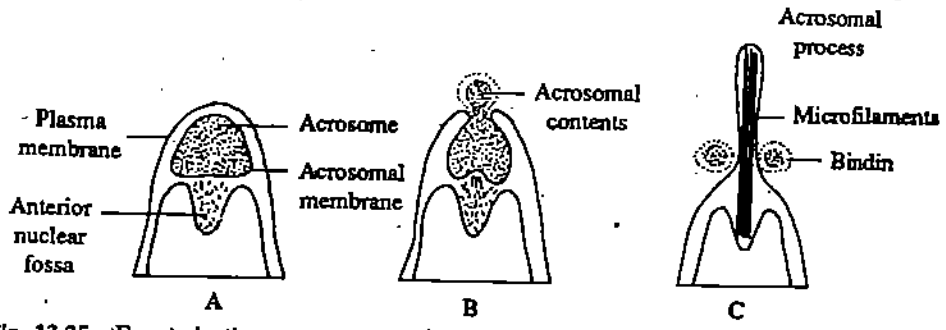

Fig. 13.25: Events in the acrosome reaction in sea urich sperm.

a) **A sperm head before the beginning of acrosome reaction. The acrosome is intact and is surrounded by plasma membrane of the sperm.**

b) **At the beginning of acrosome reaction, there is partial fusion of plasma membrane with the acrosome membrane. Also the acrosomal contents are released and this includes the protein bindin.**

c) **Later stage of arosome reaction in which acrosome has disappeared and the acrosomal process has extended further exposing bindin at the base.**

Summarising the events occuring prior to the fusion of the egg and the sperm nucleus the following can be said. There is an interaction with the activator and receptor molecules before and during the penetration of the sperm into the egg. In mammals this activator and receptor are shown to be glycoproteins. The role of the activator and receptor molecules is to trigger ion exchanges that are responsible for acrosome reaction and species-specific penetration of the egg surface coats.

13.5.2 Fusion of Sperm and the Egg

After the penetration of the extracellular layers by sperm, there occurs the fusion of the sperm plasma membrane with that of the egg. In mammals the fusion begins at the equatorial region of the sperm head. The plasma membrane of the two cells become continuous to form a cytoplasmic bridge through which the sperm nucleus enters the egg cytoplasm. Usually the entire sperm including the nucleus, centrioles, mitochondria, plasma membrane and even the flagellar axoneme enters the egg cytoplasm. Once the sperm enters the egg there is the formation of the fertilization cone. The fertilization cone is an extension of the egg cytoplasm around the entering sperm head. Microfilaments in the fertilization cone virtually draw the sperm into the egg. Inhibitors of microfilament formation such as cytochalasin B inhibit the formation of fertilization cone and sperm entry into the egg. Fig. 13.26 shows the events occurring during fertilization in sea urchin egg.

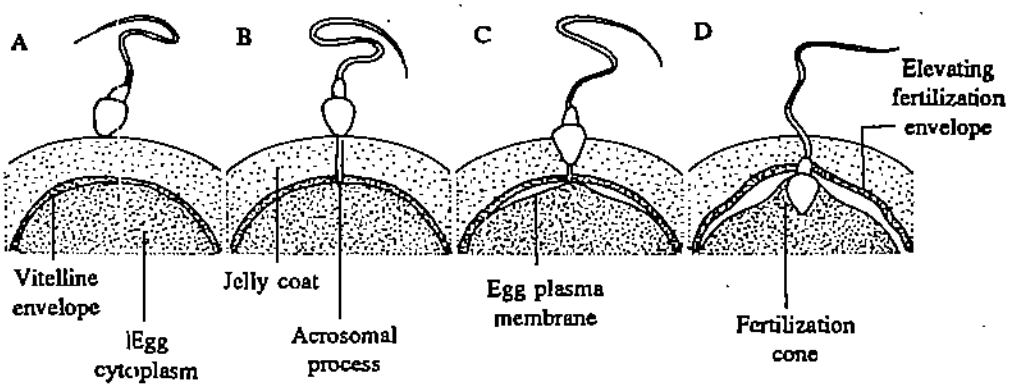

Fig. 13.26: Events that occur during the sperm-egg fusion in sea urchin.

13.5.3 Events after Sperm and Egg Fusion

The entry of the sperm into the egg activates the egg. The activation of egg results in two types of responses. (1) The early responses and (2) the late responses. The early response for the entry of the sperm into the egg is the **prevention of polyspermy**, i.e. the entry of or fusion of more than one sperm into the egg. Polyspermy may result in several abnormalites such as polyploidy, abnormal mechanism of chromosomal separation during cell division and ultimate death of the embryo.

Organisms have evolved different types of mechanisms for prevention of entry of more than one sperm into egg. Generally any one of the three different strategies may be involved in the prevention of polyspermy. For instance in fishes the sperm can enter into the egg only through the narrow opening the micropyle, the rest of the egg being covered by the impermeable chorion. In sea urchins and mammals there is a restriction on the number of sperm that are able to penetrate the extracellular coats and fuse with the egg. In mammals the sperm have to migrate the long female reproductive tract to reach the egg and further, the structural changes in zona pellucida block polyspermy. Certain animals such as salamanders do permit the entry several sperm into the egg but only the nucleus of one of the sperm can fuse with the egg to form the zygote nucleus; other sperm nuclei degenerate.

Prevention of polyspermy occurs in two phases (1) the **fast block** and (2) the **slow block**. The fast block is a temporary measure which is mediated by the electrical depolarization of the egg plasma membrane known as **fertilization potential.** In sea urchins the fertilization potential causes temporary change in the voltage across the plasma membrane from -70 to +10 mV (Fig. 13.27). Such potential changes prevent the fusion of more than one sperm with the egg. Recent studies have shown that the potential change is brought about by the insertion of a positively charged fusion protein which promotes sperm-egg fusion and the fertilization potential. Once the egg membrane becomes positive, the insertion of a positive fusion protein by another sperm is not possible.

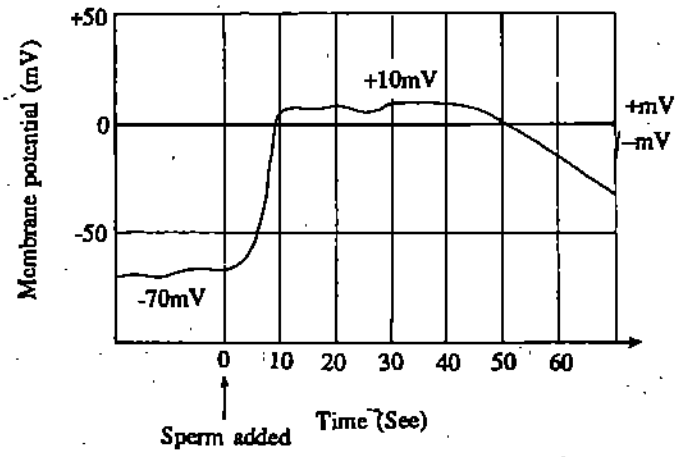

Fig. 13.27: Membrane potential of sea urchin egg before and after fertilisation

The slow block to polyspermy is achieved by **cortical reaction.** The slow block is necessitated by the fact that the fertilization potential is only a temporary phenomenon and the potential of the egg membrane soon returns to the negative. The cortical reaction (Fig. 13.28) consists of a wave of exocytosis of cortical granules which fuse with the egg membrane and release the contents into the perivitelline space. This space lies between the plasma membrane and the vitelline envelope. The reaction begins at the point of the entry of the sperm into the egg and spreads around the surface of the egg in one minute. The perivitelline space is filled with hydrated proteins and mucopolysaccharides which along with the vitelline envelope form the **fertilization membrane.** The fertilization membrane acts in three ways, (1) The membrane increases the distance between the extra sperm attached to its outer surface and the egg plasma membrane. (2) The peroxidases and the peroxides released by the cortical granules harden this fertilization membrane and make it resistant to sperm proteases. (3) The proteases of cortical granules destroy the glycoprotein receptors of the vitelline membrane thereby dislodging extra sperm.

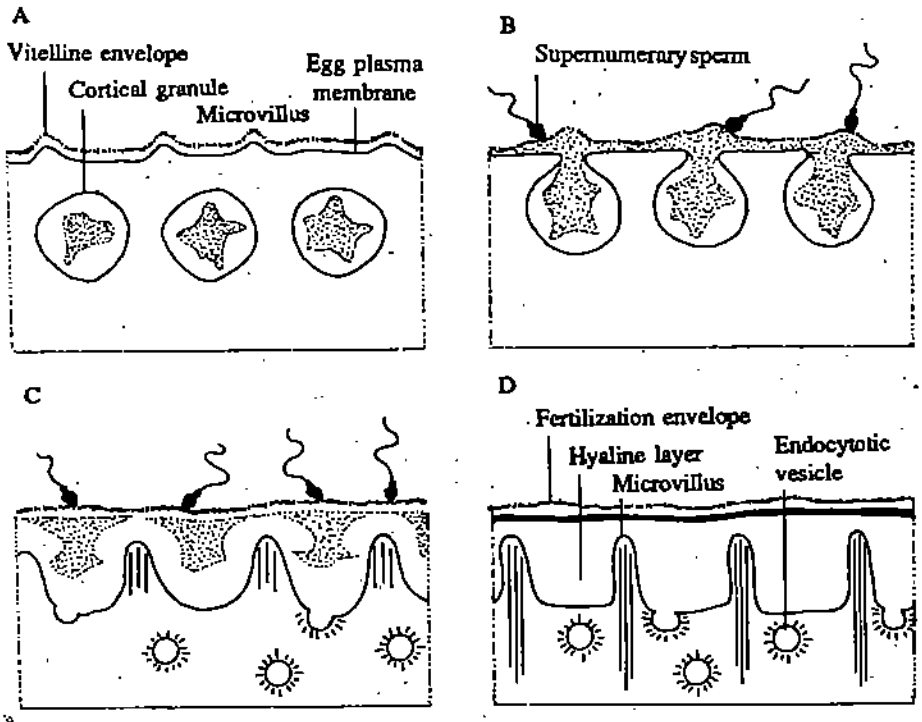

Fig. 13.28: Diagram showing cortical reaction and fertilization membrane formation in sea urchin.

a) **Unfertilised egg with cortical granules, vitelline membrane and plasma membrane with microvilli.**

b) **Just fertilized egg showing exocytosis of cortical granules and beginning of formation fertilization membrane.**

c) **Elevation of fertilization membrane and completion of cortical granule exocytosis.**

d) **Fertilized zygote showing elevated fertilization membrane, fully elongated microvilli and hyaline layer.**

In mammals the cortical granules contain hydrolytic enzymes which are released into the perivitelline space during cortical reaction. This hardens the zona pellucida which becomes refractive to penetration by extra sperm. The changes in the zona pellucida are called zona reaction.

13.5.4 Fusion of Egg and Sperm Pro-Nuclei

The fusion of the sperm and egg which is the beginning of the fertilization process is completed with the fusion of the male and female pronuclei to from the zygote nucleus called **synkaryon**. Usually the nuclear fusion occurs between 10 to 20 minutes after attachment of the sperms to the egg. In sea urchins the completion of second meiotic division occurs after the entry of the sperm and the resulting haploid egg nucleus is known as **female pronucleus**. The sperm nucleus after its entry into the ovum is known as **male pronucleus**. After its entry into the ovum there is a breakdown in the sperm nuclear envelop, decondensation of chromatin and the formation of the pronuclear envelope.

In sea urchins at the completion of meiosis the female pronucleus is located in the central region of the egg and the male nuclesus is located initially in the cortical region of the egg at the site of its entry. Before the fusion of the pronuclei, the sperm pronucleus has to migrate a considerable distance through the egg cytoplasm. In sea urchins a structure called **sperm aster** mediates this movement of the pronucleus (fig. 13.29). The sperm aster is a complex of long microtubules that radiate from the paired sperm centrioles. The centrioles which enter into the egg along with the sperm nucleus form the microtubule organising centre for the sperm aster. The microtubules of the sperm aster push the male pronucleus towards the centre of the egg. The astral microtubules also contact female pronucleus and pull it abruptly towards the male pronucleus. This activity continues until the two pronuclei are displaced to the centre of the egg where they fuse to form the synkaryon.

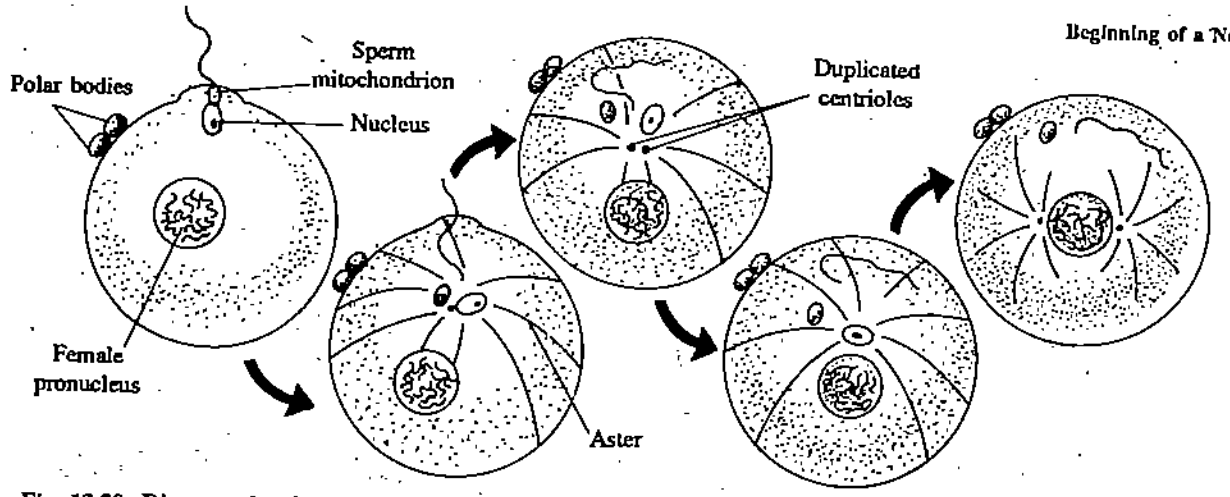

Fig. 13.29: Digrams showing the movement of pronuclei during fertilization in sea urchin.

In mammals the synkaryon formation does not depend upon pronuclear fusion. Here by a process called approximation the two prouclei migrate toward each other, become apposed to each other but do not fuse. They remain adjacent to each other until the meiotic division, then their nuclear envelops break down and the paternal and maternal chromosomes mix at a common metaphase plate.

13.5.5 The Initiation of Development

In the earlier section you have learnt the various events collectively called the activation programme. These events are directly related to the formation of the embryo. You have further learnt that the early response to the activation programme is the preventation of polyspermy. The late responses include many metabolic changes such as the activation of potassium ions and amino acid transport, an increase in the rate of the protein synthesis, initiation of DNA replication and several major regulatory events. These events include production of inositol triphosphate, diacyl glycerol, release of cytoplasmic free calcium ions and rise in hydrogen ion concentration. Fig 13.30, summarises the various events that occur during the activation programme of the egg. The egg which has been under a metabolic arrest before fertilization is released from this arrest on the entry of sperm. This initiates the long process of development into a new individual by active protein and DNA synthesis in the egg leading to the beginning of cleavage.

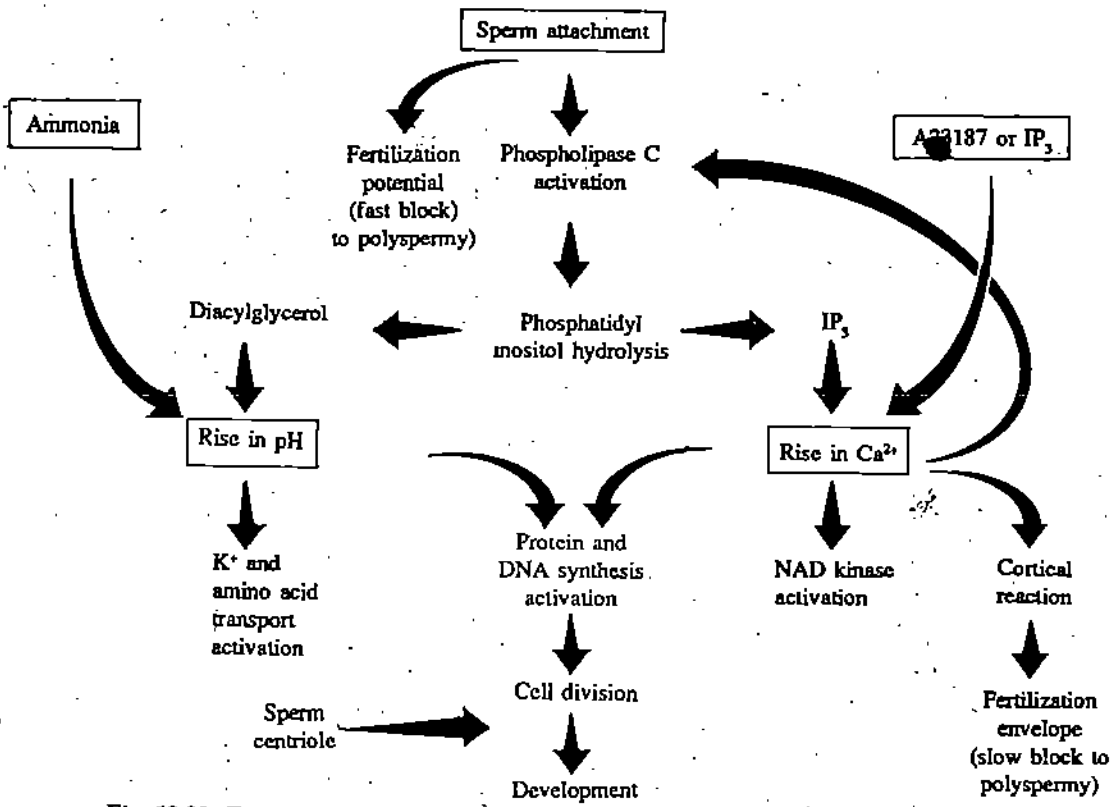

Fig. 13.30: Events leading to initiation of development in sea urchin egg.

SAQ 5

I) Choose the correct answer from the alternatives provided.

a) Fertilization is responsible for the activation/arrest of development.

b) Activation of the sperm ensures/does not ensure that sperm will meet the egg.

c) In organisms with external/internal fertilization, chemotactic mechanisms have been evolved to attract the sperm towards the egg.

d) A period of maturation in the female reproductive tract required for the transformation of sperm is known as activation/capacitation.

e) Sperm using an enzyme called acrosin/hyaluronidase penetrate their way through zona pellucida.

f) The acrosome reaction in mammals in initiated by an influx of calcium ions/ glycoproteins.

II) Fill in the blanks with suitable words.

a) ..... is the extension of egg cytoplasm around the entering sperm head.

b) Inhibitor of microfilament formation such as ..... prevent the formation of fertilization cone.

c) The early response for the entry of sperm into the egg is prevention of .....

d) The ..... for polyspermy is mediated by the electrical depolarisation of egg plasma memebrane.

e) The slow block to polyspermy is achieved by ..... reaction.

f) ..... is the fusion nucleus of female and male pronuclei.

13.6 SUMMARY

In this Unit, you have studied:

- Developmental biology is the study of ontogenetic development—both embryogenesis and blastogenesis. The important phases of ontogenetic development in sexually reproducting metazoans are — gemetogenesis, fertilization, cleavage and blastulation, gastrulation, growth, differentiation and morphogenesis.
- Protozoans reproduce by sexual and asexual means of reproduction but the occurrence of meiosis and fertilization may be variable. In some cases meiosis takes place immediately after fertilization while in others it is delayed. However, in protozoa sexual reproduction is mostly concerned with the revitalization of protoplasm.
- Sperm or male gametes are produced and differentiated in the seminiferous tubules of testes. Sperm consist of the head, middle piece and tail. Sperm head carries the sperm nucleus and acrosome; middle piece has centrioles and energy supplying power plant in the form of mitochondria. Sperm tail consists of typical flagellum or cilium. The basic purpose of sperm organization is to carry the paternal information to the egg and hence the motility is acquired.
- Egg or ovum or oocyte or female gamete is a large cell produced in lesser numbers. It contains nutrient materials for developing embryo. Yolk is the chief form of reserve food material; the yolk is simply a morphological term consisting mainly of proteins, phospolipids, neutral fats etc. The protein part is made up of two types of proteins - phosvitin and lipovitellin.
- Fertilization brings about the activation of quiescent and stationary egg and restores the diploid state in the zygote for the next generation. The spermatozoa reach the egg by chance or in some cases as a result of chemical attraction. The release of

acrosomal contents clears the passage through the egg envelopes and sperm nucleus eventually passes through the acrosomal tubules to the egg cytoplasm, being called as male pronucleus. The male and female pronuclei fuse, thus, completing the process of fertilization.

- Polyspermy is blocked by the depolarization of egg plasma membrane and formation of fertilization membrane resulting from the cortical reactions in most of the cases.
- Activation of egg involves the removal of inhibitory factors from the cytoplasm as a result of increase in the Ca^{2+} and rise in the pH. The cumulative effects of such changes bring about the sudden burst in protein synthesis, increase in oxygen consumption and ultimately initiation of cleavage of fertilized egg.

13.7 TERMINAL QUESTIONS

1) Briefly describe the spermiogenesis process.

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2) What are the basic purposes of fertilization?

a) .....

b) .....

c) .....

d) .....

e) .....

3) What are the major differences in the development and differentiation of an egg and a sperm?

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4) What do you understand by the activation of egg after fertilization?

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5) Describe the mechanisms evolved by eggs to prevent polyspermy.

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13.8 ANSWERS

SAQ 1

1) Developmental Biology is concerned with the progressive changes that a single fertilized cell undergoes to emerge as an adult organism.

2) Reproduction: The continued generation of new individuals of a species.

Growth: The increase in size of tissues and organs constitute growth.

Differentiation: The generation of cellular diversity is known as differentiation.

Morphogenesis: The organisation of differentiated cells into tissues and organs.

3) a) Isogamy b) Anisogamy

c) Conjugation d) Autogamy

e) Cytogamy

SAQ 2

i) Spermatogenesis

ii) Seminiferous

iii) Multiplication, growth and maturation

iv) Primary spermatocytes, spermatids

v) Spermiogenesis

SAQ 3

a) Head (with acrosome, nucleus) middle piece (with mitochondria and centrioles) and tail.

b) mRNA, ribosomes, rough endoplasmic reticulum, Golgi bodies

c) to approach the stationary egg

d) mitochondria in middle piece.

SAQ 4

a) True b) False c) False

d) True e) False f) False

g) True h) True i) True

SAQ 5

I) a) activation b) does not ensure

c) external d) capacitation

e) acrosin f) calcium ions

II) a) Fertilization cone b) cytochalasin B

c) polyspermy d) fast Block

e) cortical f) Synkaryon

TERMINAL QUESTIONS

1) Refer to Subsection 13.2.3

2) Activation of stationary and quiescent egg, amphimixis, restoration of diploid state, generate genetic variations, revitalization of cytoplasm.

3) Spermatozoa	Ovum
i) After the completion of maturation division the spermatids undergo the phase of differentiation (spermiogenesis) so that haploid male gamete becomes functional.	Even before the completion of maturation division, the female gamete is functional, and in some cases, no differentiation is needed after maturation division.
ii) Only short duration of growth.	Growth period is much prolonged.
iii) All the four spermatids become functional male gametes.	Only one ovum becomes functional female gamete and rest three are discarded as polar bodies.

3) Refer to Subsection 13.5.3 and 13.5.5

4) Refer to Section 13.5.5

UNIT 14 CLEAVAGE AND GASTRULATION

Structure

14.1 Introduction
- Objectives

14.2 Cleavage
- Yolk and Cleavage
- Planes of Cleavage
- Patterns of Cleavage
- Products of Cleavage (Morula and Blastula)
- Mechanism of Cleavage

14.3 Gastrulation
- Fate Maps
- Morphogenetic Movements
- Gastrulation in Some Animals

14.4 Summary

14.5 Terminal Questions

14.6 Answers

14.1 INTRODUCTION

In Unit 13 of this Block you have studied that spermatozoa reach the ovum by either chance or chemical attraction. Eventually, one spermatozoon fuses with the ovum to restore the diploid genomic condition and activates all the potentials in the fertilised egg cell or zygote to develop into a new individual of the next generation. But the zygote is one cell and the adult body in the Metazoa is constituted of many cells - from a few hundred to many billions of cells. It implies that the unicellular zygote must enter the phase of rapid divisions in quick succession to convert itself into a multicellular body. Such a series of divisions of the zygote is known as cleavage or segmentation. In this multicellular structure formed as a result of cleavage the various cells or cell groups later become rearranged as layers and sublayers during a process called gastrulation.

Cleavage and gastrulation are significant phases in the ontogenetic development because cleavage transforms the unicellular zygote into a multicellular body and gastrulation lays the foundation of primary organ rudiments so as to initiate the formation of organs according to the body plan of the particular metazoan group of animals to which the particular individual belongs.

Objectives

After you have read this unit you should be able to:

- explain the various planes of cleavage furrows
- list different cleavage patterns
- explain the purpose of gastrulation
- discuss the process and mechanism of gastrulation in some animals
- describe the influence and the role of yolk in determining the pattern and course of cleavage and gastrulation.

14.2 CLEAVAGE

Cleavage or segmentation is a series of cell divisions of the fertilised egg through which it is converted into a multicellular structure, called blastula. The main characteristics of cleavage include:

i) The unicellular fertilised egg is transformed by consecutive mitotic divisions into a multicellular body.

ii) Practically no growth takes place during cleavage.

The cell divisions in the somatic cells is mitotic. The daughter cells or blastomeres or cleavage cells are also derived as a result of miototic divisions of the zygote. We may ask whether there are any differences between mitotic divisions of somatic cells and of the zygote and the blastomeres derived from it during cleavage. From the following you will learn that the mitosis in the phase of cleavage has spme striking peculiarities:

a) Synchronisation of cell divisions of blastomeres: The early blastomeres divide simultaneously (synchronously) producing two blastomeres from zygote followed by 4,8,16,32 and so on, in most cases. However, such synchronisation is lost, during later cleavage divisions.

b) No interphase between two successive divisions, i.e. there is no growth in the amount of cytoplasm in the derived blastomeres with the result that the size of daughter blastomeres continues to decrease during successive cleavages.

c) The size of the nucleus remains practically unchanged. Therefore, the nucleus: cytoplasm ratio, which is very small in the fertilized egg cell or zygote, continues to increase in the blastomeres derived from successive cleavage divisions.

d) Rate of cell divisions is very rapid and very large number of cells are produced during cleavage (Fig. 14.1). This is possible due to absence of interphase. The rate, slows down later on

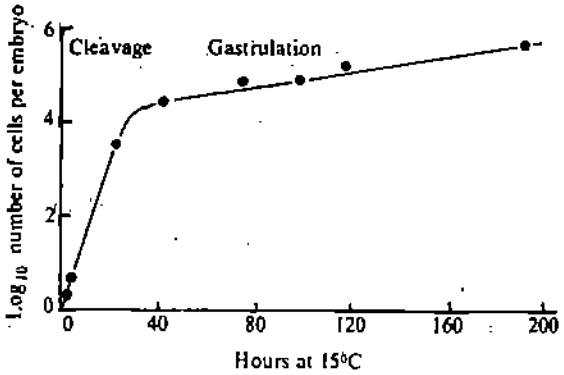


Fig. 14.1: Increase in the number of cells during early development of frog. Note the difference in the rate of cell divisions during cleavage and gastrulation.

14.2.1 Yolk and Cleavage

Apart from the importance of yolk as nutritive material for the developing embryo, yolk or deutoplasm determines the type and structure of the egg, and it also influences the rate and pattern of its cleavage. In other words, cleavage depends, to a large extent, upon the amount, distribution and orientation of yolk in the egg.

Types of Eggs

Depending on the amount of yolk, the eggs in various animal groups are of the following types (Fig. 14.2):

i) Alecithal or yolkless eggs as in the eutherian mammals. (Fig. 14.2 A).

ii) Microlecithal or oligolecithal eggs have little yolk in the form of granules, e.g., echinoderms, Amphioxus, molluscs (except cephalopods), annelids, flatworms (Fig. 14.2 B).

iii) Mesolecithal eggs with moderate amount of yolk, e.g. tunicates and amphibians (Fig. 14.2 C).

iv) Megalecithal or macrolecithal or heavily yolked eggs, e.g. cephalopod molluscs, bony fishes, reptiles, birds and egg laying mammals. The yolk occupies almost the whole of the interior of the egg with a small disc-shaped clear area of cytoplasm near the animal pole where the germinal vesicle (or nucleus) lies. Most of such eggs are large sized (Fig. 14.2 E).

Based on the placement and orientation of yolk, the egg may be:

a) Isolecithal with more or less evenly distributed yolk e.g., (echinoderms, Amphioxus, molluscs (except cephalopods), annelids. (Fig. 14.2 B).

b) Telolecithal with yolk granules or yolk mass occupying the vegetal hemisphere. In highly telolacithal eggs the yolk fills up almost the entire interior of the egg leaving only a small disc of clear cytoplasm containing the germinal vesicle (nucleus) near the animal pole of the egg. (Figs. 14.2 C, D, E).

c) Centrolecithal — In insects the yolk granules are concentrated in the interior of the egg whereas the cytoplasm is distributed as a thin peripheral layer around the yolk. There is also an island of cytoplasm in the centre of egg. This island surrounded by yolk on all sides contains the nucleus of the egg cell (Fig. 14.2 E).

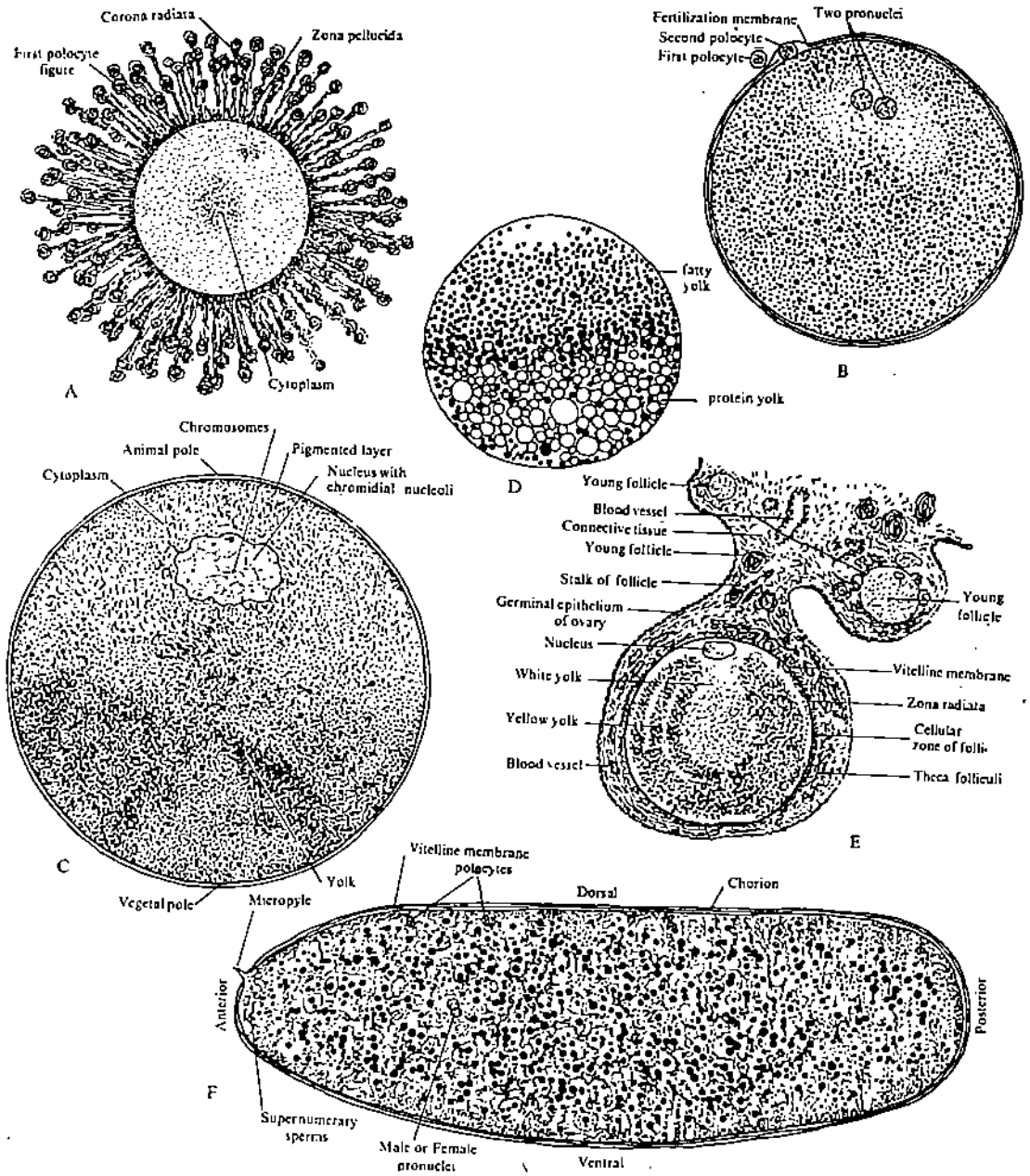


Fig. 14.2: Different types of eggs. A—human egg surrounded by follicle cells; B—microlecithal egg of Amphioxus; C—mesolecithal egg of frog; D—telolecithal egg of the mollusc Apalysia limacina; E—macrolecithal egg of hen inside the ovarian follicle and F—centrolecithal egg of insects.

Influence of Yolk on Cleavage

Though biological significance of yolk is to provide nourishment to the developing embryo, it is not part of the active cytoplasm. Yolk is dead and inert component not participating in the cellular activities, but, it influences cleavage in the following ways:

i) With gradual increase in the amount of stored yolk, the total amount of the active cytoplasm tends to decrease.

ii) Cell division is the activity of only the nucleus and cytoplasm. With increase in the yolk amount the formation of spindles, cell membranes and cleavage furrows takes place in the active cytoplasm which is restricted to relatively smaller areas of the zygote and its daughter blastomeres.

iii) The speed of cleavage is inversely proportional to the amount of yolk present. In the telolecithal eggs, blastomeres nearer to the animal pole divide at a faster rate than the blastomeres located towards the vegetal pole because the passive behaviour of the inert yolk in the yolky parts of the zygote and its daughter blastomeres obstructs the formation of cleavage furrows.

Therefore, the nature of various metabolic activities of the egg and the blastomeres derived from it depends upon the amount and placement of yolk mass.

Principles Governing Cleavage

a) The nucleus and mitotic achromatic figure tend to occupy the centre of active cytoplasmic density of the dividing cells, e.g., in isolecithal eggs or microlecithal eggs, the spindle is formed centrally in the cell while in the telolecithal eggs, it is formed nearer the animal pole.

b) Each new cleavage furrow tends to intersect the plane of the preceeding cleavage furrow at right angle.

c) The cells or blastomeres tend to divide into two equal sized daughter cells unless yolk is unevenly distributed.

d) Free sides of the blastomeres tend to become rounded.

SAQ 1

Fill in the blanks with appropriate words:

i) During cleavage zygote and blastomeres divide by ..... .

ii) There is no ..... or ..... between two consecutive divisions of blastomeres during cleavage.

iii) The egg of frog is described as ..... and ..... .

iv) Large size of hen's egg is due to ..... amount of yolk.

v) Achromatic figure or ..... tends to be formed in the centre of cytoplasmic .....

14.2.2 Planes of Cleavage

The ova of most of the animal groups (except some specific cases like insects) are spherical or nearly spherical having their own actual centre comparable to earth shape. Similar to north and south poles on earth, the egg has animal and vegetal poles. The yolk platelets have more density than active cytoplasm and are concentrated more towards vegetal hemisphere. Therefore, when the egg lies in any fluid medium (the fundamental feature of most of the eggs even in the apparently terrestrial eggs like those of birds etc.), the vegetal pole tends to face the centre of gravity and animal pole away from it.

With this picture in mind, we can now define the planes of cleavage of zygote or blastomeres, keeping in mind the imaginary lines (latitudes and longitudes) drawn on the earth surface (Fig. 14.3).

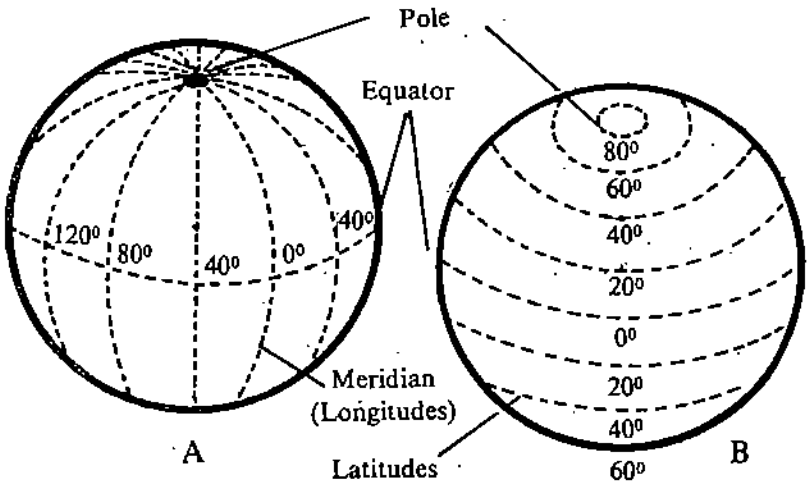


Fig. 14.3: A—Meridians (longitudes), B—Latitudes (imaginary lines on the earth surface which are comparable to the cleavage planes of a spherical egg).

The basic planes along which the egg and its daughter blastomeres are divided during early cleavage are:

i) Meridional Plane — the cleavage furrow passes from the animal pole to the vegetal pole through the centre of the spherical egg or the blastomere so as to divide the egg into two equal halves, e.g., first cleavage furrow in the chick and first as well as second cleavage furrows in the frog's egg (Fig. 14.5 A, B).

ii) Vertical Plane — the cleavage furrow forms parallel to animal-vegetal axis but a little away from the centre of the blastomers, e.g., third cleavage plane of chick blastoderm (Fig. 31 C).

iii) Equatorial Plane — the cleavage furrow bisects the egg at right angle to the median axis exactly half way between the animal and vegetal poles. The cleavage furrow appears along the equator of the spherical egg., e.g., the third cleavage plane of sea urchin (Fig. 14.5 C).

iv) Latitudinal or transverse or horizontal plane — it is like equatorial but the cleavage furrow passes through the egg cytoplasm on either side of equator along the latitudes of the egg sphere, e.g., third cleavage plane of amphibian eggs (Fig. 14.6 C).

14.2.3 Patters of Cleavage

In most of the animal groups with spherical or almost spherical egg and little or moderate amount of yolk (micro-or mesolecithal eggs), the first and second divisions result in four blastomeres of almost equal size (Fig. 14.5, A, B). Because of greater concentration of yolk platelets in the vegetal hemisphere, the third cleavage divides the 4 blastomeres in latitudinal plane giving rise to 8 cells arranged in two tiers of 4 blastomeres each including one tier of 4 small blastomeres (micromeres) in the animal hemisphere and the second tier of 4 large blastomeres (macromeres) in the vegetal hemisphere (Fig. 14.6 C). The arrangement of blastomeres in these two tiers is very distinct and on this basis, the cleavage may be of:

a) **Radial type:** If each of the blastomeres of upper tier lies exactly over the corresponding blastomere of the lower tier the pattern of cleavage is radially symmetrical i.e. blastomeres are arranged along the radii of the sphere, e.g. echinoderms, **Amphioxus,** amphibians (Fig. 14.5, D-F).

b) **Spiral type:** The upper tier of blastomeres of 8-cell stage embryo may be shifted with respect to the lower tier so that radially symmetrical arrangement of blastomeres is distorted in various degrees. In such a case the blastomeres of the upper tier do not lie exactly over the corresponding blastomeres of the lower tier. Instead, all the blastomeres of upper tier are shifted in the same direction with respect to all the blastomeres of the lower tier. This position results from the oblique position of mitotic spindle so that, from the start, two daughter blastomeres

do not lie one above the other, e.g., annelids, molluscs, some helminths of order polycladida (Fig. 14.4).

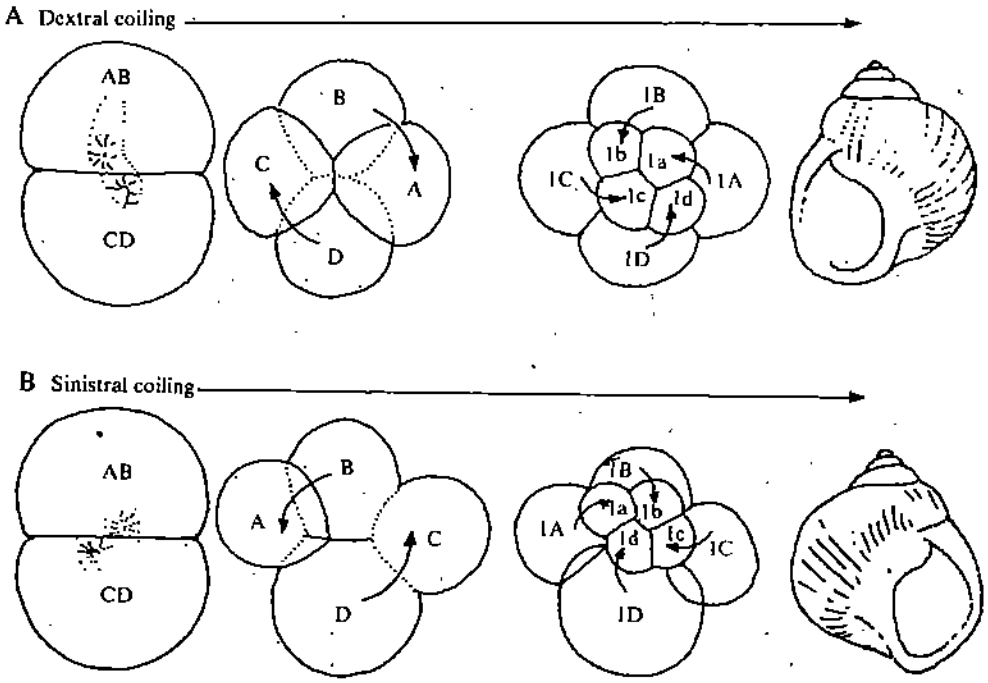


Fig. 14.4: Spiral cleavage in mollusc snails. Looking down upon the animal pole, the blastomeres are arranged either A). clockwise (dextral) B) anti-clock wise (sinistral). It happens to be a genetic character resulting in dextral or sinistral coiling of the shell of snails.

The turn of spiral as seen from the above may be in a clockwise direction (dextral) or counter clockwise (sinistral) direction, called dextral or sinistral, respectively (Fig. 14.4 A, B). In many animals such as snails, it is a genetic character.

c) Bilateral cleavage: In some animals (e.g., tunicates and nematodes, although in different manner), the arrangement of 4 blastomeres after second cleavage is almost radially symmetrical as in the radial type of cleavage, but two of these are larger as compared to the other two blastomeres establishing a plane of bilateral symmetry in the developing embryo. During subsequent cleavages the bilateral arrangement of blastomeres may be still more obvious.

Further, based on whether a particular cleavage furrow may divide the egg completely or partially, cleavage has been described as:

a) Holoblastic or complete: Each cleavage furrow divides the entire egg completely in the particular plane. It may be:

   i) Equal holoblastic cleavage: It occurs in alecithal (eutherian mammals) or microlecithal (Amphioxus, echinoderms) eggs where each cleavage furrow divides the egg so as to produce blastomeres of approximately equal size (Fig. 14.4, A-D).

   ii) Unequal holoblastic cleavage: This takes place in mesolecithal and moderately telolectithal eggs (lower groups of bony fishes and amphibians), in which the yolk is largely concentrated in the vegetal hemisphere. In these eggs the first and second cleavage divisions take place along meridional planes producing 4 equal blastomeres. But, because of the yolk being concentrated in the vegetal region, the third cleavage furrow is latitudinal above the equator and closer to the animal pole. The furrow divides each of the 4 blastomeres completely but unequally into a small and a larger daughter blastomeres. The third cleavage is therefore, unequal holoblastic producing 4 small blastomeres (micromeres) in the animal region and 4 large blastomeres (macromeres) in the vegetal region (Figs. 14.6, 14.7). Subsequently the micromeres containing relatively less yolk divide at a much faster rate than the large yolky macromeres.

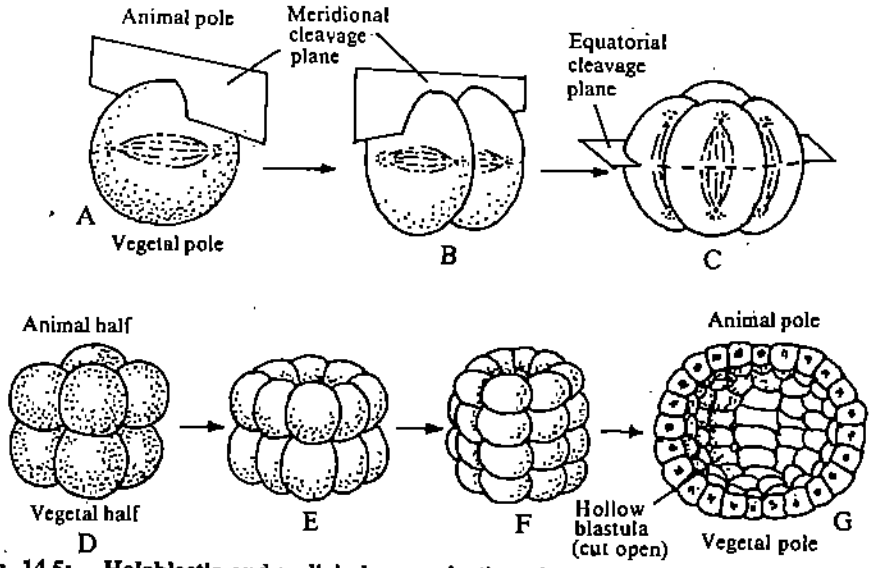


Fig. 14.5: Holoblastic and radial cleavage in the microlecithal egg of Synapta digita (Echinoderm) leading to the hollow blastula (G). A-B indicate the meridional planes of 1st and 2nd cleavage; C-equatorial plane (3rd cleavage); D-G radial arrangement of blastomeres.

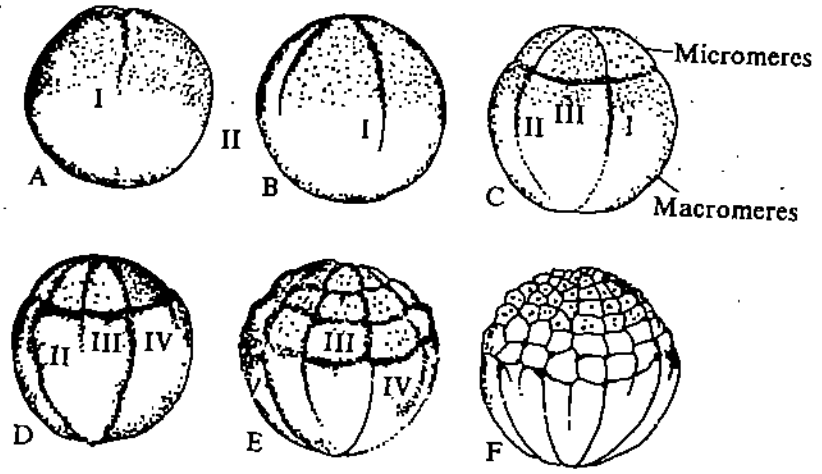


Fig. 14.6: Unequal Holoblastic Cleavage in frog's egg (A-F) cleavage furrows are designated, by Roman numerals indicating the order of appearance; G—amphibian blastula in section

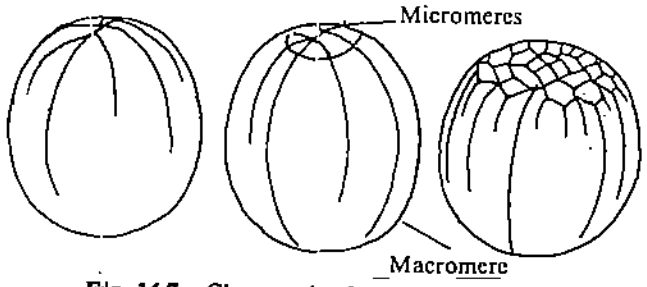


Fig. 14.7: Cleavage in the ganoid fish Amia.

b) Meroblastic or partial cleavage: The egg does not divide completely because divisions are restricted to only a part of the egg while the rest of the egg remains entirely uncleaved. It is of two types:

i) Discoidal meroblastic cleavage: It takes place in the heavily yolked macrolecithal and highly telolecithal eggs, as for example in cephalopod molluscs, reptiles, birds (Fig. 14.8) and monotremes (egg laying mammals). The cleavage is restricted to the cytoplasmic germinal disc situated at the animal pole. Even the germinal disc divides incompletely while the entire yolk mass remains undivided.

ii) Superficial meroblastic cleavage: This occurs in the centrolecithal eggs of insects. Cell divisions are restricted to the peripheral cytoplasmic layer while the centrally located yolky is left undivided (Fig. 14.9).

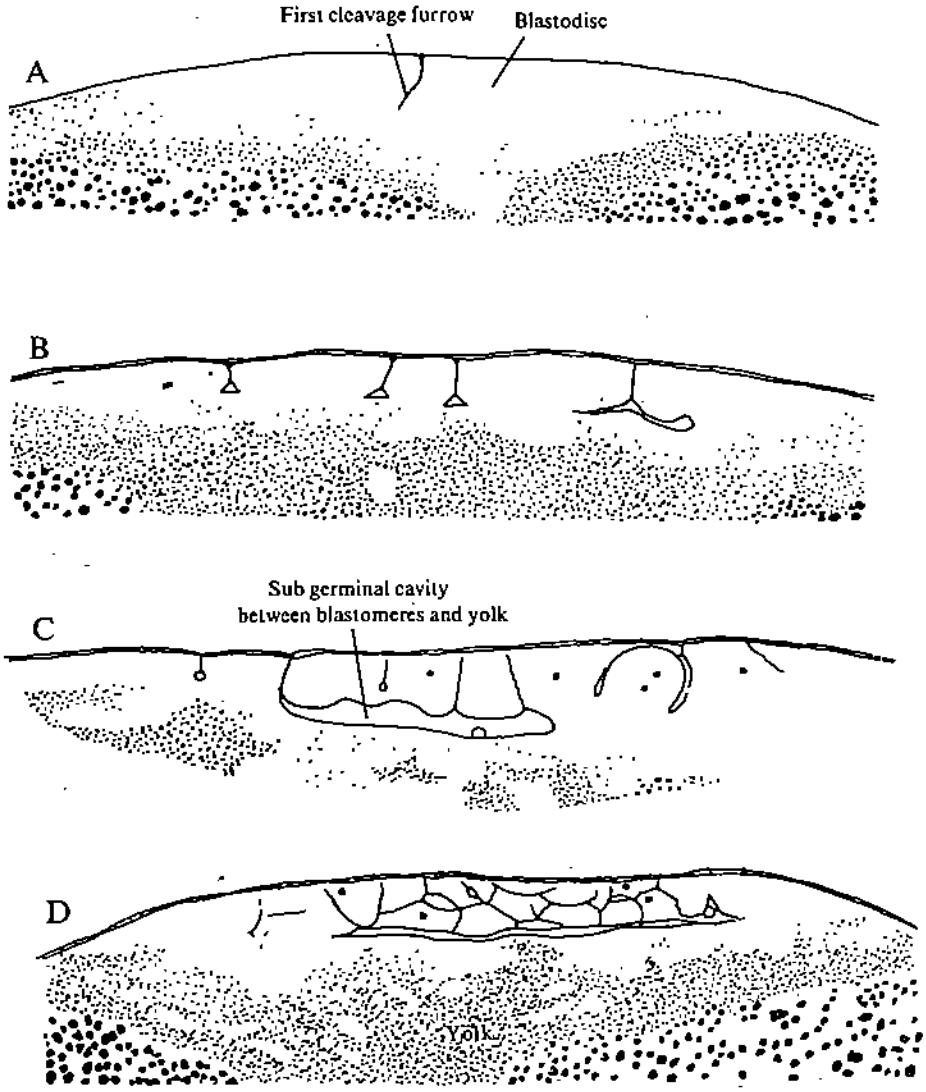


Fig. 14.8: Diagrams of sections of the fertilized chick egg. Discoidal meroblastic cleavage in its blastodisc lying on top of yolk.

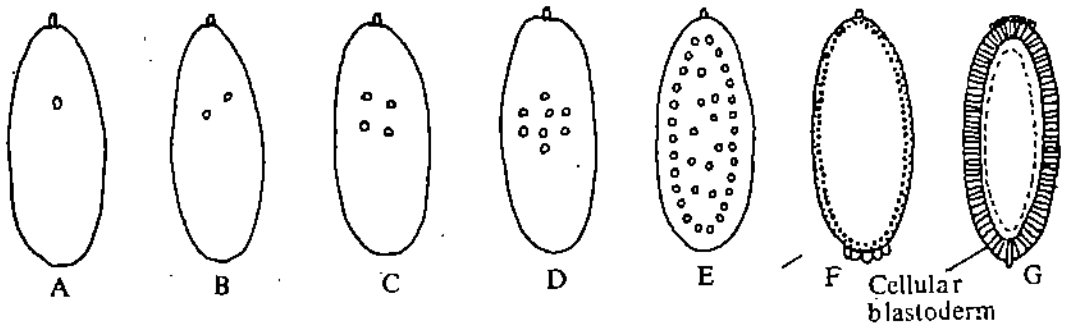


Fig. 14.9: Diagramatic representation of superficial cleavage in insect embryo. (A) Undivided zygote nucleus in the yolk. (B) - (E) After 1st, 2nd, 3rd and more divisions of the zygote nucleus. (F) Daughter nuclei have migrated from interior of the egg to peripheral cytoplasm which is still undivided. (G) Peripheral cytoplasm divided into separate cells to form cellular blastoderm around undivided yolk.

We can now summarise the various types of cleavage according to Table 14.1:

Table 14.1: Summary of Cleavage Types

Cleavage Patterns	Position of Yolk	Cleavage Symmetry	Representative Animals
HOLOBLASTIC (Complete-cleavage)	Isolecithal (oligolecithal)	Radial Spiral	Echinoderms, Amphioxus Most molluscs, Annelids, flatworms, round worms.
	Sparse, evenly distributed yolk	Bilateral Rotational	Tunicates Eutherian Mammals
	Mesolecithal, moderately telo-lecithal	Radial	Amphibians, lower bony fishes
MEROBLASTIC (Incomplete-cleavage)	Highly Telolecithal (dense yolk)	Bilateral Discoidal	Cephalopod Molluscs Reptiles, bony fishes, birds, egg laying mammals.
	Centrolecithal (Yolk concentrated in the centre of egg).	Superficial	Arthropods especially insects.

SAQ 2

i) List out various planes of cleavage:

.....

.....

.....

.....

ii) Fill in the blanks with appropriate word:

Meroblastic cleavage occurs in the ..... and ..... eggs. The ..... eggs of insects undergo ..... cleavage because the cell division is restricted to the ..... layer of ..... whereas ..... located yolk remains undivided.

14.2.4 Products of Cleavage (Morula and blastula)

In most cases, the blastomeres in early cleaving stages tend to assume spherical shape like that of the egg before cleavage. Although their mutual pressure (resulting from limited available space within the egg envelopes) flattens the surface of blastomeres in contact with each other, the free surfaces of each blastomere remain spherical. As a result, after some cleavage divisions have taken place the embryo has a shape resembling mulberry. Because of this superficial resemblance this stage of embryonic development of many animals has been referred to as Morula (Latin for mulberry).

As cleavage divisions continue the subsequent arrangement of the blastomeres in the morula may vary in different groups of animals. In some the blastomeres are packed together without any space between them, or a small cavity appears but is soon obliterated. In both cases the result is the formation of a solid blastula called stereoblastula e.g. some flatworms, annelids, molluscs and coelenterates. In such blastulae some of the blastomeres lie externally and others in the interior.

But, in most animals the cavity appearing between the blastomeres persists and may enlarge, This cavity is called blastocoel. As the cleavage progresses, the adhesion of the blastomeres to one another increases and they arrange themselves as an epithelial layer around the blastocoel. This stage of embryonic development is called blastula and such blastula is called coeloblastula (hollow blastula). The layer of blastomeres is referred to as blastoderm.

The structure of blastula becomes modified in various animal groups. The modifications are related to the amount of yolk deposited in the egg, as you will see from the accounts of blastula structure in some deuterostome animals.

The blastula of the echinoderm sea cucumbers consists of a fluid filled blastocoel surrounded by a single layer of cuboidal blastomeres, which constitute the simple epithelial blastoderm. In the blastulae of sea urchin and **Auphioxus,** the blastoderm surrounding the blastocoel is an epithelium consisting of a single layer of columnar cells (blastomeres). However, the vegetal blastomeres are larger than animal blastomeres so that the epithelium is thicker at the vegetal pole and thinner at the animal pole. Thus the polarity of the egg persists in the blastula (Fig. 14.10, a, b).

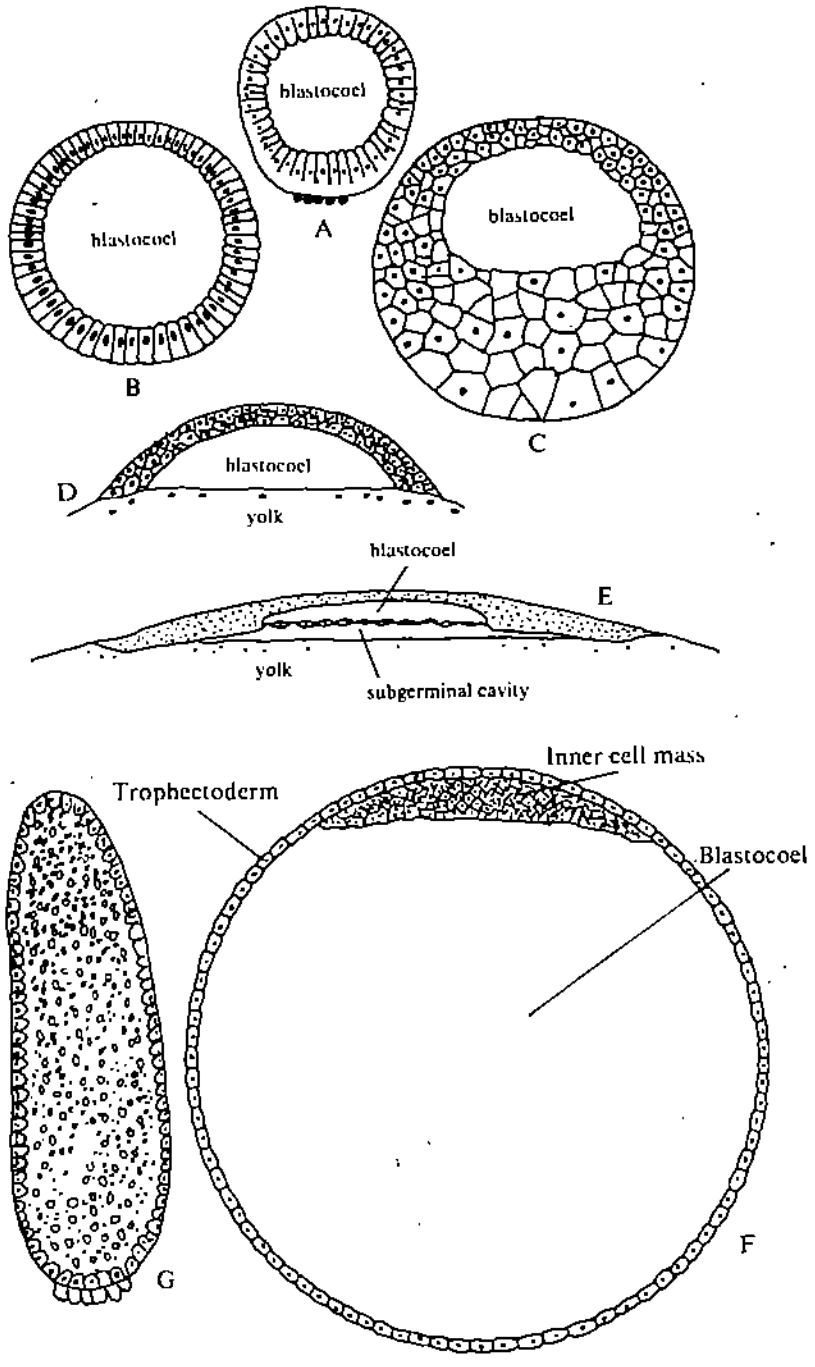


Fig. 14.10: Diagrammatic comparison of blastulae of echinoderm. (A) Amphioxus (B) Amphibian (C) bony fish (D) bird (E) blastocyst of mammals (F) and insect (G).

The blastulae of the echinoderms and **Amphioxus** have been described as coeloblastula.

Animals with larger amount of yolk (e.g. amphibians) show considerable differences in size among the blastomeres of blastular blastoderm, and the blastocoel is distinctly eccentric nearer to the animal pole instead of being in the centre. The blastoderm is not a simple epithelium of a single layer of cells but is two or more cells thick (Fig. 14.10,

c). The cells of the inner side of blastoderm are loosely connected to one another but those at the external surface adhere with each other very firmly because of the presence of tight junctions between them. The blastoderm at the animal pole and most of the animal hemisphere is made up of micromeres forming the dome-shaped roof of the blastocoel while the blastomeres of the vegetal hemisphere form the floor of the blastocoel (Macromeres). The amphibian blastula is also a coeloblastula but it is modified as described above due to larger quantity of yolk mostly located in the vegetal hemisphere of the egg (Fig. 14.10, c).

The embryonic stage comparable to blastula occurs in a still more modified form in the sharks, bony fishes, reptiles, birds and egg laying mammals, all with macrolecithal and highly telolecithal eggs. In the egg laying amniotes reptiles, birds, monotremes the active cytoplasm is restricted to a small disc (cytoplasmic germinal disc) on top of the yolk near the animal pole. Cleavage is meroblastic occuring only in the germinal disc and gives rise to a disc-shaped blastoderm made of several layers of cells lying on top of the uncleaved yolk. Such a blastula is called discoblastula. Between the blastoderm and yolk there is a narrow space called sub-germinal space (or segmentation cavity), which is not comparable to blastocoel. In the birds a true blastocoel appears later between the upper layer of blastoderm (epiblast) and the lower layer (hypoblast) formed by cells migrating from the blastoderm (Fig. 14.10 e).

In the insects having centrolecithal eggs, the blastula stage does not have any cavity. It is characterized by one cell thick epithelial blastoderm enclosing the yolk filled interior. Such a blastula is called superficial blastula (Fig. 14.10 f).

Cleavage of the yolkless eggs of eutherian mammals gives rise to a solid ball of cells (morula). Fluid is secreted into the space between the cells of morula which grows in size to become the blastocyst. This is the blastula stage of the embryos of eutherian mammals. Structurally it consists of a single layer of cells (trophectoderm) enclosing a large fluid filled blastocoel. At one end of the blastocoel pressed up against the inner surface of trophectoderm there is a group of cells referred to as the inner cell mass (ICM). The entire body of the embryo is formed from cells of ICM (Fig. 14.10, g).

SAQ 3

i) In spite of little or no yolk in the eggs of echinoderms and eutherian mammals, the cleavage follows entirely different courses in two groups, why?

.....

.....

.....

.....

ii) There is a list of various animal given below. Mention the type of cleavage and the resultant blastulae:

Animals	Types of Cleavage	Type of blastula
a) Ciona (Tunicate)	.....	.....
b) Rat	.....	.....
c) Labeo rohita (rohu)	.....	.....
d) Scoliodon (dogfish)	.....	.....
e) Rana tigrina	.....	.....
f) Calotes versicolar (garden lizzard)	.....	.....
g) Pigeon	.....	.....
h) Sea Urchin	.....	.....
i) Nereis (Annelida)	.....	.....

14.2.5 Mechanism of Cleavage

Like the mitotic division in any cell, cleavage is the result of two events: mitotic nuclear division (Karyokinesis) followed by cytoplasmic division (Cytokinesis). The two events involve numerous metabolic processes. The egg cytoplasm contains all the requirements for these processes before the egg leaves the ovary. They are synthesized and stored in its cytoplasm during oogenesis as a result of the activity of maternal genes present in the oocyte. Fertilization by sperm activates the metabolic processes in the egg cytoplasm and initiates cleavage according to the programme already set by the maternal genes during oogenesis. There is much evidence to prove that cleavage is guided by the genetic information received by the egg cytoplasm from the mother during oogenesis. There is little or no transcriptional activity in the zygotic nucleus during early cleavage. Therefore, the effects of paternal genes that come into the egg with sperm nucleus are manifested only later.

The regulative factors for such biphasic segmentation are said to lie in the egg cytoplasm itself.

The Cytosketal Mechanism

There are two co-ordinated processes in the cleavage (or cell division) shown in Table 2, Fig. 14.11.

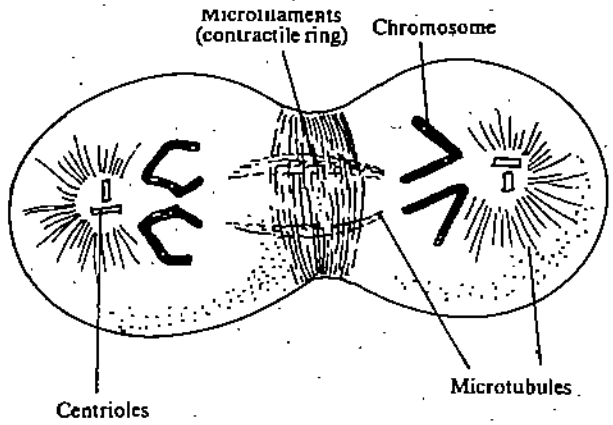


Fig. 14.11: Role of microtubules and microfilaments in cell divisions. In the telophase cell shown here, the chromosomes are being drawn to the centrioles by microtubules while cytoplasm pinches in through the contraction of microfilaments.

1. **Karyokinesis:** The mitotic division of nucleus depends upon the formation of mitotic spindle which is constituted by microtubules of which the tubulin protein is the structural unit. Treatment of the egg with the drug colchicine disrupts the microtubules and arrests karyokinesis at metaphase (Table 14.2).
2. **Cytokinesis:** Division of the cell depends upon the contractile microfilaments of which protein actin is the structural unit. A ring of microfilament appears in the cortex around the cell where the cleavage furrow is formed. Contraction of the microfilament ring in the purse-string manner deepens the furrow ultimately cutting the cell into two (Fig. 14.11). Treatment of the egg with cytochalasin B inhibits the organisation of contractile ring of microfilaments so that cleavage furrow is not formed and cytokinesis does not take place (Table 14.2).

Table 14.2: Comparison of Karyokinesis and Cytokinesis

Process	Mechanical agent	Major protein composition	location	Major disruptive agents
Karyokinesis	Mitotic spindle	Tubulin-microtubules	Central cytoplasm	Colchicine
Cytokinesis	Contractile ring	Actin macro-filaments	Cortical-cytoplasm	Cytochalasin B

Karyokinesis and cytokinesis are co-ordinated processes with the latter following the former. However, exact mechanism which brings about this coordination is not known

so far. The available evidence suggests that mitotic spindle dictates the location of cleavage furrows. The furrow always forms perpendicular to the long axis of the spindle.

Formation of the cleavage furrow depends upon the presence of a pair of asters, one at either end of the spindle. Disruption of astral rays inhibits the formation of the furrow and hence cytokinesis.

Although karyokinesis and cytokinesis are coordinated they are independent processes. Nuclear divisions can take place without being followed by cytoplasmic division. As you have learnt cytokinesis can be inhibited by treatment with cytochalasin B but the nuclear division proceeds to completion resulting in binucleate or multinucleate cells. It occurs in nature also, e.g., in the insect egg the zygotic nucleus and its daughter nuclei divide mitotically many times to produce hundreds of nuclei but the cytoplasmic divisions take place only later when all these nuclei have migrated to the peripheral cytoplasm (Refer to Fig. 14.9). Similarly cleavage of cytoplasm can take place even if karyokinesis is blocked, e.g. if the zygotic nucleus of a fertilized egg is removed the enucleate egg cytoplasm undergoes cleavage divisions upto about blastula stage.

The Formation of New Membranes

Divisions of the egg or a blastomere increase the total surface area of the two daughter cells to be covered by membrane at each cleavage. The existing membrane of the parent cell is insufficient. From the evidence available so far, it is indicated that this insufficiency of membranes for daughter blastomeres during cleavage is made up from two sources:

i) A portion of the membranes covering the daughter cells is provided by stretching and extension of the original plasma membrane of the zygote or the blastomeres.

ii) A portion of the cell membrane is newly synthesized by the daughter cells.

Thus, the furrow membranes are a mosaic of different parts.

SAQ 4

i) Define:

a) Karyokinesis .....

.....

b) Cytokinesis .....

.....

ii) What are the basic structural units of:

a) Spindle fibres:

b) Contractile ring of microfilaments:

c) Astral rays.

.....

.....

iii) How is cleavage division affected by treatment of the egg with:

a) Colchicine

b) Cytochalasin B.

.....

.....

.....

.....

14.3 GASTRULATION

The end of cleavage of the unicellular zygote results in the formation of multicellular blalstula, which may be a solid structure without a cavity (stereoblastula), or its cells

may be arranged in th form of a one cell or several cells thick epithelium around a cavity (coeloblastula) or around or on top of the yolk, (Superficial blastula; Discoblastula). In either case the blastula has no resemblances to the shape or organization of the body. Therefore, through the subsequent developmental stage the simple blastula should transform itself into a more complex embryonic structure (gastrula) upon which the adult like body may be built up. Such a process of transformation is known as gastrulation. It is a very significant phase of ontogenetic development, which marks the beginning of the development of form and organization of adult body.

In the metazoans (except in sponges and coelenterates), the various tissues and organs of the body develop from cells which become arranged in the form of three layers, the outer ectoderm, the inner endoderm and the mesoderm between these two layers. The three layers are called the germinal layers. With the exception of some parasitic flatworms a new cavity called the archenteron (future alimentary canal) is formed surrounded by endoderm.

In the blastula all the cells are located on the surface forming the blastoderm. During gastrulation there occurs displacement of the parts of blastoderm so that the presumptive endodermal and mesodermal cells are removed from the surface of blastula and brought into the interior of embryo where the respective organs are formed in the course of further development. The cells of the presumptive ectoderm remain on the surface. Thus, the single layer of cells, the blastoderm, gives rise to three germinal layers viz. ectoderm, mesoderm and endoderm. Therefore, gastrulation is a dynamic process involving large scale movement of blastula cells resulting in their arrangement in a way which establishes the basic body plan according to which the embryo has to develop further. Since these movements lay the foundation of the form and organization of the body they are called morphogenetic movements. They involve movements of epithelial layers of cells as a whole as well as independent movements of cells which break loose from epithelium and become mosenchymal.

The extent of morphogenetic movements during gastrulation depends, to a certain degree, on the number of cells in the blastoderm of the completed blastula. For example, relatively simple and less movements occur during gastrulation in the ascidian styela (tunicate) in which there are only about 100 cells at the end of blastula stage. In animals such as frog in which the blastoderm consists of many thousands of cells already at blastula stage very large scale and complicated movements are required during gastrulation for their rearrangement into the three germinal layers.

The important features of the gastrulation are:

a) rearrangement of cells of the embryo by means of morphogenetic movements

b) rhythm of cell divisions slows down (Fig. 14.1)

c) growth, if any, is insignificant.

d) there is intensification of oxidation.

e) the nuclei become more active in controlling the activities of the embryonic cells. The influence of paternal genes becomes evident during gastrulation.

f) proteins of many new types that were not present in the egg or blastula begin to be synthesized.

14.3.1 Fate Maps

The details of the process of gastrulation are not easy to understand without the knowledge of positions of the cells of the future germinal layers in the blastula. A chart or diagram showing the prospective fate of each part of blastula or embryo at any stage of development is called a "fate map".

There are various ways of constructing the fate maps of blastulae or any other developmental stage of different animals.

By Natural Markings

For some animals it is possible to make use of the peculiarities of cytoplasm in certain parts of egg, such as pigment granules in the cytoplasm. The descendent blastomeres of such cytoplasmic areas tend to carry the pigment granules where ever they happen to be present in the later stage. Pigment granules of various colours are present in different cytoplasmic areas of the egg and in different blastomeres subsequent to cleavage in

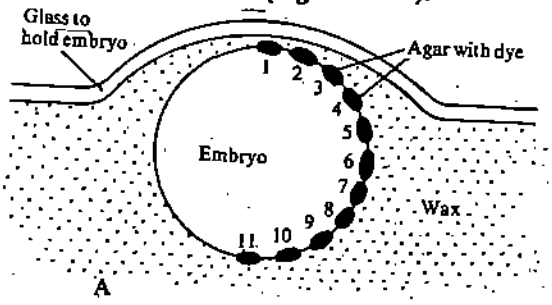
various animals, e.g., Dentalium (mollusca), sea urchins (echinodermata), ascidians, amphibians etc. But, the peculiarities of pigmentation are not sufficient enough to make it possible to reconstruct detailed fate maps of the pigmented parts or whole of the embryo.

Some of the artificial methods devised to ascertain the fates of some or all parts of an early embryo to construct their fate maps are given below:

* Vital Dye Staining

In this method, a piece of agar or cellophane soaked in the solution of a vital dye (Nile Blue Sulphate, Neutral Red, Bismarck Brown etc.) is applied to the surface of embryo at the required position. The dye diffuses from agar (or cellophane) to the cells of the embryo at that position (Fig. 14.12 A). The embryo continues to develop normally. The stained blastomeres and their descendent cells carry the dye for quite a long time and there is little or no diffusion from them to nonstained cells. The ultimate position of stained cells in the later differentiated embryo indicates the fate of the originally stained blastomeres.

With this method, the detailed fate maps of amphibian blastula and some other embryonic stages have been constructed. (Fig. 14.12 B).



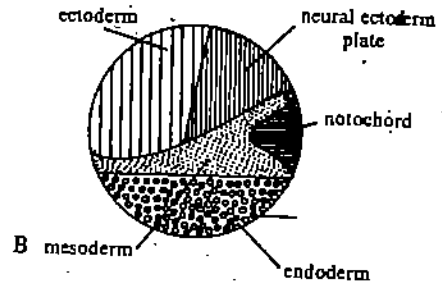


Fig. 14,12: Diagrammatic view of Vital Dye Staining Method of amphibian embryo.

A: Vogt's method for marking specific areas of the embryo surface with vital dyes.

B: Fate Map of amphibian blastula showing prospective areas of germ layers. Arrow points to site of future blastopore.

* Carbon Particle Marking Method

Tiny carbon particles are applied to the surface of developing embryo. The particles stick to the surface of the cells and serve as markers to follow the movements of these cells during further embryonic development so as to ascertain their ultimate position (fate). This method has been used to construct fate maps of the blastoderm of chick embryos (Fig. 14.13).

* Radioactive Labelling

Radioactive labelling method has been successfully applied on the chick blastoderm. The method involves labelling one embryo (donor) and grafting a part of it in the same position on another unlabelled embryo of the same stage (Host). An explanted blastodermis immersed in a medium containing the radioactive tritiated (H^3) thymidine.

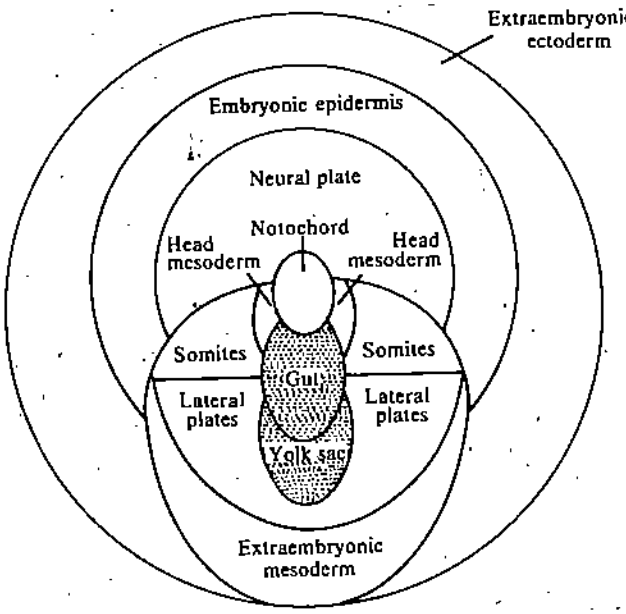


Fig. 14.13: Fate map of chick blastoderm immediately prior to gastrulation. The primitive streak is not yet formed but it will extend eventually to the region of the notochord.

In three to eight hours the tritiated thymidine is incorporated into the chromosomal DNA of dividing blastodermal cells. The embryo labelled in such a way by tritiated thymidine serves as a donor. Another embryo at the same stage of development as attained by the labelled donor in the meantime is then selected to serve as the host. A small area of the host embryo is excised and replaced by a corresponding piece from the donor of which the fate is to be determined. (Fig. 14.14). Healing usually occurs quickly and the development is not impaired if the operation has been done carefully. The thymidine does not pass out of the nuclei of the labelled cells but remains in the chromosomes of their descendents. Although the radioactive thymidine present in the DNA is gradually diluted with each subsequent chromosomal replication radioactivity remains for a considerable time. Such composite embryo (partly from donor and partly from the host) is tested at a later stage of development for radioactivity by special techniques such as autoradiography etc. Only the part(s) or structure(s) developed from the grafted piece show the presence of radioactivity thus establishing the fate of particular area taken from the donor.

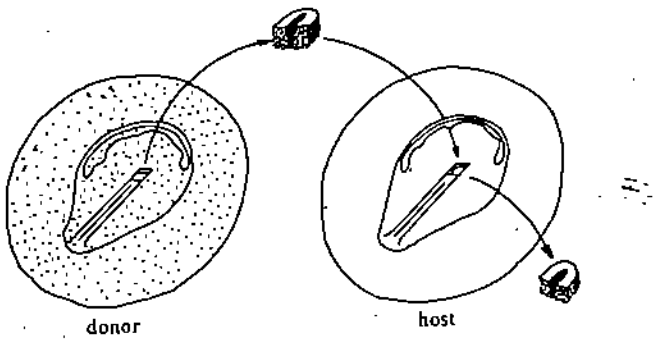


Fig. 14.14: Diagram explaining the method of testing the fate of particular part of a chick blastoderm by implanting a corresponding part from a tritiated thymidine labelled donor into unlabelled recipienthost.

14.3.2 Morphogenetic Movements

Gastrulation is a dynamic process involving a variety of coordinated movements of cells of different areas of the blastula.

The movements of cells in the embryo from one place to another to establish a particular form or structural arrangement are referred to as morphogenetic movement. (Morphos = shape; genesis = formation). Such movements occur during embryonic development (from the beginning of gastrulation onwards) as well as in the adult body. In the adult body, these are reversible but the movements occurring during gastrulation are irreversible.

Gastrulation begins and proceeds as a result of the onset of various types of morphogenetic movements which are inherrent to the particular category of cells. For the sake of convenience, these are described separately but it should be understood that two or more of them may occur simultaneously. Broadly, there are two groups of morphogenetic movements in embryonic development i.e., Epiboly and Emboly.

i) Epiboly or Epibolic Morphogenetic Movements

Epiboly means to throw on or to extend upon (Fig. 14.15). It occurs only in the presumptive ectodermal blastomeres (epidermal and neural areas). The cells of this area have an inherrent property of flattening, expansion and stretching. The cells of the presumptive ectodermal areas expand and extend but they remain on the surface eventually forming the outer layer covering the entire embryo and enveloping the inwardy migrating presumptive mesodermal and endodermal blastomeres.

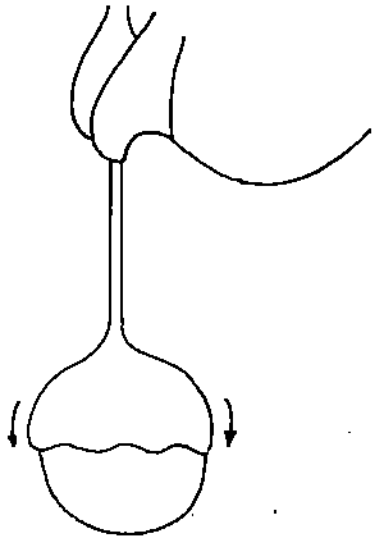


Fig. 14.15: Diagram showing the analogy with epiboly. Viscous liquid poured over a sphere slowly spreads covering its surface.

ii) Emboly or Embolic Morphogenetic Movements

Emboly means to throw in or to thrust in. Such movements bring about the migration of presumptive mesodermal and endodermal cells from the external surface of the embryo into its interior.

Emboly includes several different types of movements:

Invagination

It specifically includes the process of insinking or infolding of presumptive endodermal areas (Fig. 14.16) and is the most widely observed embolic movement during gastrulation in most animals, e.g. echinoderms, **Amphioxus** and amphibians etc. Invagination may be:

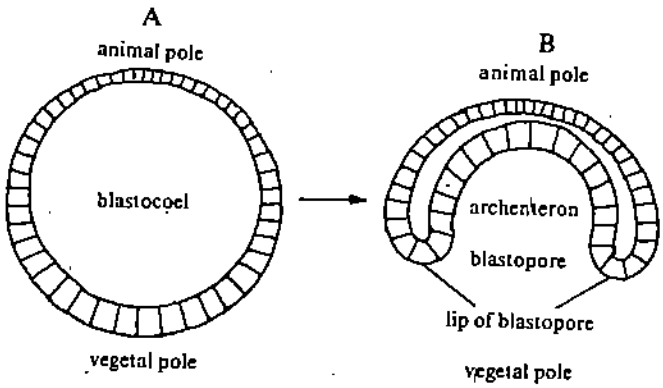


Fig. 14.16: Diagrammatic representation of gastrulation by invagination. Sections of blastula (A) and gastrula (B).

a) passive, occurring as a result of the activity of other cells, or

b) active, resulting from the inherrent forces within the invaginating cells.

Various causes have been attributed to the process of invagination:

- Absorption of blastocoelic fluid by certain cells.
- Differences in the characteristics of blastocoelic fluid and external medium.
- Higher relative alkalinity of blastocoelic fluid which causes local surface tension changes in the membrane of certain cells.

It should be kept in mind that not any one factor causes invagination but a combination of different factors may be involved in various animals.

Involution

It denotes turning in or rolling over. Involution of mesodermal blastomeres has been observed in Amphioxus, amphibians, birds, reptiles, monotremes and even in some eutherian mammals. During gastrulation in the amphibians the blastomeres located on the margin of the lip of blastopore roll over the lip (involute) to become internal (Fig. 14.25, 14.28). In the bird embryo the cells located on the posterior margin of the blastoderm roll over (involute) to from the lower layer called the hypoblast (Fig. 14.17) and the presumptive mesodermal and endodermal cells located on the surface of the blastoderm reach the interior by turning in through the groove of the primitive streak (Fig. 14.34).

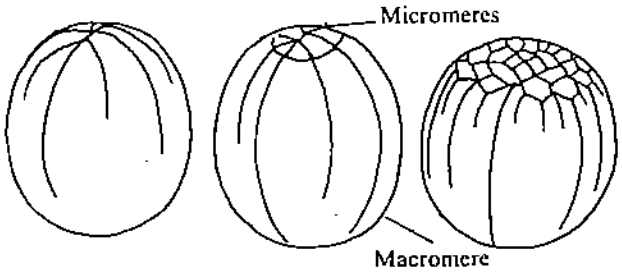


Fig. 14.17: Schematic representation of formation of hypoblast by involution of cells from the posterior margin of blastoderm in a heavily yolked egg. A = anterior; B = posterior. Arrow indicates the direction of hypoblast cells.

Convergence

It means to move towards one point from many places. Movement of the presumptive chorda-mesodermal cells located on the surface of the blastula of amphibian embryo towards the rim of blastopore during gastrulation is an example of convergence. (Fig. 14.18).

Similarly, in chick there occurs the convergence of presumptive mesodermal cells towards the primative streak followed by their involution through the groove of the primitive streak to the inside (Fig. 14.19). Infact, involution and convergence go on simultaneously.

Divergences

It is the opposite of convergence, i.e., movements or extension away from a common point. After the chorda-mesodermal cells have converged and then involuted over the blastoporal rim or through the primitive streak in gastrulating embryo, the cells continue to migrate diverging to their future positions within the embryo away from the blastoporal rim or the primitive streak. (Figs. 14.18, 14.19).

Delamination

It denotes the separation of groups of cells from other cell groups to form separate cell layers. It includes splitting of a pre-existing sheet (layer) of cells into two more or less parallel sheets usually with a space separating them.

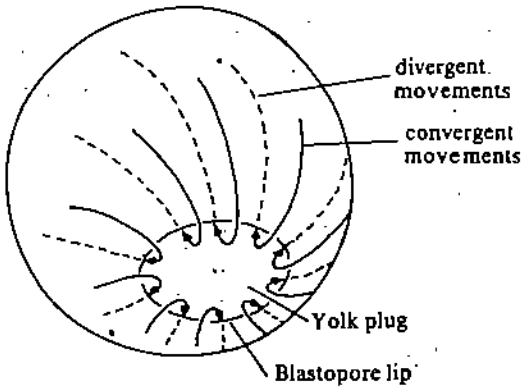


Fig. 14.18: Convergent and divergent morphogenetic movements of cells migrating into the blastopore and then under the surface in gastrulating amphibian embryo.

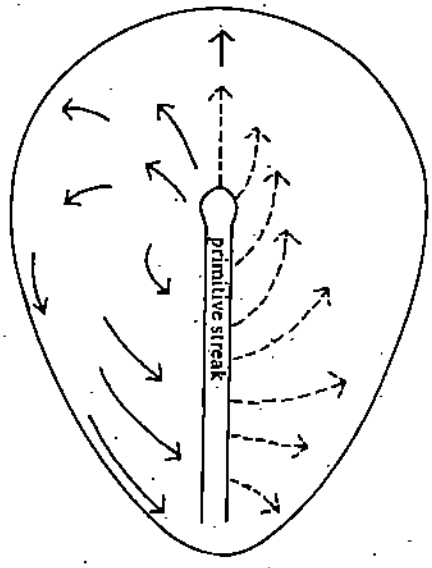


Fig. 14.19: Morphogenetic movement during gastrulation in chick embryo, continuous lines (on left side) show movement in the epiblast and broken lines (on right side) indicate the cellular movements which have immigrated into the interior through the primitive streak.

Polyinvagination or Ingression

In this process, individual cells or small groups of cells in different parts of the blastoderm or blastodisc invaginate (or ingress) and migrate into the cavity or spaces developed within the embryo (Fig. 14.20). Since such invagination may occur at different points at the same time it is also called polyinvagination. Ingression and polyinvagination have similar meanings. Primary mesodermal cells of sea urchin embryo become internal by this process.

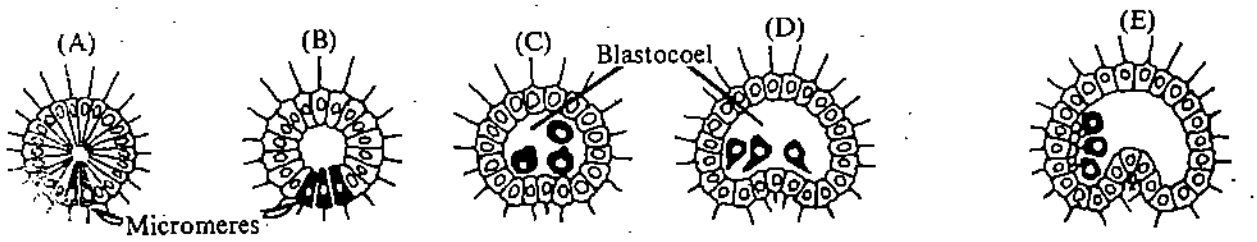


Fig. 14.20: Diagrammatic representation of the ingression of primary mesenchymal cells (black) in sea urchin embryo. (A) Early blastula with ciliated micromeres. (B) Late blastula with cilia being withdrawn from micromeres and the cell rounding up. (C) Mesenchyme blastula, with micromeres detached from the hyaline layer and entered the blastocoel as primary mesenchyme cells. (D) Early gastrula with mobile cells. (E) Midgastrula, with formation of syncytium prior to deposition of skeletal matrix.

i) Fill in the blanks with appropriate words:

Gastrulation is ..... process caused by the ..... of blastomeres from the surface of ..... . The cytoplasmic areas of blastula practically show the same ..... as that of ..... . As a result of ....., single layered blastula is ..... into a two layered or ..... layered ..... .

(ii) List the various morphogenetic movements through which the gastrulation may take place.

(iii) List the various methods of constructing the fate maps of blastoderm of embryos in different animal groups.

14.3.3 Gastrulation in some Animals

As mentioned earlier gastrulation marks the beginning of morphogenesis i.e. development of body form and organisation of cells in the embryo. By the end of this process the groups of cells destined to form different tissues and organs are arranged in their respective proper positions within the three germinal layers (ectoderm, mesoderm, endoderm) and the primitive basic body plan of the animal is established. However, in different groups of animals gastrulation takes place in different ways determined mainly by the type of egg and subsequent pattern of cleavage and the structure of blastula.

In this section you will study gastrulation as it occurs in echinoderms, amphibians, birds and eutherian mammals. The description will help you to understand the difference in the method of gastrulation due to the influence of the amount of yolk and pattern of its distribution in the egg, subsequent pattern of cleavage and the ultimate structure of the blastula.

i) Gastrulation in Sea Urchin

The small, isolecithal eggs of sea urchin have very little yolk and undergo holoblastic cleavage. The resultant free swimming ciliated blastula is a sphere consisting of a single layer of cells surrounding a large blastocoel (Fig. 14.21).

Formation of Primary Mesencychyme

Gastrulation is initiated by flattening of the cells of vegetal region forming a vegetal plate (Fig. 14.21H). Small cells in the centre of this plate lose the cilia of their external surface, show pulsating movements at the inner end which becomes rounded and attachment with adjacent cells is lost. Eventually, these cells separate from the vegetal plate and migrate as individual cells into the blastocoel by ingression. They move around in the blastocoel for some time before setting down near the vegetal plate (Fig. 14.21 I). These cells constitute the primary mesenchyme which gives rise to skeleton of the larva (Fig. 14.21 J, M).

First Stage of Invagination

The large endodermal cells remaining in the vegetal plate move laterally towards the centre of the plate and fill the gap in the vegetal plate caused by ingression of the primary mesenchyme cells. As a result, the vegetal plate becomes even more flattened. Soon thereafter the plate bends inwards (invagination) in the centre initiating the formation of a new cavity called the archenteron (primitive gut). Its opening at the vegetal pole is the blastopore. Invagination proceeds until the archenteron extends into the blastocoel about one third the distance between the vegetal and animal poles and then stops (Fig. 41.21 J). The endodermal cells invaginate even if the vegetal plate is isolated and cultured in vitro indicating that invagination occurs due to intrinsic

properties of these cells and no external force is involved. Invagination probably occurs as a result of constrictive activity of microfilaments at the outer ends of the cells of the vegetal plate.

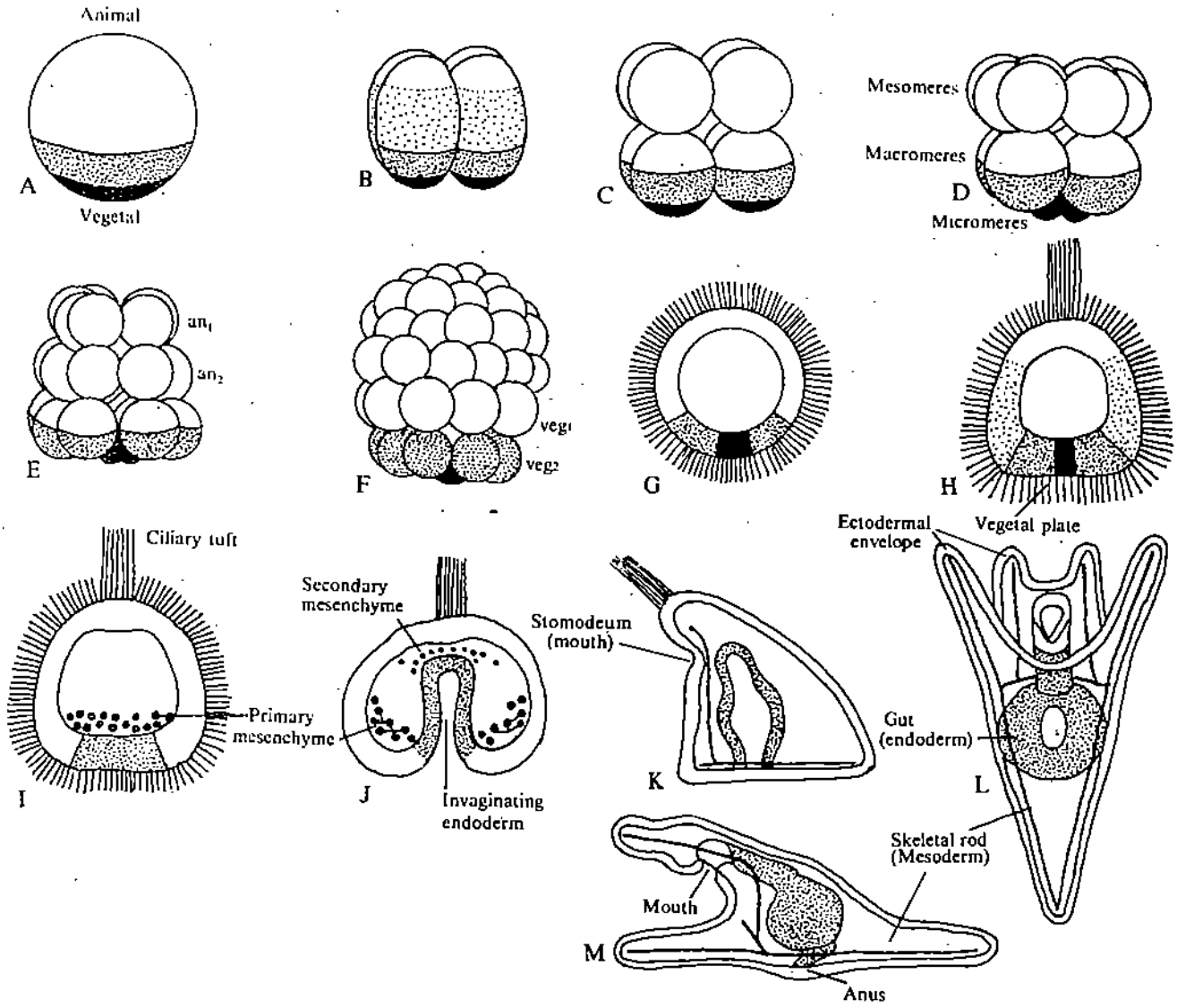


Fig. 14.21: Diagrams showing sea urchin development, (A-F) Cleavage through the 64-cell stage (2-cell stage omitted); (G) early blastula with cilia; (H) late blastula with ciliary tuft and flattened vegetal plate; (I) blastrula with primary mesenchyme; (J) gastrula with secondary mesenchyme; (K) prism-stage larva; (L, M) pluteus larvae. Fates of the zygote cytoplasm can be followed through the variations of shading.

Second Stage of Invagination

The second stage of invagination begins with release of secondary mesenchyme from the

tip of archenteron into the blastocoel. The cells of secondary mesenchyme remain where they are released at the tip of archenteron but their filopodia extend upto and attach to the inner side of the wall of blastocoel opposite to blastopore. Contractions of these filopodia pull the archenteron towards the animal pole causing further invagination of the endoderm. Eventually, the archenteron bends and its tip fuses with the blastocoel wall on the ventral side where the mouth opens later. The secondary mesenchyme then disperses in the blastocoel and gives rise to mesodermal organs. (Fig. 14.21)

Major features of gastrulation in sea urchin are:

i) gastrulation begins at the vegetal pole.

ii) Ingression of primary mesenchyme

iii) Initial invagination of endoderm occurs due to forces intrinsic to its cells.

iv) Completion of invagination and archenteron formation with external help provided by secondary mesenchyme.

v) Origin of secondary mesenchyme (mesoderm) from endoderm.

SAQ 6

i) Fill up the blanks:

a) Gastrulation in sea urchin begins with ..... of ..... in ..... region.

b) Vegetal plate in gastrulating sea urchin embryo consists of presumptive ..... and ..... .

c) Primary mesenchyme cells in sea urchin embryo enter ..... by ..... .

d) Archenteron in sea urchin gastrula is formed by ..... of endodermal cells at ..... pole.

c) In sea urchin embryo secondary mesenchyme arises from ..... at the tip of ..... .

ii) List major features of gastrulation in sea urchin embryos.

.....

.....

ii) Gastrulation in Amphibians

Ambhibians have a large and moderately telolecithal egg. Cleavage is holoblastic and unequal producing a spherical blastula with a reduced eccentric blastocoel shifted towards the animal pole. The blastocoel has a thin roof of several layers of small yolk-poor cells and a floor of large yolk-laden cells several layers deep extending down to the vegetal pole (Fig. 14.22 A). The process of gastrulation starting with such a blastula is complicated because it is impossible for the huge mass of large yolk cells in the floor of the reduced blastocoel of the cells to invaginate from the vegetal pole inwards as occurs in sea urchin. Instead, invagination begins on the future dorsal side of of the embryo where cells are small and less yolky (Fig. 14.22 B). Moreover, invagination is very limited and only initiates gastrulation; rest of the gastrulation process involves a variety of cell movements including convergence, divergence, involution, epiboly etc.

Fate map

Gastrulation can be better described and understood with reference to the fate map of different areas of the blastula. The first such map was constructed by a German embryologist, Vogt. in 1929 for a urodele amphibian using the method of lebelling the embryonic cells with agar chips soaked in vital dyes of different colours (See section 14.3.1). According to this map (Fig. 14.23) the surface of amphibian blastula is divisible into three major zones:

i) A large area around the animal pole (Animal zone) contains the presumptive ectoderm including the epidermal and neural ectoderm;

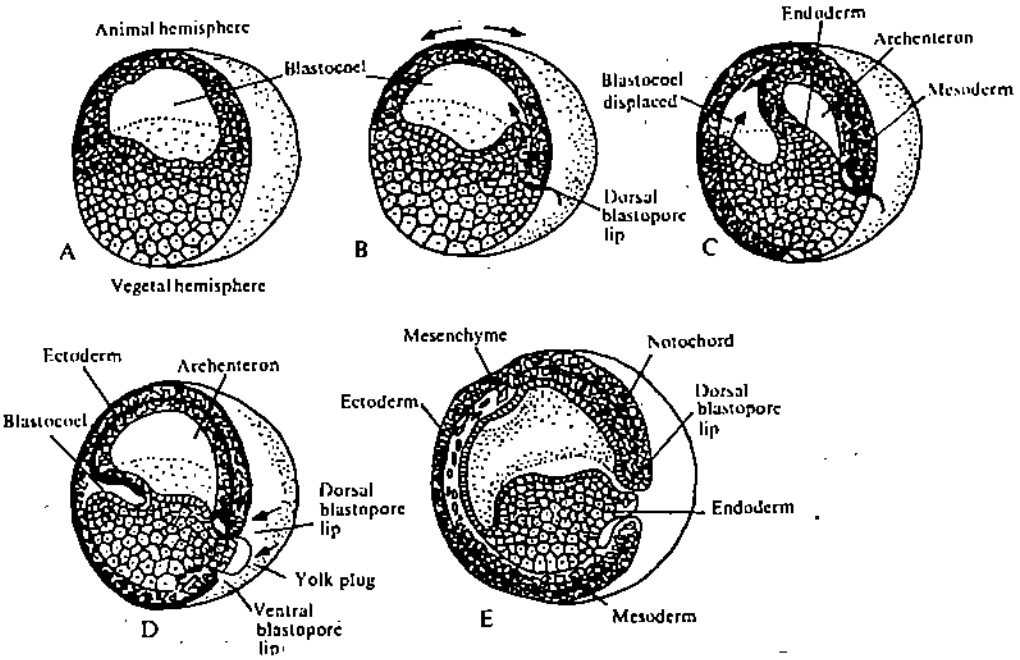


Fig. 14.22: Cell movements during frog gastrulation. The sections are cut through the centre with the dorsal surface towards the viewer. Major cell movements are indicated with arrows, and cells originally on the animal hemisphere surface are shaded. (A) Blastula. (B) Gastrulation begins as cells move inward to form the dorsal lip of the blastopore. (C) Involution of cells through the dorsal lip and under the roof of the blastocoel creates the archenteron and displaces the blastocoel. (D) Cells involute through ventral and lateral blastopore lips as well as through the dorsal lip; the ectodermal precursors migrate over the vegetal hemisphere. The yolk plug represents the only endoderm visible on the surface. (E) Gastrulation continues until the entire embryo is surrounded by ectoderm, the endoderm has been internalized, and the mesodermal cells are brought between them.

ii) The area around the vegetal pole (vegetal zone) contains the presumptive endoderm;

iii) A broad band like intermediate area around the equatorial region between the two zones (intermediate of marginal zone) contains the presumptive mesoderm. Within this the presumptive mesoderm for head mesoderm and notochord (Chorda-mesoderm) lies in the dorsal marginal zone (gray crescent), and that for the somites in the lateral and the laterla plate mesoderm in the ventral areas of the lateral (Fig. 14.23) marginal zone, respectively.

In many ambhibian species the three zone can be distinguished by differences in pigmentation so that the animal zone has deeply pigmented cells, those of the marginal zone are grayish and those of the vegetal zone are white. During gastrulation the cells of the marginal and then the vegetal zones move to interior while the cells of the animal zone remain on the outside and cover the embryo.

The fate maps constructed by Vogt for a salamander (urodele amphibian) has been very useful for a variety of studies on gastrulation and other aspects in amphibian embryo. However, while this map helps to trace the movements and fates of cells located on the surface of blastula it does not provide information about the cells lying in deep layers beneath the superficial layer. According to recent studies of Keller (1975) on the embryos of African clawed frog, **Xenopus laevis** using the same vital dye staining method the superficial layer in the marginal zone is entirely endodermal and the cells of the presumptive mesoderm including chorda-mesoderm lie in deeper layers beneath it. Keller has constructed fate maps for both the outer and inner surfaces of Xenopus blastula (Fig. 14.24). The account of the process of gastrulation that follows is mainly based on the description given by Keller for the frog Xenopus. It is possible there are differences among various species in this respect.

Initiation of Gastrulation — Invagination

Gastrulation begins with the appearance of a shallow groove on the future dorsal side of the embryo below the equator at the lower edge of gray crescent which marks the border between the vegetal zone and the dorsal marginal zone. (Fig.14.25A). The

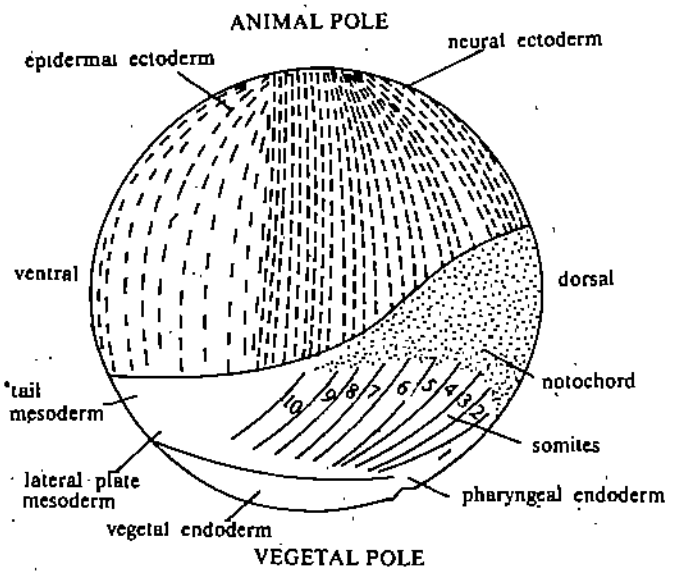


Fig. 14.23: Fate map of a urodele amphibian embryo. Prospective fates of cells present on the surface of embryo at the beginning of gastrulation.

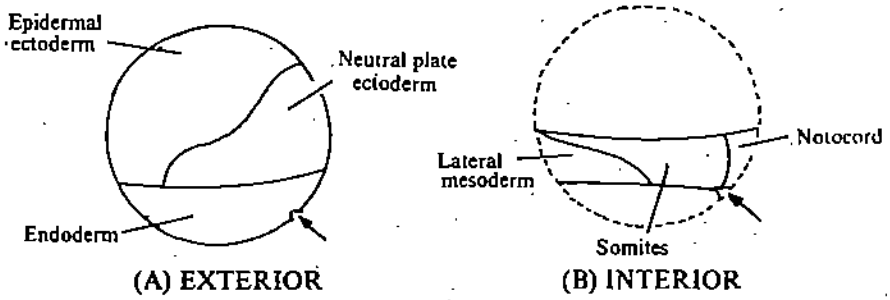


Fig. 14.24: Fate map of the embryo of the frog Xenopus leavis. Fate map for the exterior (A) and interior (B) cells of the blastula, indicating that most of the mesodermal derivatives are formed from the interior cells. The point at which the dorsal blastopore lip forms is indicated by an arrow.

groove is formed as some small endodermal cells derived from the superficial layer of gray crescent, undergo a striking change in shape and sink in (invaginate) beneath the surface. As a result of the shape change the cells become flask-like with a round bulbous inner part and an outer long narrow neck and are called "Bottle Cells" (Fig. 14.26). However, unlike the primary mesenchyme cells of sea urchin gastrula the bottle cells of amphibians remain bound to the surface via their apical ends of the narrow necks and never form pseudopodia at the inner ends. The initial groove is probably due to a coordinated contraction of the outer ends of "Bottle Cells" in a plane perpendicular to their long axis as occurs in the invaginating epithelia in general.

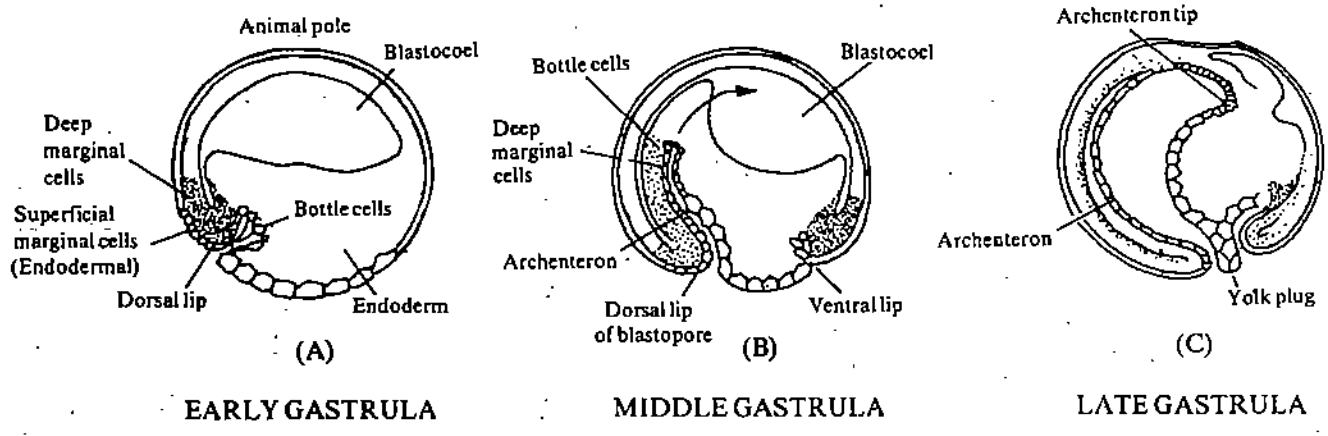


Fig. 14.25: Diagramatic representation of gastrulation in the frog Xenopus laevis.

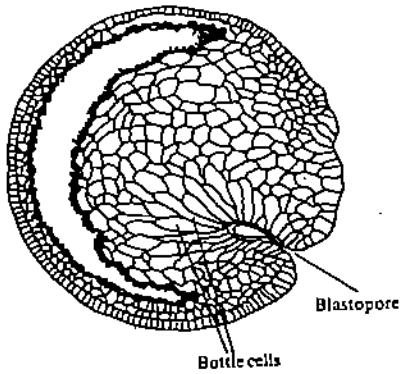


Fig. 14.26: **Diagram of cells seen in a section of gastrulating amphibian embryo, showing the extension of the bottle cells from the blastopore.**

Formation of Blastopore

The slit like opening of the initial groove is the opening of the developing blastopore and its dorsal rim is called the dorsal lip of blastopore . As gastrulation proceeds the two ends of the groove extend on either side horizontally in a wide arc along the border between the lateral marginal and vegetal zones. Ultimately they meet at the mid-line of the future ventral side of the embryo thus completing the blastopore with the formation of its lateral and ventral lips (Fig. 14.27 A). The mass of yolky endodermal cells of vegetal zone, now encircled by the four blastoporal lips, protrudes through the blastopore and is called the yolk plug (Fig. 14.27 D). Later, the lateral lips of blastopore grow towards each other over the yolky plug completely enclosing the yolky endoderm as the blastopore is reduced to a narrow vertical slit by the end of gastrulation. (Fig. 14.27 E).

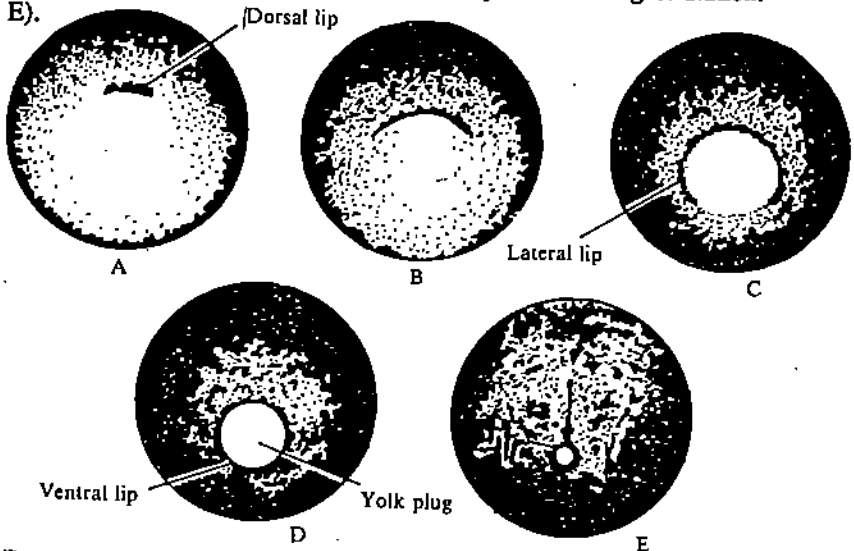


Fig. 14.27: **Formation of blastopore and epiboly of the ectoderm and changes in the region around the blastopore, as the dorsal, lateral, and ventral lips are formed in succession. When the ventral lip completes the circle, the endoderm becomes progressively internalized.**

Involution: Internalization of endoderm and formation of archenteron

The initial shallow groove is the precursor of archenteron. As the bottle cells which invaginate to form the groove leave the dorsal lip their place is taken by new groups of cells derived from the more anterior regions of the marginal zone (gray crescent). They roll over the dorsal lip (involute) and continue to migrate inward deepening the groove. They are followed by successive streams of others until all the cells of this zone have involuted over the dorsal lip and migrated inward. Among these the cells that come from the superficial layer are endodermal and they form the roof of the deepening groove which becomes the tubular archenteron. Continued inward migration of involuted cells extends the archenteron into the blastocoel anteriorly until its tip reaches upto the inner surface of the animal pole region (Fig. 14.25). Meanwhile, the endodermal cells of the lateral marginal cells also involute over the lateral lips of the blstopore and become internal.

The bottle cells are passively carried along attached to the advancing tip of archenteron (Fig. 14.28), ultimately they flatten and become part of the endodermal tissue of the pharynx.

The large yolky cells of the vegetal zone also perform slow inward streaming movements so that the cells initially situated in the vegetal zone just below the initial groove reach the anterior part of the archenteron floor and those from the vegetal pole region come to be located in its posterior part.

With the reduction of blastopore to a slit and archenteron formation all the presumptive endoderm of the blastula surface becomes internal. The endodermal cells coming in from the dorsal marginal zone by initial invagination and then by involution from the lateral marginal zone from the roof and walls of archenteron, and the yolky cell of vegetal zone form its floor.

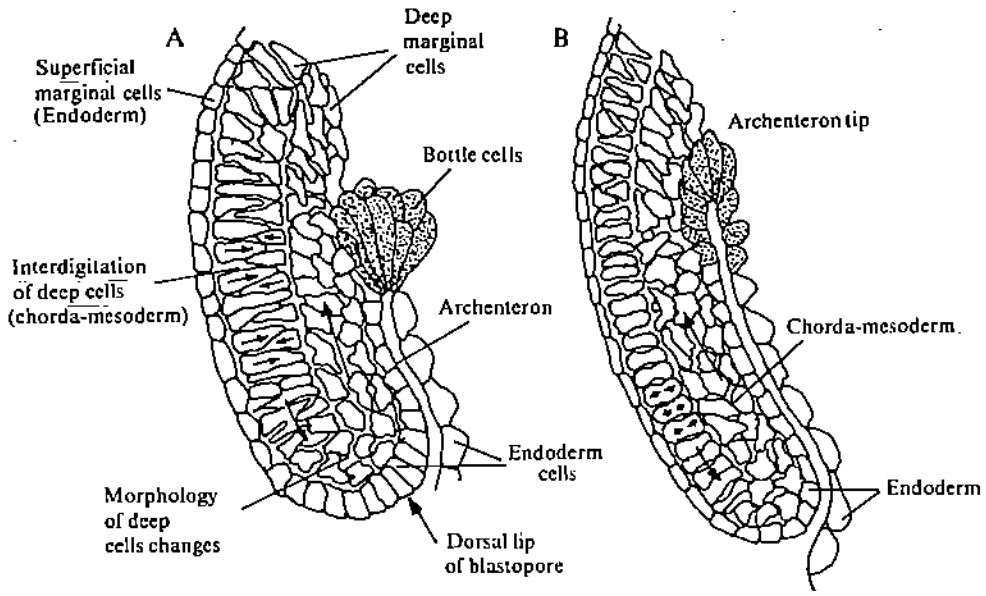


Fig. 14.28: Cell movements during gastrulation in Xenopus. (A) Early gastrulation is characterized by the interdigitation of the marginal deep layers and by involution. (B) In later gastrula, the deep marginal cells flatten and the formerly superficial cells form the wall of the archenteron. Bottle cells are darkly stippled.

As the archenteron lengthens and becomes increasingly spacious blastocoel is pushed forward reducing to a small space which is also obliterated at a later stage.

Involution: Internalization of Mesoderm

As already mentioned, in the frog **Xenopus** (and probably in other amphibian species also) the cells of presumptive mesoderm are in the deeper layers of the dorsal and lateral marginal zones beneath the superficial layer which is presumptive endodermal. The entire mesoderm is internalized by involution over the blastoporal lips. Simultaneously with the involution of endoderm of the superficial layer the chorda-mesoderm located in the dorsal marginal zone (gray crescent) involutes over the dorsal lip, the presumptive somitic mesoderm over the lateral lips and the presumptive lateral plate mesoderm over the ventral lip. Since the dorsal lip is formed first and ventral lip last the involution first begins in the region of the dorsal marginal zone followed successively in the lateral and ventral regions of the lateral marginal zone.

The mesodermal cells of deep layers first interdigitate forming a single thick layer in which cell division also occurs. The layer along with the overlying superficial endodermal layer converges towards the blastoporal lips, where the edges of the two layers break up into separate cells, which now, involute individually and move into the interior. The involuting mesodermal cells retain their deep position during and after involution. (Fig. 14.28).

The cells of the presumptive chorda-mesoderm involute over the dorsal lip in successive waves and once inside they migrate actively as streams anteriorly towards the animal

pole. The path of migration is along the mid-dorsal line between the non-involuted layer of the surface of animal zone above and the layer of involuted endodermal cells below (Fig. 14.28). The cells of first such streams reach the future head region where they form the head mesoderm. They are followed by other successive streams of involuted cell which form the notochord above the archenteron.

Internalization of the presumptive somitic and lateral plate mesoderm of the lateral marginal zone occurs in the same way. After involution over the lateral and ventral blastoporal lips respectively, these cells move in an anterior direction to form a loosely organized layer called mesodermal mantle on either side of archenteron (Figs. 14.29 and 14.30).

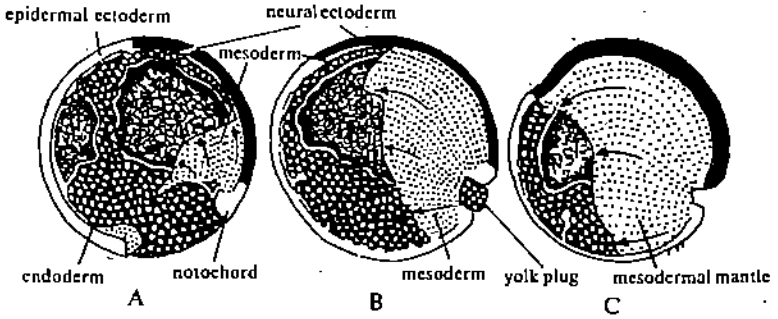


Fig. 14.29: Anterior movement of the mesoderm from the blastoporal lip area after involution in an amphibian embryo. A-early gastrula. B-Late gstrula. C-Early neurula.

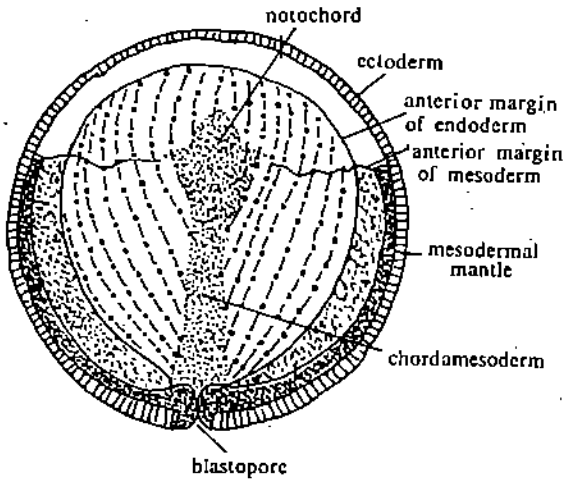


Fig. 14.30: Diagramatic view of late gastrula of frog from the dorsal side after removal of dorsal ectoderm. Mesodermal mantle is shaded. Endoderm (broken lines) is below the mesodermal mantle.

Epiboly: Expansion of Ectoderm

The presumptive ectoderm (including epidermal and neural ectoderm) in the blastula is represented by the several layers of cells in the large area in the animal zone around the animal pole (Figs. 14.12 and 14.23).

During gastrulation this area gradually extends towards the vegetal pole from the periphery of this zone and ultimately surrounds the entire embryo completely. This is brought about by epiboly which means expansion of the presumtive ectoderm as a sheet. Epiboly does not involve movement of individual separate cells. The expansion involves three processes that occur within the presumtive ectoderm. They are:

i) Increase in the number of cells in the superficial as well as deep layers.

ii) Integration of all layers into a single one cell thick layer

iii) Flattening and stretching of cells.

Combined result of the three processes is progressive increase in the surface area of the ectodermal layer which thus extends and occupies the space on the surface vacated by the marginal zone cells as they move towards blastoporal lips, involute and disappear into the interior.

Epiboly of ectoderm is very clearly seen in the frog embryo in which the animal zone is deeply pigmented, marginal zone is grayish and vegetal zone is white. As gastrulation proceeds the pigmented area gradually shifts in the vegetal direction. By the yolk plug stage it extends upto all the four blastoporal lips. By continued expansion vegetally the pigmented ectoderm ultimately encloses the white large yolk endodermal cells of the vegetal zone also and the blastopore is reduced to a vertical slit (Fig. 14.27).

Major features of amphibian gastrulation are:

i) Gastrulation begins on the future dorsal surface of the embryo.

ii) Gastrulation is initiated by a limited invagination of endodermal cells.

iii) Formation of archenteron is completed as a result of involution of endoderm.

iv) Mesoderm and most endoderm are internalized by involution over dorsal, lateral and ventral lips of blastopore.

v) Ectoderm surrounds the embryo by epiboly.

vi) Blastocoel is finally obliterated.

SAQ 7

i) Fill in the blanks:

a) According to Keller presumptive mesoderm in xenopus blastula is represented by ..... cells in the ..... zone.

b) Gastrulation in amphibians begins on the future ..... side and not at the ..... pole because of large quantity of ..... .

c) Initially invaginating cells in amphibian gastrula change ..... to become ..... .

d) During gastrulation in amphibians internalization of mesoderm occurs by ..... over ....., ... and ..... lips of ..... .

e) In amphibian gastrula archenteron formation involves initial limited ..... and subsequent ..... of endoderm.

ii) List the three processes involved in expansion of ectoderm during epiboly.

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iii) Describe the formation of blastopore in gastulating amphibian embryos.

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iii) Gastrulation in Amniotes

You have already learnt that among the amniotes the reptiles, birds and monotremes (egg laying mammals) have very yolky (macrolecithal) and highly telolecithal eggs.

Meroblastic cleavage in such eggs is restricted to a small disc of cytoplasm (blastodisc) at the animal pole. It results in the formation of a disc-shaped blastula (discoidal blastula or simply discoblastula) lying on top of the undivided yolk. In such a blastula the inert yolk-mass imposes severe restrictions on cell movements during gastrulation so that invagination is inhibited and an archenteron is not formed. Consequently, the method of gastrulation in these animals is different from that which you have studied in the case of sea urchin and frog. Among the amniotes the viviparous higher mammals including marsupials and eutherians have secondarily lost the yolk and have alecithal (yolk less) eggs but they have retained the same method of gastrulation as followed by

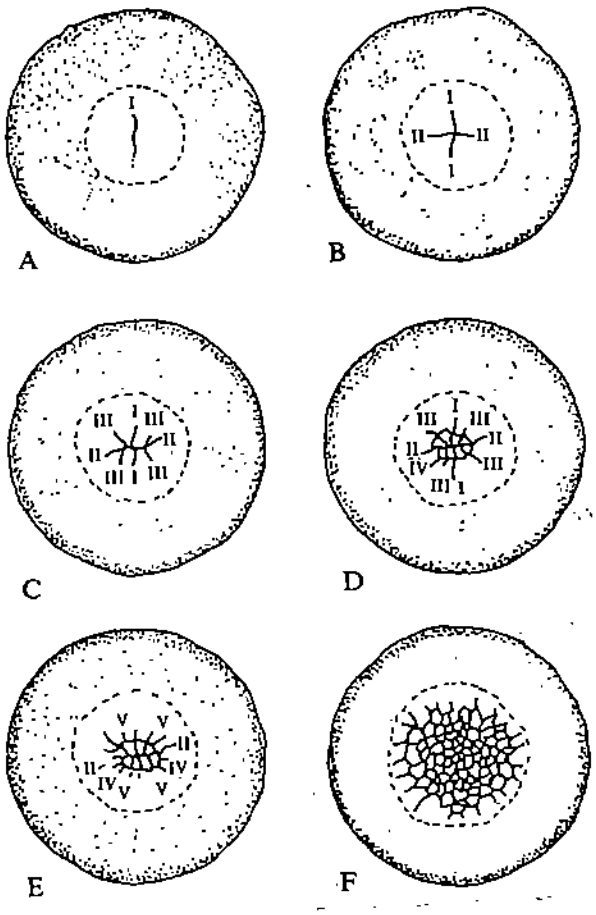

Fig. 14.31: Schematic diagrams of various stages of cleavage in the blastodisc of fertilized chick egg. Cleavage furrows do not penetrate the yolk and a disc-like blastoderm of single layer of cell (F) is formed on top of the yolk. Roman numerals indicate the order in which cleavage furrows appear.

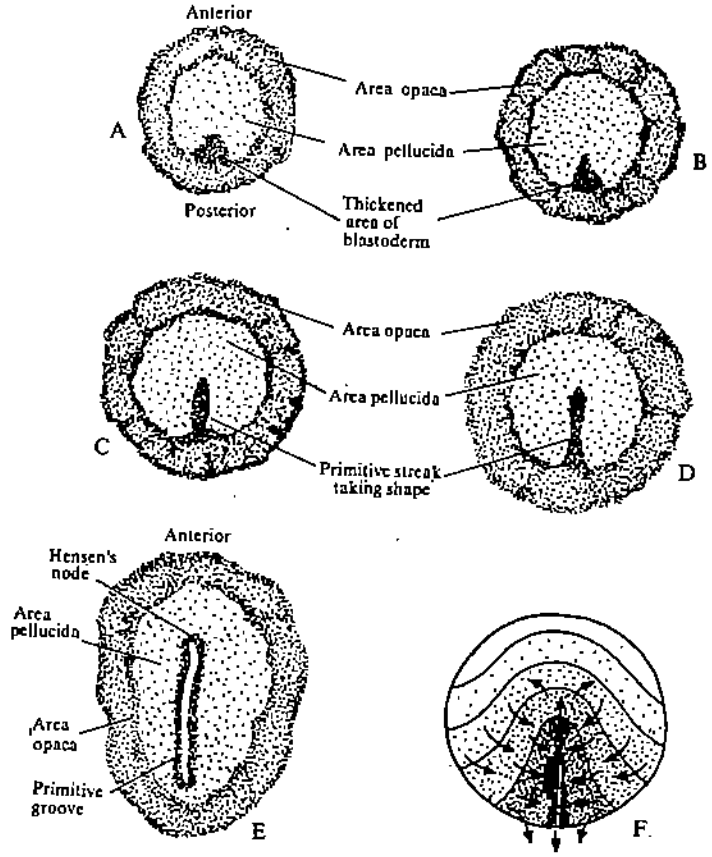

Fig. 14.32: Cell movements forming the primitive streak of the chick embryo. Dorsal view of chick blastoderm at (A) 3-4 hours, (B) 5-6 hours, (C) 7-8 hours, (D) 10-12 hours, (E) 15-16 hours. A summary of these cell movements is shown in (F).

the yolky embryos of reptiles and birds. In this section we will describe gastrulation in a bird (chick) embryo as an example of gastrulation in the egg laying amniotes followed by a description of gastrulation in the eutherian mammals. You will notice that while there are many differences between gastrulation in amniotes and gastrulation in sea urchin and frog there are also many similarities.

A) Gastrulation in Check

Cleavage in the fertilised egg takes place during its passage through the oviduct to cloaca of the hen (Fig. 14.31). By the time it is laid meroblastic cleavage has resulted in the formation of a several layered disc-like blastoderm (discoblastula) in which two distinct regions can be recognised: i) the clear central circular **area pellucida** separated from the underlying yolk by a space called the **sub-germinal space** or cavity and ii) the peripheral **area opaca** in which the cells of the lower layer are in intimate contact with the yolk making this region dark and opaque. The inner border of area opaca immediately adjoining the area pellucida is the **marginal zone** and its remaining peripheral part is called the **germ wall.** A thickened region of marginal zone due to great density of cells indicates the future posterior side of the embryo (Figs. 14.31, 14.32, A).

In chick, gastrulation is a prolonged process. It is initiated soon after the beginning of incubation after laying of the egg and is completed in about 4 days. The actual process of gastrulation is preceded by some pre-gastrular movements of certain cells resulting in their separation from the blastoderm and formation of a lower layer called the **hypoblast.** Most of the cells, however, remain in the upper layers of the blstoderm which now constitute the **epiblast.** (Fig. 14.33).

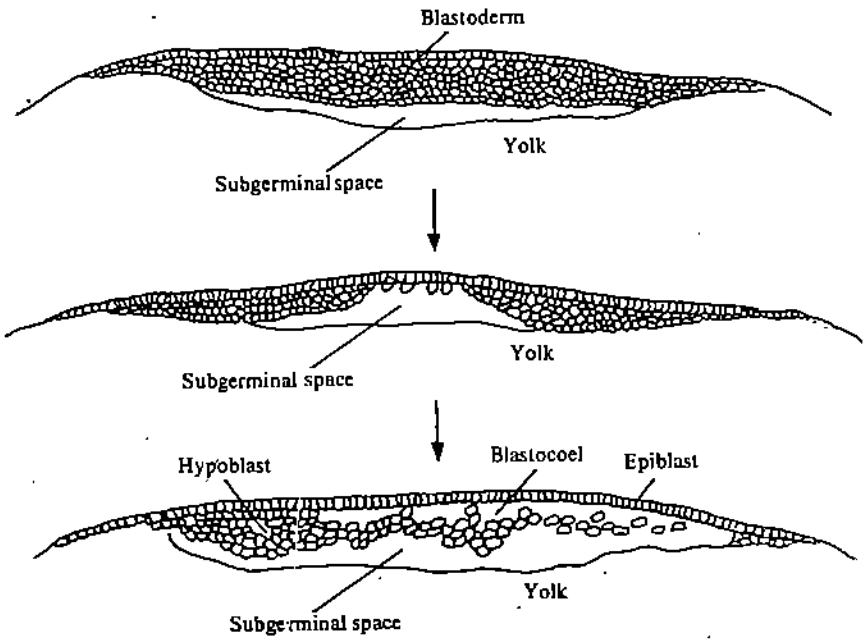

Fig. 14.33: Formation of the hypoblast in the avian egg.

Formation and Role of Hypoblast

Hypoblast is formed as a result of two processes. First, some cells individually leave the blastoderm and move down by polyinvagination into the sub-germinal space to form a thin layer. Soon thereafter this layer is joined by a sheet of cells which separates from the posterior marginal one of the blastoderm and moves anteriorly in the sub-germinal cavity. The hypoblast thus formed expands and spreads peripherally to give rise to a complete thin layers below the remaining part of blastoderm now called the epiblast. The hypoblast and epiblast are joined together at the margin of area opaca and the space between them is the blastocoel. The structure of the embryo is now somewhat similar to that of the frog blastula but the hypoblast is not the precursor of either ectoderm, mesoderm or endoderm. It is later displaced by endodermal cells derived from epiblast.

Hypoblast contributes cells for parts of some extra-embryonic membranes but none at all for the formation of the body of the embryo. However, the hypoblast has an important role in development of the embryo. Its removal at an early stage stops all further development until a new hypoblast is regenerated from the epiblast. Hypoblast induces the formation of the primary embryonic axis (the primitive streak) in the epiblast and determines its orientation.

Fate Map

All the three primary germinal layers (ectoderm, mesoderm, endoderm) are formed from cells initially located in the epiblast within area pellucida. Therefore, the entire body of chick and most parts of the extra-embryonic membranes (yolk sac, amnion, chorion, allantois) are developed from cells derived from the epiblast. Hence, the fate maps constructed for the blastoderm of chick embryo refer to the various presumptive organ forming areas in the epiblast only. A generalised fate map for the blastoderm in the area pellucida is presented in Fig. 14.13. This map is based on the results of a number of studies by different workers using a variety of **in ovo** and **in vitro** techniques including staining with vital dyes, marking with carbon particles, labelling with tritiated thymidine and subsequent autoradiography, grafting and transplantation of specific areas etc. The map shows the location and the extent of different areas in the blastoderm (epiblast) from where the presumptive cells are derived for the three germinal layers: i) Ectoderm (including epidermal, neural and extra-embryonic ectoderm) ii) Mesoderm (including mesoderm for notochord, head, somites, lateral plate and extra-embryonic mesoderm) and iii) Endoderm (including gut and extra-embryonic yolk sac endoderm).

As a result of morphogenetic movements during gastrulation the cells from the various areas reach their respective specific destinations forming the ectoderm on the surface, endoderm below and mesoderm between the two.

The Gastrulation Process: Formation of Primitive Streak

Gastrulation in all amniotes including eutherian mammals is related to a characteristic structure called the **primitive streak** formed on the epiblast surface during the first 10-18 hours of incubation at 38.5°C temperature (Fig. 14.34). It forms as a result of convergence of epiblast cells to the dorsal midline of the blastoderm. The beginning of primitive streak formation is first indicated by a thickening in the central posterior region of area pellucida immediately after the formation of hypoblast. The thickening narrows and elongates growing anteriorly in the centre of area pellucida. When fully formed the primitive streak is a narrow structure with a groove (Primitive groove) in its floor along its length flanked by a fold (or ridge) on either side. It extends anteriorly upon about three fourth the length of area pellucida where it ends in a deep pit called **Hensen's Node** with thick borders (Fig. 14.34).

During the formation of primitive streak the shape of area pellucida gradually changes from circular to pea shaped with the broad side anterior and narrow side posterior. This change is due to convergence of cells toward dorsal midline beginning at posterior end and progressing anteriorly but stopping where the Hensen's Node is formed. With reference to the fate map (Fig. 14.13) it should be noted that the posterior end of the primitive streak would be in the centre of the posterior edge of the area of presumptive extra-embryonic mesoderm, and its anterior end (Hensen's Node) would be located within the presumptive endodermal area posterior to notochordal area. Primitive streak marks the median anterior-posterior axis of the embryo and establishes bilateral symmetry (Fig. 14.34).

Movements of Epiblast Cells

The cells for the presumptive mesodermal and endodermal organ areas pass from the epiblast into the blastocoel below through the Hensen's Node or primitive groove of the primitive streak. This occurs by the cells sheets converging toward the Hensen's Node or the streak. On reaching there the cells change shape becoming "bottle cells", the sheet breaks up into separate cells which pass into the blastocoel individually by involution through the Hensen's Node or the primitive streak. Once inside the blastocoel the cells flatten and continue to migrate as streams of loosely connected mesenchyme vertically down or laterally and anteriorly.

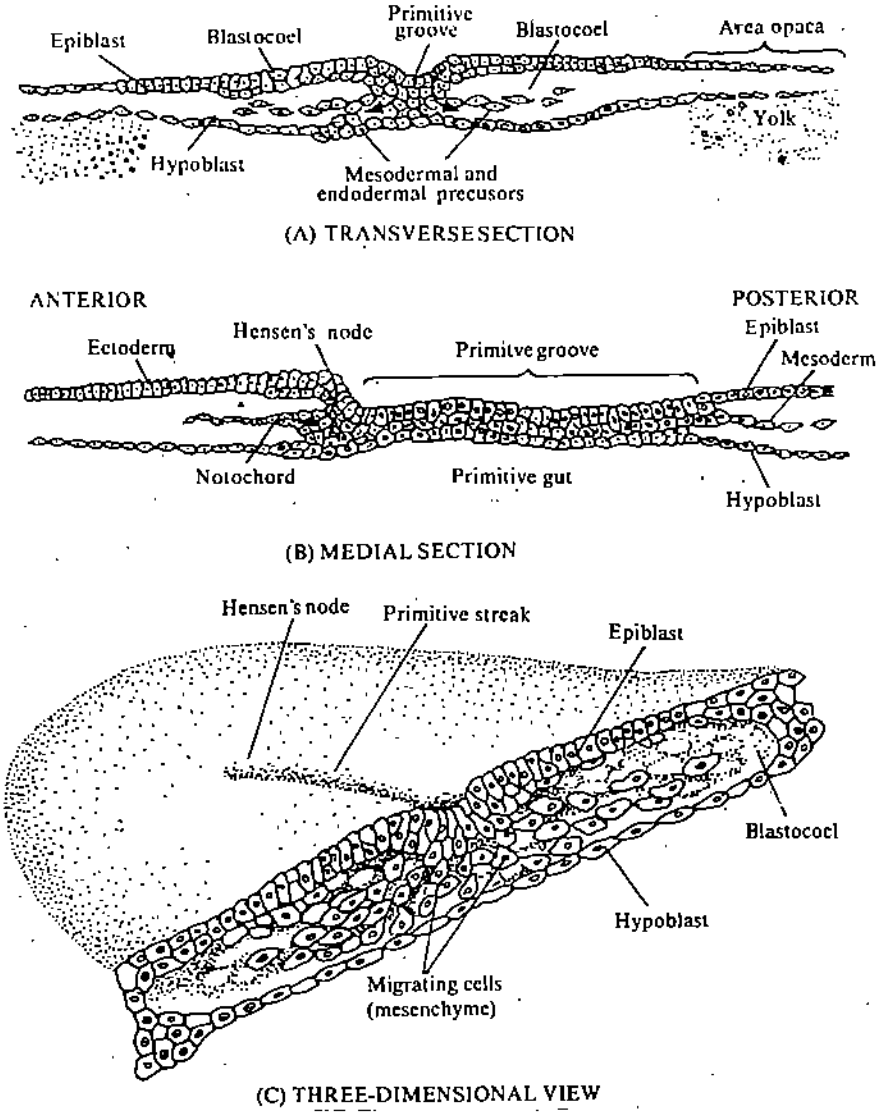

Fig. 14.34: Migration of endodermal and mesodermal cells through the primitive streak. (A) Diagram of transverse section through a 17-hour embryo, illustrating the lateral movement of endodermal and mesodermal cells passing into the blastocoel. (B) Diagram of a medial section through the same embryo, showing that those cells migrating through Hensen's node condense to form the notochord (head process). (C) Stereogram of a gastrulating chick embryo, showing the relationship of the primitive streak, the migrating cells, and the two original layers (epiblast and hypoblast) of the blastoderm.

The first cells to enter blastocoel are those of the presumptive foregut endoderm located immediately around the Hensen's Node through which they move in. On entering the blastocoel they move anteriorly and ventrally and displace the cells of the anterior part of hypoblast. Later, infolding of this endodermal layer forms the foregut.

Next, the cell of presumptive chorda-mesoderm migrate into the blastocoel also through the node and then move anteriorly in the mid line just below the overlying epiblast to form the head mesoderm and anterior part of notochord called the **head process**. Later, the remaining endodermal and mesodermal cells of the epiblast migrate through the anterior and posterior regions of the streak, respectively. Once inside the blastocoel they form two streams of migrating cells. One stream contains the endodermal cells which move down into the hypoblast, displacing it and forming a continuous sheet with that of the foregut endoderm. The other stream contains cells of presumptive somite and lateral plate mesoderm. They remain within the blastocoel, move laterally and anteriorly to take up positions on either side of the forming notochord as a loose sheet of mesoderm between the epiblast and hypoblast.

The movement of cells within the blastocoel is facilitated by hyaluronic acid secreted by the ectodermal cells of epiblast. This polysaccharide accumulates in the blastocoel and coats the surface of the incoming cells which keeps them separate allowing their migration as individual cells.

Regression of Primitive Streak

As the inward migration of cells through the primitive streak ends progressively along its anterior to posterior regions the streak gradually regresses in the same direction. As the streak shortens the Hensen's Node, which forms its anterior end, also moves in a posterior direction (Fig. 14.35). Simultaneously, the presumptive notochordal cells still remaining in the epiblast migrate inward through the posteriorly moving node adding to the length of the notochord. The regressing streak thus leaves behind in its path the dorsal axis of the embryo represented by the posteriorly lengthening notochord (Fig. 14.35). By the time the streak disappears and the Hensen's Node arrives at the final position at the posterior border of area pellucida all the presumptive endodermal and mesodermal cells have left the epiblast which now consists of only the ectodermal cells. Regression of streak begins after about 22 hours of incubation and is completed in the next about 20 hours. It should be noted that unlike amphibian gastrulation differentiation of axial structures and some organs such as neural tube, somites, foregut, heart, occurs cephalo-caudally along with the progress of later stages of gastrulation in chick embryos (Fig. 14.36).

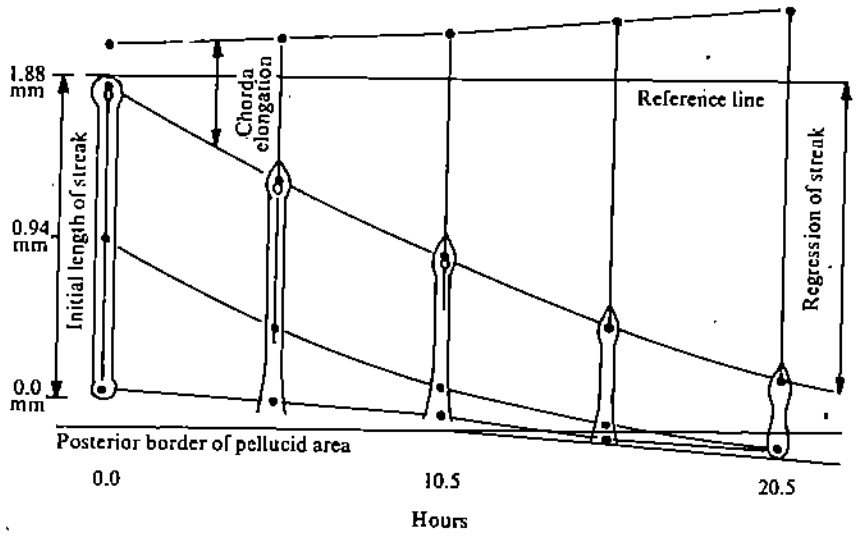

Fig. 14.35: Regression of the primitive streak, leaving notochord in its wake. Time represents hours after achieving maximum length by the primitive streak.

Epiboly of Ectoderm

As the presumptive endodermal, notochordal and mesodermal cells migrate inward the areas in the epiblast vacated by them are occupied by expansion (epiboly) of the embryonic epidermal and neural ectoderm. Simultaneously with gastrulation the ectodermal cells expand as a sheet beneath the vitelline membrane outward from the area opaca ultimately encircling the yolk. The upper surfaces of ectodermal cells of area opaca are in firm contact with the inner surface of vitelline membrane and they spread along this inner surface of the membrane. Also, these cells extend pseudopodial processes which attach to inner side of the vitelline membrane. It appears that these marginal cells of area opaca migrate outward along the inner surface of the vitelline membrane with the help of these pseudopodia and at the same time drag along the expanding sheet of ectodermal cells of which they are the leading members. Removal of the vitelline membrane inhibits epibolic movements and expansion of ectoderm.

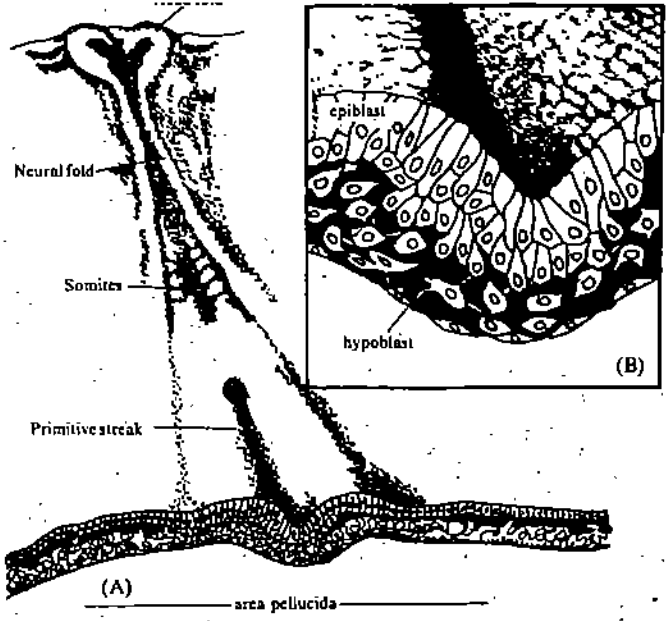

Fig. 14.36: (A) Gastrulating chick embryo of 3 pairs of somites transected about midway along the regressing primitive streak. (B) Enlargement of section to show cells from epiblast migrating through primitive streak inward. Note differentiation occurring in the cephalic region while gastrulation still occurring in the posterior region.

Characteristic Features of Avian Gastrulation

i) Formation of hypoblast and its important role in formation of the axis and orientation of embryo.

ii) Presence of the cells of all the three germinal areas in the epiblast.

iii) Formation of the primitive streak and its regression in later stages of gastrulation.

iv) Absence of archenteron formation.

v) Gastrular cell movements include polyinvagination, involution, convergence, divergence and epiboly.

vi) Cephalo-caudal differentiation of axial structures and growth of the embryo during gastrulation.

Comparison with Amphibian Gastrulation

On comparison of chick gastrulation with amphibian gastrulation you will find many similarities along with important differences. Primitive streak is analogous to the blastopore although there is no actual pore. The Hensen's Node represents the dorsal lip and the folds on the lateral sides of primitive groove represent the lateral lips. However, there is no ventral lip. As in amphibians, the prechordal endoderm and chorda mesoderm of chick migrate inward through the Hensen's Node (dorsal lip) and the remaining endoderm and mesoderm do so by involution over the lateral folds of the streak (lateral lips). The topographic locations of the presumptive ectodermal, chordamesodermal; mesodermal and endodermal areas relative to each other in the epiblast are nearly the same as on the surface of amphibian blastula. As in amphibians, the involuting mesodermal and endodermal cells change shape to become bottle cells in the gastrulating chick embryos also.

SAQ 8

i) List the types of morphogenetic movements of cells that occur during gastrulation in chick embryos.

ii) What is the role of hypoblast in chick embryos?

iii) Cells of which presumptive germinal layers are constituents of the epiblast in chick embryo.

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iv) How do mesodermal and endodermal cells migrate inward?

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v) What structures are formed from cells of areas opaca?

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vi) List similarities between gastrulation in chick and amphibians.

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Gastrulation in Mammals (especially eutherians)

With the disappearance of the yolk, mammalian eggs have reverted to complete (holoblastic) cleavage, but subsequent development bears ample evidence of the former presence of yolk, and in many respects the morphegenetic processes resemble those in meroblastic eggs with a discoidal type of cleavage.

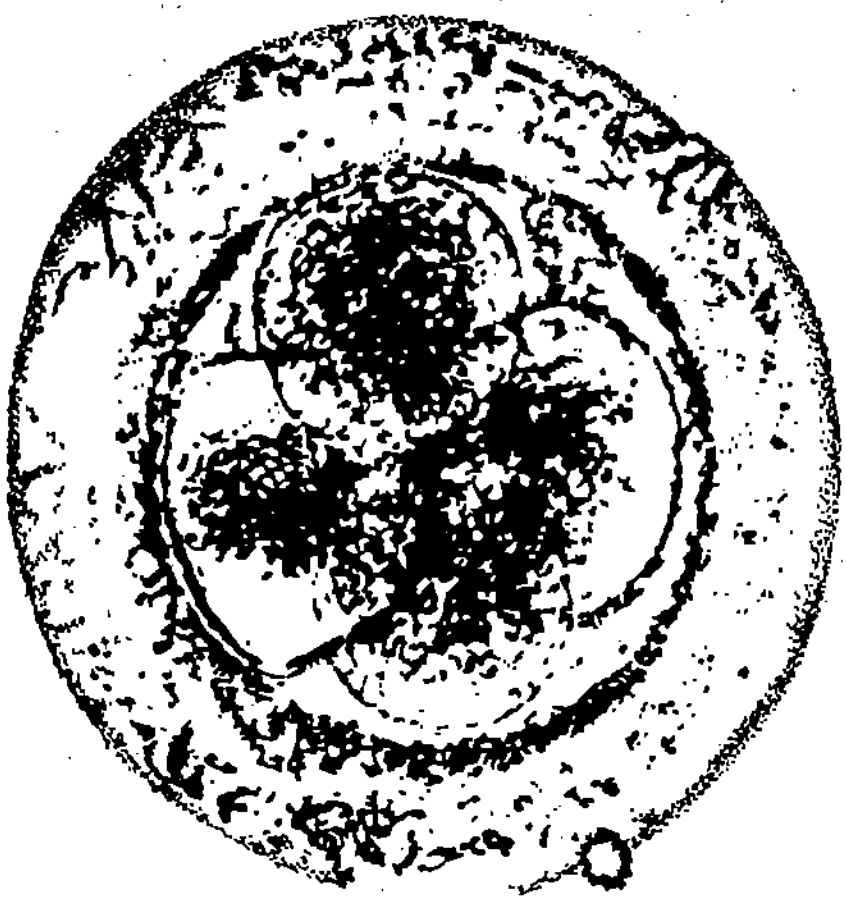

Fig. 14.37: Human egg. Cleaving: stage of four blastomeres. Numerous spermatozoa outside of zona pellucida.

Cleavage and Blastocyst

Although the cleavage is complete and all the blastomeres are of more or less equal size (Fig. 14.37), synchronization of mitoses in the blastomeres is lost very early. Even the first two blastomeres may cleave at different rates; consequently, a three cell stage and subsequently stages of five, six, seven blastomeres and so forth are found (Fig. 14.38). The result of the cleavage is a solid mass of cells, a morula, in which some cells are

superficial and others lie inside, completely cut off from the surface by the enveloping cells (Fig. 14.39). In due course the superficial cells join to form a distinct epithelial layer. This layer gives rise to most of the extraembryonic parts (the embryonic membranes), serves to attach the embryo to the uterine wall, and mediates in the supply of nourishment to the embryo from the maternal body via the placenta. This outer layer of the mammalian embryo is known as the trophoblast (the term trophe meaning nourishment). The cells lying in the interior are known as the inner cell mass (ICM), and it is these cells which provide material for the formation of the embryo proper. They may, therefore, be referred to as the formative cells. Sooner or later a cavity appears inside the compact mass of cells of the morula. The cavity is formed of crevices which appear between the inner cell mass and the cells of the trophoblast. Fluid is imbided into this cavity, so that it enlarges. The trophoblast becomes lifted off most of the inner cell mass remaining attached to it on one side only. This side corresponds later to the dorsal side of the embryo. A mammalian embryo at this stage is called a blastocyst (Fig. 14.40)

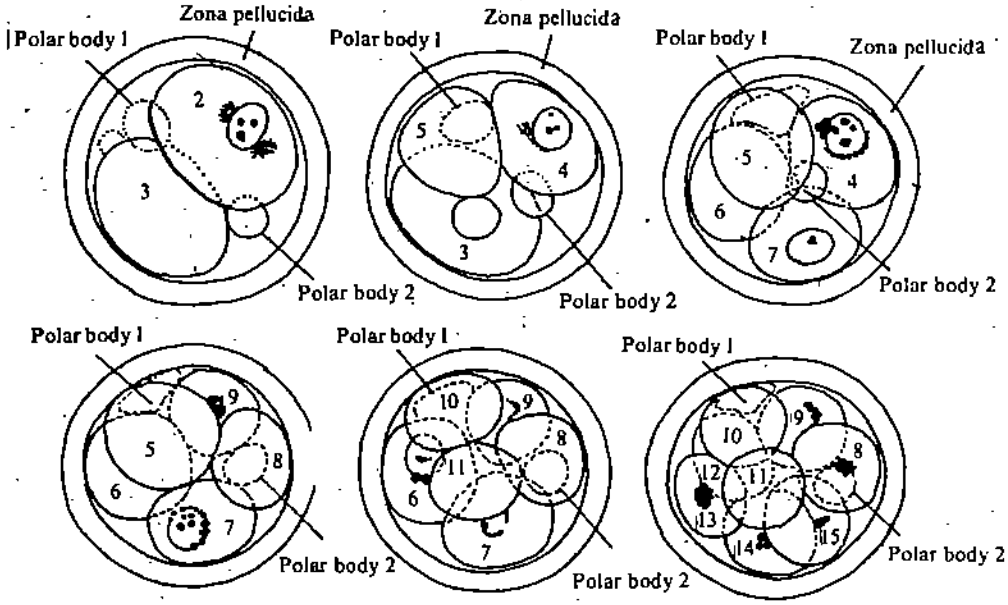

Fig. 14.38: Cleavage of the egg of a monkey (Macacus rhesus).

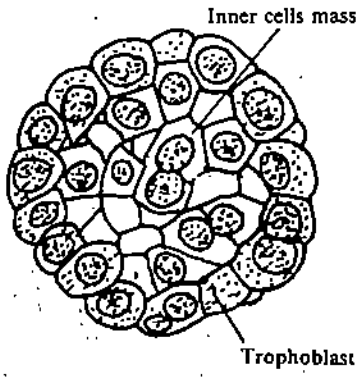

Fig. 14.39: Morula of rat.

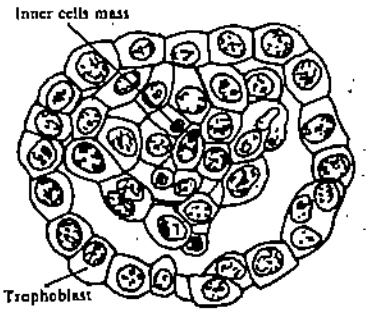

Fig. 14.40: An early blastocyst of a bat, showing differentiation into a inner cell mass and the trophoblast.

Formation of Germinal layers

The cavity of the blastocyst may be compared to the blastocoel, but the embryo as a whole differs from a blastula, since its cells are already differentiated into two types: the inner cell mass and the cells of the trophoblast. The first segregation of cells within the inner cell mass involves the formation of the Hypoblast (sometimes called the primitive endoderm) layer (Fig. 14.41). These cells separate from the inner cell mass to line the blastocoel cavity, where they give rise to the yolk sac endoderm. As in avian embryos,

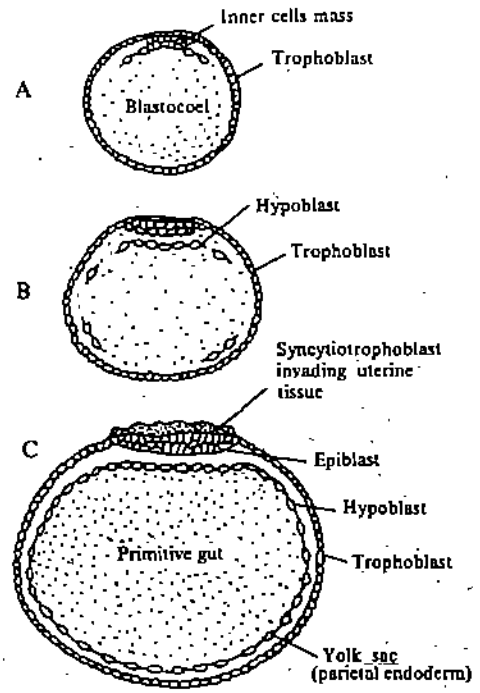

Fig. 14.41: Mammalian blastocyst immediately prior to gastrulation. The inner cell mass delaminates hypoblast cells that line the trophoblast, thereby forming the primitive gut (A—C). The remaining inner cell mass constitutes the epiblast.

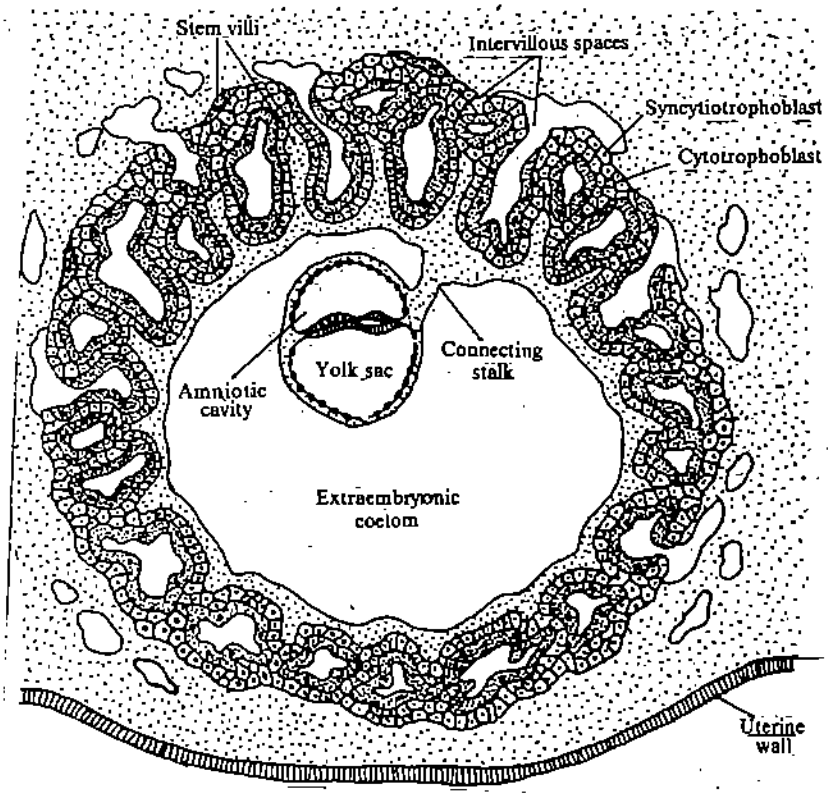

Fig. 14.42: Diagram of gastrulating human embryo at the end of the third week of gestation. The trophoblast cells forming the placenta are coming into contact with the blood vessels of the uterus. The embryo is connected to the trophoblast by a connecting stalk of extraembryonic mesoderm that will shortly carry the fetal blood vessels to the placenta.

these cells do not produce any part of the newborn organism. The remaining inner cell mass tissue above the hypoblast is now referred to as the epiblast. The epiblast cells are split by small clefts that eventually coalesce to divide the epiblast into two lyers. One of the layers is the embryonic epiblast and the cells of the other layer form the lining of the Amnion (Fig. 14.42). Once the lining of the epiblast amnion is completed, it fills with a secretion called Amniotic fluid, which serves as a "shock absorber" to the developing embryo and also prevents its dessication.

The embryonic epiblast is believed to contain all the cells that will generate the actual embryo. It is similar to the avian epiblast. At the posterior margin of the embryonic epiblast a localized thickening occurs, eventually producing a primitive streak through which the endodermal and mesodermal precursors migrate (Fig. 14.43). As in avian embryos, the cells migrating between the hypoblast and epiblast layers appear to be coated with hyaluronic acid, which is first synthesized at the time of primitive streak formation.

While the embryonic epiblast is undergoing cell movements, the extra-embryonic cells are making the distinctly mammalian tissues that enable the fetus to survive in the maternal uterus. Although the initial trophoblastic cells appear normal they give rise to a population of cells wherein nuclear division occurs in the absence of cytokinesis. The first type of cells constitute a layer called the **cytotrophoblast**, whereas the second type of cells form the **syncytiotrophoblast**. This latter tissue invades the uterine lining, embedding the embryo within the uterus. The uterus, in turn, sends blood vessels into this area, where they eventually contact the syncytiotrophoblast. Shortly thereafter mesodermal tissue extends outward from the gastrulating embryo (Fig. 14.42). It has been shown that this tissue had migrated through the primitive streak but becomes extraembryonic rather than embryonic mesoderm and joins the trophoblastic extensions. This extraembryonic mesoderm gives rise to the blood vessels that carry the nutrients from the mother to the embryo. The narrow connecting stalk of the extraembryonic mesoderm that connects embryo to the trophoblast eventually forms the vessels of the umbilical cord. The fully developed organ, consisting of trophoblast tissue and blood vessel containing mesoderm is called the **chorion** which fuses with uterine wall to form the placenta. The chorion may be very closely opposed to maternal uterine tissues while still being readily separable (as in the contact placenta of the pig) or it may be so intimately integrated that the two tissues cannot be separated without damage to both the mother and the developing fetus (as in the Deciduous placenta of most mammals, including humans).

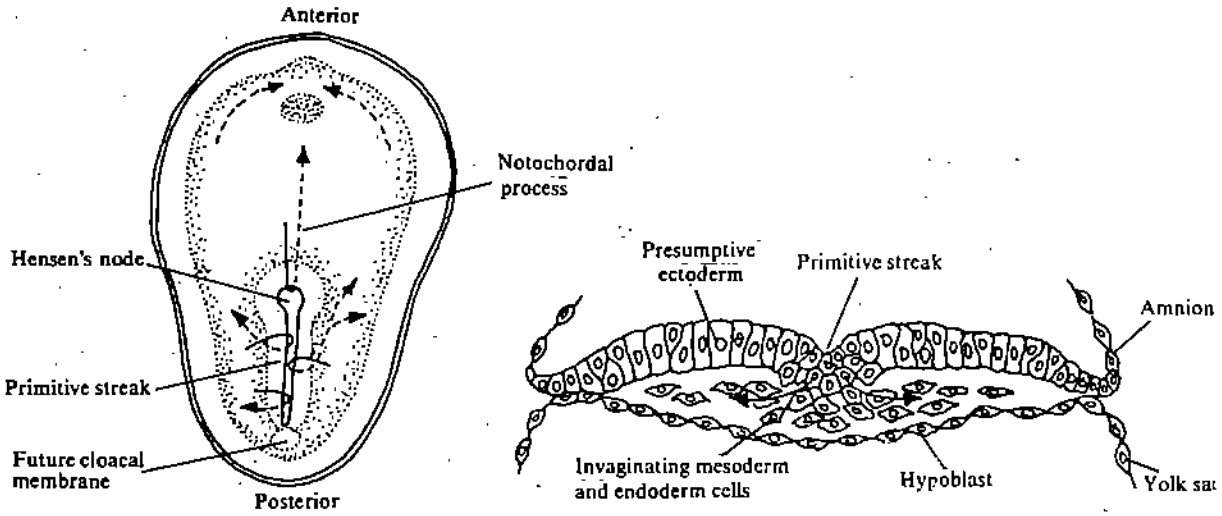

Fig. 14.43: Cell movements during mammalian gastrulation. (A) Schematic diagram showing the dorsal surface of the embryonic epiblast (amniotic ectoderm removed). As in chick embryos, cells migrating through Hensen's node travel anteriorly (cephalad) to form the notochord, while the remaining cells travelling through the streak migrate laterally to become the mesoderm and endoderm precursors. Dotted lines indicate internal migrations. (B) Transverse section of the embryo showing inward migration of presumptive mesodermal and endodermal cells through the primitive streak.

SAQ 9

i) Describe the formation of mammalian blastocyst.

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ii) What are the fates of trophoblast and Inner Cell Mass?

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iii) What is the source of hypoblast cells in mammalian embryos?

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iv) In what features gastrulation in mammals resembles gastrulation in chick?

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v) What is the embryological basis of the idea that both birds and mammals may have evolved from reptilian ancestors?

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14.4 SUMMARY

- Cleavage transforms the unicellular zygote into a multicellular structure. The divisions during cleavage are essentially mitotic but they lack G_1 and G_2 phases (or collectively interphase). The important characteristics of cleavage are synchronization, little or no displacement of cytoplasmic substances, no change in the embryonic shape and considerably high nuclear/cytoplasmic ratio.
- The amount of yolk determines the structure of the egg which may be alecithal (eutherian mammals), microlecithal (Amphioxus), mesolecithal (amphibians) and macrolecithal (bony fishes, cephalopod molluscs, reptiles birds and egg laying mammals) depending upon the amount of the yolk; or isolecithal (sea urchin), teloecithal (amphibian, reptiles, aves, and egg laying mammals) and centrolecithal (insects) depending on the placement of the yolk materials within the egg.
- The amount of yolk influences the size of egg, passage of cleavage furrows, rate of cleavage as well as the size of resultant blastomeres.
- The general shape of the egg is spherical or nearly spherical (except in insects). Hence, the planes of cleavage are meriodional, vertical, equatorial or latitudinal.
- Based on the arrangement of the blastomeres, the cleavage may be described as radial, spiral or bilateral. According to whether cleavage furrows divide the egg completely or partially, the cleavage is either holoblastic or meroblastic or superficial.
- Cleavage produces morula which is converted into blastula or a blastula-like structure. The organization of blastula is dependent on the amount of yolk originally deposited in the egg.
- The amount of yolk influences the whole course of development and structure of embryonic stages. Such an influence continues even during subsequent stages of gastrulation.

- The fate maps of various groups of blastomeres are constructed by different methods — natural pigmentation, vital dye staining technique, marking with carbon particles and by the use of radioactive traces etc.
- Rearrangement of various groups of blastomeres is brought about during gastrulation which is a dynamic process, i.e. the blastomeres and their derivatives actually move from one place to another.
- Gastrulation is effected by many morphogenetic movements of blastomeres — epiboly e.g. cell proliferation, thinning out, spreading, expansion and extension of ectoderm and emboly e.g. invagination, involution, convergence, divergence, concrescence, delamination, infilteration, ingression and polyinvagination of mesoderm and endoderm.
- Gastrulation in sea urchin is a simple process involving polyinvagination of primary mesenchymal cells from the surface of the blastula at the vegetal pole into blastocoel followed by initial invagination of endoderm to form the archenteron and formation of the blastopore.
- Gastrulation in amphibians begins with invagination of endoderm on the dorsal side to initiate the formation of archenteron. Mesoderm and endoderm are internalized by involution over the dorsal, lateral and ventral lips of the blastopore. Ectoderm covers the embryo on the outside by epiboly.
- During the gastrulation of aves (also in the reptiles, egg laying mammals), the primitive streak provides the passage for the movement of presumptive notochordal, mesodermal as well as endodermal cells. Hence the primitive streak may be called the closed blastopore.
- In eutherian mammals although the egg is small and alecithal and cleavage is holoblastic, the process of gastrulation is essentially similar to that in birds.
- Similarities in the early ontogenic development of the reptiles, birds and egg laying mammals on one hand and metatherian as well as eutherian mammals on the other, provide strong evidence to support the idea that all amniotes share a common ancestry.

14.5 TERMINAL QUESTIONS

1. If the alecithal egg of a eutherian mammal is filled with extremely large amount of yolk and enclosed inside a hard porous shell will the following statements qualify such an imaginary situation (put 'T' for true statement and 'F' for false ones):
   a) It will behave as the macrolecithal egg of reptiles, birds and egg laying mammals. ( )
   b) It will behave as the macrolecithal eggs of bony fishes or cephalopod molluscs. ( )
   c) Such a situation is impossible in case of eutherian mammals according to the law of irreversibility of evolution. ( )
   d) It will surpass the limits of general purview of amniote ontogeny. ( )
2. What are the important factors governing the early ontogenetic development (embryogenesis) of an animal:
   a) .....
   b) .....
   c) .....
3. Write 'T' for true statement and 'F' for false statement:
   a) Embryo is not a living entity. It becomes alive at the moment of hatching or birth as the case may be. ( )

b) Different embryonic stages are the different phenotypic expressions of the same genome at various stages of ontogenetic development. ( )

c) Because of dissimilarities in cleavage, it is artificial to club reptiles birds, monotremes and eutherian mammals as 'Amniota'. ( )

d) The amount of yolk in the egg may determine the events of hatching or birth. ( )

4. Define the following terms:

a) Blastoderm

b) Morphogenetic movements

c) Meroblastic cleavage

d) Fate maps

e) Coeloblastula

f) Epiblast

g) Inner cell mass.

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5. What is the fate of presumptive notochordal area of blastula of the vertebrate in the adult stage?

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14.6 ANSWERS

Self Assessment Questions

SAQ 1: i) mitotic cell division

ii) cytoplasmic growth or G_1 and G_2 phases

iii) mesolecithal, telophase

iv) large

v) spindle, density.

SAQ 2: i) meridional, vertical, equatorial, latitudinal

ii) macrolecithal, centrolecithal, centrolecithal, superficial, superficial, cytoplasm, centrally, cytoplasm.

SAQ 3: i) In eutherian mammals, the alecithal eggs are secondarily derived from ancestral macrolecithal eggs of reptilean ancestor. Therefore, it tries to behaves (recapitulate) the ancestral types.

ii)

Animals	Types of cleavage	Types of blastula
a), c), f), g)	— Radial, holoblastic	Coeloblastula discoblastula
b),	— rotational holoblastic	blastocyst
d)	— discoidal meroblastic	discoblastula
e)	— radial holoblastic (unequal)	coeloblastula

h)	—	radial holoblastic (equal)	coeloblastula
i)	—	spiral	stereoblastula

SAQ 4: For (i) refer to Subsection 14.2.5

ii) a) microtubules b) microfilaments

c) microtubules.

iii) a) Arrests karyokiness

b) Inhibits cytokinesis.

SAQ 5: i) dynamic, migration, blastula, organization, undivided egg, gastrulation, transformed, three, gastrula.

ii) refer to Subsection 14.3.2.

iii) refer to Subsection 14.3.1.

SAQ 6: i) a) flattening, blastomeres, vegetal

b) primary mesenchyme, endoderm

c) blastocoel, ingression

d) invagination, vegetal

e) endoderm, archenteron

ii) Refer to subsection 14.3.3-(i).

SAQ 7: i) a) deep, intermediate or marginal zone

b) dorsal, vegetal, yolk

c) shape, bottle cells

d) involution, dorsal, lateral, ventral, blastopore

e) invagination, involution

ii) Increase in cell number, integration of all cells into one cell thick sheet, flattening and stretching of cells.

iii) Refer to subsection 14.3.3–(ii)

SAQ 8: i) Convergence, divergence, involution, polyinvagination, epiboly

ii) Influences formation of antero–posterior medial axis and orientation of embryo.

iii) ectoderm, endoderm, mesoderm.

iv) individually through primitive streak.

v) Extra embryonic membranes (yolk sac, amnion, allantois, chorion).

vi) Refer to subsection 14.3.3-(iii).

SAQ 9: i) Refer to subsection 14.3.3–(iv).

ii) Trophoblast participates in formation of placenta; Inner cell mass is the source of cells for the body of fetus, yolk sac, allantois stalk and amnion.

iii) Blastodisc.

iv) Hypoblast, epiblast, primitive streak, amnion, yolk sac, allantois, involuntary movements of endodermal and mesodermal cells from epiblast through primitive streak.

v) Pattern of early embryonic development in reptiles, birds and mammals is similar.

Terminal Questions

1. a) T b) F c) T d) F

2. a) The phylogenetic group to which the particular animal (species) belongs.

   b) Amount of yolk deposited in the egg during the growth of oocyte.

   c) Site of development (whether inside the maternal body or external environment).

3. a) F b) T c) F d) T

4. Refer to the relevant parts of the text.

5. The presumptive notochordal area of zygote of a vertebrate forms notochord in the early embryonic stages but it is destined to be obliterated completely by the adult stage except in the cyclostomes.

UNIT 15 MORPHOGENESIS AND TISSUE ORGANISATION

Structure

15.1 Introduction
Objectives
15.2 Morphogenetic Processes
Types of Morphogenetic Processes
Modes of Cell Movement
The Role of Cytoskeletal Structures in Cell Movement
Adhesion of Cells to Extracellular Matrix
15.3 Morphogenesis of an Ectodermal Derivative
Neurulation in Amphibians
Neurulation in Chick
Mechanisms of Neural Plate Formation
15.4 Morphogenesis of Mesodermal Derivatives
Development of Heart in Amphibians
Development of Heart in Chick
Development of Blood Cells
15.5 Morphogenesis of Endodermal Derivatives
Origin of Endodermal Organs
Origin and Migration of Primordial Germ Cells (PGC) in Frog
Origin and Migration of PGC in Chick and Mammals
15.6 Summary
15.7 Terminal Questions
15.8 Answers

15.1 INTRODUCTION

In the previous unit you have learnt about different patterns of cleavage, mechanism of cleavage and how a single layered blastula gets converted into a gastrula with three germ layers. In this unit, we shall discuss the postgastrulation changes which i) result in the formation of definite body form by rearrangement of cells of the embryo, and ii) the differentiation of organs and organ systems from the germ layers - processes which are collectively known as **morphogenesis.**

Morphogenesis is a Greek word meaning generation or organisation of form.

Further, in this unit, you will also learn how different types of morphogenetic processes lead to the formation of ectodermal, mesodermal and endodermal derivatives. It would be interesting to know that there is a constancy about the germ layers and the organs derived from them, irrespective of whether the organism is a fish, a frog, a chick or a mammal. The morphogenesis of certain structures of frog and chick will be discussed in detail in this unit.

Objectives

After you have studied the unit, you should be able to:

- list the various types of morphogenetic processes and movements of cells,
- describe the morphogenetic processes that lead to the formation of neural tube in amphibians and chick.
- explain the formation of heart as a mesodermal derivative.
- discuss that the germ cells do not arise from the gonad itself, but the precursors of germ cells, the primordial germ cells, originate in the endoderm and migrate into developing gonads.

15.2 MORPHOGENETIC PROCESSES

In unit 14, we discussed the process by which a fertilised egg assumes the shape of a ball or a sheet of cells around a cavity, called blastula. Subsequently, the cells in a blastula actively rearrange themselves and take up positions in the gastrula from which they give rise to adult organs. Essentially, there are three layers of cells, the ectoderm, endoderm and mesoderm. Ectodermal cells will give rise to skin and its derivatives and central nervous system. The cells that are aligned in the interior of the embryo, the endodermal cells, will develop into alimentary canal and its derivatives; and the cells which lie in between ectoderm and endoderm of a gastrula, the mesodermal cells, will give rise to muscles, skeleton, connective tissue, urinogenital system etc. (Table 15.1).

Table 15.1 Major Derivatives of the Three Germ Layers In Vertebrates

Ectoderm
Nervous tissue
Sense organs
Epidermis of skin and derivatives
Mesoderm
Dermis of skin
Skeleton
Muscle
Circulatory system
Excretory system
Reproductive system (except germ cells)
Connective tissue
Endoderm
Digestive system linings
Digestive glands
Lung and respiratory tract linings
Primordial germ cells

It should be obvious to you that a single cell, the fertilised egg, gives rise to various structures that we mentioned. Such a generation of cellular diversity is called **differentiation.** The differentiation is a broad term which includes i) cytodifferentiation, ii) histodifferentiation and iii) development of shapes of organs. The process of differentiation which results in the formation of diverse organs and tissues involves various types of cell movements termed as morphogenetic processes. The morphogenetic processes are responsible for rearrangements of cell positions relative to each other. Further there is the shaping and realignment of individual cells as well. The various morphogenetic processes can be said to belong to either one of the six following processes. i) the direction and amount of cell divisions, ii) cell shape changes, iii) cell migration, iv) cell growth, v) cell death and vi) changes in cell membrane and extracellular matrix.

15.2.1 Types of Morphogenetic Processes

During early changes in the embryonic form and in the formation of organ rudiments, the epithelial cells undergo a variety of folding and spreading movements, the processes which shape the embryo. Transformation in the epithelial cells due to morphogenetic processes may involve either the folding or branching or spreading of epithelial sheets. The morphogenetic movements which bring about the required transformation are as follows:

- Any change in the epithelial sheet is preceded by a thickening of epithelium known as palisading. Palisading occurs due to the elongation of cells (Fig. 15.1) and can

be seen in the formation of neural plate and ectodermal placodes such as lens, ear and nasal rudiments.

Fig. 15.1: Elongation of epithelial cells called palisading, a process that precedes any morphogenetic event in epithelial cells.

- Epithelium can be folded either inward or outward. When the epithelium bends outwards from the surface of the embryo it is called evagination. On the other hand when the folding is inwards, into the embryo or into a cavity, the phenomenon is known as invagination.

i) Folding along a line will give rise to a groove (Fig. 15.2). The formation of neural tube and laryngo-tracheal tube are examples of such a folding process.

Fig. 15.2: Formation of a groove (such as neural groove) by folding along a line of thickened epithelium.

ii) The formation of lens vesicle or otic vesicle from their respective thickenings illustrate inpocketing or infolding of the epithelium to form pouches (Fig. 15.3).

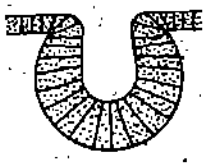

Fig. 15.3: The inpocketing or infolding of epithelium to form a pouch as in the case of optic cup.

- Folds and pouches undergo modification to form branched structures. The formation of various glands depends on the fold or cleft appearing at the epithelial outpocket (Fig. 15.4).

Fig. 15.4: The branching of pouches as in the case of the formation of glands.

- Folding or bending of a sheet of cells may result in changes in the shape of the cell within an epithelium. For instance, the narrowing of columnar epithelial cells at the apical end results in the formation of pyramidal cells (Fig. 15.5). This in turn results

apical end results in the formation of pyramidal cells (Fig. 15.5). This in turn results in the differences in the surface area on the two ends of epithelium and the bending of the entire sheet.

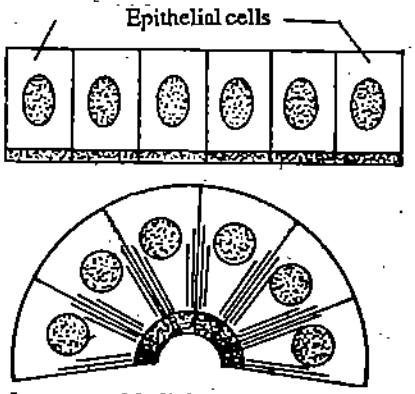

Fig. 15.5: Narrowing of the columnar epithelial cells and the formation of pyramidal cells.

- You may recall that while discussing gastrulation in amphibians, we described a process called epiboly. In this process, there is a spreading of cells. The prospective ectoderm spreads to cover the embryo. Such spreading of cells also occurs at other times during development. For example, during neurulation the epidermal ectoderm (prospective skin) spreads to cover the area left vacant by the convergence of neural epithelium toward dorsal midline. Spreading may be accompanied by a change in cell shape such as thinning and flattening of individual cells (Fig. 15.6).

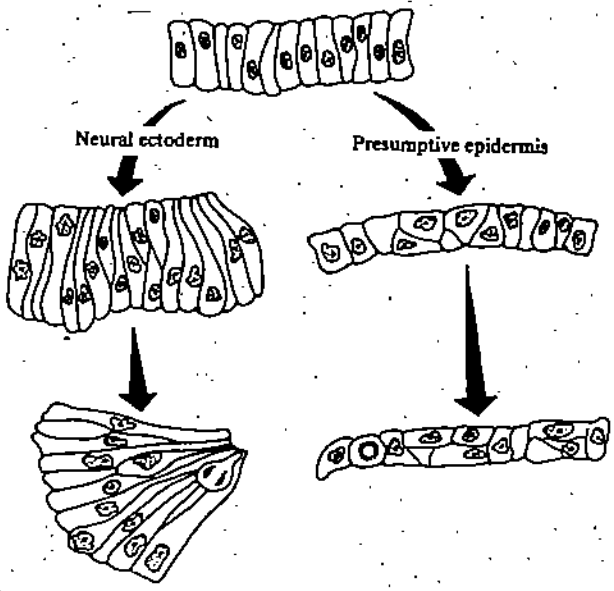

Fig. 15.6: The process of flattening and spreading of epithelial cells as it occurs during neurulation process and future epidermis formation.

- Also, individual cells such as mesenchymal cells or primordial germ cells detach from major cell layers and migrate to new locations where they develop into structures that they are programmed to develop.
- In addition to various types of cell movements that are involved in morphogenesis, selective death of cells plays an important role in shaping various structures of developing embryo. Brain, limb and palate are the structures where cell death is witnessed in certain regions during development. Fig. 15.7 shows the necrotic region or regions of cell death between developing digits of a chick that would ultimately result in separate digits.

15.2.2 Modes of Cell Movement

In the previous subsection, we described the various types of morphogenetic processes. Such processes are indicative of mobile nature of embryonic cells, and cell movement is basic to morphogenetic process. In this subsection, we shall discuss the changes in the shape of cells during morphogenetic process, as well as the factors that guide the cells to their location.

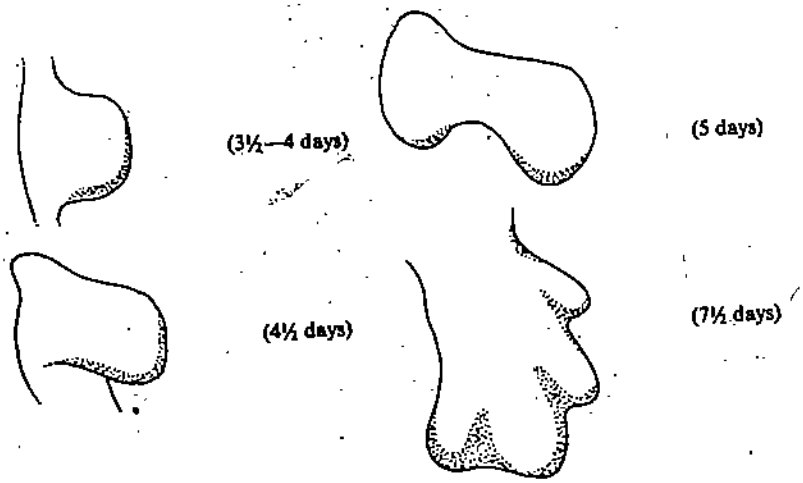

Fig. 15.7: Necrotic zones in chick leg at various stages of development. Shaded areas are necrotic zones where cells die at specific times during development. ANZ, anterior necrotic zone, INZ — interdigital necrotic zone, PNZ — posterior necrotic zone.

Fibroblasts which give rise to connective tissue have been studied extensively to understand the mechanism of cell mobility. The movement of fibroblasts occurs in two phases: i) An adhesion phase in which the cell stretches itself to the limits of its plasma membrane and ii) a detachment phase in which the hinder part of the cell is pulled forward. The cells propel themselves forward by generating regions that are quite thin and fan shaped. Such regions of the cell are called the **lamellae** (Fig. 15.8). The lamella bears a very thin and flat extension called **lamellipodium.** When the lamellipodium protrudes foward, part of it gets detached from the substratum and folds back on itself, thus giving a ruffled appearance.

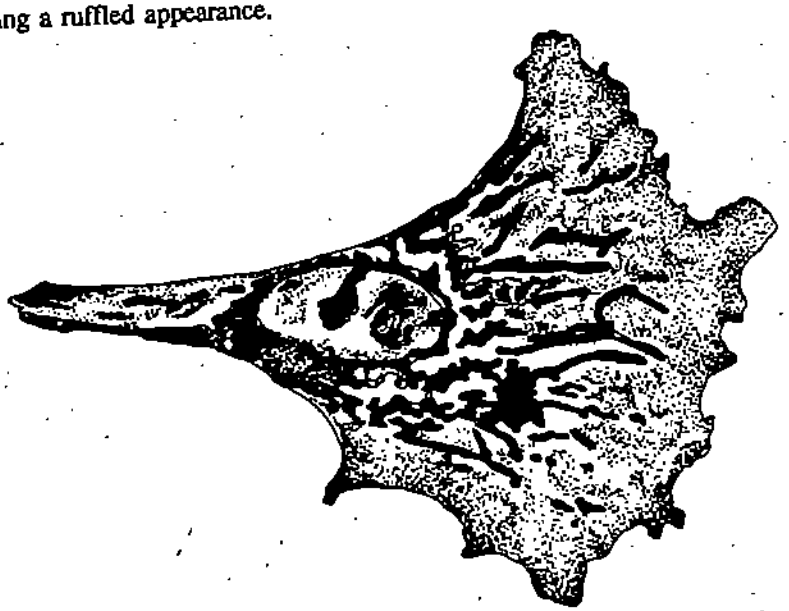

Fig. 15.8: A migrating fibroblast. The cell moves in the direction in which the fan shaped lamella with lamellipodia is formed.

The leading edges of such moving cells are termed as **ruffled lamellipodia.** The lamellipodia are continued to be produced at the leading edge till the cell gets larger and larger, and the hind end detaches itself from the substratum.

Since the cells move to and reach specific positions, we have to ask, what are the mechanisms that direct the movement of cells to specific location in the embryo? It is suggested that different mechanisms may be responsible for the guided movement of cells. They are a) **chemotaxis** b) **haptotaxis** c) **galvanotaxis** and d) **contact guidance.** We shall briefly look into each one of these processes.

a) **Chemotaxis:** Chemotaxis refers to the directed movement of cells in response to a concentration gradient of a chemical factor in solution. An example of chemotaxis during morphogenesis of the embryo is the migration of embryonic lymphocytes from bone marrow to embryonic thymus. The compound responsible for the directed

movement of cells is shown to be a heat-stable peptide with a molecular weight of 1000 to 4000 daltons. The peptide is probably produced by the thymus. The gradient of the peptide would be highest in the thymus towards which the cells move.

b) **Haptotaxis:** Haptotaxis refers to the directed movement of cells in response to a concentration gradient of an adhesive molecule that may be present in the extra cellular maxtrix. The adhesive material is not in solution. The cell would constantly make or break adhesions with such molecules and move from the region of low concentration of the molecule to the place of its higher concentration. Poole and Stemberg (1982) provided evidence that pronephric duct cells in salamander embryos move under the regulation of haptotaxis. The pronephric duct rudiment separates from the dorsal mesoderm as a solid cord of cells and it is at first seen near the head of the embryo. With further development of embryo, this rudiment ultimately elongates towards the cloaca where urine is excreted. Studies have shown that the enzyme alkaline phosphatase may be the adhesive molecule that regulates the migration of propheric duct rudiment.

c) **Galvanotaxis:** Galvanotaxis refers to the movement of cells in response to a potential difference between cells. It is suggested that there are voltage differences between embryonic regions that could play a significant role in morphogenesis. Minute electric fields of the order of 10 to 100 mv/mm appear to be sufficient to alter the direction of nerve growth. Nuccitelli and Erickson (1984) showed that embryonic chick fibroblasts migrate toward negative pole when cultured in small steady electrical field. Early chick embryos, regenerating blastema of amphibians and moth ovaries where transportation of yolk proteins occurs, are some of the structures where large electric currents have been detected.

d) **Contact guidance:** Besides chemical or ionic factors that regulate cell movement in developing embryos, physical factors also appear to play a role in morphogenetic processes. A phenomenon called contact guidance influences cell movements in cultures. The cell is influenced by the physical surface over which it passes and is directed towards its location (Fig. 15.9). Weiss (1934) demonstrated that cells can detect discontinuties in their substratum and migrate after aligning themselves along such features as fibres. They could even move along scratches at the bottom of petridish. In a recent study Harris (1980) showed that fibroblasts cultured on silicone rubber or collagen change the shape of the substrate by the stress generated by them, and move along such changed substrate fields. Contact guidance is assumed to be the cause for the migration of mesenchymal cells inside the fish fin.

Fig. 15.9: The mammalian cells shown aligning themselves on the grooved surfaces.

15.2.3 The Role of Cytoskeletal Structures in Cell Movement

The term cytoskeleton refers collectively to a complex array of fibres in the cytoplasm that mediate changes in cell shape and produce cell movements in embryos. The system consists of three types of fibres i) microtubules ii) microfilaments and iii) intermediate filaments.

i) **Microtubules:** All animal cells have microtubules as vital parts of basic cellular structure and machinary (refer to Unit 3 of Block 1 of LSE-01; Cell Biology

Course). Microtubules are hollow cylinderical rods and are 25 nm in diameter. Each microtubule is formed of 13 rows of solid protofilaments which run parallel to the long axis of microtubule. Each protofilament is a protein dimer composed of one α and one β tubulin chain. The protofilaments are assembled concomitantly side by side to produce a hollow microtubule. Microtubules have a mechanical role in the elongation or palisading of epithelial cells.

ii) **Microfilaments:** Microfilaments are also present in all animal cells. They are found singly throughout the cytoplasm organised as a meshwork and measure 6 nm in diameter. These are the polymers of the contractile protein actin. **F actin** *or* **filamentous actin** is the name given to the assembled polymers of actin. In subsection 15.2.1, you have learnt that folding of cells brings about changes in the cell shape within the cytoplasm and the apical surfaces of cells become narrower. The narrowing of apical surfaces is brought about by contraction of microfilaments made up of actin.

iii) **Intermediate filaments:** These filaments are intermediate in size between microtubules and microfilaments and are 10 nm in diameter. Five classes of intermediate filaments are known i) **keratin filaments** found in epithelial cells of ectoderm and endoderm ii) **vimentin filaments** found in mesoderm derived tissues such as bone and cartilage iii) **nerve filaments** found in nerve cells iv) **glial filaments** found in glial cells v) **desmin filaments** found in all types of muscle tissues. The functions of different types of intermediate filaments are not clear. It is proposed that they may act with cellular organelles in organising and maintaining their 3-dimensional structure.

15.2.4 Adhesion of Cells to Extracellular Cell Matrix

In subsection 15.2.2, you have learnt that the cells in order to move or change shape, adhere to each other or to the substratum in their environment. Generally the cells adhere to complex environmental surfaces formed of molecules found in extracellular matrix. Such molecules are known as **extracellular matrix molecules** or **ECM molecules.** These molecules are secreted into spaces between cells and assemble into a meshwork. Some molecules can form a dense sheet on the basal surface of epithelial cells called basal lamina. The ECM molecules are capable of self-aggregation or self-assembly and hence are able to form the extracellular matrix. Proteoglycans, collagens and certain other glycoproteins belong to the category of ECM molecules. The binding of cells to extracellular matrix is mediated by receptor proteins located in the plasma membrane. Receptor proteins that mediate adhesion to several ECM molecules have also been isolated.

Here, we end our discussion on certain of the general aspects of morphogenesis. Before we start describing the morphogenesis of specific structures from their respective germ layers, you may answer the following self-assessment questions.

SAQ 1

a. Define the terms i) differentiation and ii) morphogenesis.

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b. Match the items in A with the ones found in B.

A		B	
i)	Thickening of epithelium prior to the changes in epithelial sheet	a)	invagination
ii)	Bending of the epithelium away from the surface of the embryo	b)	cell death

iii)	Folding of the epithelium that results in the infolding into the embryo	c) epiboly
iv)	The spreading of one layer of germ cells over the other	d) palisading
v)	Removal of specific cells by necrotic process during development	e) evagination

c. Fill in the blanks with suitable words.

i) The directed movement of cells in response to a concentration gradient of a chemical factor in solution is known as _____ .

ii) _____ refers to the movement of cells along a concentration gradient of an adhesive molecule present in the extracellular matrix.

iii) Movement of eukaryotic cells in response to a potential difference between them is referred to as _____ .

iv) The movement of cells influenced by the physical surface over which they pass is termed as _____ .

v) The complex array of fibres in cytoplasm that mediates changes in cell shape and promotes cell movement in embryos is called _____ .

15.3 MORPHOGENESIS OF AN ECTODERMAL DERIVATIVE

During the development of vertebrate body the different regions in the three germ layers of the gastrula become segregated from each other to form the rudiments of future organs and tissues. In this section we shall discuss the partitioning of ectoderm as well as the formation and inward displacement of neural tube in frog and chick. The process is called **neurulation.** We have chosen to discuss the neurulation process because the external morphology of developing embryo at this stage is dominated by the developing nervous system and the embryo itself is known as **neurula** at this stage. We shall begin with the separation of ectoderm into subpopulations of cells, each of which will develop into distinct ectodermal organ. The ectodermal layer essentially separates into i) **epidermal ectoderm** from which the skin is formed, ii) **neural ectoderm** which gives rise to central nervous system and iii) **neural crest** which gives rise to a portion of peripheral nervous system and a variety of other tissues.

15.3.1 Neurulation in Amphibians

The first step in the neurulation process is the flattening and thickening of dorsal ectoderm to form neural plate (Fig. 15.10a). The plate of cells differs from the rest of ecotoderm cells in that they have changed shape and appear more columnar. The edges of the neural plate then rise above along the rest of the neural plate to form neural folds (Fig. 15.10b) . The neural folds are now found along the two flanks of a central depression called neural groove (Fig. 15.10c). The neural groove extends along the entire middorsal line of the embryo. Eventually the neural folds meet in the middle above the deepening neural groove and fuse to form the neural tube (Fig. 15. 10d, e). At the time of rolling of neural plate to form the neural tube, the neuroepithelial cells at the lateral folds of the neural plate develop constrictions at their apical edges. This changes the shape of columnar eithelial cells into pyramidal cones. As the surface area of apical end of neural epithelial cells becomes smaller relative to the basal surface, the rolling up of the neural plate and formation of neural tube takes place. With the fusion of neural folds along the middorsal line, the neural ectoderm separates from epidermal ectoderm which now covers the neural tube and completely surrounds the embryo. The change in the shape of cells in the neural tube results in the regionalisation of neural tube. In the cephalic end, i.e. the end which will differentiate into brain, the wall of the tube is broad and thick and a series of swellings and constrictions develop that define

the various divisions of brain. Caudally, towards the posterior end, the tube is simple and narrow extending into the tail.

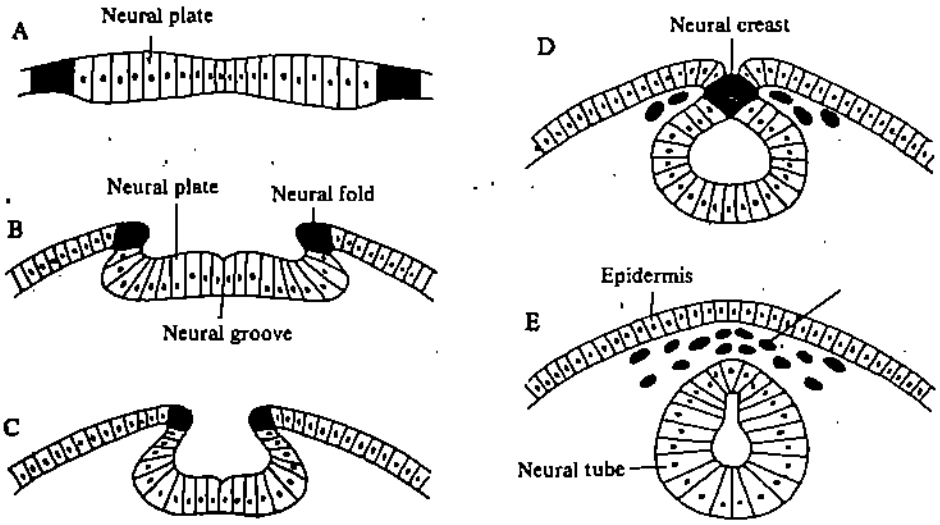

Fig. 15.10: Stages in neurulation in frog. A) thickening of dorsal ectoderm to form neural plate B) formation of neural folds and neural groove C) deepening of neural groove D) fusion of neural folds and formation of a neural tube E) neural tube has separated and is covered over by epidermis. Neural crest cells have separated from neural folds.

With the formation of neural tube, a second population of cells separate from the neural ectoderm and lie between the neural tube and epidermis. This group of cells is the neural crest cells (Fig. 15.10e). The neural crest cells subsequently migrate into various parts of body to give rise to a variety of tissues in different parts of the body.

15.3.2 Neurulation in Chick

Neurulation in chick is similar to that of amphibians but there are certain differences. In amphibians, the formation of neural tube occurs simultaneously along the entire length of the embryo. In birds, reptiles and mammals even as the neurulation has begun in the anterior part of the embryo, the posteior region is still in the process of gastrulation. At a time when the neural folds are just about to form in the posterior region, in the anterior region the neural folds have already started fusing forming the neural tube (Fig. 15.11)

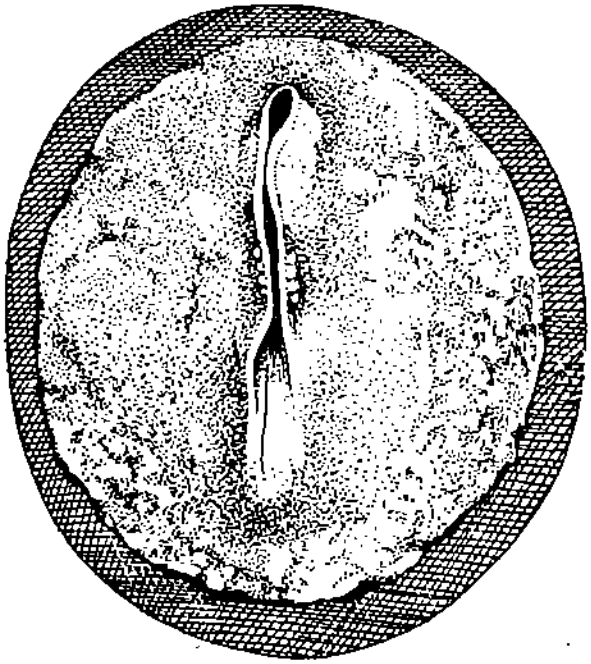

Fig. 15.11: Dorsal view of a chick embryo of 25-26 hours with 5 pairs of somites. Neural folds approaching each other fuse and form neural tube in the cephalic region; but in the posterior region gastrulation is still occurring and primitive streak and neural folds are yet to form.

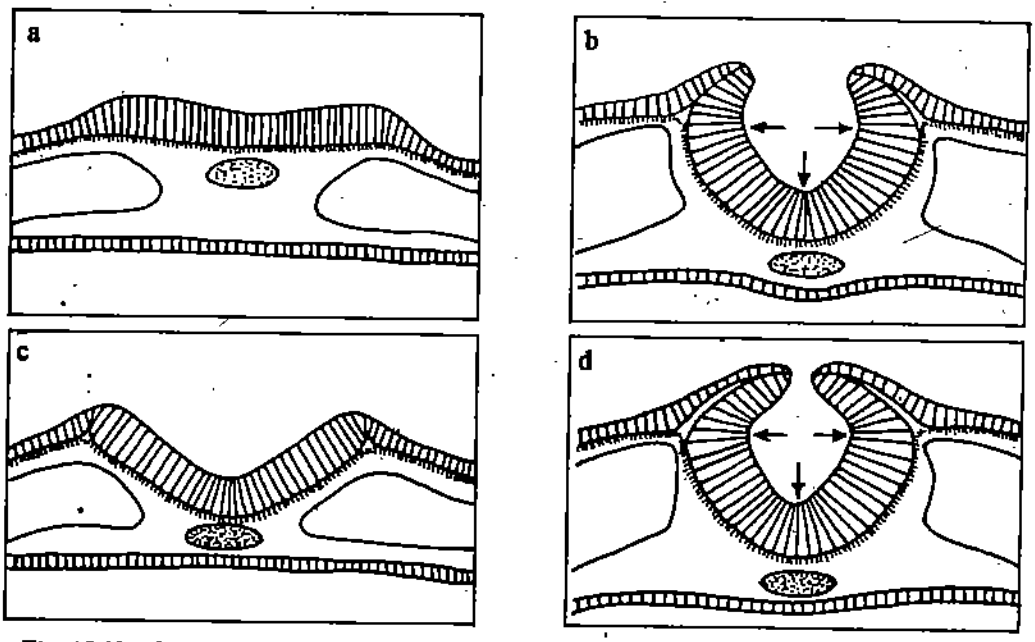

Fig. 15.12: Stages in the neurulation of chick. a) Neural plate formation (b, c, d) The bending of plate in three locations (indicated by arrows), just above the notochord (N) and on each side of the neural plate, just below the tips of neural folds.

We described two important changes in cell shape while discussing neurulation in amphibians. Such changes occur in chick embryo as well. One relates to changes in neural ectoderm cells from cuboidal to columnar shape that results in narrowing and thickening of neural plate. The second relates to narrowing of the apical ends of some neural epithelial cells when the neural plate rolls up to form neural tube (Fig. 15.12b, c, d).

15.3.3 Mechanisms of Neural Plate Formation

You are now familar with the concept that the neurulation comprises of two processes i) the neural plate formaton and ii) the neural tube formation. The mechanisms responsible for these two processes are not very clear.

The shaping of neural plate from an oval to a long, narrow plate is believed to occur by the elongation of neural epithelial cells and the accompanying apical shrinkage. Although it was earlier believed that microtubules (refer to subsection 15.2.3) may be involved in the elongation process, evidence is inconclusive to suggest a role for microtubules in the elongation process. The role of microtubule appears to be important in stabilising the elongated state of the cells rather than in the elongation process itself.

Several hypothesis have been put forward to explain the bending of neural plate to form neural tube. The more acceptable one is that during the rolling of neural plate, the apical surface of neural epithelial cells contract at the site of bending because of the contraction of actin microfilaments (refer to section 15.2.3).

Before we proceed to discuss the mesodermal derivatives attempt the following SAQ.

SAQ 2

State wether the following statements are true or false.

i) During post-gastrulation development, different germ layers of the gastrula become segregated from each other to form tissues and organs.

ii) The term neurulation refers to the partitioning of ectoderm, and the formation and inward displacement of neural tube.

iii) Neurulation results in the separation of ectoderm into epidermal ectoderm, neural ectoderm and neural crest cells.

iv) Neurulation process in chick is entirely different from that of amphibians.

v) During neural plate formation, the neuroepithelial cells change in shape from columnar to pyramidal ones.

vi) Microtubules play a significant role during the rolling up of the neural plate into neural tube.

vii) In chick the neurulation process occurs simultaneously through out the length of the embryo.

15.4 MORPHOGENESIS OF MESODERMAL DERIVATIVES

In this section, you will study the early development of an organ derived from mesoderm. In fact, all the organs that lie between ectoderm and endoderm tissues arise from mesoderm. In the neurula stage of the embryo, the mesoderm cells are arranged in five distinct regions. The five regions of the mesoderm and the organs derived from them are as follows:

- **Chordamesoderm:** Chordamesoderm separates as a middorsal strip from the rest of the mesodermal tissue and establishes the body axis of the embryo. The anterior part gives rise to head mesoderm and the remaining part to notochord (Fig. 15.13B).
- **Dorsal mesoderm or paraxial mesoderm:** Tissues developing from this region will be in the back of the embryo, on either side of spinal cord. It segments into blocks of tissues called somites. (Fig. 15.13C, D). Connective tissues and associated structures such as muscles, cartilage and dermis arise from this region.
- **Intermediate mesoderm:** Intermediate mesoderm is located as a thin stalk connecting paraxial mesoderm with the rest of the mesodermal sheet. The urinogentital system arises from intermediate mesoderm (Fig. 15.13C, D).

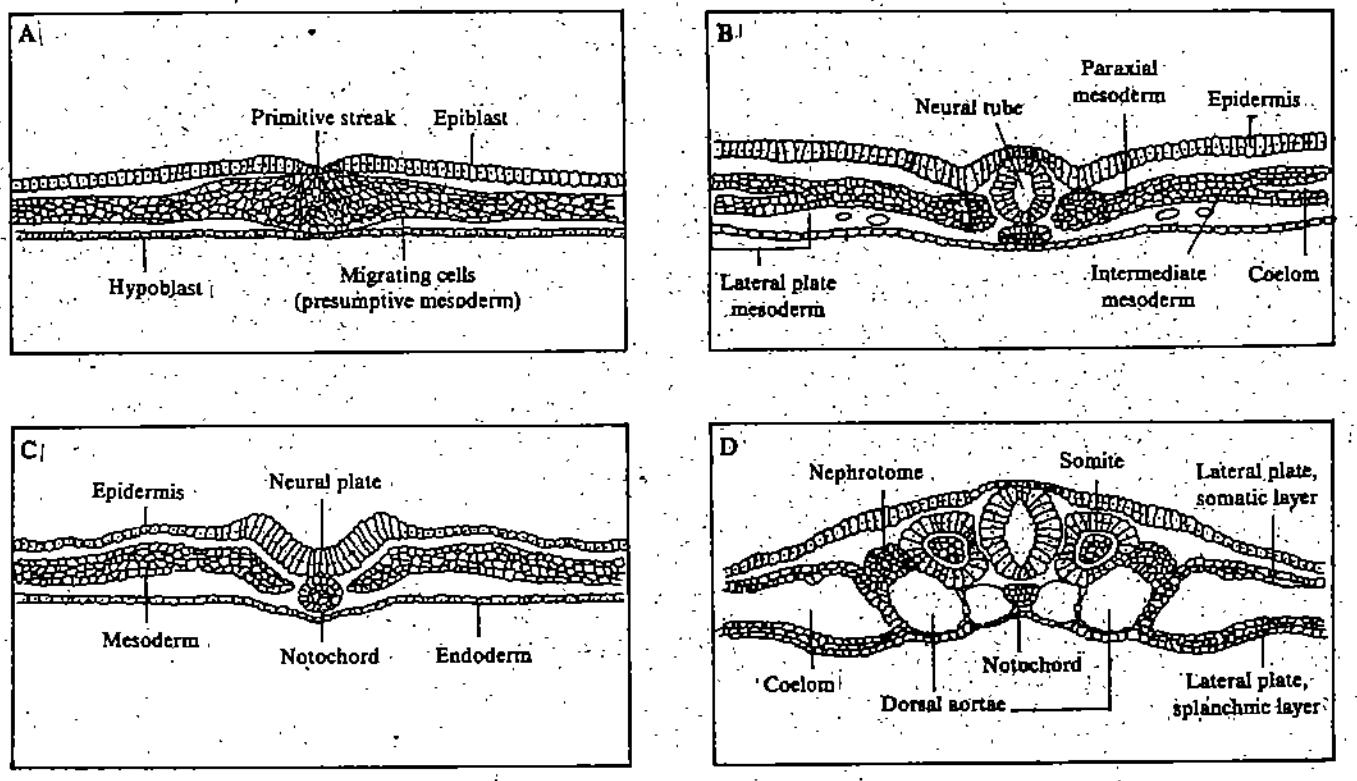

Fig. 15.13: Stages in the development of mesoderm shown in transverse sections of chick embryo at trunk level.

- **Lateral plate mesoderm** (Fig. 15.13D): Lateral plate mesoderm is a sheet of loosely connected cells on either side of gut (Fig. 15.13C). This loose sheet of epithelial cells splits into two layers, the somatic mesoderm which becomes closely associated with ectoderm and the splanchnic mesoderm which becomes closely

associated with endoderm. The space between the two regions of mesoderm is the future coelomic cavity (Fig. 15.13D). Lateral plate mesoderm gives rise to heart, blood vessels and blood cells and the lining of the body cavities. All the components of limb except muscles are derived from lateral plate mesoderm.

- **Head mesoderm** The head mesoderm located in the head region will give rise to head muscles.

We shall now describe the development of one of the organ rudiments derived from mesoderm. It is a common practice to describe the development of limb rudiment as an example of an organ differentiating from lateral mesoderm. In fact, all the processes of development can be seen in the formation of a limb and the major features of limb development are common to all vertebrates. Since the development of limb is discussed in detail in Unit 17 of this block, in this section we shall describe the development of two other mesoderm derivatives, the heart and the blood cells. The circulatory system is the first functional system in the developing embryo and the heart is the first functional organ. The development of circulatory system with heart, blood cells and a network of blood vessels is more complex than the development of other systems. As examples, we shall discuss the process of development of heart in amphibians among lower vertebrates and chick among the amniotes. The differences between the two are largely determined by the differences in the amount of yolk in their eggs.

15.4.1 Development of Heart in Amphibians

The heart and the surrounding pericardial cavity develop from lateral mesoderm. After gastrulation, the mesodermal mantles continue to grow anteriorly and ventrally. At the ventral ends of these mantles below the gut and in the space between them there occurs proliferation of cells (Fig. 15.14A) which initially form a cord (Fig. 15.14B) but subsequently the cord of cells hollows out to form a tube (Fig. 15.14C). The tube made of endothelial cells is called **endocardium**, meaning the inner lining of heart. Similar endothelial tubes are present anterior and posterior to endocardium and these tubes will become the major blood vessels to and from heart.

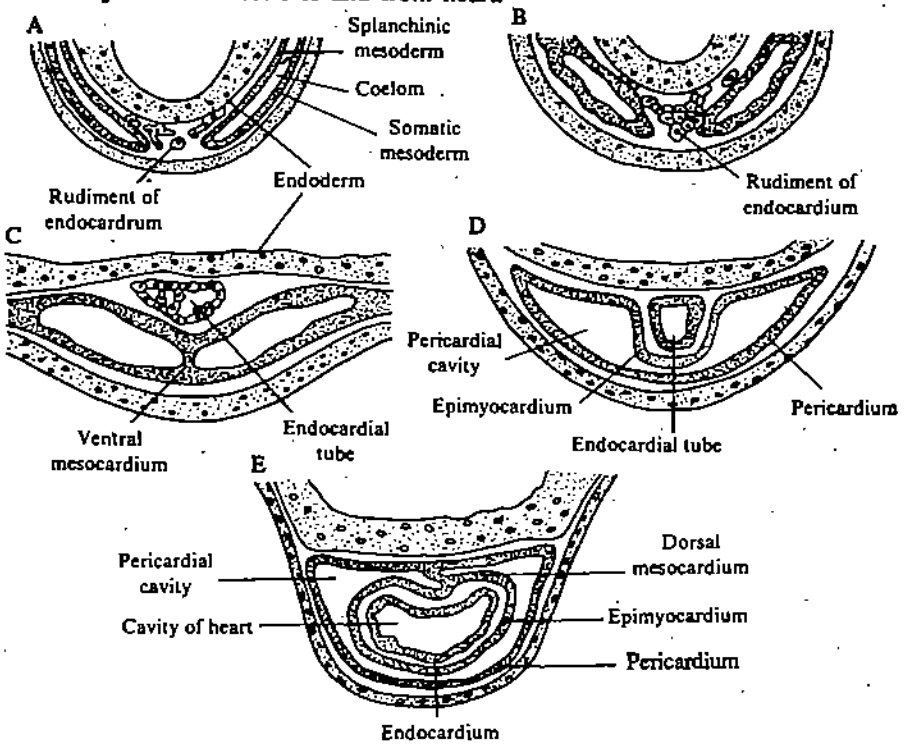

Fig. 15.14: Cross section of frog embryo showing stages in the development of heart.

Once the endocardium is formed, the mantles of mesoderm meet and fuse in the middle, ventral to the endocardium. The splanchnic mesodermal layer then spreads dorsally on either side of the endocardium ultimately surrounding the endocardium and joining above it. The portion of splanchnic mesodermal layer surrounding the endocardium is the prospective muscle layer of the heart, the **epimyocardium** (Fig. 15.14D, E). The regions of the mesoderm above and below the heart, where the fusion of the mesoderm

of the two sides have taken place, become the mesenteries that suspend the heart in pericardial cavity (Fig. 15.14E). The ventral mesentery disappears later. The coelomic cavity now becomes the pericardial cavity and the somatic layer of mesoderm becomes the lining of the pericardial cavity, the **pericardium.**

15.4.2 Development of Heart in Chick

You have learnt earlier that in amniotes because of the presence of a large amount of yolk, the germ layers are found as flattened sheets on the yolk. In chick the heart develops first as a pair of tubes and then the two tubes fuse to form a single tube. During the head fold stage of embryo, the cells of the splanchnic mesoderm detach from the epithelium and migrate to lie below the foregut endoderm to form two groups of cells. In each group, the cells coalesce and form a thin walled tube (Fig. 15.15A, B, C).

The two tubes then fuse in the midline below the gut to form the **endocardium** (Fig. 15.15C, D). As in amphibians, here also the regions of splanchnic mesoderm that lie dorsal and ventral to endocardium fuse forming the **myocardium** (Fig. 15.15C, d). The development of chick heart takes place in the anterior-posterior direction which means

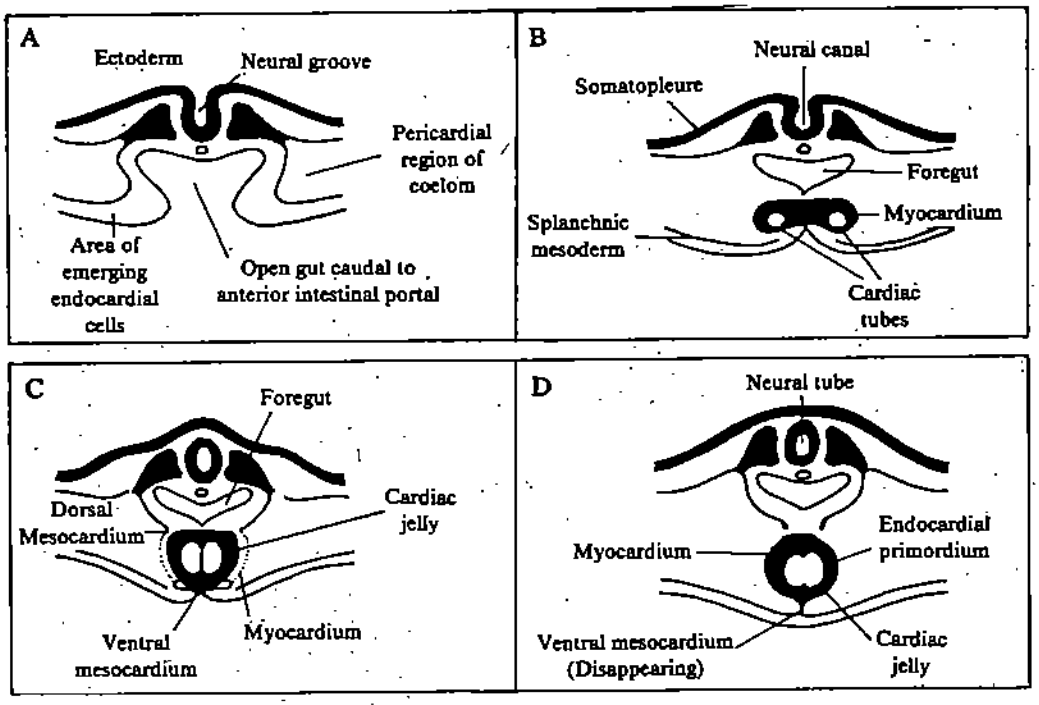

Fig. 15.15: Stages in the development of heart of chick from splanchnic mesoderm.

the fusion of the endocardial tubes takes place initially anteriorly and subsequently posteriorly (Fig. 15.16A to D). In a 33 hour old chick embryo, a **tubular heart** is formed but the chambers have not yet developed. The heart is already beating with the blood entering the heart at the posterior end which is the future **atrium or auricle.** The blood is then pumped into **ventricle** from where it flows out through developing **aortic arches.** The coelom enlarges to form the **pericardial cavity** surrounding the heart and is lined with **pericardium.**

The tubular heart of an early embryo develops into an adult heart around 120 hours of embryonic life. The development of a four chambered heart from a tubular structure with the separation of pulmonary and systemic circulation depends on two significant morphogenetic events. i) The atrium is brought dorsal to the ventricles by the process of looping and bending and ii) division of the tube into chambers is achieved by the formation of septa. Figs. 15.17A to F show the process of looping that transforms the tubular heart into a chambered one. The transformation appears to be an **endogenous property** of the heart and also involves certain cell shape changes in the myocardial epithelium. The details of the forces that bring about the transformation are not clearly understood.

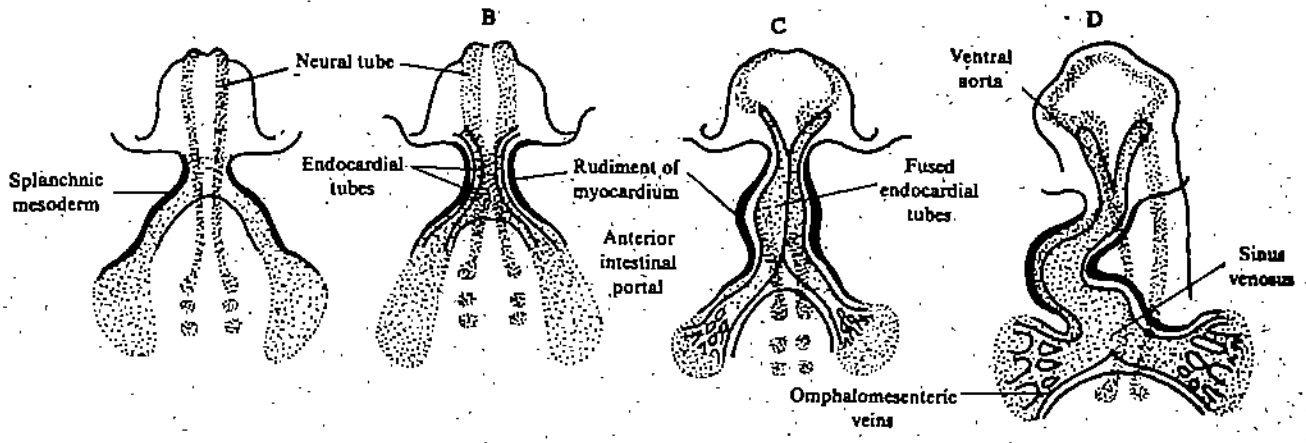

Fig. 15.16: Stages in the development of the heart in the chick embryo shown from the ventral side. Note the antero-postelor fusion of the paired cardiac primordia.

15.4.3 Development of Blood Cells

In this subsection we shall discuss the development of blood cells, mainly erythrocytes or red blood cells (RBCs). Erythrocytes are the most numerous cell type in the blood

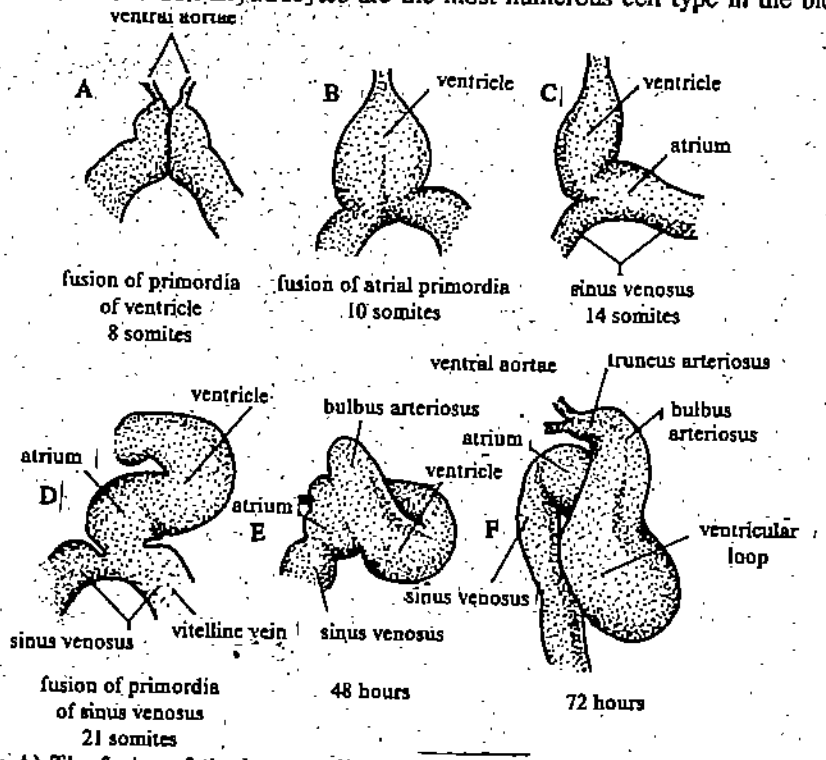

Fig. 15.17: A) The fusion of the heart rudiments of the chick embryo (ventral views). The heart rudiments consist of two tubes which fuse to form a single tubular heart. This fusion begins at the level of the ventricles (28 hours) and continues progressively in a posterior direction. At 30 hours the paired rudiments of the atria begin to fuse, and by 34 hours a single atrium is formed. The sinus venosus is still present as two primordia at 34 hours. B) Form changes in the heart of the chick embryo (dorsal view). At 40 hours the paired rudiments of the sinus venosus are fusing and the ventricle becomes bent into a loop. Further bending of the ventricle at 48 hours places it in a position lateral to the atrium, and at 72 hours the ventricle is posterior to the atrium.

which also contains the different types of white blood cells or leucocytes including granulocytes, monocytes, platelets, plasma cells and lymphocytes. Our knowledge about the development of blood cells is derived mainly from studies on birds and mammals. All types of blood cells have a limited life span. For example, a human RBC survives for only about 120 days and in a healthy person trillions of RBCs and other blood cells are lost every day and replaced by continuous production of new cells in the bone marrow from the **haematopoietic** (blood forming) **stem cells.**

A stem cell is an undifferentiated cell, capable of extensive proliferation that can generate differentiated cells as well as more undifferentiated, embryonic cells of its own type. Thus a population of such embryonic stem cells is maintained even in the adult that guarantees continuous replacement of differentiated cells of specific type that are dying and are lost throughout life of the animal.

Experimental evidence indicates that in birds and mammals all types of blood cells are derived ultimately from one type of stem cell called **CFU – M, L (myeloid and lymphoid colony forming unit).** This cell is pluripotent and generates both red and white blood cells in addition to itself.

However, CFU – M, L does not give rise to various blood cell types directly. Instead, in addition to reproducing its own kind it produces two other types of stem cells called **CFU – S** and **CFU – L.** These are also pluripotent but with lesser potentiality than that of the parent CFU – M, L. Thus, while CFU – S can generate erythrocytes, granulocytes, monocytes and platelets but not lymphocytes; the CFU – L can give rise to only lymphocytes and plasma cells.

In its turn, CFU – S generates, in addition to more of itself, five other types of stem cells (**BFU – E, Ba – CFC, GM – CFC** and **Meg – CFC**). Each of these is capable of generating differentiated cells of some specific type(s) in addition to replacing itself. Each of these cells is therefore, called a **committed stem cell.** Among them the stem cells BFU – E (Blood forming unit — erythroid) are committed to the erythroid pathway leading to the formation of erythrocytes only (Fig. 15.18).

By repeated divisions the BFU – E cell eventually produces a population of cells called **proerythroblasts.** In mammals the proerythroblast cell passes through a series of stages ultimately becoming a fully differentiated and functional **erythrocyte** (Figs. 15.18, 15.19). During this process many changes take place in the cell and each stage is characterised by certain features of its own (Fig. 15.19).

Proerythroblast stage	:	Active RNA synthesis and proliferation
Erythroblast stage	:	Chromosomal condensation; beginning of hemoglobin synthesis
Polychromatophilic stage	:	Increased synthesis and accumulation of hemoglobin; decreased RNA synthesis
Orthochromatic stage	:	Nucleus completely inactivated; division no longer possible
Reticulocyte stage	:	Nucleus extruded; some hemoglobin synthesis still occurring; cell enters blood stream
Erythrocyte	:	No more synthetic activity of any kind; cell is a membranous bag filled with hemoglobin solution

Transformation of the progeny of BFU – E into proerythroblast cells and the subsequent process of their differentiation into erythrocytes occur under the influence of a hormone, **erythropoietin,** secreted in the kidneys. If O_2 supply is deficient, production of this hormone is enhanced leading to increased number of BFU – E progeny transforming into proerythroblasts. Ultimately the proerythroblasts are terminally differentiated into functional red blood cells.

In adult mammals the major site of the formation of blood cells is the bone marrow. Experimental studies show that in embryos the first pluripotent haematopoietic stem cells (CFU – M, L) originate in the mesodermal blood islands of yolk sac. They reach and colonize successively the liver, and then the spleen and bone marrow during later life of the fetus. In bird embryos these master stem cells appear to originate in the yolk sac as well as the blood vessels within the embryo proper.

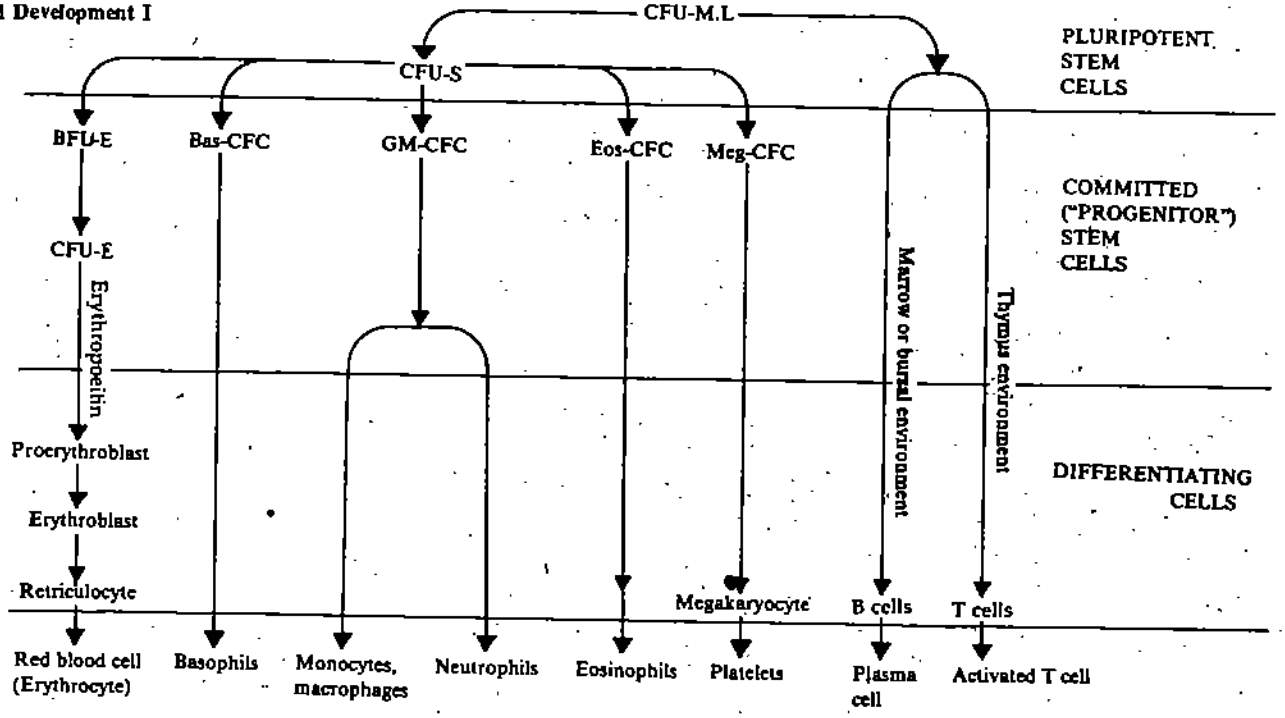

Fig. 15.18: Schematic diagram showing the origin and developmental pathways of blood and lymphoid cells.

Differentiation of blood cells begins first in the yolk sac of the embryo, later in the fetal liver and last in the bone marrow. In mouse it begins in the yolk sac on 8th day, in the fetal liver on 12th day and in the bone marrow it occurs from 16th day of gestation onward. In the human fetus blood cell differentiation begins in the yolk sac on 9th day and in the bone marrow after the first trimester.

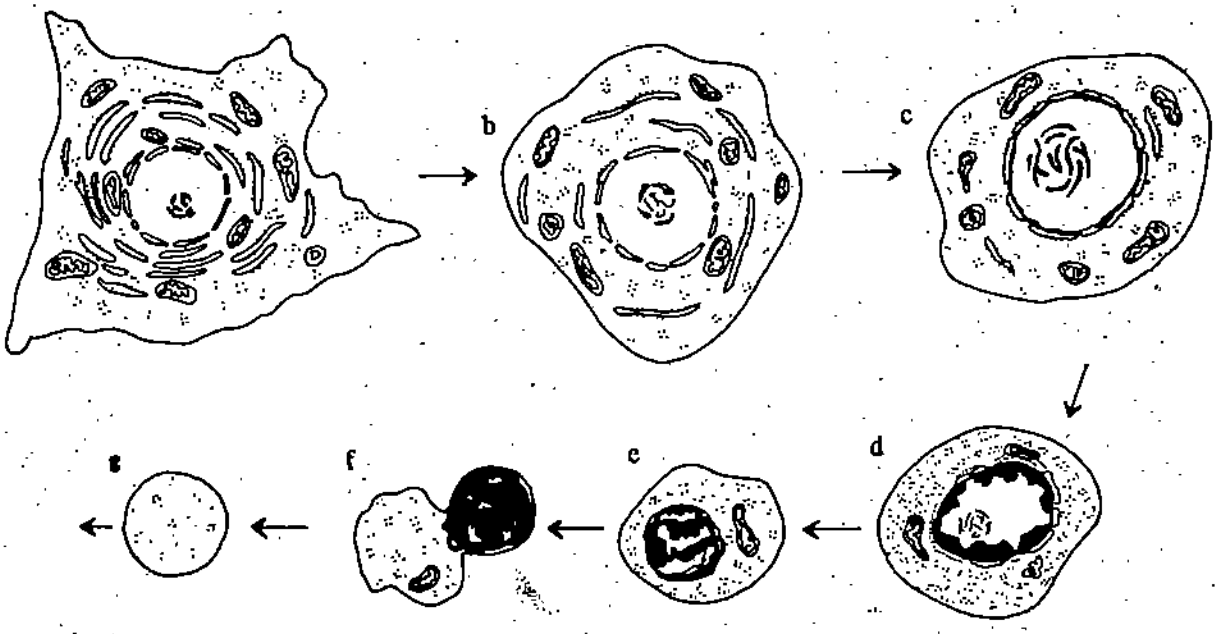

Fig. 15.19: Diagrammatic representation of the stages in the differentiation of erythrocyte (RBC). a) The mesenchymal pluripotent CFU – S which can give rise to more than one type of stem cell. b) The hemocytoblast, (BFU – E), the stem cell of erythroid line. c) Proerythroblast d) Erythroblast e) Polychromatophilic erythroblast f) Orthochromatic erythroblast. g) Reticulocyte. Terminally differentiated erythrocyte stage (RBC) follows the reticulocyte stage.

SAQ 3

a) Match correctly the region of the mesoderm (A) with the organs derived from it (B).

A	B
i) Intermediate mesoderm	a) notochord
ii) Dorsal mesoderm	b) heart and limb
iii) Head mesoderm	c) muscles, cartilage and dermis
iv) Lateral plate mesoderm	d) muscles of the face
v) Chordamesoderm	e) urinogenital ducts

b) Choose the correct answer from the alternatives provided.

i) The first functional system in a developing embryo is nervous system/ circulatory system.

ii) The heart and the pericardial cavity develop from dorsal/lateral mesoderm.

iii) The proliferating epithelial/endothelial cells of mesoderm ultimately form the heart tube called pericardium/endocardium.

iv) The epimyocardium which gives rise to the muscle layers of the heart differentiates from somatic/splanchnic mesoderm.

v) In a 30 hr/120 hr embryo a tubular heart is formed but the chambers are not yet developed.

vi) The coelom enlarges to form the chambers of the heart/pericardial cavity.

vii) In the conversion of tubular heart into a chambered one the future atria/ ventricles are brought dorsal to the atria/ventricles by processes of looping and bending.

c) State whether the following statements are true or false.

i) The development of chick heart takes place in the posterior-anterior direction.

ii) Both in amphibians and chick the somatic mesoderm that lie dorsal and ventral to endocardium fuse forming myocardium.

iii) In the tubular embryonic heart the blood enters at the posterior end, pumped into the ventricle and flows out through the developing aortic arches.

iv) The transformation of tubular heart into a chambered one depends exclusively on cell shape changes in myocardial epithelium.

v) The oxygen supply to the developing embryos of chick is through lungs.

vi) The CFU – S and CFU – L are commited cells which develop into a specific cell type.

15.5 MORPHOGENESIS OF ENDODERMAL DERIVATIVES

Thus far we have discussed the morphogeneis of organ rudiments derived from ectoderm and mesoderm. In this section, we shall discuss about endoderm and its derivatives.

The third and the innermost germ layer endoderm mainly gives rise to gut tube and its accessory organs, respiratory apparatus and primordial germ cells (PGC). The development processes of endoderm includes long distance migration of germ cells, prominent foldings and a number of evaginations from the digestive tube.

15.5.1 Origin of Endodermal Organs

Following major endodermal organs arise as evaginations of the digestive tube.

- In pharyngeal region paired pouches bulge out laterally, meet ectodermal invaginations and form gill slits which in the higher vertebrates give rise to cords of

cells that form among other structures, the **eustachian tube, thymus** and **parathyroid gland.**

- From the floor of pharynx cords of endodermal cells penetrate ventrally into the underlying mesoderm forming **thyroid gland.**
- A midventral groove (laryngo – tracheal groove) from the floor of pharynx gives rise to **trachea** and **lungs.**
- Further, posteriorly at the level of the future duodenum, the other evaginations initiate the development of **liver, pancreas** and **gall bladder.**

The organs that bud off from the pharynx and gut are not purely endodermal in their final state; mesoderm invests them and supplies blood vessels; and a framework of connective tissue shapes each organ into its definitive structure.

- The **primordial germ** cells that migrate from endoerm deserve special attention because the gonads in which they are ultimately found are mesodermal in origin.

In this section you will learn about the origin of primordial germ cells and their subsequent migration to specific destination in vertebrates.

15.5.1 Origin and Migration of Primordial Germ Cells in Frog

In frog eggs the germplasm present as granular material is located in the cytoplasm near the vegetal pole of the egg. During cleavage the germinal granules are found in the endodermal blastomeres of this region (Fig. 15.20A). Later, the descendents of these cells known as primordial germ cells (PGC) migrate dorsally around the posterior gut region and enter into mesodermal region (Fig. 15.20B – E). The mesodermal region into which PGC enter are the genital ridges (Fig. 15.20F). The gonads develop from these ridges. In the developing gonad the PGC derived from endoderm divide and form gonial cells which undergo meiosis to produce gametes.

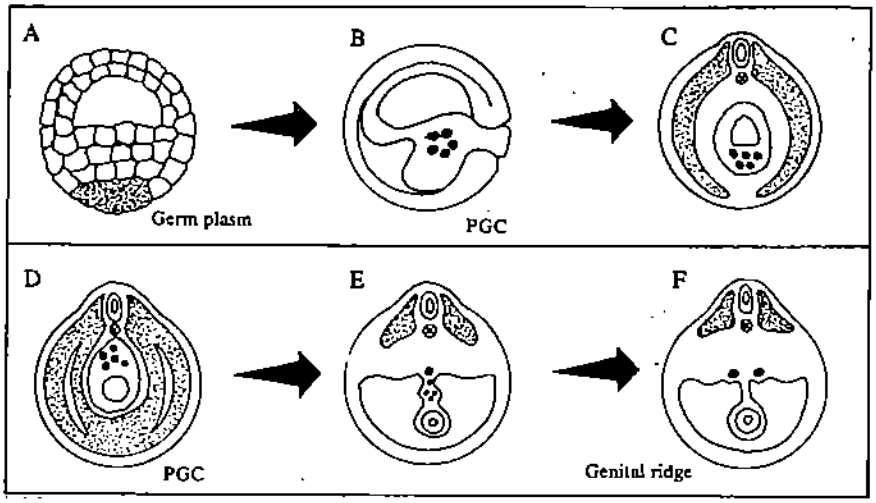

Fig. 15.20: Diagram showing the distribution of primordial germ cells (PGC) in the vegetal blastomeres and their subsequent migration to the genital ridge in frog embryos.

At about the end of gastrulation the primordial germ cells are found on the floor of archenteron (gut endoderm). The migrating primordial germ cells of frog (*Xenopus*) embryos leave the floor of the gut, move laterally and are then found aligned with dorsal mesentery. The PGCs migrate along this mesentery until they reach the developing gonads. Such an alignment suggests that contact guidance (see section 15.2.2) directs the movement of germ cells to gonads. It is suggested that adhesion of PGCs to the substratum is mediated by fibronectin produced by the mesentery cells. The frog PGCs adhere with and align on the fibronectin fibrils when they begin their migration into gonads.

15.5.2 Origin and Migration PGCs in Amniotes

In birds and reptiles the primordial germ cells originate in the epiblast (see marginal remarks), and move into underlying hypoblast (see marginal remarks) at the anterior

border of area pellucida of the primitive streak stage embryo. This region is known as germinal crescent region. Here the PGCs appear larger than the other embryonic cells (Fig. 15.21). In the chick the PGCs can be identified cytochemically by their large glycogon content. During further development PGCs enter the space between hypoblast and epiblast and finally invade the developing blood vessels. They are carried passively in the blood stream through the blood vessels until they reach the the genital ridges. Form the genital ridge they migrate through the mosoderm cells to reach the developing gonads.

Epiblast is the layer of cells lying above the blastocoel in a discoidal embryo such as chick embryo.

Hypoblast is the layer of cells below the blastocoel in a discoidal embryo.

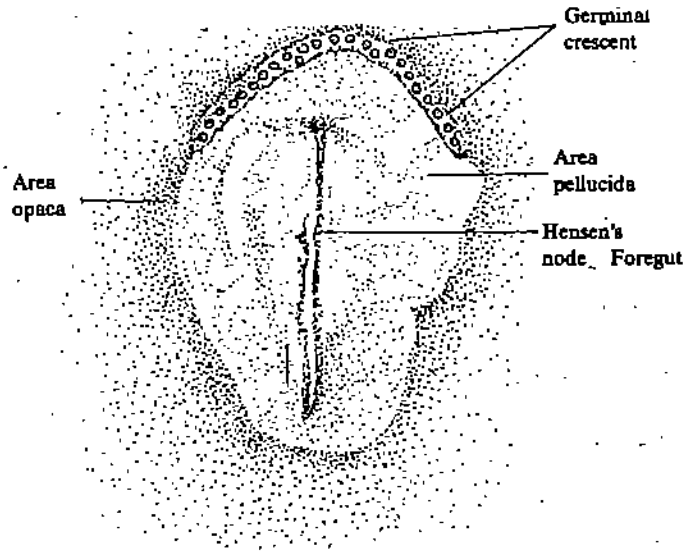

Fig. 15.21: Primitive streak stage of chick embryo showing primordial germ cells in the crescent at the anterior border between area pellucida and area opaca.

From the genital ridge to the gonads the movement is mediated by chemotaxis (see Section 15.2.2). Experimental studies have indicated that gonad tissues release a diffusible chemotactic molecule that attracts and directs the movement of PGC to their sites of location.

In mammals, the PGCs have high concentration of the enzyme, alkaline phosphatase and can be distinguished from other embryonic cells by staining for this enzyme. They are first seen in the yolk sac endoderm near the base of embryonic allantois (Fig. 15.22A). Here, they break up into two streams, each of which migrates through the developing gut into the dorsal mesentery and finally into the genital ridge of its respective side (Fig. 15.22B). In mouse it has been shown that besides the active movements by filopodia and chemotaxis, contact guidance also plays a part in the movement of PGCs

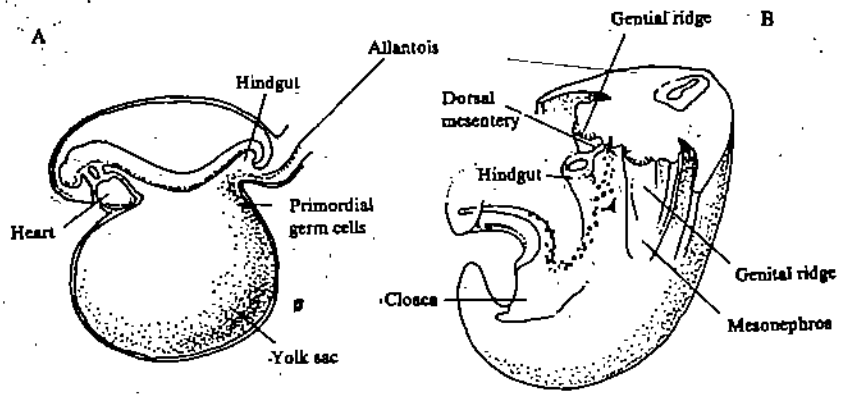

Fig. 15.22: Origin and migration of mammalian primordial germ cells. A) Primordial germ cells first recognised in the yolk sac. B) Migration through gut and dorsally up the dorsal mesentery into the genital ridge.

We end our discussion here on the morphogenetic processes and the morphogenesis of specific organs from their germ layers. You may attempt the following SAQ to check your understanding of this section of the unit.

SAQ 4

Fill in the blanks with appropriate words.

i) Endoderm gives rise to _____ tube and its accessory organs, _____ apparatus and _____ .

ii) Gonads arise from the _____ layer and primordial germ cells from _____ .

iii) The border between area pellucida and area opaca where the primordial germ cells are first seen is known as _____ .

iv) In *Xenopus* embryos the migration of primordial germ cells to the gonads is mediated by _____ .

v) In chick the migration of PGCs from genital ridges to gonads is mediated by _____ .

vi) In mammals the migration of PGC from the site of their origin to the gonads is mediated by _____ , _____ and _____ .

15.6 SUMMARY

- In this unit you have learnt the meaning of the word morphogenesis, the different types of morphogenetic processes and the mechanisms which mediate the cell movements in vertebrate embryos.
- We discussed the role of cytoskeletal components such as microtubules, microfilaments and intermediate filaments in cell movement during morphogenesis. You have also learnt about the nature of molecules to which embryonic cells adhere when they undergo change in their shape in order to effect morphogenetic movements.
- In this unit we also discussed the morphogenesis of organ rudiments from three germ layers of the embryo viz. the ectoderm, mesoderm and endoderm. We discussed the neurulation process which results in the separation of ectoderm into epidermal ectoderm and neural ectoderm and the subsequent separation of neural ectoderm into neural tube and neural crest cells. These processes are described in amphibians and in chick. Neurulation consists of, first the neural plate formation and then the neural tube formation. The neural plate is formed by changes in the shape of the cells by the cell elongation and accompanying apical shrinkage. The rolling of neural plate into neural tube occurs due to changes in cell shape brought about by the contraction of actin filaments.
- We have listed the five distinct regions of mesoderm found in neurula stage and the organs derived from them. We have discussed in detail the development of heart in amphibians and chick as the derivatives of lateral mesoderm. The differentiation of different blood cells from the pluripotent hematopoietic stem cell is also discussed.
- Finally, the various organ rudiments derived from endoderm are listed. In amphibians, chick as well as mammals, the gonads are derived from mesoderm but the primordial germ cells (PGCs) arise from endodermal cells. From the site of their origin the PGCs migrate to the genital ridge from where they find their way into gonads to differentiate into gametes.

15.7 TERMINAL QUESTIONS

1. What are the six different processes that characterise morphogenesis in vertebrates?

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2. Explain briefly the following terms in relation to morphogenesis i) palisading ii) invagination iii) evagination iv) cell death.

.....

.....

3. Describe the following types of movements exhibited by cells of a developing embryo.

   a) Chemotaxis b) Contact guidance

4. Describe the process of neurulation in chick.

5. Discuss the differentiation of an erythrocyte from a pluripotent CFU – S cell.

6. Describe briefly the origin and migration of PGC in chick embryos.

15.8 ANSWERS

SAQ 1 a) i) Differentiation: The process of generation of cellular diversity is referred to as differentiation. The process by which the single cell, the fertilised egg gives rise to muscle cells, skin cells, nerves, lymphocytes, blood cells and other cell types is known as differentiation.

ii) Morphogenesis: The process by which embryonic form and structure are produced is known as morphogenesis. Morphogeneis is a Greek work meaning generation of form or structure.

b) i) d ii) e iii) a iv) c v) b

c) i) chemotaxis

ii) haptotaxis

iii) galvanotaxis

iv) contact guidance

v) cytoskeletal structures

2. i) False

ii) True

iii) True

iv) False

v) True

vi) False

vii) False

3. a) i) e ii) c iii) d iv) b v) a

b) i) Circulatory

ii) lateral

iii) endothelial, endocardium

iv) splanchnic

v) 30 hr

vi) pericardial cavity

vii) atria, ventricles

c) i) True

ii) True

iii) True

iv) False

v) False

vi) False

4. i) Digestive, respiratory, primordial germ cells

ii) mesoderm, endoderm

iii) germinal crescent

iv) contact guidance

v) chemotaxis

vi) filopodia, chemotaxis, contact guidance

TERMINAL QUESTIONS

1. i) direction and amount of cell divisions

   ii) cell change shapes

   iii) cell migration

   iv) cell growth

   v) cell death

   vi) changes in the composition of cell membrane and extracellular matrix.

2. Refer to the text in subsection 15.2.1.
3. Refer to the text in subsection 15.2.2.
4. Refer to the text in subsection 15.3.2.
5. Refer to the text in subsection 15.4.3.
6. Refer to the text in subsection 15.5.2.

UNIT 16 MECHANISMS OF CELL INTERACTION

Structure

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16.1 INTRODUCTION

In the previous unit you have studied that differentiation means the production of numerous functionally distinct cell types from a single cell — the fertilised egg. The single diploid nucleus in the cytoplasm of a fertilised egg is the genetic material for generating a multitude of diverse cell types. More specifically, all the genes that are contained in this nucleus designate the properties of each and every cell type in the body. The relevant questions are, how does a single genome contained in the embryo is utilised to produce various cell types for performing different functions? Are different portions of the genome selected by different embryonic cells for expression in the various differentiated cell types? What determines the fate of different embryonic cells to develop into specific cell types? Do interactions exist between neighbouring cells of the embryo and if so, what is the role of such interactions in realizing the eventual fate of a population of cells to form a specific tissue? Each of these questions is directly related to mechanisms of cell interaction and cell differentiation. In this unit we shall discuss the answers to the above questions as suggested by results of recent studies on this subject.

Objectives

After studying this unit, you should be able to

- explain the concepts of totipotency, pluripotency and cell determination and that the embryonic nuclei possess genomic equivalence
- discuss that as embryonic development proceeds from zygote stage onwards there is

a progressive restriction in the potency of the nuclei to initiate and support normal development and promote differentiation of various alternate cell types

- describe that cell determination is regulated either by intrinsic factors located in the embryonic cells or by extrinsic factors by way of cell interactions
- distinguish the terms mosaic and regulative embryos and comprehend that the terms relate to the time at which restrictions in development are imposed on the blastomeres
- comprehend the phenomenon of embryonic induction and that instructive and permissive interactions between neighbouring populations of cells stimulate differentiation of specific tissues.

16.2 TOTIPOTENCY AND PLURIPOTENCY

In the beginning we said that the fertilised egg cell (zygote) has the capacity or potentiality to give rise to all kinds of cell types, such as a blood cell or a bone cell or a muscle cell etc. In embryological terminology such a cell is said to be **totipotent.** The zygote cleaves and forms a large number of blastomeres. The blastomeres may also remain totipotent upto a certain stage and have the capacity to form every cell type of the organism. However, as development proceeds the various blastomeres lose totipotency but may still be **pluripotent.** By pluripotent it is meant that they are able to form several but not all of the various cell types or tissues. Ultimately, with further development, even the pluripotency is lost and different groups of cells of the embryo are now capable of forming only one particular cell type or tissue. For example, upto a certain stage of embryonic development prospective ectodermal cells can form either epidermis or neural tissue, i.e. they are pluripotent. But at a later stage cells of one region of ectoderm can form only epidermal tissue and those of another region can differentiate only into neural tissue. What may be the cause of progressive restriction of potency in different embryonic cells although they are all descendents of the same single cell, zygote? Each one of these embryonic cells contain a nucleus which is also the descendent of the same zygotic nucleus. Does the restriction in potency in various embryonic cells result from differential distribution of specific cytoplasmic substances among different blastomeres during cleavage of zygote and subsequent divisions? We may ask a similar question with regards to the nucleus which contains the genes that direct the differentiation into specific tissues and cells types.

You know that the diploid zygote nucleus contains all the information located in its genes (or genome) required for the development of the entire body of the individual; and it directs and supports normal development from the egg and formation of all kinds of tissues and cell types. In other words, it has not only a complete genome but it is also totipotent in its capacity to support normal development from zygote stage onwards. Is this also true for the nuclei of all the cells of the embryo which are descendents of the original zygote nucleus? Do nuclei of all the cells of the embryo and later of the adult contain the entire genome like the zygote nucleus? Do the nuclei of embryonic and/or adult cells remain totipotent to support complete and normal development of all tissue and cell types like the zygotic nucleus? Or, does the potency of nuclei become progressively restricted, reduced or disappear as cells derived from the zygote diverge towards different pathways of differentiation? In the following sub-sections, we shall discuss the information available from experiments made to find answers to these questions.

16.2.1 Analysis of Genomic Equivalence of Nuclei

Towards the end of 19th century August Weismann had proposed that during cleavage the genetic determinants (later shown to be chromosomes) were parceled out among different blastomeres in some manner that determined the path of differentiation that a given cell might take. According to this theory only the germ cells contained all the genetic determinants and the various somatic cell types differed in the genetic determinants that they contained. In other words, the nuclei of only the germ cells contain all the genes and have a complete genome, while the nuclei of different somatic

cells may contain only those genes that are needed for their particular type of differentiation. However, on the basis of many studies it is believed that within an organism nuclei of all cells of the body contain a complete and identical set of all the genes like that in the original nucleus of the zygote. It means that the genomes of all cells and the zygote are equivalent. Development involves utilisation of different genes from this common genome by each cell type for its own particular type of differentiation. This belief in genomic equivalence of nuclei of all cells is supported by a fair amount of evidence of both genetic and embryological nature.

It has been found that specific number of chromosomes, on which genes are arranged, is constant throughout all the different tissues of the adult organism. The DNA, the carrier of genetic information, extracted from different somatic tissues has similar composition and properties. It has been shown that in the larvae of *Chironomus* and also *Drosophila* the number of chromosomes and their banding pattern (indicating the number of genes) are constant in all cells (Fig.16.1).

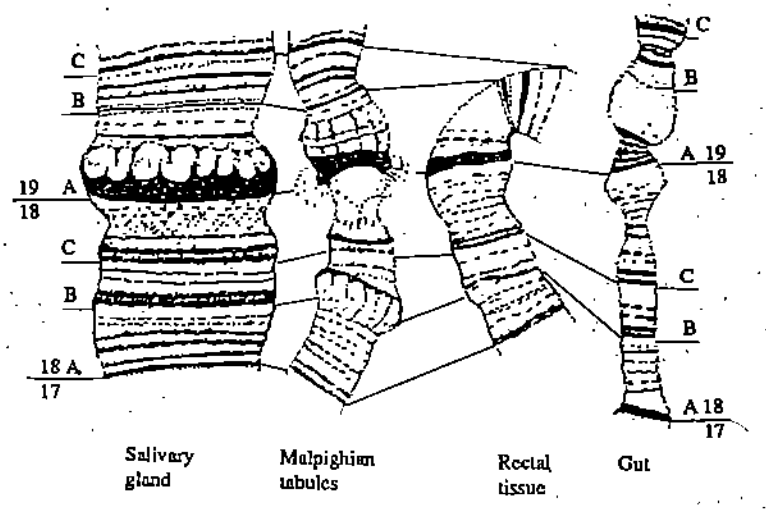

Fig 16.1: A region of polytene chromosomes of the midge *Chironomus tentans* from various tissues. Note the constancy of band number in different tissues.

In the salamander if the lens is removed, a new lens is regenerated from the dorsal iris. The cells of the new lens derived from differentiated iris cells, synthesise all the usual crystalline proteins characteristic of lens. You know that synthesis of each particular protein is controlled by a specific gene located in the nucleus. It means that the genes responsible for synthesis of lens proteins are present in the nuclei of iris cells which are therefore, genetically equivalent to the nuclei of lens cells; but these specific genes are not utilised in the iris cells. However, when iris cells are transformed into lens cells these genes become active and cause synthesis of lens proteins.

16.2.2 Analysis of Developmental Potency of Nuclei by Nuclear Transplantation in Eggs

The examples given above and many other observations and experimental results of this nature provide strong support to the hypothesis that within an organism all cells regardless of the type of their differentiation and functional assignment, are genetically equivalent; that is the nuclei of all of them contain a complete genome identical to that of the zygote nucleus. But are the nuclei of differentiated cells also capable of directing normal development and generating of cells of all types like the nucleus of a zygote? In other words, do the nuclei of cells derived from the zygote retain the totipotency of their nucleus or do they undergo some irreversible functional restrictions in the course of differentiation of cells? The most direct method of seeking an answer to this question involves the use of the technique of nuclear transplantation in suitably designed experiments.

i) Transplantation of Embryonic Nuclei

It was Hans Spemann (1936) who first suggested a procedure for testing the nuclear potency. The procedure consisted of isolation of nuclei from older embryos and implanting them into enucleated eggs (i.e. eggs whose own nucleus had been removed or killed). Fourteen years later Briggs and King (1952) successfully performed the first

nuclear transplantation experiments. They isolated the nucleus of a somatic cell and transplanted it into an enucleated egg (see box for details) in the leopard frog *Rana pipiens* (Fig. 16.2). When nuclei isolated from blastula were transplanted into enucleated eggs, 55% of them underwent normal cleavage and developed into blastula, 80% of the remaining developed into tadpoles and 70% of the remaining reached metamorphosis stage. In experiments by McKinnel (1962) transplantation of blastula nuclei into enucleated eggs resulted in development of normal postmetamorphic frogs. These experiments essentially suggested that upto blastula stage the nuclei of blastomeres have not lost the potency to promote normal development and are equivalent to zygote nucleus in every respect.

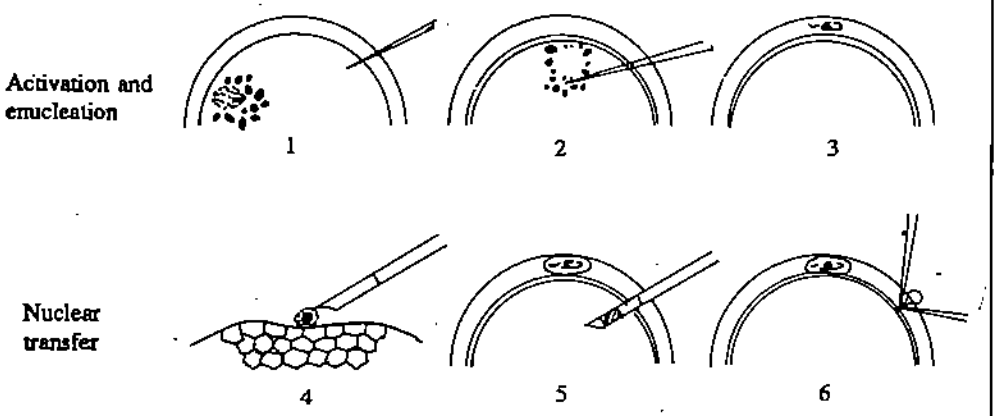

Fig. 16.2: Steps in nuclear transplantation experiments in frog eggs.

1) Activation of eggs with a glass needle
2) Enucleation
3) Enucleated eggs
4) Dissociation of donor cells and drawing of an individual nucleus into a micropipette
5) Insertion of the nucleus into the enucleated eggs.
6) Transplanted egg

The transplantation experiments consist of 1) preparation of a recipient egg of the frog and 2) isolation of a donor cell and transfer of its nucleus into the enucleated egg. The recipient eggs are obtained from females by inducing ovulation in them. You may recall from Unit 13 that activation is an essential component of fertilisation process. Here activation is achieved by pricking the egg surface with a glass needle. This results in the movement of nucleus to animal pole. With a second glass needle the nucleus is removed. Donor cells are first dissociated in a dissociation solution which does not contain Ca^{++} needed for normal cell adhesion. The individual cell is then drawn into micropiptette with a slightly smaller diameter than the cell. The cell breaks and the nucleus with surrounding cytoplasm is drawn into the micropipette. This nucleus is then injected into the activated, enucleated egg. The transplanted egg is now ready for development.

In another set of experiments, nuclei from gastrula were tested in a similar manner. Early gastrula nuclei were as effective as blastula nuclei in promoting cleavage and development. But definite restrictions were observed in the developmental potential of the nuclei of later stages. Enucleated eggs receiving nuclei from the endoderm of late gastrula and post-gastrula stages did exhibit further development; but deficiencies were found in the size and extent of differentiation of ectodermal and mesodermal derivatives. The endodermal derivatives developed normally but not the mesodermal and ectodermal derivatives. When the endoderm nuclei from the tail bud stage embryo were chosen for transplantation, they did not support normal development, indicating that the nuclei have become increasingly specialised as endoderm nuclei and are restricted in their ability to support development. But occassionally nuclei from postgastrula stages were also found to promote normal development in some cases. Thus, transplantation of nuclei from the

ectodermal cells of the neural plate of frog neurula did produce a low percentage of normal larvae. Here also, as in the case of endodermal nuclear transplants, the percentage of normal development decreased as the nuclei were derived from neural ectoderm of progressively older embryos. Moreover, the aberrant tadpoles that developed from eggs containing transplanted neural cell nuclei had good neural differentiation but lacked endodermal structures.

Such experimental studies involving transplantation of nuclei from cells of progressively older embryos to enucleated eggs have also been made on another frog species, *Xenopus laevis*, by Gurdon and co-workers. As in the case of *Rana pipiens* a progressive loss of nuclear potency with increasing development has been found in *Xenopus laevis* also (Fig. 16.3).

Briggs and King have shown that the kind of restriction imposed on the potency of nuclei of embryonic cells as development proceeds is stable and tissue specific. For this purpose they used the technique of serial nuclear transplantation. In this technique one nucleus is transferred into an enucleated egg which is allowed to develop upto blastula stage consisting of thousands of cells having identical nuclei, each having been derived from the same transplanted nucleus through many cleavage divisions. These blastula nuclei are than transferred singly into more enucleated eggs, which are again allowed to develop upto blastula stage to provide another generation of thousands of nuclei which are identical copies of the single originally transplanted nucleus, and so on. This is called nuclear cloning (Fig. 16.4). Using these many identical copies of the original nucleus the potential of that nucleus can be tested and evaluated in large number of cases through several generations of cloned nuclei. With this technique it was observed that the nuclei cloned from a single endoderm nucleus of a late gastrula transplanted into enucleated eggs produced similar deficiencies in the resultant embryos through several generations. They had endodermal structures (mainly, the gut) but lacked ectodermal and mesodermal structures. Similar tissue specific restriction in the potency was observed when nuclei from neural ectoderm were cloned and transplanted into enucleated eggs. The resultant embryos were abnormal and contained mainly ectodermal structures.

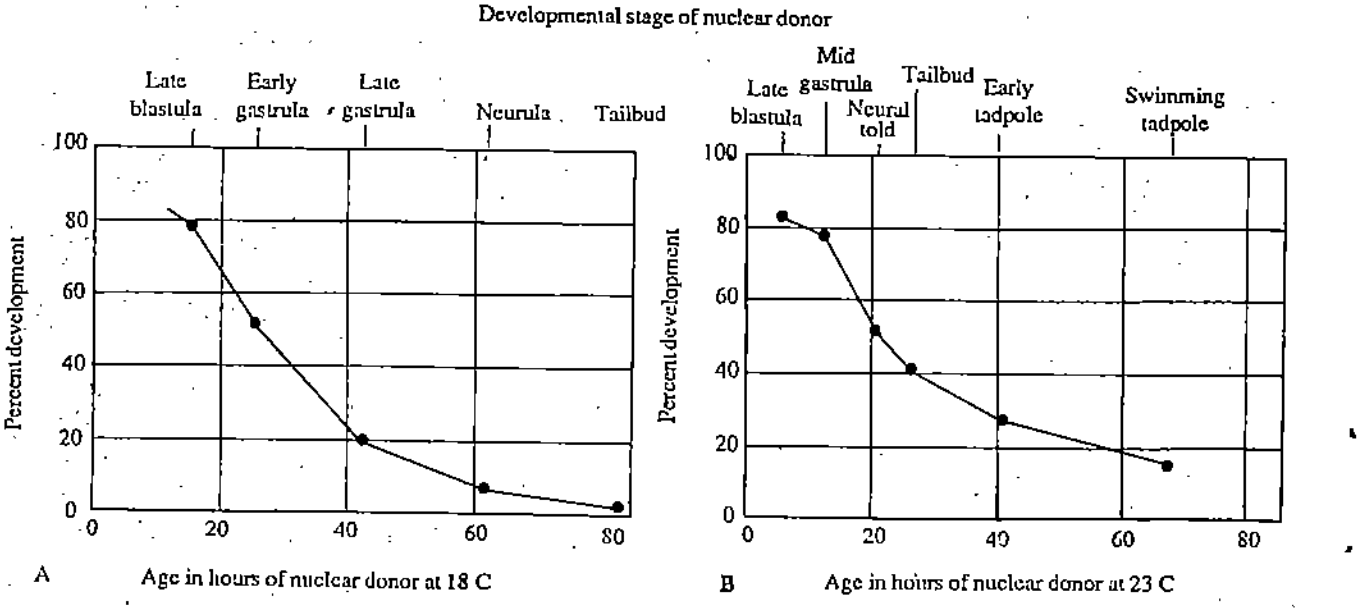

Fig. 16.3: Relationship between the age of the donor and the ability of their nuclei to promote normal development. (A) *Rana pipiens* (B) *Xenopus laevis*. You may observe from the figures that although *Rana pipiens* develops more slowly than *Xenopus laevis* the loss of potency occurs at a similar rate in both the species.

ii) Transplantation of Postembryonic Nuclei

So far, we have discussed the nuclear transplantation experiments involving embryonic nuclei. Let us now briefly look into the results of transplantation studies involving postembryonic nuclei in amphibian eggs.

You have learnt that as the embryo develops, there is a progressive reduction in nuclear

potency. But this is not always true. Experiments have shown that the nuclei of cells from post-embryonic stages can promote considerable development in enucleated eggs. One such type of cell is the primordial germ cell (PGC). When the nuclei of PGC from young tadpoles were transplanted into the enucleated eggs of frog, complete tadpoles developed in 40% cases. Since PGCs are precursors of gametes, a high degree of potency must exist in their nuclei, which have an intact totipotent genome capable of directing normal and complete development of an embryo. But when later stage germ cells such as spermatogonial cells from juvenile and adult frogs were transplanted into enucleated eggs, development did not proceed beyond blastula stage. Of the 13 blastulae, only 3 developed into gastrula stage and one into an abnormal larva that died just after commencing feeding. These results suggest that developmental totipotency is different from genetic totipotency. Genetically totipotent nuclei need not be developmentally totipotent. Probably, although the germ cell line retains the genome intact it appears that beyond the primordial germ cell stage, the nuclei of PGCs undergo a developmental restriction which limits the utilisation of the genome for promotion of development in the egg into which they are transplanted.

Extensive nuclear transplantation experiments have been carried out to test the potency of the nuclei of fully differentiated somatic cells. Gurdon (1962) transplanted nuclei from the differentiated intestinal epithelial cells of *Xenopus* tadpoles into enucleated eggs of the same species. A very small number, 10 out of 726, of the recipient eggs developed into adult frogs; but in a large percentage of cases the nuclear recipients could go through at least some cleavage divisions producing arrested blastulae. The failure of further development was considered to be possibly due to damage to the nuclei during transplantation or to incomplete chromosomal replication of transplanted nuclei. To overcome these problems Gurdon also used the technique of serial transplantation developed by King and Briggs which we have described earlier in this subsection (see Fig. 16.4). Using this method, several nuclei isolated from such abnormal arrested embryos were injected singly into enucleated eggs and their development followed. Some of the serial transfer embryos developed into feeding tadpoles increasing the total number of feeding tadpoles to 7%. In many of them the nuclei from intestinal epithelial cells had succeeded in generating all cell types-neurons, blood cells, beating heart, muscles etc. Moreover, seven of these tadpoles developed beyond metamorphosis into fertile adult frogs. These results indicated that the nuclei of the intestinal epithelial cells of feeding stage tadpoles of *Xenopus* are totipotent capable of promoting complete and normal development.

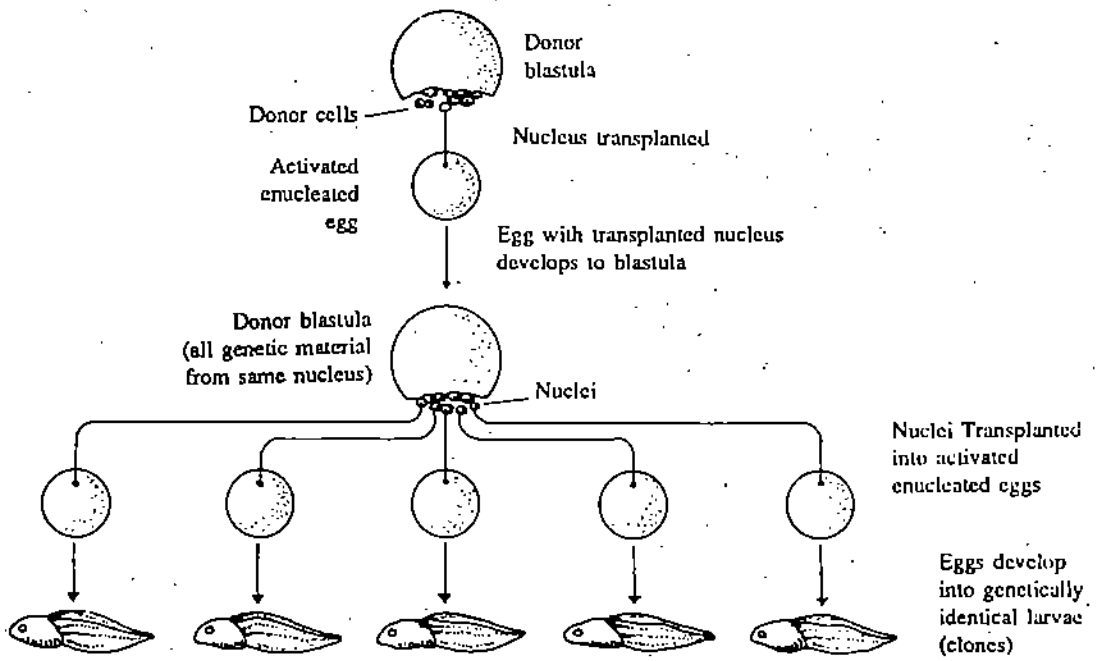

Fig. 16.4 **Testing potency by serial nuclear transplantation. Nuclei of donor cells from a blastula are placed into activated, enucleated eggs. The blastula produced by such a transplant is used as a source of nuclei for a second generation of transplants, a process called nuclear cloning.**

Gurdon and his colleagues have used the technique of serial transplantation with the

nuclei of differentiated epidermal cells obtained from cultured webbing of the foot of adult frog. These cells contained keratin showing they were differentiated. Keratin is the characteristic protein of differentiated adult skin cells. By serial transplantation of these nuclei numerous tadpoles developed from recipient eggs. They contained functional muscle and nerve, beating heart, circulating blood, eyes with lenses and other types of differentiated cells.

Similar results have been obtained by serial transplantation of nuclei from lymphocytes of adult *Xenopus* into enucleated eggs.

Two possible conclusions emerge from the results of nuclear transplantation experiments in amphibians that we have discussed above:

i) The genome contained in the nucleus of a differentiated cell is remarkably potent in its ability to promote differentiation of several other different cell types.

ii) There is a general restriction in the potency of nuclei to promote normal development as embryonic development progresses from zygote onwards.

In the opinion of Gurdon, if a nucleus from a particular cell type is found to promote differentiation of only some but not all cell types other than its own type it should not be assumed to have lost any potency. The nuclei of older embryos and differentiated cells may not be able to support normal development in most cases not because of irreversible alterations in the nuclei but because they are probably unable to adjust to the strange cytoplasmic environment of the recipient egg. Cytoplasm may even cause damage to the transplanted nucleus and its chromosomes.

In any case, even if there is controversy about the totipotency of nuclei of differentiated cells, there is no doubt they are extremely pluripotent. Many unused genes latent in the nuclei of differentiated cells of one type can be reactivated to produce, cells of several other types.

Other experiments have shown that in insects also the nuclei atleast upto gastrula stage remain capable of promoting normal development of enucleated eggs. In the case of mammals the nuclei isolated from the inner cell mass of blastocyst and injected into enucleated eggs are reported to support development to fetal stages.

In this section we discussed the question of genomic equivalence and potency of the nuclei of cells as embryonic development progresses and cells differenitate into different types. We also mentioned that the embryonic cells or blastomeres, which upto a certain stage of development are totipotent, gradually become pluripotent, and ultimately remain capable of forming only one cell type. When this happens they are said to have become determined. In an embryo, cell determination is a progressive process involving a sequence of decisions that gradually restrict the cell fate. In the next section, we shall discuss the problem of cell determination, the methods of studying the process as well as the role of cytoplasm in the process. Before we do so, answer the following SAQs to check your understanding of what we have discussed so far.

SAQ 1

i) Define the terms totipotency and pluripotency.

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.....

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.....

ii) State whether the following statements are true or false by ticking T or F, T/F.

a) Blastula nuclei of frogs do not exhibit any loss of potency. T/F

b) There are no restrictions in the development potential of the nuclei of gastrula stage of frog. T/F

c) Nuclei obtained from tail bud endoderm of the gastrula stage of frog when transplanted into enucleated eggs showed normal development. T/F

d) Nuclei of more highly differentiated cells have progressively lost the potency to promote development of reciepient eggs. T/F

e) Primordial germ cells as precursors of gametes are highly determined cells. T/F

f) Developmental totipotency and genetic totipotency mean one and the same thing. T/F

g) Fully differentiated somatic cells are as potent as the cells from a blastula. T/F

h) Nuclei from some of the postembryonic cells retain the potency to promote partial development of enucleated eggs. T/F

16.3 CELL DETERMINATION

Cell determination is a process by which portions of embryonic genome are selected for expression in specific embryonic cells. Determination to follow a specific pathway of differentiation precedes the appearance of any visible change in biochemistry or morphology of the cell. We know of two major ways by which determination takes place.

In one way certain factors which reside within the cytoplasm of embryonic cells (intrinsic factors) control cell determination. These factors are referred to as ooplasmic or **cytoplasmic determinants.** During cleavage they are passed on to specific blastomeres whose future differentiation is thus finally decided. This process is also called determination by cytoplasmic segregation.

In the other method, the final cell fate is determined by extrinsic factors that originate outside the cells. The extrinsic factors include:

1) The instructions obtained as a function of the position of blastomeres within the embryo,

2) signals that are transmitted between the blastomeres.

This second process is known as embryonic induction in which the cell fate is determined by signals received from different cells. The role of embryonic induction in cell determination is discussed in section 16.5.

In different species determination occurs at different times during development. It may be very early or it may occur at relatively much later stages. In quite a number of animal groups, such as annelids, molluscs, tunicates etc. the fates of the blastomeres resulting from the first few cleavage divisions are already determined. Such eggs and embryos are called **determinate** or **mosaic.** In others such as echinoderms and vertebrates final determination occurs much later, even as late as during or after gastrulation, and they are said to have **indeterminate** or **regulative** eggs.

In this section we shall first describe determinate (mosaic) and indeterminate (regulative) eggs. We shall then discuss the role of ooplasmic or cytoplasmic determinants (intrinsic factors) in cell determination process.

16.3.1 Mosaic and Regulative Eggs

Eggs as well as early cleavage embryos are sometimes divided into two groups: i) **mosaic** or **determinate** embryos and ii) **regulative** or **indeterminate** embryos. The terms mosaic and regulative relate to the time at which the restrictions in development are imposed on the blastomeres. For example, the embryos of tunicates belong to mosaic or determinate category. In these organisms the various blastomeres become restricted to

form only specific structures as soon as they are formed or during the first few cleavages. In other words, the respective fates of different blastomeres are finally determined early in mosaic embryos. As a result, the removal or loss of certain blastomeres can not be compensated by the other blastomeres and the embryos will be defective in structure which are normally derived from the missing blastomeres.

In regulative or indeterminate embryos the restrictions on blastomeres occur later in development. Amphibians and sea-urchins belong to this category. Since the cell fate of blastomeres is finally determined later, any removal or destruction of blastomeres early in development can be compensated by the other blastomeres which are able to form all the structures.

It is also believed that the differences between mosaic and regulative embryos are directly related to the extent of their usage of extrinsic and intrinsic mechanisms for cell determination. The mosaic embryos depend upon ooplasmic determinants, that is, intrinsic factors for cell fate determination. In constrast, the regulative embryos mainly rely upon extrinsic factors derived from cell interaction for cell determination. In the following section we shall look into the details of extrinsic and intrinsic factors that determine the cell fate with suitable examples.

16.4 CELL INTERACTIONS AND OOPLASMIC DETERMINANTS

Miscroscopic observations of egg cytoplasm suggests that it is not homogenous in appearance. The observable differences in the cytoplasmic regions of the egg are referred to as **cytoplasmic localisations**. The cytoplasmic localisations do not always reflect the presence of ooplasmic determinants but only help to identify specific regions of cytoplasm. As we have mentioned earlier ooplasmic determinants (intrinsic factors) are apportioned unequally to different blastomeres during cleavage. In other words, the initial determination of cell fate is controlled by the specific ooplasmic determinants which a blastomere receives. The determination of both the future germ cells and somatic cells lineage is decided by ooplasmic determinants. We shall now look into the role of ooplasmic determinants in the determination of germ cell lineage in nematodes and somatic cell lineage in tunicates.

16.4.1 Ooplasmic Determinants and Germ Cell Lineage in the Eggs of *Ascaris* (Nematode)

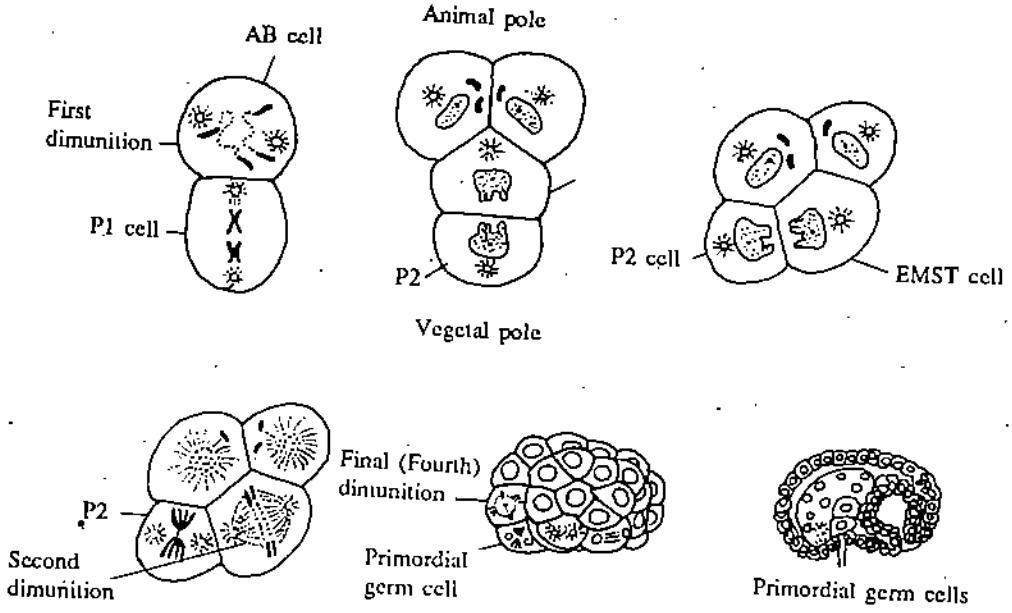


Fig. 16.5: Chromatin dimunition and primordial germ cell determination in the early development of *Ascaris* (See text for explanation)

Many studies have shown that the germ cells become distinct from somatic cells during

early development. In certain cases, e.g. vertebrates, the prospective germ cells show a morphology in terms of size, shape and most importantly, cytoplasmic granules, distinct from prospective somatic cells. The specialised cytoplasmic granules are confined to the primordial germ cells during development and these are possibly the ooplasmic determinants that regulate germ cell determination (refer to Unit 15).

In *Ascaris* (a nematode worm) the blastomeres destined to develop into somatic cells lose specific parts of chromosomes - a process known as **chromatin dimunition**, but the germ cells retain the complete set of chromosomes and full complement of nuclear DNA. Studies have shown that the somatic cells do not lose any structural genes but only repeated DNA sequences. The chromatin dimunition is a distinct cytological marker for distinguishing the primordial germ cells from somatic cells in this nematode.

Fig. 16.5 shows cleavage and early development in *Ascaris*. You can observe that first cleavage is equatorial and it divides the egg into an animal hemisphere blastomere known as **AB** cell and a vegetal hemisphere blastomere the **PI** cell (Fig.16.5A). Before the second cleavage, the mitotic apparatus of one of the cells rotates to a position perpendicular to the other mitotic apparatus. Now, the AB cell cleaves meridionally and the PI cell equatorially. The four cells of the embryo are arranged in a T shape (Fig. 16.5B). During the second division, chromatin dimunition occurs in the AB cell. This happens by some parts of the chromosomes fragmenting. These fragments are not included in the daughter nuclei and remain outside it in the cytoplasm. Thus, the nuclei of the daughter cells of AB become deficient in chromatin. The descendents of AB cell form the somatic cell. No such dimunition occurs in PI cell, and its two daughters are designated as **EMST** and **P2**, both with intact chromosomes. The T shaped four cell embryo transforms into a trapezoid shaped embryo (Fig. 16.5C) and its cells undergo the third cleavage. During this division one of the daughters of PI cell (termed EMST cell) also undergoes chromatin dimunition (Fig. 16.5D); its daughter cells and subsequent descendents are all deficient in chromatin and they develop as somatic cells. In the other daughter cell of P1 (i.e., the P2 cell) there is no dimunition of chromatin and it divides into 2 daughter cells, one of which is a somatic cell that loses some chromatin during next division, the other daughter of P2 (P3 cell) which retains the intact genome is the precursor of the future germ cells. During the next (4th) cleavage P3 produces two cells, one of which (the P4 cell) retains the intact genome with no chromatin loss and becomes the primordial germ cell (Fig. 16.5E). The other daughter cell loses some more chromatin during the next division and its descendents become somatic cells. During the next (5th) cleavage the P4 cell divides to produce two primordial germ cells, both of which retain the intact chromosomes (Fig. 16.5F), both of which migrate into gonads where they proliferate and differentiate into germ cells. There is no chromatin dimunition in any descendent of primordial germ cells. Thus, by the end of 4th cleavage all the precursors of somatic cells have reduced chromatin and only the germ cells retain the full set of intact chromosomes. The entire process is presented in a schematic manner in Fig. 16.7.

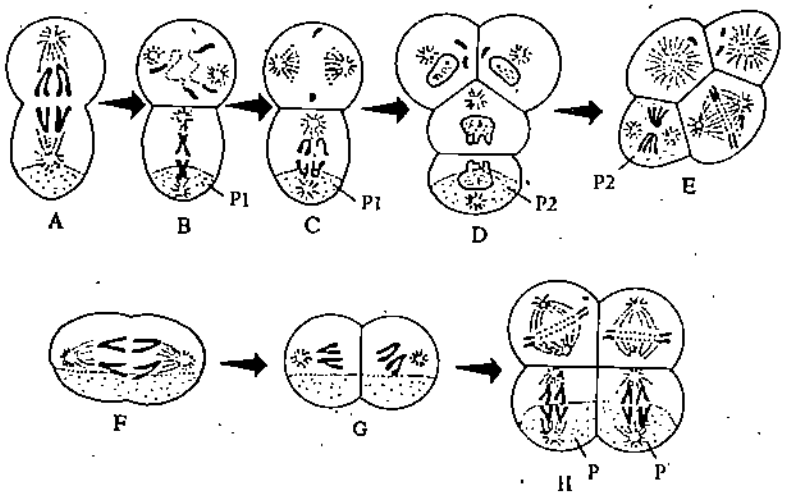


Fig. 16.6 A-G : Vegetal cytoplasm of *Ascaris* and its role in regulation of germ cell determination. A-E: Cleavage in normal eggs. Note that vegetal cytoplasm (shaded) is restricted to cells P1 and P2 in which chromatin dimuntion does not occur. F-H : these figures show results of centrifugation of egg before first cleavage in an experiment by Boveri (for explanation refer to text).

Boveri (1910) in a series of experiments showed that some ooplasmic determinants located in the vegetal cytoplasm of the egg regulate the germ cell determination in *Ascaris* (Fig. 16.6. A-E). By centrifuging *Ascaris* eggs and shifting the orientation of the first mitotic spindle the plane of division was changed so that the first cleavage of the egg became vertical instead of being equatorial (Figs. 16.6 F). As a result the vegetal cytoplasm gets distributed equally to both the blastomeres after the first cleavage division; and chromatin dimunition did not occur in either of the first two blastomeres (Fig. 16.6 G). The next cleavage was equatorial so that at 4 cell stage only the two vegetal blastomeres got all the vegetal cytoplasm and it was lacking in the two animal blastomeres. While chromatin dimunition occurred in the latter, the chromosomes remained intact in the former (Fig. 16.6 H). It was obvious that the vegetal cytoplasm contained some substance (determinants) which protected the chromosomes from fragmentation and chromatin dimunition.

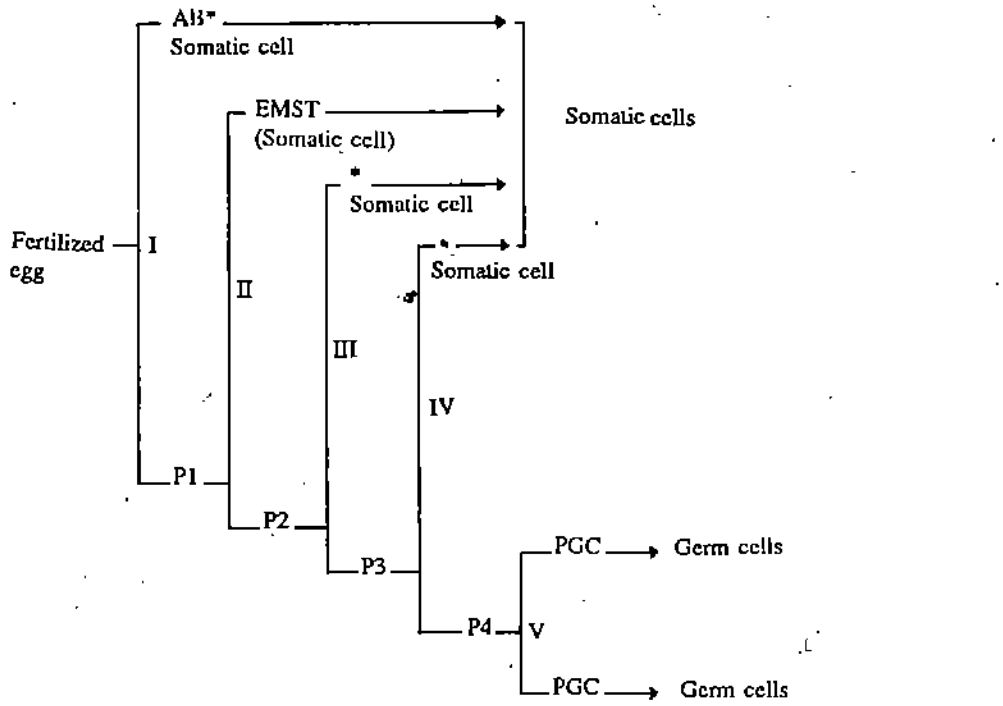


Fig. 16.7 Schematic representation of first 5 cleavage divisions of *Ascaris* eggs. Cells marked * undergo chromatin dimunition. P, P2, P3, P4 and PGC retain intact chromosomes. P3 is the precursor of the first primordial germ cell (P4) which gives rise to 2 primordial germ cells from which all germ cells with intact genome arise. Roman numerals I-V indicate the serial numbers of cleavage.

16.4.2 Ooplasmic Determinants and Somatic Cell Determination in Tunicates

Eggs of tunicates undergo typically mosaic development in which the various blastomeres become determined to follow their specific paths of differentiation very early after fertilization. The determination is signalled by determinants present in the egg cytoplasm which become segregated in specific blastomeres during the first three cleavage divisions. Intensive descriptive, experimental and biochemical studies on some tunicate species such as *Styela* and *Ciona* have confirmed that determination is brought about under the influence of intrinisic cytoplasmic (ooplasmic) determinants and not by interaction between blastomeres or alteration in the genome in the nuclei of different blastomeres. This is especially true for the determination of blastomeres which give rise to muscle cells of the tadpole that hatches from the egg.

In the previous sub-section, you have studied determination of germ cells in the mosaic eggs of *Ascaris* by cytoplasmic determinants. Now, we will discuss muscle cell determination in tunicates based on the studies on *Styela* and *Ciona*. This is a good example of determination of a somatic cell type (muscle) by cytoplasmic determinants in mosaic eggs.

Eggs of tunicates contain coloured cytoplasmic regions. For instance, *Styela* eggs contain

an yellow coloured region, the myoplasm. In another tunicate *Boltenia* the myoplasm region is orange coloured. Besides, the egg contains ectoplasm and endoplasm regions as well. Cytoplasmic movements known as ooplasmic segregation localise these regions in eggs after fertilisation. In *Styela* and *Boltenia* the ooplasmic segregation is evident because of the coloured regions of the cytoplasm. In the unfertilised egg of *Styela* the clear ectoplasm is present in the form of germinal vescicle near the animal pole (Fig. 16.8A). The grey yolky endoplasm occupies the entire vegetal half and the yellow myoplasm is confined to the peripheral cortical region. Immediately after fertilisation there occurs displacement of the three materials (plasms) in the zygote. The yellow myoplasm flows down to the vegetal pole. The germinal vesicle breaks and the clear ectoplasm moves into the vegetal hemisphere. At the same time the endoplasm is displaced into the animal half of the egg. (Fig. 16.8B). This arrangement of cytoplasmic contents of the zygote does not last for much time and soon by a second phase of segregation the three cytoplasmic materials are rearranged again. The myoplasm moves to a position below the equator of the egg and forms a crescentic region on the future posterior side of the egg (Fig. 16.8C). It is known as the yellow crescent. Most of the grey yolky endoplasm moves to the future anterior half and the ectoplasm to the animal half of the egg simultaneously. Opposite to the yellow crescent, a fourth cytoplasmic region, the chordoplasm is formed (Fig. 16.8D). This is also a crescent located beneath the equator on the anterior side of the vegetal half of the egg. This arrangement of various cytoplasmic regions clearly shows the bilateral organisation of zygote contents.

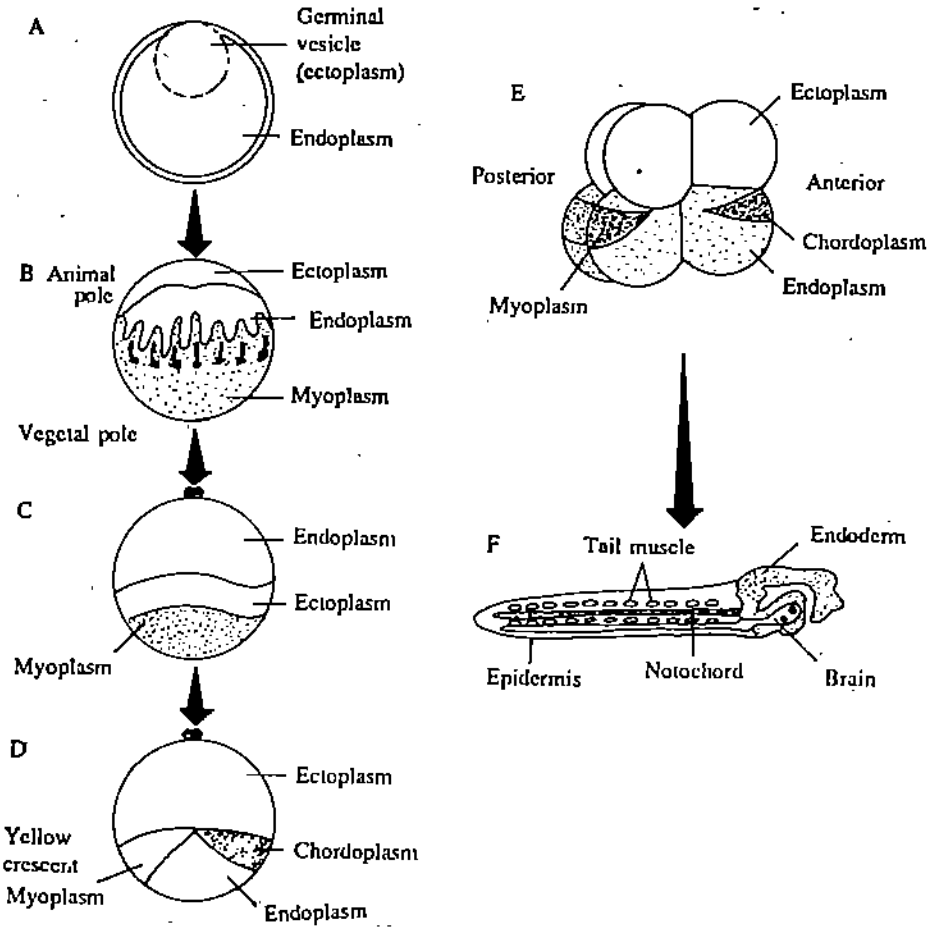


Fig. 16.8 : Diagram showing distribution of coloured cytoplasmic regions during cytoplasmic segregation and at 8-cell stage of *Styela*. See the text for explanation.

At the time of the cleavage, the coloured regions of the egg are distributed to different blastomeres. The first cleavage ocurs in the meridional plane passing along the axis of bilateral symmetry and produces two equal blastomeres each containing parts of the two crescents. The second cleavage is also meridional and perpendicular to the first. Now the two anterior blastomeres receive the chordoplasm and the two posterior blastomeres receive the yellow crescent. The equatorial third cleavage produces eight blastomeres. At this stage the ectoplasm is confined to four animal cells, the endoplasm to the four vegetal cells (2 anterior and 2 posterior), the myoplasm to the two posterior vegetal cells and the chordoplasm to the two anterior vegetal cells (16.8E). The fates of these

coloured blastomeres could be further followed and it was found that the blastomeres containing ectoplasm give rise to epidermis and neural tissue, those containing endoplasm give rise to gut, the chrodoplasm containing blastomeres form the notochord and the cells containing myoplasm give rise to tail muscles (Fig. 16.8F).

The enzyme acetyl cholinesterase is found only in the muscles of tunicate larvae. Whittaker (1973) demonstrated that in cleavage arrested embryos this enzyme is produced in both blastomeres at 2-cell stage, only in two posterior blastomeres at 4-cell stage; and only in the two posterior vegetal blastomeres at 8-cell stage. It is these two cells into which the myoplasm (yellow crescent material in *Styela*) becomes localised by 8-cell stage. No other blastomere produces acetyl cholinesterase. If the yellow crescent bearing blastomeres are removed the remaining 6-cells form defective larva which does not have muscles. The results indicated that the muscle specific enzyme acetyl cholinesterase is produced under the influence yellow crescent material (myoplasm) which becomes localised in the two posterior vegetal cells that are thus determined to form muscle cells. Later, Whittaker (1980) by another elegant experiment confirmed that the muscle determinants are cytoplasmic in origin. Just before third cleavage the normal distribution of myoplasm was changed by compressing the embryo between two glass coverslips. Usually, the third cleavage in tunicates is equatorial and the myoplasm is partitioned to two posterior vegetal cells (Fig. 16.9A-E). But after compression of the egg between the two coverslips the third cleavage occurred along a meridional plane. As a result the myoplasm got distributed to four blastomeres instead of the usual two (Fig. 16.9F, G). There was no change in the nuclei of the blastomeres. Each of the eight blastomeres received the same nucleus which it would have if the cleavage was equatorial as usual. Now, further cleavage was arrested by treating the embryo with cytochalasin B. This drug inhibits cytokinesis but allows nuclear divisions to continue, and the cells also continue to proceed whith their specific type of cytoplasmic differentiation. Later, such cleavage arrested embryos showed acetyl choline sterase production in all four cells which had received the myoplasm (Fig. 16.9G). The two extra acetyl cholinesterase producing cells obtained the same nuclei as they would have obtained under normal cleavage, but it was the presence of myoplasm in them that conferred on them the properties of muscles

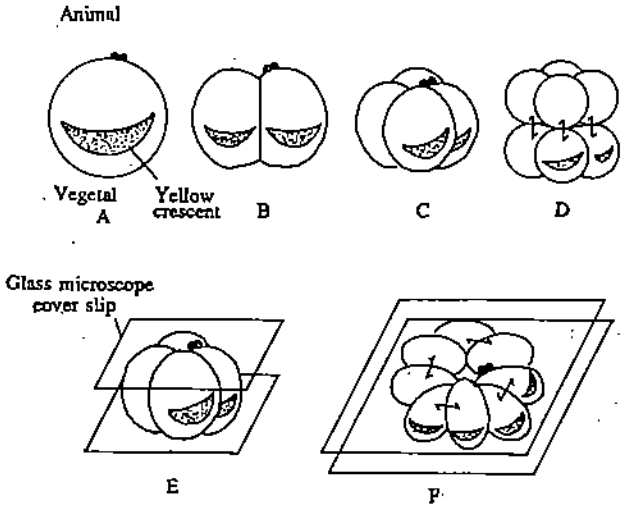


Fig. 16.9 Diagram showing the distribution of myoplasm (yellow crescent material) in normal (A-D) and compressed (F,G) embryos (see text for explanation).

Thus far, we have discussed the role of ooplasmic determinants in germ cell determination in the case of mosaic eggs of *Ascaris*, and somatic cell (muscle) determination in the case of mosaic eggs of ascidians. Attempt the following SAQs before you proceed to read about embryonic induction.

SAQ 2

Fill in the blanks with suitable words.

a) The process in which selective portions of embryonic genome are expressed in specific embryonic cells is known as_____ _____

b) The process of cell fate determination by transmission of signals between

blastomeres is referred to as _____ _____.

c) Eggs in which restrictions in development are imposed on blastomeres during the first few cleavages are _____.

d) _____ eggs are those in which restrictions in development occur later in development.

e) _____ _____ are the intrinsic factors that determine the cell fate in mosaic embryos.

j) Regulative eggs rely upon _____ _____, the extrinsic factors, for cell determination.

g) _____ _____ are observable differences in the cytoplasmic regions of the egg.

h) The process of loss of specific parts of chromosomes in the blastomeres during development is known as _____ _____.

i) _____ _____ located in the _____ _____ of the egg regulate germ cell determination in *Ascaris*.

j) The localisation of different regions in the eggs of tunicates is achieved by cytoplasmic movements known as _____ _____.

k) In tunicates the posterior part of vegetal hemisphere where myoplasm is located is known as _____ _____.

l) The four regions obtained in the eggs of tunicates after the second phase of ooplasmic segregation are _____ _____ _____ and _____.

m) The muscle determinants in tunicates are _____ in origin and not _____.

16.5 EMBRYONIC INDUCTION AND CELL DETERMINATION

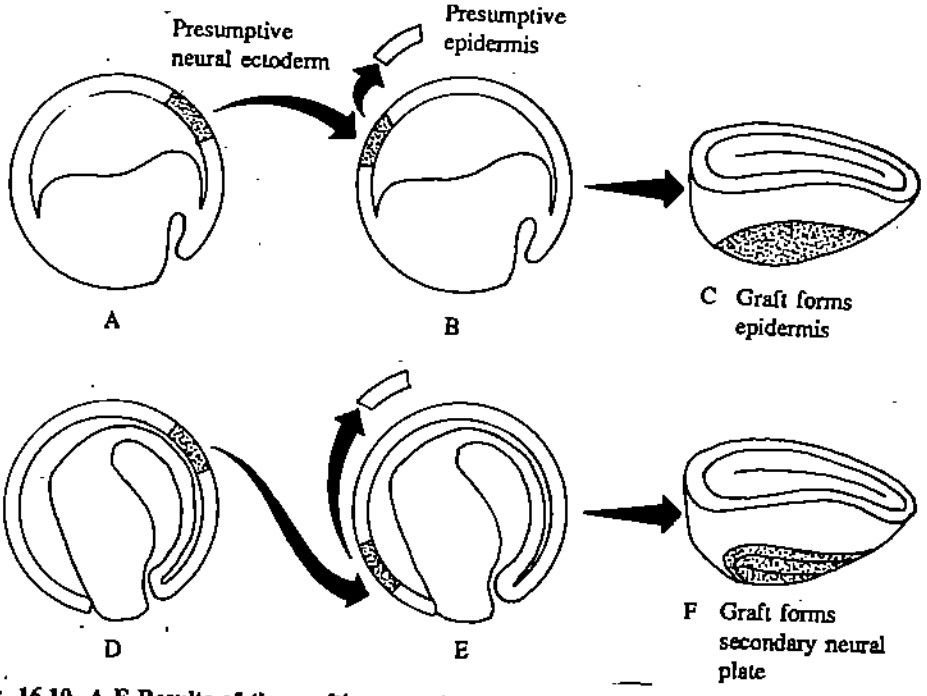
In section 16.3, we mentioned that cell determination or fate of embryonic cells is regulated by factors which may reside within the embryonic cells or by the factors that originate outside the cell. In fact most differentiated tissues arise by a process that requires interactions with neighbouring cells of the embryo. These interactions limit or restrict the possible fate of localised regions of the embryo. **The stimulation of a population of cells to differentiate in a specific direction by another group of cells is known as induction.** The concept of embryonic induction was first put forward by the German embryologist Hans Spemann and his co-workers early in this century. Hans Spemann and Hilde Mangold did a series of grafting experiments using amphibian embryos. They reported that the interaction between neural ectoderm and the underlying chordamesoderm determines the eventual fate of the neural ectoderm tissue. Subsequent experiments established that earlier to induction of neural ectoderm, the mesoderm itself is induced during blastula stage by the prospective endodermal cells of the vegetal hemisphere. We shall begin with the description of the Spemann's experiment and then briefly look into mesoderm induction in amphibians.

16.5.1 Determination of Neural Ectoderm by Induction from Dorsal Mesoderm : Primary Embryonic Induction

We mentioned earlier that the dorsal mesoderm induces the ectoderm to differentiate into neural tissue. Spemann and his co-workers conducted their experiments in newts, a group of amphibians. Two species of newts whose cells differed in pigmentation were chosen for the experiments. These included the non-pigmented *Triturus cristatus* and the pigmented *Triturus taeniatus*. He then exchanged pieces of presumptive epidermal and neural ectoderms between early embryos of the two species and followed the

development of grafted tissue in the respective hosts with pigment as the marker. When a piece of prospective neural ectoderm from an early gastrula stage donor embryo was grafted into a region of the host embryo where belly skin should develop, the graft developed into belly ectoderm (Fig. 16.10 A-C). Similarly, when the prospective epidermal ectoderm was grafted into a region where a neural plate should develop, it developed into a neural plate.

Such exchange of embryonic tissues made in early gastrula stages resulted in the development of structures according to their new surroundings. These results clearly showed that the fate of neither the prospective epidermal nor of neural ectoderm are not determined at the early gastrula stage embryos of the two species of newts on which the experiments were made.



Figs. 16.10 A-F Results of the grafting experiments of Spemann and his co-workers. A-C. Prospective neural ectoderm (A) grafted into a region of epidermal ectoderm in an early newt gastrula (B) developed into epidermis (C).

D-F : When the same experiment is repeated in a late gastrula (D,E), the prospective neural ectoderm forms neural tissue (F), thereby showing that the fate of neural ectoderm was fixed by this time.

However, entirely different results were obtained when such graftings were done in embryos that had completed the gastrula stage. Neural ectoderm grafted into prospective belly region differentiated into neural plate (Figs. 16.10 D-F). In the converse experiment the presumptive epidermis grafted in the neural ectodermal region developed into epidermis irrespective of its new position. Obviously these results suggest that the respective fates of neural and epidermal ectoderms were determined during gastrulation. Spemann and Hilde Mangold (1924) then asked what determines the fate of neural ectoderm. Results of their experiments showed that the chordamesoderm underlying neural ectoderm induces it to form the neural plate.

As you know (Unit 14) the dorsal lip region of gastrula in amphibians contains cells of prospective chordamesoderm. During gastrulation these migrate inward by involution over the dorsal lip of the blastopore, come to lie under the prospective neural ectoderm and subsequently form the notochord. Even when the dorsal lip of an early gastrula is grafted to the ventral region of the same or another embryo of the same stage its cells migrate inwards by involution and come to lie under the overlying ectoderm.

Spemann and Mangold grafted the dorsal lip of blastopore from the early gastrula of the unpigmented embryo of the newt *Triturus cristatus*, to the ventral region of the early gastrula of the pigmented *Triturus taeniatus*. The dorsal of *T.cristatus* lip grafted to the ventral region of *T. taeniatus* embryo, involuted and a secondary embryo was formed in the ventral region of the host; and it contained an entire set of secondary organs

including a gut, neural tube, notochord, renal tubules and somites (Fig. 16.11). Microscopic examination of these secondary organs revealed that the gut and renal tubules and also the neural tube were formed entirely from the pigmented host cells. The somites contained both pigmented and unpigmented cells which must have been contributed by the pigmented host mesoderm as well as the unpigmented grafted dorsal lip. The notochord contained only unpigmented cells that must have come from the grafted dorsal lip.

The formation of notochord and somites from the transplanted dorsal lip was expected; these are normally derived from cells of the dorsal lip. Even the formation of gut could be explained since the process of involution of the transplanted dorsal lip would produce a secondary archenteron. But the most surprising feature was the formation of neural tube which is not a derivative of dorsal mesoderm (a tissue that differentiates from cells of the dorsal lip) but is formed from ectodermal cells. Microscopic examination had shown that the neural tube had developed from the non-pigmented cells of the host ectoderm that were otherwise destined to develop into belly skin. Based on these results, Spemann concluded that the dorsal lip material had induced the overlying prospective epidermal ectoderm to become neural tissue. During gastrulation involution of chordamesoderm (dorsal lip) brings this mesoderm into contact with the overlying ectoderm (Fig. 16.12). This contact induces the ectoderm which now becomes determined to develop into neural tissue. The other regions of the ectoderm that do not come into contact with the chordamesoderm develop into epidermis and its derivatives.

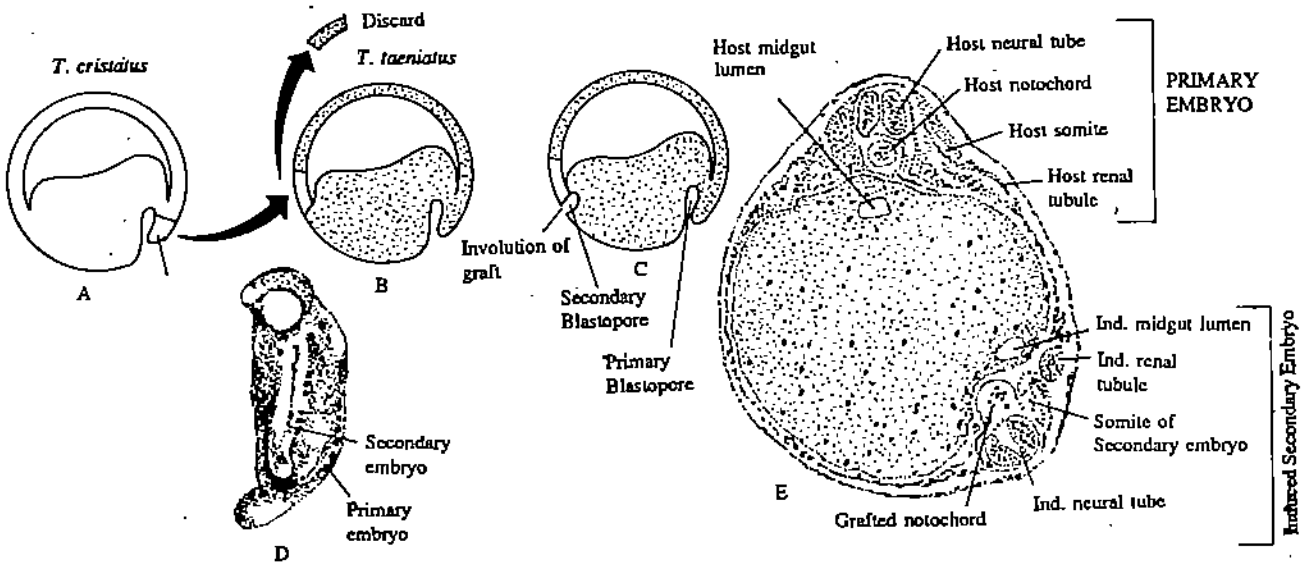


Fig. 16.11 A-E: Spemann's experiment in which he grafted a piece of dorsal lip of blastopore from an unpigmented species of newt to a pigmented species. A: Early gastrula of unpigmented *Triturus cristatus* B: Transplantation of the dorsal lip of *T. cristatus* to the ventral side of early gastrula of pigmented *T.taeniatus* C: Involution of grafted dorsal lip in the host gastrula. D: Result of the original experiment of Spemann and Mangold. Note the formation of a secondary embryo on the ventral side of the host embryo. E: Cross section of the host embryo at the tail-bud stage showing different induced dorsal structures such as neural tube, somites, notochord and gut in the ventral side also.

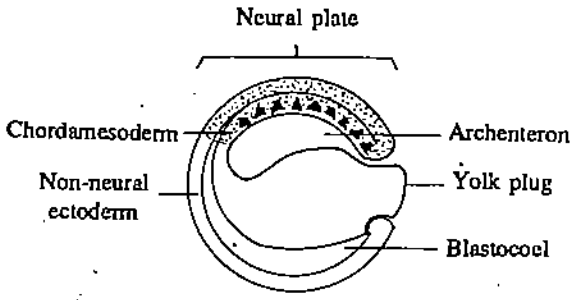


Fig. 16. 12: Late gastrula stage of an amphibian embryo showing relative positions of chordamesoderm and neural ectoderm. The arrow heads indicate the route of inductive signals.

The induction of the presumptive neural ectoderm leading to the formation of neural tube is termed as **primary induction** because earlier it was believed to be the first inductive event during embryogenesis. But now it is known that another inductive event, namely, the mesoderm induction occurs earlier to neural ectoderm induction. Nevertheless, neural ectoderm induction is even today referred to as primary induction. The induction of the neural ectoderm by dorsal lip of the blastopore also organises the anteroposterior axis of the embryo with the neural tube above, gut below and somites on each lateral side of the notochord. Hence the dorsal lip of blastopore is designated as the **primary organiser**. Precisely for this work Hans Spemann was awarded the Nobel Prize in 1935.

To date the nature of natural inducer is not known, although a variety of substances both of biological origin and non-biological nature such as methylene blue, toluene, steroids as well as acidic and basic solutions can elicit an inductive response. However, numerous experiments performed by many biologists since the discovery of the phenomenon of neural induction have confirmed that in all vertebrates the differentiation of neural tube from ectoderm depends upon the instructive induction from the underlying chordamesoderm. With suitable and carefully designed experiments based on the principles of primary indiction it has been possible to induce the formation of complete secondary embryos in amphibians (Fig. 16.13).

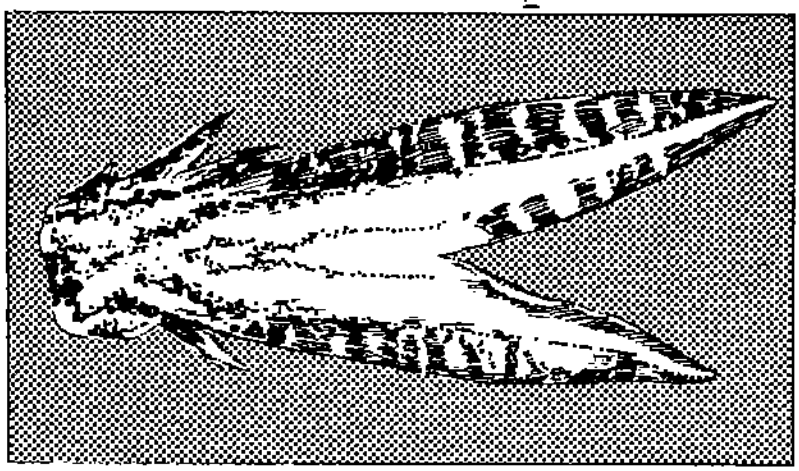


Fig. 16.13: Dorsal blastoporal lip of an early newt gastrula transplanted into the blastocoel of another resulted in the formation of a complete secondary embryo.

16.5.2 Induction of Mesoderm in Amphibians

In the last subsection, we said that an event which precedes neural ectoderm induction in amphibians is the induction of mesoderm. We shall briefly look into this aspect before we proceed to discuss secondary induction.

Until 32 cell stage in amphibian blastulae there are only two cell types. In animal hemisphere there are small ectodermal cells and in the vegetal hemisphere there are large endodermal cells. A third cell type from which mesoderm develops is formed in the equatorial region of the embryo during early blastula stage. Fate map of *Xenopus* embryo (Fig. 16.14) reveals that equatorial cells in contact with both animal and vegetal cells in the equator of blastula are precursors of mesoderm.

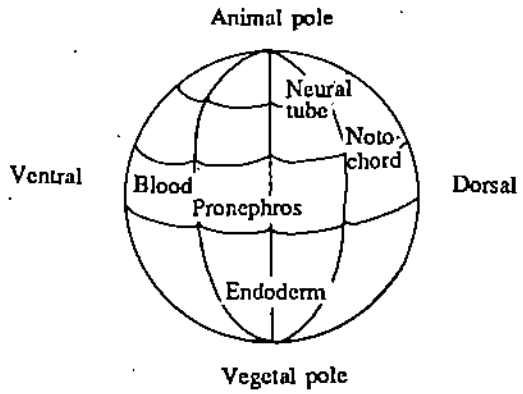


Fig. 16.14: Fate map of *Xenopus* embryo at 32 cell stage.

How is the fate of the equatorial cells as prospective mesoderm cells determined? Either the ooplasmic determinants as intrinsic factors or cell interactions as extrinsic factors may play a role in such a determination. To test one of the two possibilities, the blastulae were divided into explants of animal, equatorial and vegetal cells and cultured independently (Fig. 16.15). Animal cells developed into ectoderm cells, vegetal cells into endoderm cells and the equatorial cells into mesoderm cells. However, if the animal cells were recombined with vegetal cells and cultured, they formed mesoderm cells also suggesting the possibility that mesoderm formation is not exclusively dependent on ooplasmic determinants.

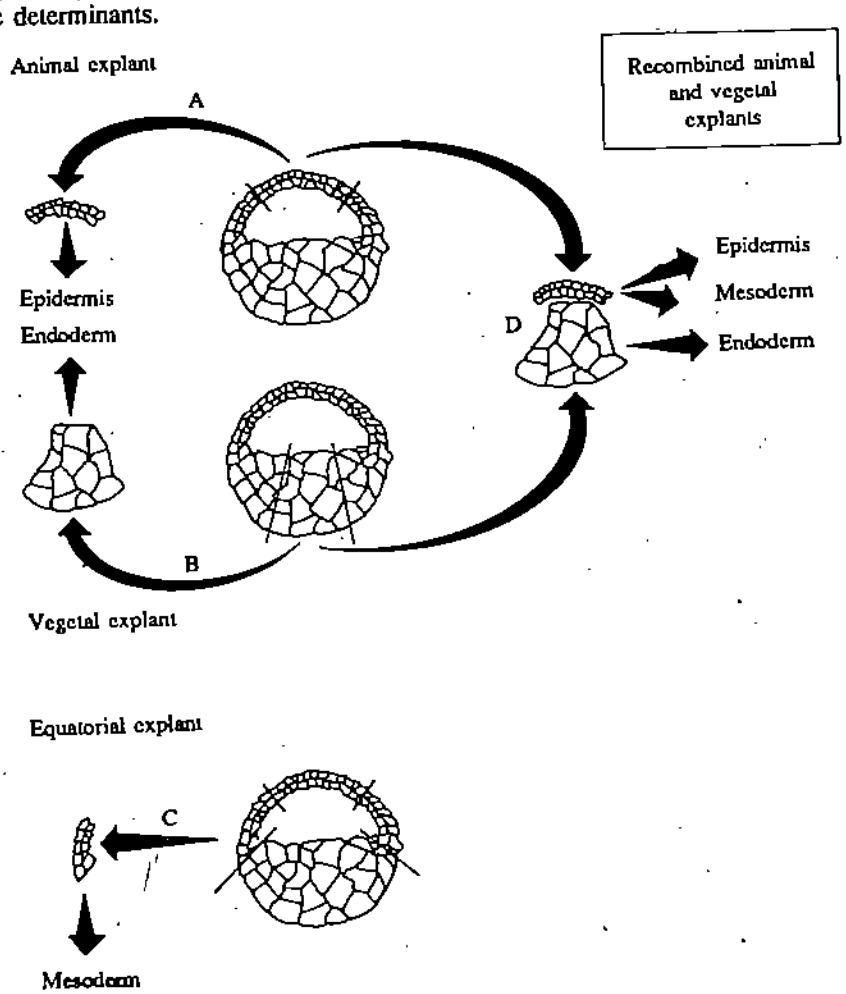


Fig. 1615: Diagram showing the design of explant experiments and the results obtained in *Xenopus* embryos. A: Isolated animal cells develop into ectoderm. B: Isolated vegetal cells develop into endoderm. C: Cells from equatorial region differentiate into mesoderm cells. D: Animal and vegetal cells when cultured together produce mesoderm cells as well.

The equatorial cells develop into mesoderm cells possibly because the adjacent vegetal cells secrete some inducing substance which influences the adjacent cells of the equatorial region inducing them to become mesodermal cells. In the normal blastula, the animal hemisphere cells differentiate only into ectoderm since they are widely separated

from the vegetal cells by the blastocoel and thus are too far from the source of inducer.

16.5.3 Secondary Induction

The two primary inductive events which we have described so far are important in the patterning of early embryo. But there are other such signalling events called **secondary induction** events which are important for two reasons.

1) Secondary induction events position the differentiated cells in precise locations in the embryo and

2) Secondary inductions which could be of sequential in nature, produce diverse cell types from relatively less distinct precursor cells.

The secondary inductions are classified into two types.

1) **Instructive interaction**

2) **Permissive interaction**

1) Instructive Interaction: In such an interaction the inducing tissue gives instructions to commit cells to a specific pathway of development.

2) Permissive interaction : Here, the responding cells are already determined and are ready to commence differentiation in a particular direction but wait for a signal from the inducing tissue. In other words, they require induction from a different tissue so that their committed potential could be expressed.

Whether it is instructive interaction or permissive one the inducing tissue has a critical role to play in the developmental process. It should be remembered that in the inductive interactions two tissues are involved : one, which provides the inductive stimulus (the inducer) and the other which is induced to develop in a particular way (the responding tissue). Therefore, the responding tissue must also be competent enough to receive the induction signals. In many instances the competence of responding tissue to the induction signals is limited to a specific time period during development. We shall now analyse instructive and permissive interactions each with one example.

16.5.4 Instructive Interaction between Ectoderm and Mesenchymal cells

The cell types that differentiate from epidermal ectoderm depend on instructions from the underlying dermis. The epidermal ectoderm differentiates into epidermis and the

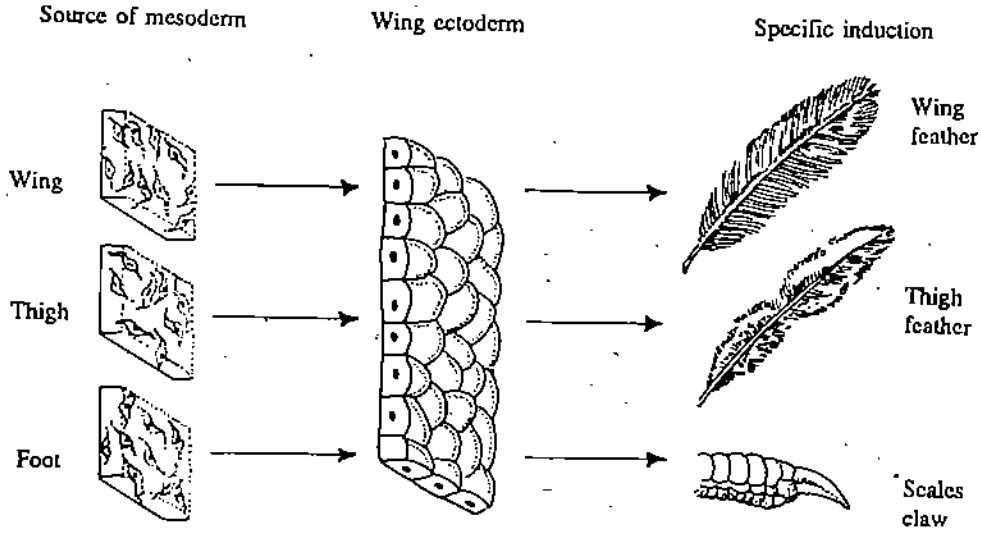


Fig. 16.16 **Regional specificity of induction. When cells of the dermis (mesoderm) are recombined with the epidermis (ectoderm) in the chick, the type of cutaneous structure made by the ectoderm is determined by the original location of the mesoderm.**

underlying dermis differentiates from mesoderm. The epidermis and dermis together form the skin. Prior to differentiation, the dermis is organised as a loose segregation of cells, generally known as mesenchyme cells. The interaction between the mesenchyme and the overlying epithelial ectoderm is an example of **epithelio-mesenchymal interaction.**

We shall now discuss one such epithelio-mesenchymal interaction in the differentiation of three cutaneous structures of the chick, viz. feathers of the wing, narrow feathers of the thigh and scales and claws of feet. All the three are epidermal structures entirely derived from ectoderm; but their development depends upon induction of the ectoderm by the underlying mesenchyme (mesoderm). The ectoderm is competent to form any of these structures, but what it will form is determined by the type of mesoderm lying beneath and inducing it (Fig.16.16). The development of the three epidermal structures provides examples of the phenomenon of secondary induction of instructive nature and also of regionally specific inductions.

If a piece of thigh mesoderm is transplanted under the wing ectoderm, the wing ectoderm develops thigh feathers under the instructions from the grafted thigh mesoderm. Similarly, if foot mesoderm is combined with wing ectoderm, scales and claws are formed. Contrarily if leg ectoderm is combined with wing mesoderm broad feathers typical of a wing would develop. These experimental results illustrate the following ideas:

i) A responding tissue may have the general competence to form several kinds of structures; but what it will actually form depends on specific instruction from the inducer with which it interacts.

ii) Mesoderm from different regions of the embryo has different properties and provides specifically different inductive signals or instructions to the responding tissue concerned in different regions.

iii) Regional differentiation may be induced by regionally specific inducer tissue.

16.5.5 Permissive Interaction - Pancreas Development

Figure 16.17 illustrates the development of pancreas in a mammal. In an organism like mouse the pancreas is first seen as a diverticulum of embryonic gut around 9th day of development (Fig. 16.17 B). As the diverticulum grows it pushes into mesenchyme and branches forming blind pockets called acini (Fig. 16.17 B & D). The cells of these pancreatic acini differentiate into exocrine cells that produce digestive enzymes—the proteases, peptidases, amylase, lipase etc. Also, certain cells that separate out from pancreatic epithelium form clusters of cells surrounded by mesoderm. These cells are the endocrine cells, the islets of Langerhans (Fig.16.17 C.E.), producing hormones such as insulin, glucagon and somatostatin. Thus the exocrine and endocine cells arise from epithelium of embryonic gut.

Development of pancreas depends on the associated mesoderm. If in a 9-day old embryo, the pancreatic mesoderm and ectoderm are separated from one another, there will not be any further development. But if they are recombined in a culture medium, both exocrine and endocrine cells differentiate normally. Also, instead of the pancreatic mesenchyme, if the mesenchyme from other regions such as mesenchyme from salivary gland region is substituted normal pancreatic cells still differentiate. Somites which produce muscles and cartilage cells can also induce pancreatic endoderm to develop normally. Even extracts of embryo can induce the pancreatic endoderm. All these results suggest that by 9th day of embryonic life of mouse, the pancreatic endoderm is already determined or committed and only a non-specific signal from mesoderm is sufficient for it to complete the development. The mesoderm can be from any region but must be in proximity to the endoderm. It is such interactions that come under the category of permissive interaction.

We discussed certain of the basic concepts of embryonic induction, primary and secondary, instructive and permissive and their role in cell determination and differentiation. Attempt the following SAQ to test your comprehension of the subject.

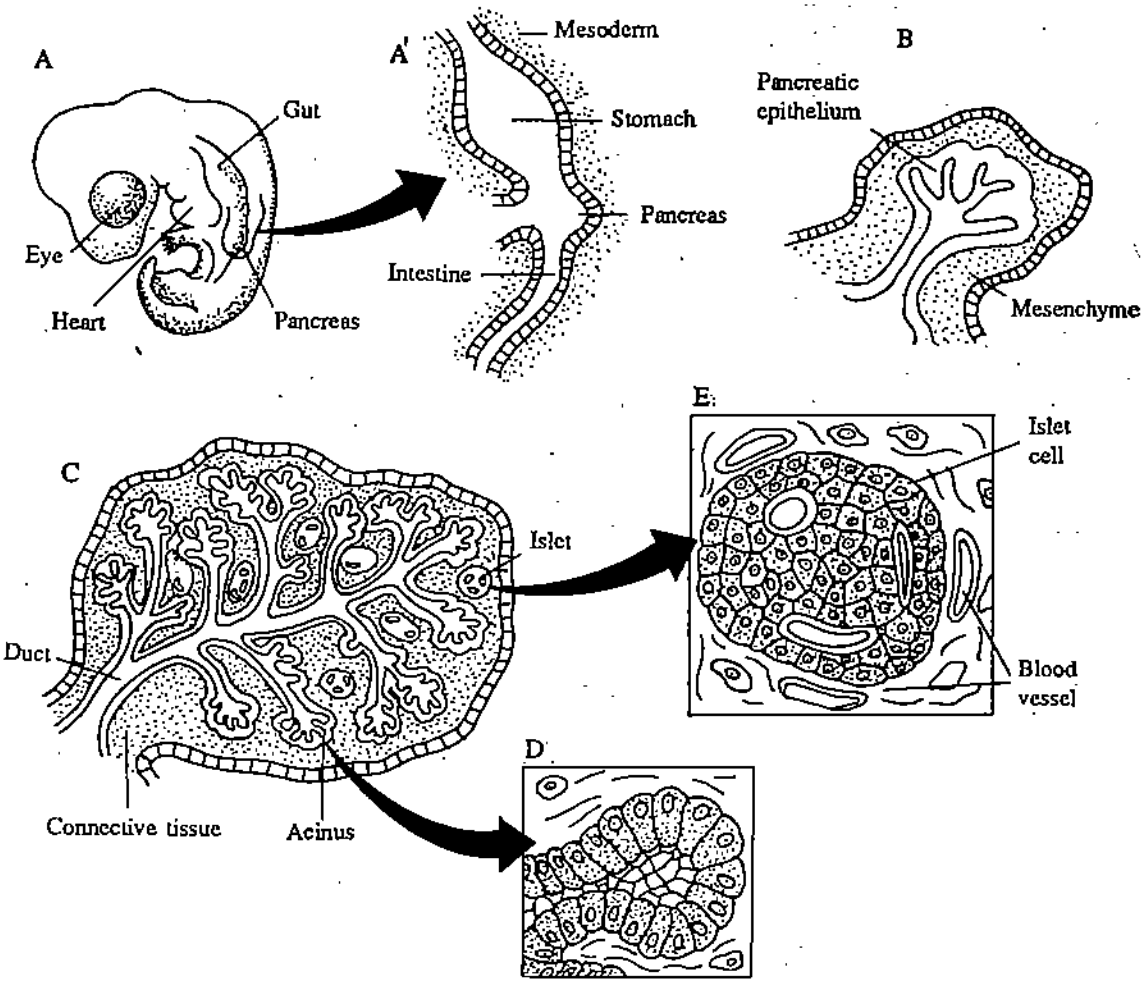


Fig. 16.17 : Development of pancreas in a mammal. A and A': Position of the gut in a 9-day old embryo and the magnification of the gut showing the formation of pancreas as an outpocketing of gut. B. In a 12-day old embryo, the pancreatic epithelium grows into adjacent mesoderm and branches out. C. By day 15, the acinar cells of exocrine function (D) and islet cells (E) of endocrine function develop.

SAQ 3

Answer the following questions briefly.

1. Define the term embryonic induction.

   .....

   .....

   .....

   .....

2. What would be the result of transplantation of a piece of prospective neural ectoderm of an early gastrula to the region of belly ectoderm?

   .....

   .....

   .....

   .....

3. What would be the result of transplantation of a piece of neural ectoderm of a late gastrula of a newt to the region of belly ectoderm?

   .....

4. How would you interpret the results of experiments stated in questions (2) and (3)?

5. In Spemann's experiments, using pigmented and unpigmented embryos of newt species, which region of the embryo was shown to induce neural plate formation?

6. Why is the dorsal lip of the blastopore called a primary organiser?

7. In an early blastula stage of an amphibian where are the prospective mesoderm cells located?

8. Which cells do interact with equatorial cells for development into mesoderm cells?

9. What is the significance of secondary induction?

10. Distinguish the terms instructive and permissive induction.

16.6 SUMMARY

- In this unit we have discussed certain mechanisms of cell interaction and cell differentiation. The concepts of totipotency, pluripotency and cell determination have been explained with suitable examples. The significance of nuclear transplantation experiments in embryos has been discussed to show that nuclei obtained from different developmental stages exhibit marked differences in their potency. The nuclei obtained from later stages of development show progressive restrictions in their ability to promote development and the nuclei from some of the post-embryonic cells retain potency to promote partial development.
- Intrinsic factors such as ooplasmic determinants and extrinsic factors such as cell interactions decide whether the cell determination occurs early or late during the embryonic life. The role of ooplasmic determinants in determining the germ cell lineage in nematodes and somatic cell determination in tunicates has been described.
- We have discussed the concept of embryonic induction and its role in cell determination. This type of cell interaction is described as an extrinsic factor in the cell determination process. Spemann's classical experiments demonstrated the induction of neural plate by the underlying dorsal mesoderm. In amphibian embryos interaction between the vegetal cells and the equatorial cell of early blastula induces the latter to become mesoderm.
- The secondary inductions have important role in positioning of differentiated cells in precise locations and in producing diverse cell types.
- Instructive interaction results in the commitment of the cells to a specific pathway of differentiation and permissive interaction involves signalling of a committed cells to realise their full potential in development.

16.7 TERMINAL QUESTIONS

1. Describe Spemann's experiments to test the nuclear potency of embryonic cells.

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2. What are the advantages of serial transplantation technique?

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3. What conclusions could one draw from the results of nuclear transplantation experiments?

4. What is chromatin dimunition? What prevents chromatin dimunition in the germ cells?

5. Discuss the process of mesoderm induction in amphibian embryos.

16.8 ANSWERS

Self Assessment Questions

1. i) Totipotency : Refers to the potential of embryonic cells to support the development of a variety of cell types that constitutes an organism.

   ii) Pluripotency: Refers to the potential of embryonic cells to form fewer of various cell types.

   iii) a) True b) False c) False d) True e) False f) False g) True h) True

2. a) cell determination b) embryonic induction c) mosaic d) Regulative e) Ooplasmic determinants f) Cell interactions g) cytoplasmic localisations h) chromatin dimunition i) Ooplasmic determinants, posterior cytoplasm j) Ooplasmic segregation k) yellow crescent l) ectoplasm, endoplasm, chordoplasm and myoplasm m) cytoplasmic, nuclear

3. 1) Alteration of cell fate because of interactions with neighbouring cells is known as induction.

   2) The neural ectoderm of the early gastrula, in accordance with its new location will develop into belly skin.

   3) The neural ectoderm of the late gastrula when transplanted into the region of belly ectoderm will develop into neural tissue.

4. In the first case where the neural ectoderm of the early gastrula when transplanted into belly ectoderm develops into belly skin, it shows that, the fate of the neural ectoderm is not yet determined. And it develops into a structure depending on its new surrounding. However, in the second instance, in the late gastrula stage, the fate of the neural ectoderm is already determined.

5. Dorsal lip of the blastopore.

6. Since the dorsal lip of the blastopore organises the antero-posterior axis of the embryo, it is designated as primary organiser.

7. In the equatorial region of the blastula.

8. Vegetal cells.

9. Secondary induction i) positions the differentiated cells in precise locations in the embryo and ii) could direct the production of diverse cell types from relatively a distinct precursor cells.

10. Instructive induction: the inducing tissue gives instruction to commit cells to a specific pathway of development.

    Permissive induction : the inducing tissue provides signals to an already committed responding tissue to commence differentiation.

TERMINAL QUESTIONS

1. Refer to text in subsection 16.5.1

2. In serial nuclear transplantation experiments, an abnormal embryo which has reached blastula on preblastula stage is dissociated and the nuclei isolated from the cells are injected into the enucleated eggs. The abnormal blastula itself is a recipient of a diploid nucleus from another embryo. It developed into an abnormal embryo because of the damage it suffered during transplantation or due to incomplete chromosomal replication of transplanted nucleus. Serial transplantation technique helps to overcome these problems and percentage of transplants completing the development is much higher.

3. Refer to text in subsection 16.2.2.

4. Refer to text in subsection 16.4.1

5. Refer to text in subsection 16.5.2

UNIT 17 ORGANOGENESIS OF EYE AND LIMB

Structure

17.1 Introduction
Objectives
17.2 Vertebrate Eye
17.2.1 Adult Eye
17.2.2 Eye Field
17.2.3 Development of Eye
17.2.4 Differentiation of Retina, Lens and Cornea Tissue Interactions in Development of Eye
17.3 Vertebrate Limb
17.3.1 Basic Structural Pattern of Limbs
17.3.2 Limb Field
17.3.3 Determination of Limb Polarities
17.3.4 Pattern of Limb Development
17.3.5 Role of Mesoderm and Ectoderm in Limb Morphogenesis
17.3.6 Apical Ectodermal Ridge (AER)
17.3.7 Control of Pattern Formation
17.4 Common Features of Eye and Limb Development
17.5 Summary
17.6 Terminal Questions
17.7 Answers

17.1 INTRODUCTION

In the preceding Units you have studied the early events in the embryonic development.These events prepare the embryo for organ formation (organogenesis) by making the embryo multicellular and three layered structure with separation of ectoderm, mesoderm and endoderm. In this unit, you will study how an organ is formed from different groups of cells which interact among themselves. An organ may consist of several components made of different tissues after derived from different germinal layers. The process involves morphogenetic movements, growth, cell interaction and cell differentiation. You are advised to recapitulate regarding morphogenetic movements (Unit 15) and concepts of competence, induction and determination (Unit 16).

In order to ensure proper formation of an organ it is essential that the development of all its components must occur in a coordinated manner in time and space. This is achieved by a precise sequence of interactions between different groups of cells and tissues at successive stages of the development of the organ concerned.

In this unit you will study the development of eye and limb in vertebrates. We have chosen these two as examples of organogenesis for several reasons. The eye of all vertebrates are homologous structures, composed of same parts, built on the same architectural pattern and develop in the same manner from cells from identical sources in the embryo. This is also true for the vertebrate limb. Moreover, much information obtained by numerous experiments on these organs is now available about the processes and mechanism involved in their development.

We will first study the development of eye and then the limb. Later, a comparison will be made to highlight the common features of organogenesis of these organs.

Objectives

After the study of this unit you should be able to

- explain the concept of morphogenetic field,

- describe the development of eye and its components and explain tissue interactions in their development,
- describe the process of limb formation,
- identify the polarities of a vertebrate limb,
- explain the role of mesoderm and ectoderm in the development of eye and limb,
- explain the common features of eye and limb development.

17.2 VERTEBRATE EYE

17.2.1 Adult Eye

There are no major differences in the structure or composition of the eyes among different vertebrates. This should be clear to you from the figure 17.1. The reptilian eyes are also similar. Let us review briefly the structure of a mammalian eye (Fig. 17.1 d)

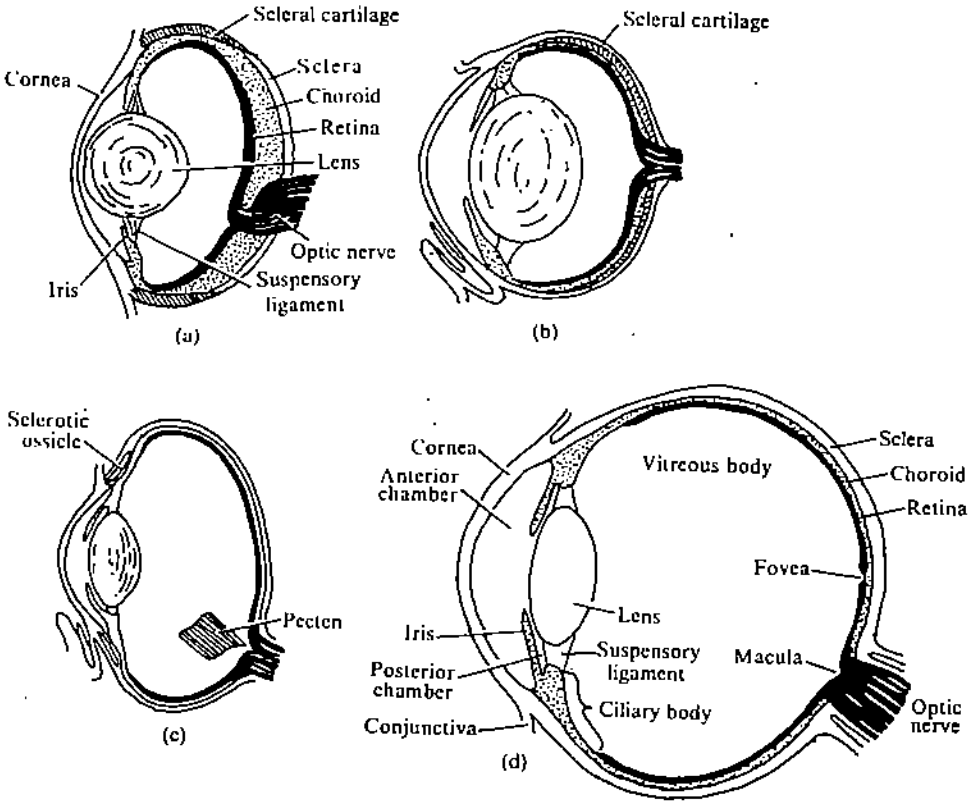


Fig. 17.1 : Diagrams of vertebrate eyes (a) Fish; (b) Frog; (c) Bird; (d) Mammal

The mammalian eye is a ball-like organ composed of three concentric layers. The outer most is the *selerotic coat* or *selera* made of tough connective tissue in mammals and birds, but it is cartilaginous in lower vertebrates. In front it is continuous with the transparent *cornea*. The middle coat is the vascular and pigmented *choroid* which is continuous with the *iris*. The perforation in the centre of iris is the *pupil* in front of the *lens*.

The inner coat is the *retina* consisting of the outer *pigmented retina* and the inner *neural retina*. The latter contains the light sensitive cells (cones and rods), ganglion cells and other neurons. The nerve fibres from ganglion cells form the *optic nerve* which connects the eye with the *brain*. Cones are concentrated at *fovea* in the neural retina and there are no light receptive cells in the region of *macula* where the optic nerve passes on way to the brain. The anterior part of retina is non-sensory and is continuous with the ***ciliary body*** which contains smooth muscles. The *lens* is located behind the iris held in place by the ligament. Contraction and relaxation of these muscle fibres alter the distance between the lens and cornea and also their convexities. The space between the cornea and iris is the ***anterior chamber***, and that between the iris and ligament is called the ***posterior chamber***; these spaces are continuous with each other and filled with a fluid.(aqueous humor). The large cavity of the eye ball between the lens and neural retina is the *vitreous* body.

The accessory structures of the eye include eye lids, nictitating membrane, conjunctiva, lacrimal glands and extrinsic intraocular muscles of the eye orbit.

As you would notice eye is an extremely complex organ. The cells that make up the various parts of the eye are contributed by ectoderm and mesoderm. The retina and lens are ectodermal; the cornea, iris and ciliary body are formed from cellular contributions from both ectoderm and mesoderm. The extrinsic intraocular muscles are purely mesodermal and so are the choroid and sclera. The eyelids and lacrimal glands are ectodermal derived from the epidermis.

17.2.2 Eye Field

The development of eyes begins with evagination of the lateral wall of the forebrain, one on each side, which form the optic vesicles. By vital dye staining it is established that at the open neural plate state in amphibians the presumptive material for the optic vesicle lies in the median anterior region of the neural plate. This region is called the eye field. Later this field gets divided into two lateral portions, one for each eye. The lens fields lie outside the neural plate in the epidermal ectoderm (Fig. 17.2).

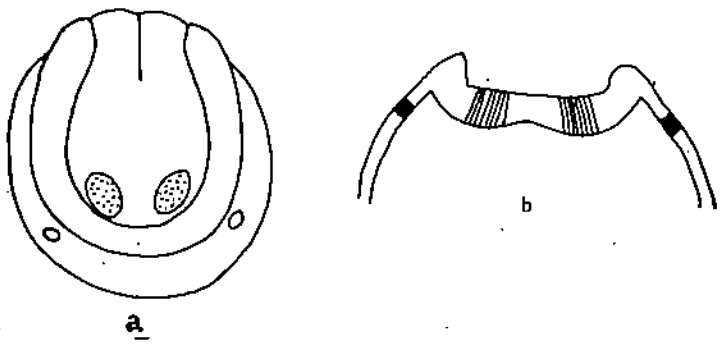


Fig. 17.2 : Location of presumptive areas of optic vesicles (dotted) and lens in open neurula stage in an amphibian as revealed by vital staining methods. Lens area (dark) is outside the neural fold . (a) Top view. (b) In section.

In the chick the eye field has been identified as a broad median area in the anterior part of epiblast in front of the Hensen's node at primitive streak stage (Fig. 17.3).

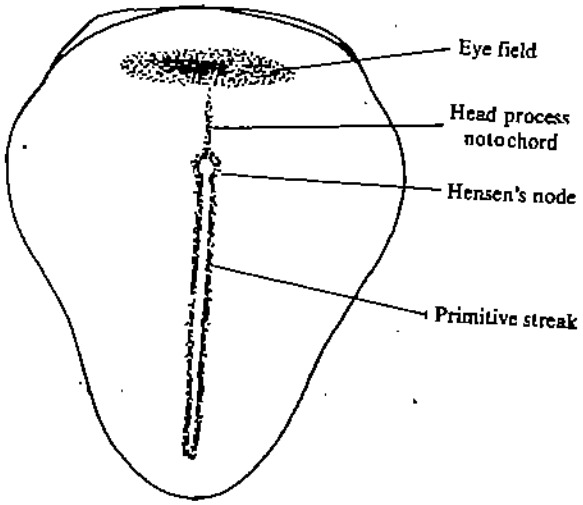


Fig. 17.3. : The eye-forming area of the chick blastoderm at the stage of the head process.

By 8-sonite stage middle part of the eye field in chick embryos has lost the capacity to form the eye, but the two lateral parts retain this capacity and each forms a complete eye. Treatment of eggs with some chemicals such as lithium chloride, choretone etc., or removal of the chorda-mesoderm from below the anterior region of neural plate prevents the division of the median eye field and a single median eye (cyclopean eye) is formed instead of two lateral eyes (Fig. 17.4).

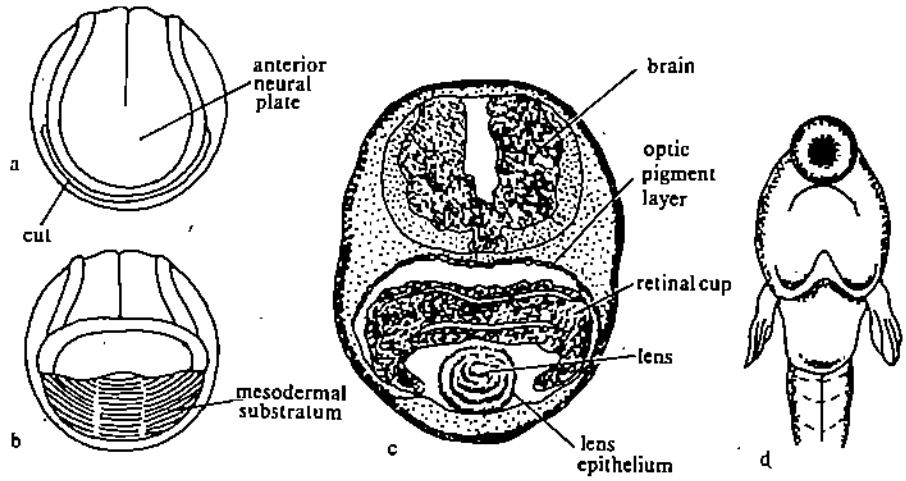


Fig. 17.4 : Cyclopia in the newt produced by mechanical defect. Excision of the mesodermal substratum of the anterior part of the medullary plate in the early neurula stage has entailed median fusion of the eyes into a single ventral eye. *a* Neurula prepared for operation by cutting around the anterior border of the medullary plate. *b* Anterior part of medullary plate is lifted. The mesodermal substratum thus exposed is then excised and the medullary flap is put back in position. *c* Cross section through the head showing the ventral eye with a single median lens. *d* Fish larva with cyclopean eye induced by magnesium (or lithium) chloride treatment of early embryo.

The term field, or more precisely the morphogenetic field of an organ may be defined as the sum total of properties of the cells of that region of the embryo from which the particular organ or structure will develop. Normally, the structure concerned forms from cells in the central part of the field. However, the properties to form that organ usually extend to some distance peripherally beyond the limits of the region that contributes cell for construction of the organ normally. Hence in the absence of the central part of the field cells around it may form the structure.

17.2.3 Development of Eye

The development of eye in vertebrates begins with the formation of two optic vesicles which arise as evaginations or sac-like protrusions, one from each lateral side of the forebrain (Prosencephalon) as shown in figures 17.5 and 17.6.

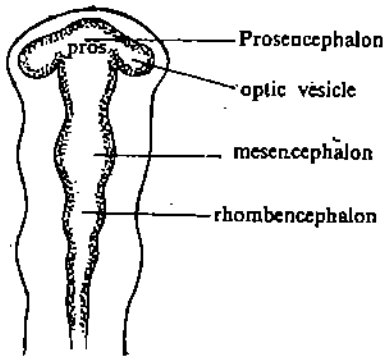


Fig. 17.5 : Primary brain vesicles and eye rudiments of a 33-hour-old chick embryo pros., Prosencephalon; mes, mesencephalon, rhomb, rhombencephalon; opt, eye vesicles.

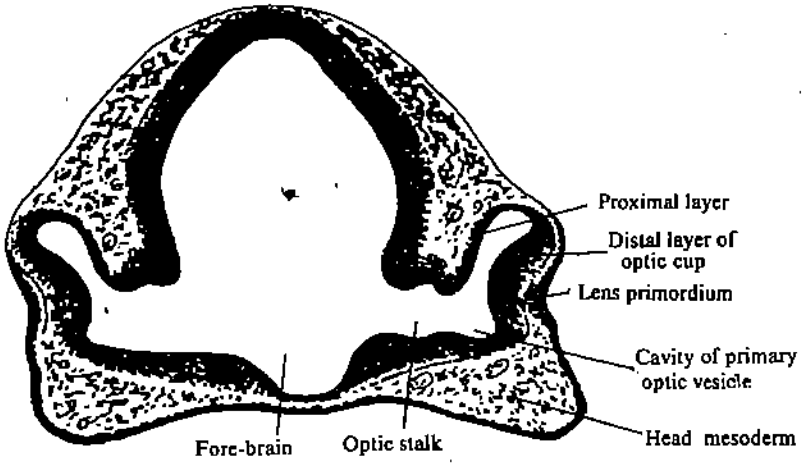


Fig. 17.6 : Section through the forebrain of a 5mm human embryo showing the early formation of the optic cups.

Further development of the eye in all vertebrates occurs according to the same sequence of events as illustrated in figure 17.7 which shows the process of eye development in the amphibian axolote. The vesicles are thus extensions of the brain. The broad opening of the optic vesicle into the brain becomes narrow due to the growth of a ventrally directed fold from the side of the diencephalon (posterior part of the prosencephalon). This narrow connection between the vesicle and the brain is the optic stalk (Fig. 17.6).

The vesicle grows in size and makes contact with the inner side of the epidermal ectoderm at the site of presumptive lens. After the vesicle is in touch with the ectoderm the latter thickens and forms the lens placode.The placode soon thereafter separates from the overlying ectoderm and becomes a rounded lens vesicle with a cavity. Simultaneously, the distal side of the optic vesicle in contact with lens placode also thickens and begins to invaginate to form a double walled optic cup.

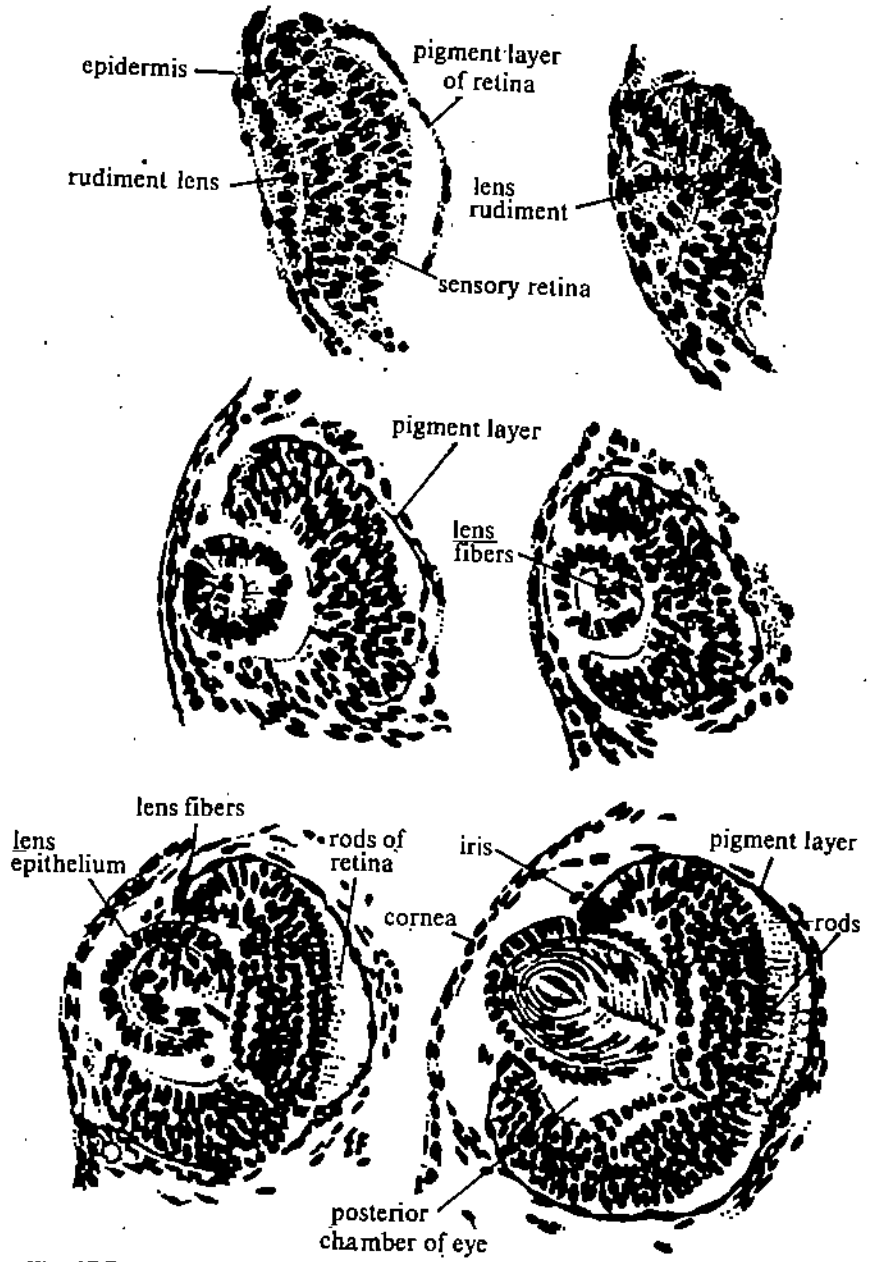


Fig. 17.7: Stages of the development of the eye in an amphibian axolotl.

The inner wall of the optic cup continues to thicken and ultimately forms the neural or sensory retina. The outer wall remains thin and forms the pigmented retina (Fig. 17.7).

The cavity of the optic cup becomes the vitreous chamber, the margin of the cup forms the pupil. Since the growth of the optic cup is unequal, a slit-like gap, called the choroid fissure, is left for sometime on the ventral side of the cup. This fissure serves as the outlet for the optic nerve and through it blood vessels enter the optic cup.

The lens placode, along with its basement membrane, invaginates to form the lens vesicle, which fits into the opening of the optic cup (Figs. 17.7). Once the lens placode has invaginated it is covered over by two cells layers from the adjacent epidermal ectoderm, which contributes to the formation of transparent cornea under the influence of the lens and the retina.

Extension of the pigmented retinal epithelium at the rim of the original optic cup and of mesodermal choroid coat participate in the formation of the iris between the lens and the

cornea. The iris is pigmented and forms the border of the pupil. Within the iris smooth muscle fibres develop which control the size of the pupil. Unlike other muscles of the body which are derived from mesoderm pupillary muscles are derived from the ectodermal non-neural region of the optic cup.

The ciliary body develops from the non-neural part of neural retina on the inner side of the rim of the optic cup. It also receives cellular contribution from the mesoderm of choroid for development of ciliary muscles.

The choroid and sclera develop from the mesoderm surrounding the optic cup. The choroid mesoderm is continuous towards the margin of the cup with ciliary muscles, the corneal seroma and the outer epithelium of the iris.

17.2.4 Differentiation of Retina, Lens and Cornea

Retina: As invagination of the optic vesicle proceeds to form the double walled optic cup the two layers of the optic cup begin to differentiate in different directions. *Pigment* is produced in the cells of the outer 1- cell thick layer which becomes the pigmented retina (Fig. 17.7 f). The cells of the inner layer proliferate rapidly, mainly in its germinal zone near the margin of the optic cup. From here the daughter cells migrate into the deeper region of the inner layer increasing its thickness. These cells ultimately differentiate into a variety of cell types including the light sensitive photoreceptor cells (cones and rods), glial cells, interneurons (bipolar, horizontal, amacrine) and ganglion cells. Collectively these cells constitute the neural retina. The proliferation then occurs only in the marginal zone of neural retina. Glial cells become the Muller's Fibres which form the packing tissue and the outer and inner limiting membranes on the outer and inner surfaces of the neural retina. The bipolar, horizontal and amacrine cells serve to connect the photoreceptor cone and rod cells with each other and the ganglion cells by way of their axons and dendrites. The axons from the ganglion cells of the neural retina meet at the base of the eye to form the optic nerve which travels down the optic stalk and connects the neural retina with the brain. The detailed cellular architecture of the neural retina is shown in figure 17.8.

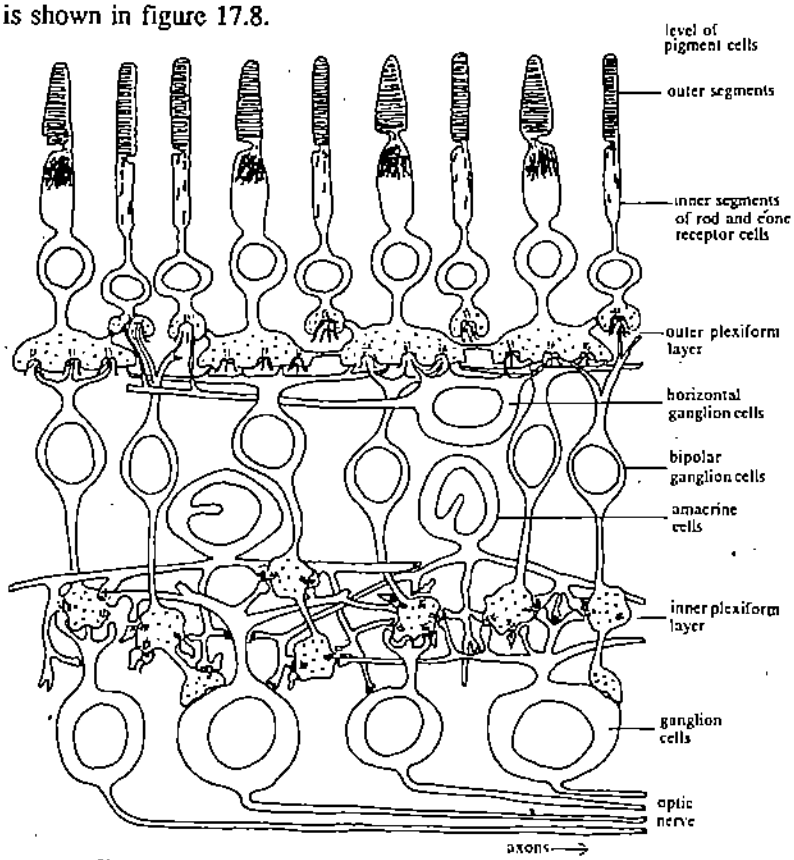


Fig. 17.8: Summary diagram of synaptic contacts between the principal types of retinal cells in the human neural retina. The upper layer consists of rods and cones, the outermost segments of which are more or less embedded in the retinal pigment cell layer (not shown). These sensory cells make synaptic contact with their neighbours and with a complex layer of bipolar and horizontal ganglion cells. These in turn make synaptic contacts with the inner ganglion cells whose axons constitute the optic nerve.

Differentiation of neural retina first involves separation of the cells of the inner layer of the optic cup into basically three zones (Fig. 17.9): (1) the outer nuclear layer (ONL), facing the pigmented retina, which contains cells that differentiate into photoreceptor cells; (2) the innermost zone facing the lens called ganglion layer (GL) whose cells differentiate into ganglia and (3) the middle zone called the inner nuclear layer (INL) whose cells differentiate into glial cells and interneurons (Fig. 17.9, c).

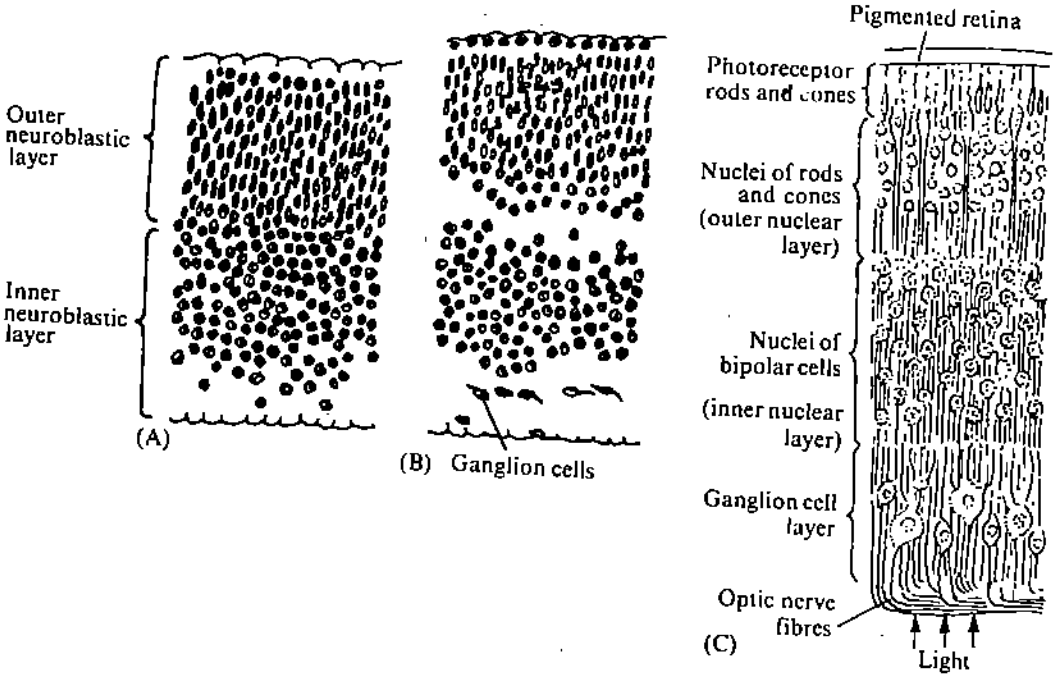


Fig. 17.9 : Development of human neural retina. Retinal cells segregate into two zones (A) and then three zones (B). A histological section of human neural retina is shown in (C). Note the difference.

Along with the waves of cells migrating from the marginal zone into the deeper regions of neural retina there also occur waves of cell death during early stages of neural retina differentiation. Probably, the death of cells in certain regions results in separation of the neural retinal cells into the three zones (ONL, INL and GL).

The differentiation of various cell types in the three zones occurs according to the following sequence :

(1) Glial cells, (2) Ganglion cells, (3) interneurons of various types and lastly (4) the photoreceptor cells.

The light sensitive rods and cones develop from the outer ends of the cells of the outer nuclear layer (Fig. 17.10). Each cell produces a cytoplasmic bud at its outer end protruding through the outer limiting membrane into the space between the neural retina and the pigmented retina. The buds elongate and first form the inner segment of the rod and cone cells containing one or more of the various specialized organelles (myoid, ellipsoid, oil droplet, paraboloid). A basal body or centriole at the distal end of the inner segment produces a non-contractile cilium which participates in the formation of the outer segment of these cells.

The outer segment arises by further distal extension of the cytoplasm in the region of the cilium. The plasma membrane surrounding this extended portion of the cells folds back upon itself and to form sacs or discs upon which the photoreceptive pigments are placed. Light induces chemical changes in these pigments that lead to change in the membrane potential of these cells which send electrical signals to ganglion cells from where they are relayed to the brain via the optic nerve. The process of differentiation of photoreceptor cells is illustrated by figure 17.10.

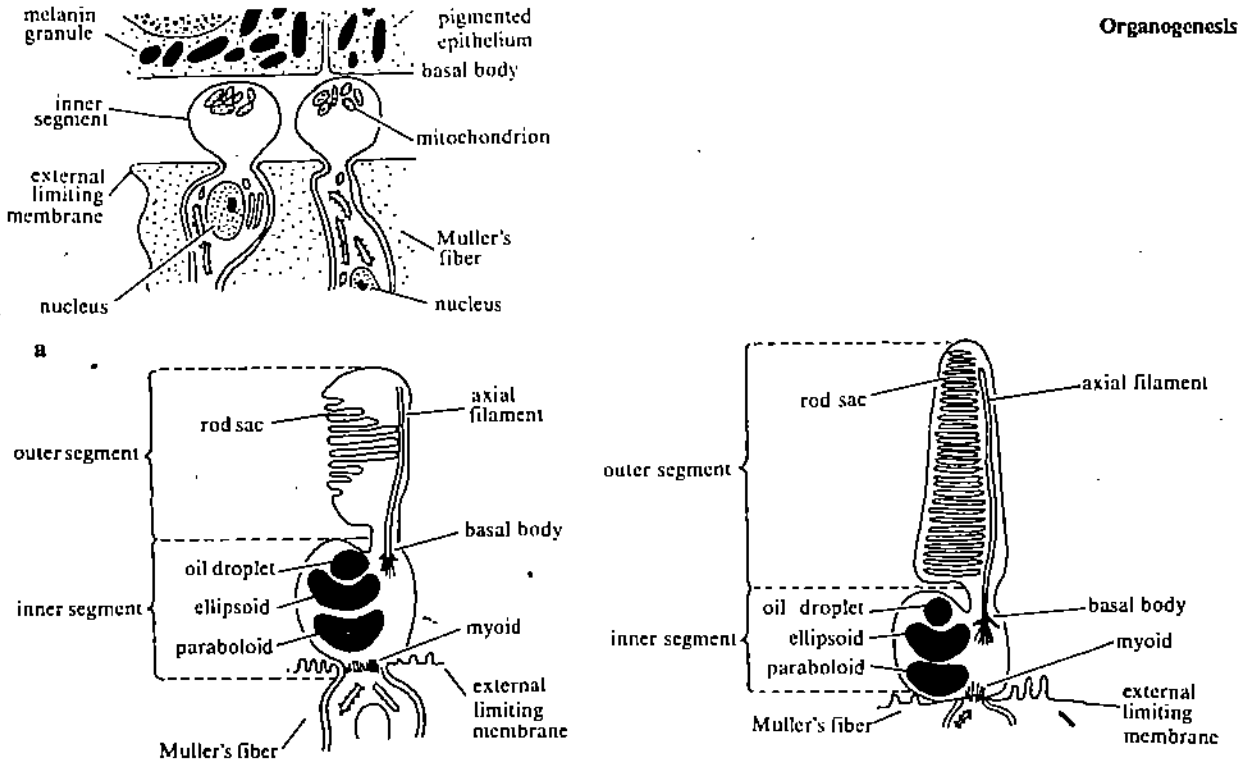


Fig. 17.10 : Rod and cone cytogenesis, illustrating the successive stages in the maturation of the cone or rod. a, cells differentiating between pigmented retinal epithelium and the external limiting membrane of the sensory retina, forming a bulbous inner segment of the prospective rod or cone. b and c, Two later stages showing development of outer segment, the folding of the plasmalemma to form a stacked series of membrane plates, and the axial filament, which is essentially a nonmotile cilium complete with basal body.

The differentiation of retina is completed when the pseudopodia are formed by the cells of pigmented retina and they invade the spaces between the outer segments of the photoreceptor cells of the neural retina (Figs. 17.7, f; 17.9, c).

LENS : On separation from the overlying epidermal ectoderm the lens placode rounds up to become a lens vesicle (Figs. 17.7, 17.8). Its cavity is surrounded by a 1—cell thick lens epithelium which is covered on the outside by a non-cellular lens capsule. The differentiation of lens vesicle into a transparent structure involves changes in the structure and shape of the cells of lens epithelium as well as synthesis of lens specific proteins called crystallins. (Fig. 17.11). The crystallins are of four types designated as α (alpha), β (beta), γ (gamma) and δ delta. The sequence of synthesis of these types varies in different vertebrate species. In most species β—crystallin forms the bulk of crystallins in the adult stage.

The cells on the inner side of the lens vesicle facing the retina elongate and form lens fibres. As these long fibre cells grow they synthesize the crystallins which eventually fill up the cell and cause extrusion of the nucleus. Ultimately the crystallin—synthesizing fibres fill the entire cavity of the lens vesicle transforming it into a transparent crystalline body.

The anterior part of lens epithelium facing the cornea is the germinative region in which the cells keep dividing. The daughter cells move towards the equator of the lens, and as they pass the equatorial region they also begin to elongate (Fig. 17.12). Thus, the lens contains three zones: an anterior zone of dividing epithelial cells, an equatorial zone of elongating cells, and a posterior and central zone of crystallin-containing fibre cells. This arrangement persists throughout the life time of the animal as new fibres are continuously laid down from the equator of the lens. The oldest fibres are in the centre and the youngest outermost. The basement membrane surrounding the lens forms the lens capsule. The differentiation phase of lens cells is very long. In the adult chicken, the differentiation from an epithelial cell to a lens fibre cell takes 2 years. In 3—day old mouse most cells of lens epithelium synthesize DNA indicating that cell division is occurring in most epithelial cells; but in 12—day old mouse DNA synthesis occurs only in the germinative region on the anterior side.

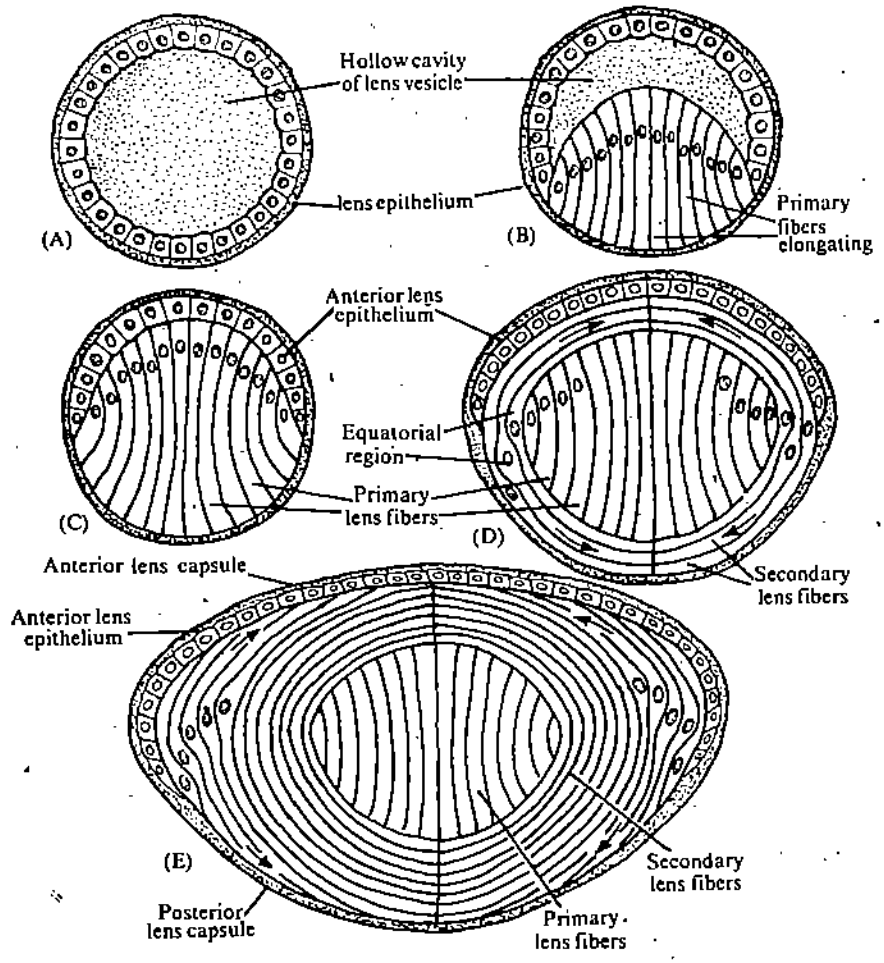


Fig. 17.11: Differentiation of the lens cells. (A) Lens vesicle. (B) Elongation of the interior cells, producing lens fibers. (C) Lens filled with crystallin-synthesizing cells. (D) New lens cells derived from anterior lens epithelium. (E) As the lens grows, new fibers differentiate.

CORNEA : The fully formed cornea consists of 2-cell layered epithelium and the stroma made of extra cellular secreted materials. The bulk of the cornea is made up of the stroma. Corneal development has been best studied in the chick. (Fig. 17.12).

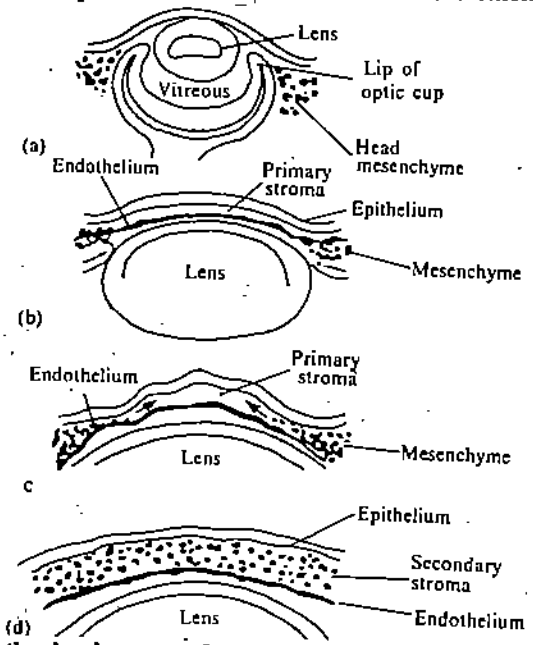


Fig. 17.12 : Stages of the development of cornea in chick. (a) Lens vesicle separates from the overlying corneal epithelium. (b) Corneal epithelium cells secrete collagen and primary stroma is formed. Mesenchymal cells enter the space between corneal epithelium and lens and form endothelium. (c) Endothelial cells secrete hyaluronic acid causing primary stroma to swell. (d) Wandering mesenchymal cells secrete secondary stroma.

The ectoderm overlying the lens vesicle, consisting of 2 layers of cells, is the presumptive cornea. The cells of the basal layer divide by mitosis throughout life to replace those of the upper layer that are shed by desquamation. Under the influence of the lens vesicle the cells of basal layer become columnar and secrete about 20 layers of type I and type II collagen. The alternate layers of collagen secreted are parallel to the corneal epithelium but at right angles to each other forming an orthogonal ply (Fig. 17.13) and constitute the primary stroma between the corneal epithelium and the lens. Meanwhile, mesenchymal cells (of neural crest origin) from blood capillaries migrate into this region and form a one-cell thick endothelium, which secretes hyaluronic acid into the primary stroma. The stroma swells up and then into this migrate two waves of mesenchymal cells also of neural crest origin. These cells then secrete layers of their own collagen forming the secondary or adult stroma (Fig. 17.12).

Beginning on 10th day of incubation in chick embryo the enzyme hyaluronidase is secreted which destroys the hyaluronic acid and causes the stroma to shrink. The stroma is dehydrated later under the influence of thyroxine secreted by the thyroid gland making it transparent.

In the amphibians the outer cornea is formed by the epidermal ectoderm and the stroma by this and by inner corneal cells of mesoderm (probably of neural crest origin). The adult cornea is completed during metamorphosis under thyroid influence.

Fig. 17.13 : Section of corneal stroma showing alternate layers of collagen fibrils at right angles to each other.

17.2.5 Tissue Interactions in Development of Eye

The vertebrate eye is a complex organ made of several components precisely adjusted to each other to ensure its efficient functioning. Such arrangement is brought about by an orderly sequence of interactions between various tissues derived from different sources in the embryo during development. We shall discuss these interactions separately in relation to the formation and further development of optic vesicles, lens and cornea.

Optic vesicle: We have described earlier that the presumptive material for the optic vesicles lies in the eye field in the anterior region of the early neural plate. Experiments on amphibian embryos have shown that if at this early open neural plate stage this material is cut out and grafted in the flank of another embryo it forms an eye cup showing that the cells of eye field are already determined to form the eye. This determination is preceded by induction of the ectoderm to form the neural plate by the invaginating chordamesoderm during gastrulation (see Unit 16). This is followed by the inductive action of the anterior part of chordamesoderm that comes to be below the prospective forebrain region of the neural plate, on the anterior part of neural plate. If this mesoderm is removed at this stage the eye field does not split and one median eye is formed. Moreover, if this anterior chordamesoderm is grafted under the prospective hind brain region of the neural plate it induces an eye field in that region. Continued association with mesoderm is also necessary for further development of the optic vesicle.

Invagination of the optic vesicle to form the double walled optic cup depends upon its contact with the presumptive lens ectoderm and the reciprocal induction from the lens

SAQ 1

1) Where is eye field located in
   a) early amphibian neurula
   b) primitive stage chick embryo?

.....

2) What tissue is responsible for division of the single eye field into two lateral parts, one for each eye in amphibian neurula?

.....

3) Why is retina considered an outpost of brain?

.....

4) Is corneal epithelium continuation of body epidermis, or sclera, or both or none?

.....

5) Name the three sources in the normal sequence from which in some species presumptive lens ectoderm receives inductive signals.

.....

6) Will an optic vesicle induce a lens from epidermis of the flank if it is transplanted under it in an amphibian at (a) neurula stage (b) hatched tadpole stage?

.....

7) What will be the effect on the lens if neural retina is removed?

.....

8) Is cornea induced by lens epithelial cells *or* lens capsule *or* both or none?

.....

9) Name the three zones of the neural retina in order from the inner to the outer side of the retina.

.....

17.3 VERTEBRATE LIMB

In the previous section you have studied how a series of sequential and coordinated interactions between different cell groups and tissues bring about the construction of a complex organ, the vertebrate eye. In this section you will study the development of another complex organ, the vertebrate limb. The paired limbs of all tetrapod vertebrates are built on a common basic pattern, develop in the same manner from cells derived from identical sources in the embryo and, therefore, they are all homologous structures.

A very large number of descriptive and experimental studies have been done on development in vertebrates during the present century. At first the work was done mainly on amphibian embryos but later the chick has been preferred for, among other reasons, the chick embryos are large, easily accessible and available throughout the year. Since recently much experimental work has been done on the development of wing of chick embryos. The wing bud is relatively a large structure and can be manipulated in various ways by the investigator. Portions of the bud can be cut out and their development studied by grafting them in the same or different embryos or on chorio-allantoic membrane of older chick embryos. Its mesodermal and ectodermal components can be separated from each other by treatment with some chemicals such as trypsin, EDTA etc or microsurgery and then they can be reassembled readily in different combinations. The two components of such synthesized buds can be from the same or different limb types, from embryos of the same or different stages, of the same or different species. The development of such combinations can be studied by grafting them on embryos or chorio-allantoic membrane or culturing them in vitro. Much of our understanding about the mechanisms involved in limb development has come from investigations on such studies on chick embryos.

Before we begin the study of limb development let us first recollect the common basic structural pattern and morphology of the limbs in tetrapod vertebrates. We shall then deal with the presumptive limb areas (limb fields) in the embryo, the problem of determination of limb axes, the origin and structure of the initial limb rudiment (limb bud) and subsequent process of morphogenesis of the limb, interactions between mesoderm and ectoderm during limb development. Finally we shall briefly discuss the information available regarding mechanisms controlling the development pattern in limbs.

17.3.1 Basic structural pattern of limbs

The common structural pattern of tetrapod limbs is very clearly expressed in their skeletal components and in the way they are organized. The limb is composed of a series of bones which develop in a proximodistal sequence and are arranged in a definite common manner. Typically, a forelimb consis of a humerus, followed distally by two parallel bones, a radius and ulna and then several carpals, metacarpals and digits made of phalangeal elements in this order. The corresponding skeletal elements of hindlimbs are femur followed by parallel tibia and fibula, several tarsals, metatarsals and digits. Since there is a bone-for-bone correspondence in the forelimbs and hindlimbs the various bones of these limbs are said to be serially homologous (Fig. 17.16).

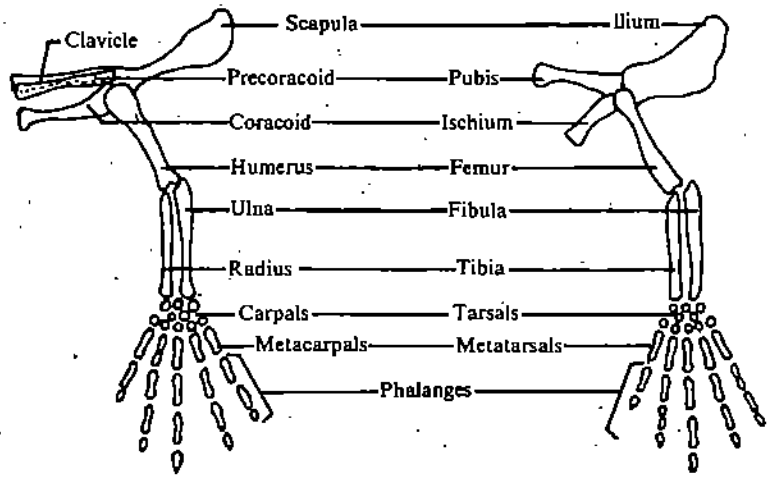


Fig. 17.16 : Scheme of serial homology of the bones of forelimbs and hindlimbs of vertebrates.

The bones are arranged in three limb segments : (1) *Stylopodium* (fore arm or upper arm of forelimb; thigh of hindlimb) is the first segment with its proximal end attached to the body wall. It contains the humerus or femur (2) *Zeugopodium* (lower arm of forelimb; shank of hindlimb) is the next segment and contains the radius and ulna or tibia and fibula. (3) *Autopodium* (wrist & hand of forelimb; ankle & foot of hindlimb) is the most distal segment. It is composed of carpals, metacarpals and digits (forelimbs) or tarsals, metatarsals and digits (hindlimbs). Primitively, the tetrapod autopodium is pentadactyl, bearing 5 digits, each made of several phalanges, and is believed to have consisted of 13 carpals (or tarsals) arranged in 3 rows, one row of 5 metacarpals (or metatarsals) and 5 digits (Fig. 17.17).

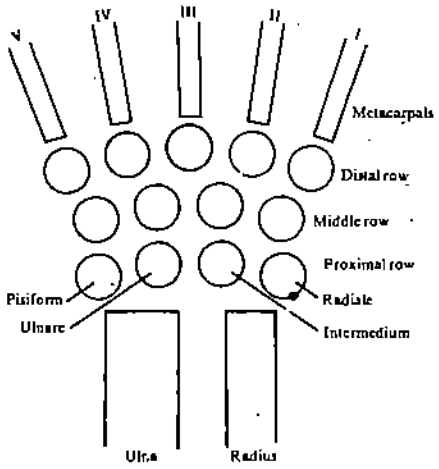


Fig. 17.17 : Scheme of the complete carpal elements in a pentadactyle forelimb.

This primitive basic structure is exhibited by both forelimbs and hindlimbs in all tetrapods with minor variations or major modifications, mainly of the autopodium. In many the pentadactyl character has been lost secondarily. In birds and many mammals and also aquatic vertebrates the autopodium has been modified in a variety of ways which has involved reduction or loss of some elements, fusion and elongation etc. of others (Fig. 17.18).

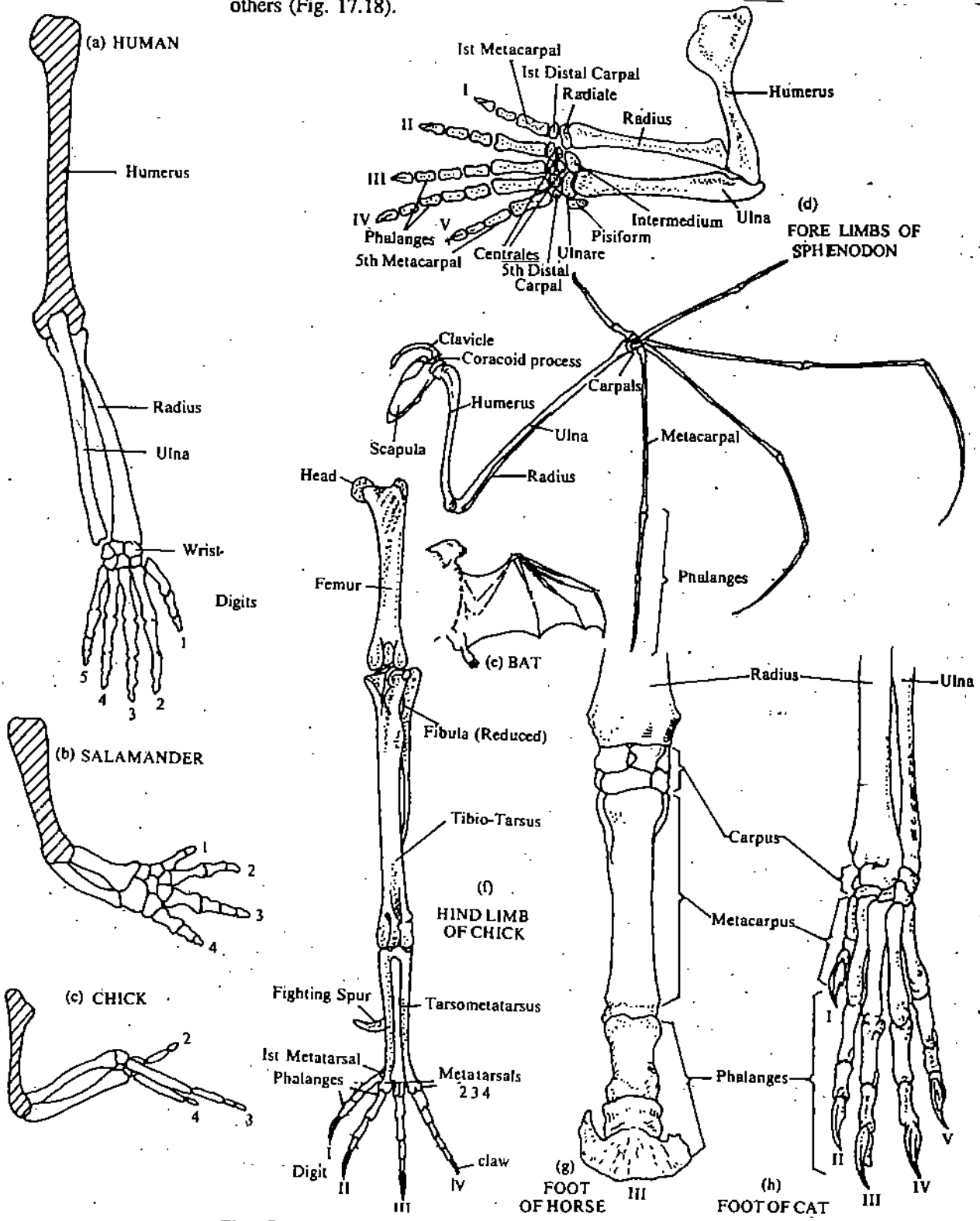


Fig. 17.18 : Fore limbs: (a) Man, (b) salamander; (Amphibian), (c) Chick wing, (d) Sphenodon; (Reptilia), (e) Bat, (f) Hindlimb of chick (male), (g) Foot of horse, (h) Foot of cat

17.3.2 Limb Field

You have already learnt (unit 14) how by various marking and transplantation methods it is possible to identify in early embryos the specific areas of groups of cells which ultimately form different organs. Identification of such areas helps to construct fate maps

of different parts of embryos at different developmental stages. Using similar methods it has been possible to identify the presumptive areas of forelimbs and hindlimbs and localize precisely in the early embryos of several vertebrates. The prospective forelimb area in the tail bud stage embryo of the salamander, *Ambystoma maculatum* (Amphibia) is shown in Figure 17.19. It is a circular disc-like area just behind the gill area consisting of ectoderm underlain by lateral plate mesoderm. The limb is actually formed from cells in the central part of this disc and those surrounding this central part give rise to peribranchial tissue and the shoulder girdle. Experiments made by many biologists since the early work by Harrison (1918) have provided the following information:

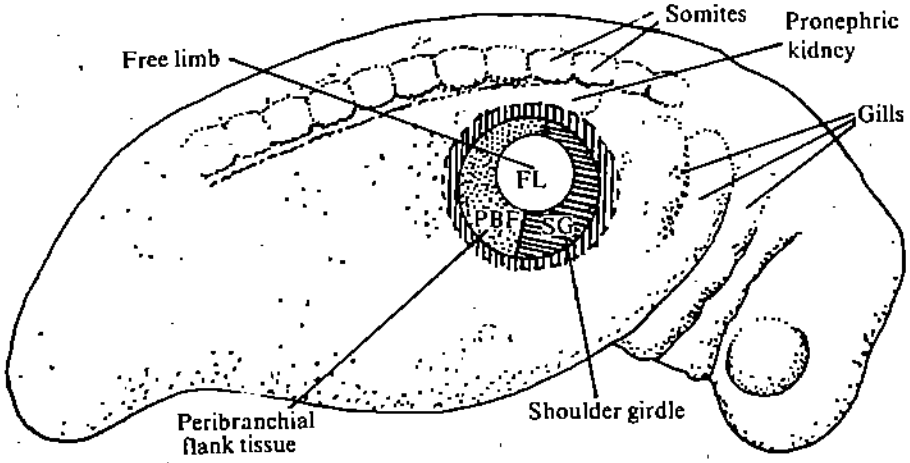


Fig. 17.19 : Prospective forelimb bud of Ambystoma a salamander. The central area contains those cells which are destined to form the free limb; the cells surrounding free limb are those which give rise to the peribranchial flank tissue and the shoulder girdle. The cells outside these regions usually are not included in limbs but can regulate to form a limb if more central tissues are removed.

1) If the central part of the disc (including mesoderm and ectoderm or mesoderm alone) is removed a normal limb forms from cells of the outer ring which otherwise the peribranchial tissue and girdle.

2) If the entire disc including the outer ring is removed the cells from an additional ring surrounding the disc move in to close the wound and form a normal limb (Fig. 17.20)

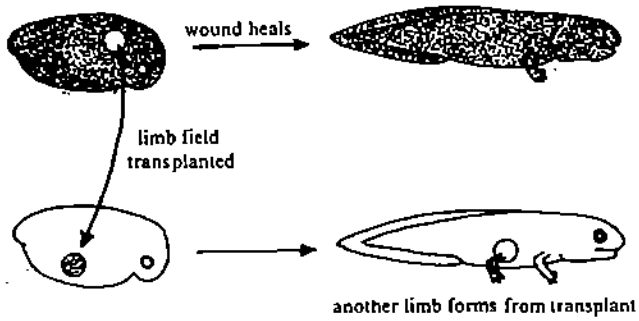


Fig. 17.20 : Two limbs from a single limb region. A disc of tissue is removed from one embryo and transplanted to another. One limb develops from the transplanted limb disc and a second limb forms from tissue which fills in the wound.

3) If this additional outer ring is also removed, no limb develops.

The larger region including the entire disc plus the outer ring of cells surrounding it constitutes the *Limb field* for the forelimb in Ambystoma (and also other amphibians) at neurula and tail bud stages. By other experiments on amphibians it has been demonstrated that :

1) When half of the field is removed and grafted at a different site (ectopic) two limbs are formed, one from the graft and the other from the remaining half of the field left in its original place.

2) When the presumptive limb area of one embryo is grafted adjacent to the limb area of another embryo the two areas fuse to form a single limb field and only one limb is formed, not two as one would expect.

3) When the field is divided in the middle and the two parts are prevented by a barrier (e.g. a membrane) from re-fusing two limb fields result and two normal limbs are formed.

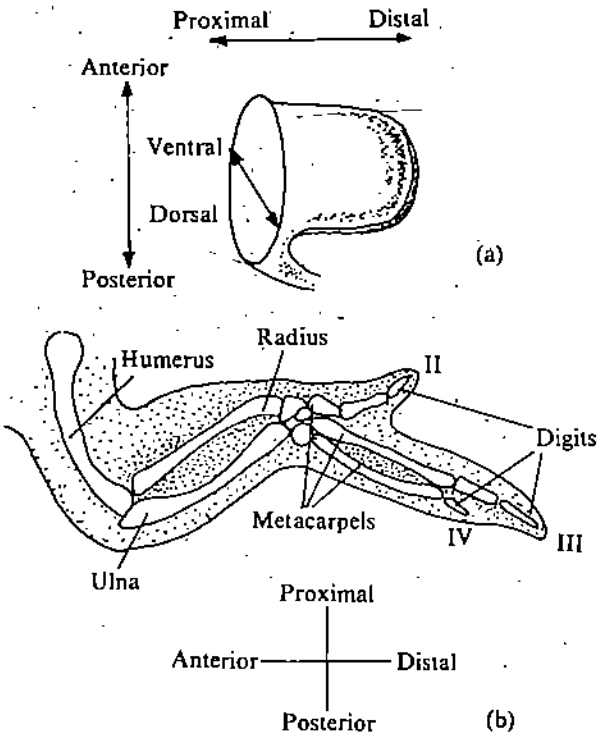
These experiments lead to the following conclusions:

1) The limb field extends to some distance beyond the actual presumptive limb area.
2) The cells of the entire limb field are capable of forming a limb.
3) The cells of the field have properties greater than those of forming the limb, the girdle and other tissues which they normally form. The individual parts of the field recognize any disruption in the field (e.g. loss of a part; addition; splitting etc) and respond according to the new situation to form a normal limb. This is called *regulation*.

These properties of limb field are similar to those of eye field which we have discussed in the previous section.

In the chick embryo the wing and leg limb fields are already localized at 2-somite stage (stage 8) and lie in the regions opposite to those of the future somites 15-20 and 26-32, respectively.

17.3.3 Determination of Limb Polarities



17.21 : The three axes (polarities) of early wing bud (a) and of mature wing (b) of chick

The limb pattern is characterized by three axes (or polarities) as shown in figure 17.21: (1) The direction from the shoulder (or hip) to the digits is the *proximal-distal (P-D) axis*. (2) The cranial (head) side of the limb is anterior and the caudal (tail) side is posterior. The direction from the former to the latter side is *anterior-posterior (A-D) axis*. (3) The third axis is the *dorsal-ventral (D-V) axis* which indicates the direction from the upper (dorsal) to the lower (ventral) side of the limb. In your own hand the thumb is anterior and little finger posterior, the back of the hand marks the dorsal and palm the ventral side. You should also notice that the respective contralateral forelimbs and hindlimbs develop as mirror images of each other. For example, your right hand and foot are mirror images of the left hand and foot, respectively.

The polarities or axes of the future limb are established in the cells of the presumptive area of a limb early in the embryo but not simultaneously. A number of experiments on *Ambystoma* forelimbs area and chick embryo wing buds have shown that A-P axis is determined first, D-V axis next and P-D axis relatively at a later stage after the limb rudiment (limb bud) has become prominent (Fig. 17.22).

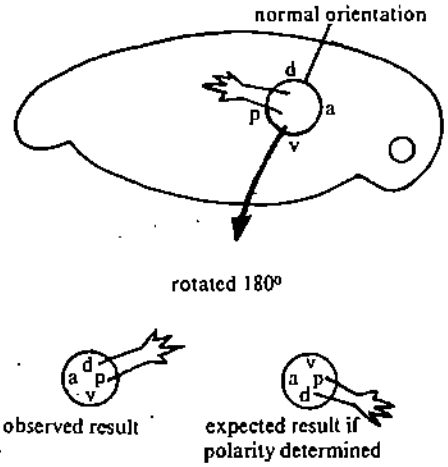


Fig. 17.22 : The polarity of the limb disc. Upper figure: An amphibian embryo, the approximate size and location of the limb disc, and the direction of out growth of the limb. Lower left: The observed result when the limb disc is cut out and rotated through 180 . A-P polarity was determined but not yet D-V polarity. Lower right: The expected results if both A-P and D-V polarities were determined. Anterior,a; posterior, p; dorsal, d: and ventral, v.

In Ambystoma, if the limb field (ectoderm + mesoderm) is cut out at gastrula stage, rotated by 180 and regrafted in the same location, a completely normal limb forms with regards to its dorsal, ventral, anterior, posterior, proximal and distal organization. However, after a similar operation at late tail bud stage both the A-P and D-V axes of the developed limb are seen to be reversed; i.e. in relation to the axes of the embryo the dorsal side of the limb faces ventrally, the ventral side dorsally, anterior side points posteriorly and the posterior side anteriorly. The P-D axis is however normal. When the rotation is carried out at an intermediate stage between gastrula and tail bud stages the limb formed is normal along D-V axis but its A-P axis reversed. It has been established that A-P axis of the prospective forelimb *Ambystoma* is determined by late gastrula (Yolk plug) stage and D-V axis a little before tail bud stage.

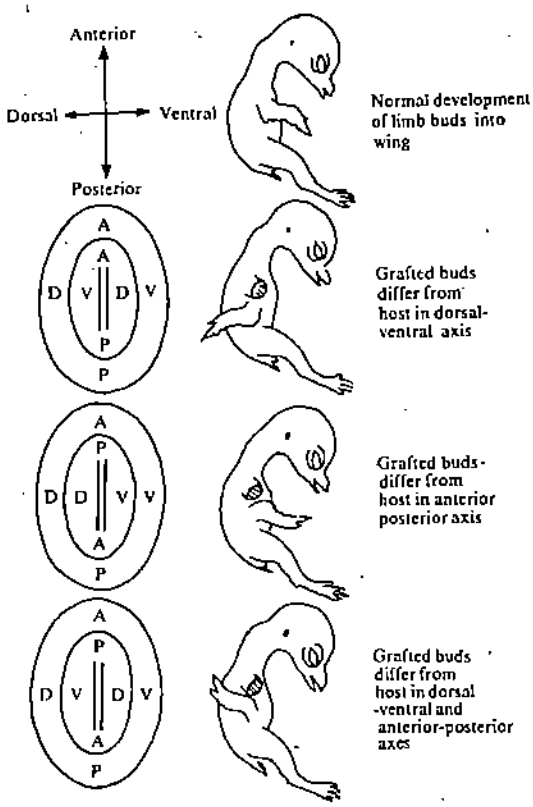


Fig. 17.23: The wing bud removed from one embryo, rotated in various ways and grafted on a host embryo where it developed in the ectopic position. Outer circles show normal axes of the host. Inner circles show the rotation done of the graft changing its A-P, D-V or both axes in relation to those host. For sake of clarity the host's own normally developed wing is not shown in the lower three figures.

Determination of polarities in fact occurs in the presumptive limb mesoderm of the limb field. This has been found by above type of experiments in which mesoderm above was isolated, rotated and grafted in the same or in an ectopic location under the ectoderm. Still the same results were obtained with regards to the axial pattern of the formed limb. The polarities reside in the mesoderm and not in the ectoderm.

In the chick's presumptive wing mesoderm A-P axis is determined by 5-somite stage and D-V axis at 13-somite stage but the P-D axis is not established until some time after the initial wing bud has become visible externally. When, the wing mesoderm of an early stage embryo is isolated, rotated by 180 and transplanted in the flank of another host embryo of the same stage the graft from the donor forms a wing according to its own previously determined A-P and D-V axes and not in live with those of the host embryo (Fig. 17.23).

That P-D polarity is not rigidly fixed until later was shown by an experiment on embryos of stages 18-21 in which (1) as much as 85% of the wing bud mesoderm was removed, and (2) after removal of the distal third of the bud a complete bud was grafted over the remainder (Fig. 17.24). In both cases regulation in the remaining cells in (1) and in the increased mass of cells in (2) occurred and the wings formed were normal along P-D axis.

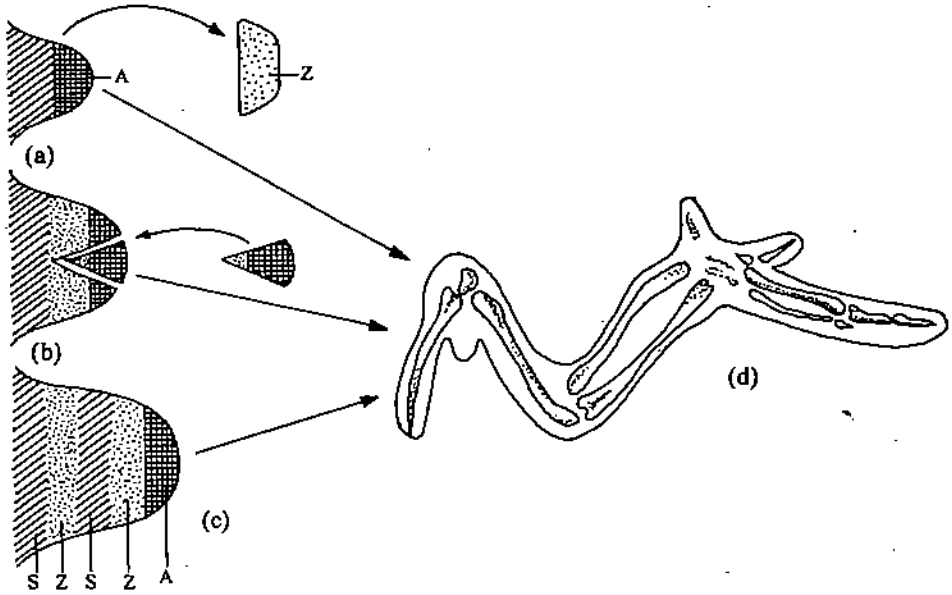


Fig. 17.24 : Regulation in chick limb development.

(a) Removal of zeugopodium or substantial part of mesoderm (stage 18-21). (d) Still normal limb development can take place) (b) Inserting a large wedge of limb tissue from another embryo. (c) A whole limb (stage 18-19) is transplanted on to a stylopodium-zeugopodium stump (stage 21-22) (d) Many times a normal limb is formed after such operations. A autopodium; Z zeugopodium.

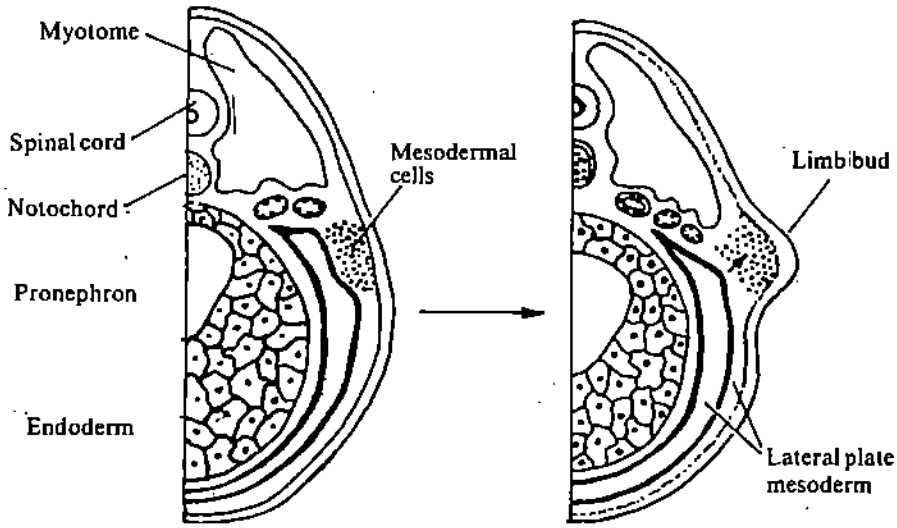


Fig. 17.25 : Diagram showing the origin of limb mesoderm in an amphibian embryo.

17.3.4 Pattern of Limb Development

The first visible sign of limb development is the appearance of ridge or thickening on each lateral side of the embryo of amniotes. The ridge called **Wolffian Ridge** extends antero-posteriorly between the brachial and pelvic regions; but only its anterior and posterior ends persist from which the forelimb and hindlimb develop respectively. In the amphibians there is no such ridge and separate pairs of ridges arise in the respective limb regions. The ridge is formed from proliferation of cells of the somatic (parietal) layer of lateral plate mesoderm. The mesodermal cells of the ridge or disc lose their epithelial connections and become mesenchymal migrate as separate cells and form a loose mass of mesenchyme beneath the embryonic ectoderm opposite the ridge or thickening. Their continued proliferation elevates the covering ectoderm and a limb bud is formed (Fig. 17.25).

The early limb bud has a core of mesoderm and a covering of ectoderm; The ectoderm is 2-cell layered. The outer, peridermal layer is of flattened cells and the inner layer has cuboidal cells, separated from the mesoderm by a basement membrane. The bud is at first a small mound but soon grows in size due to proliferation of mesodermal cells; as this happens the cells of the inner layer of ectodermal covering along its distal edge become tall columnar and form a pseudostratified layer. In the amniotes (lizard, birds, mammals) this thickening of the apical ectoderm appears as a prominent ridge running antero-posteriorly along the free distal margin of the bud. It is called the Apical Ectodermal Ridge (AER) (see Fig. 17.26). It has also been described in xenopus and atleast one frog (Rana tigrina) and may be present in other amphibians also, though not so prominently. In chick limb development AER has been found to play a very important role which we will discuss later in this section.

Fig. 17.26: Section through the leg bud of a stage-19 chick embryo showing the apical ectodermal ridge (aer) and the underlying mesenchyme (M)

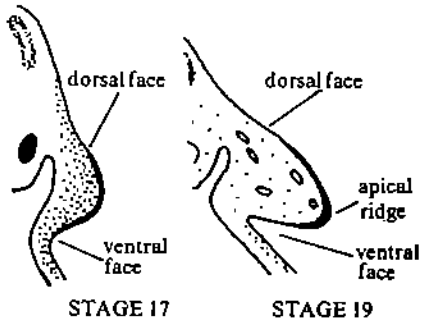


Fig. 17.27: Cross sections of chick embryos, stages 17 and 19, at the wing-bud level. There are differences in thickness of the ectoderm between ventral and dorsal faces of the wing bud

Recent findings suggest very strongly that the limb bud mesoderm is derived from two sources : lateral plate mesoderm and ventral ends of somites. The cells from the former source give rise to cartilage, (which later ossify to bones), soft tissues and muscle connective tissue, and the mesoderm, derived from somites form the muscles. However, all the mesodermal cells of the limb bud coming from whichever source are morphologically indistinguishable from each other and remain so for long until they start differentiating.

In the chick the dorsal surface of the fully developed limb bud is convex and the ventral surface is flattened with the AER at the free distal margin (Fig. 17.27). The narrow tip of the bud grows out in a slanting posterior direction instead of growing straight out away from the body. This asymmetry in bud indicates the ultimate asymmetrical pattern of the formed limb. In the amphibians the limb buds grow into a nearly conical structure. In either case, as the bud grows its distal part becomes flattened and paddle shaped; this is often referred to as paddle stage. Soon, a bend appears in the elongating bud which marks the Junction between the developing stylopodial and zeugopodial segments. Later another bend forms at the zeugopodial-autopodial Junction. From the distal edge of the flattened paddle shaped autopodial segment rudiments of digits grow out in a definite sequence at successive developmental stages (Fig. 17.28).

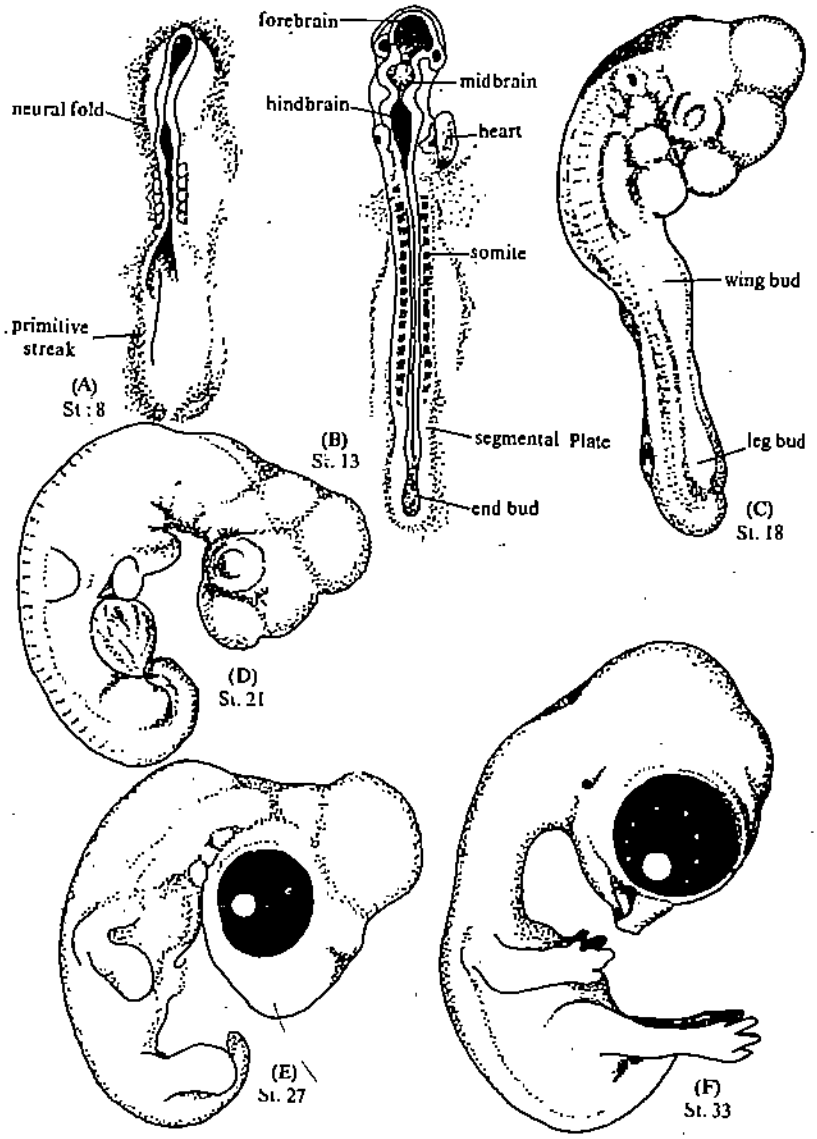


Fig. 17.28: Stages in the development of wings and legs in the chick embryo. Numbers indicate developmental stages in the Hamburger-Hamilton series. EB, end bud; FB, forebrain; H, heart; HB hindbrain; LB, leg bud; MB, midbrain; NF, neural fold; PS, primitive streak; S, somite; SP, segmental plate; TB tail bud.

Concurrently with changes in external morphology of the growing limb bud differentiation of various tissues occurs in the mesodermal interior. The mesenchymal

cells that are at first uniformly packed in the bud begin segregating in the differentiating regions. Cells for muscles (myogenic cells) and other soft tissues form loose masses in the dorsal and ventral areas; and those for the skeleton (chondrogenic cells) form dense condensation (s) in the central region, which are later converted into procartilage cells and then into cartilage. In the initial stage a common mass of condensed mesenchyme represents the skeletal tissue in each limb segment, but in the procartilage stage individual skeletal elements (such as radius and ulna, carpals etc.) become recognizable as separate concentrations of mesenchymal cells. In some cases these may fuse again later.

The mesenchymal cells of the limb bud are at first indistinguishable from each other with respect to ultrastructure, mitotic rate and synthetic activities. Using radioactive sulphate ($^{35}SO_4$) it has been noted that in the chick wing bud upto stage 22 (about $3\frac{1}{2}$ days old embryo) the level of $^{35}SO_4$ incorporation is similar in all cells. As development proceeds beyond this stage, incorporation of $^{35}SO_4$ decreases in the dorsal and ventral regions where muscles are formed; but sharply increases in the central chondrogenic core because the chondrogenic cells require large amounts of sulphate to synthesize chondroitin sulphate for the cartilage matrix. At about this time the ultrastructure of myogenic cells begins to change by accumulation of ribosomes and glycogen particles, the mitotic rate of chondrogenic cells in the core region decreases. By the end of stage 25 the differences between the two cell types become very obvious: the myogenic cells have begun to fuse to form myotubes and form myosin-action filaments while the cartilage cells have secreted a good amount of matrix (chondroitin sulphate and collagen) in the intercellular spaces. These changes take place in a sequence along the P-D axis of the developing limb (Fig. 17.29).

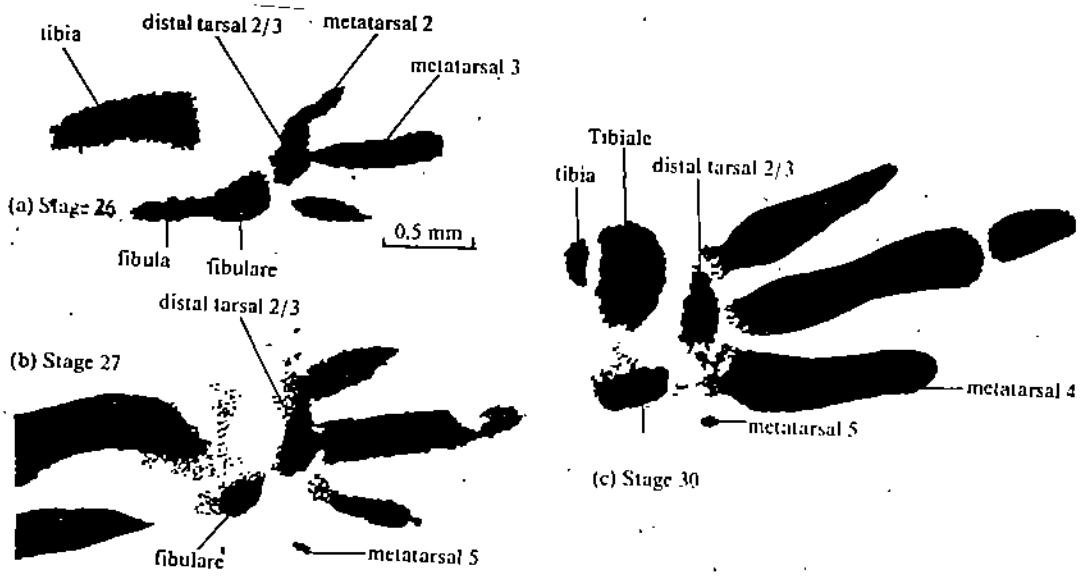


Fig. 17.29: The chondrogenic pattern of the hindlimb of a chick embryo as revealed by the incorporation of $^{35}SO_4$ into chondroitin sulfate. Autoradiographs prepared at (a) stage 26; (b) stage 27; (c) stage 30. T; Tibia; F, Fibula; f, fibulare; dt, distal tarsal; mt, metatarsal.

The differentiation of limb skeleton (as also of other tissues) proceeds in a proximo-distal direction. The stylopodial humerus (or femur) is the first to become recognizable followed by the zeugopodial elements (radius and ulna or tibia and fibula). The skeletal elements of the autopodium are laid down next also in a proximo-distal order so that the terminal phalanges of the various digits differentiate last. However, some deviation to this rule is of general occurrence; the carpals and tarsals arise later than the more distal metacarpals and metatarsals, respectively. In the amphibians girdles begin to form later after the stylopodium but in amniotes simultaneously with humerus and femur. Blood vessels appear early already in the very young limb bud.

The proximo-distal sequence of differentiation of muscles and cartilages is considered to be the consequence of the influence of AER on the rather loose mass of mesenchyme located beneath it. The AER at the tip of the growing limb persists until all parts of the limb are formed. Even at late stage the AER is present at the tip of each digit until the rudiment of the last phalange is laid down. In the mesenchyme subjacent to AER there

is much mitosis and the cells remain undifferentiated. This region is known as the Progress Zone from which cells are continuously added to the more proximal regions of the bud as it grows in length. (Fig. 17.30). So long as cells are in the progress zone they remain undifferentiated. As a result of proliferation among these cells the distance between the AER and the cells located at the proximal boundary of the progress zone increases until they pass out of the zone into the interior of the bud. Now no longer under the influence of the AER these cells start segregating in different regions and begin differentiating. In consequence, the proximal elements are formed first as their cells leave the progress zone and the more distal elements differentiate in a proximo-distal sequence as their progenitor cells are freed from AER influence in this order. However, what specific structure any population of mesodermal cells will form is a consequence of the genetic programme within those cells and is not determined by the AER.

In vitro culture experiments of AER and limb mesenchyme have shown that the AER (1) stimulates mitosis and (2) delays differentiation of mesenchyme.

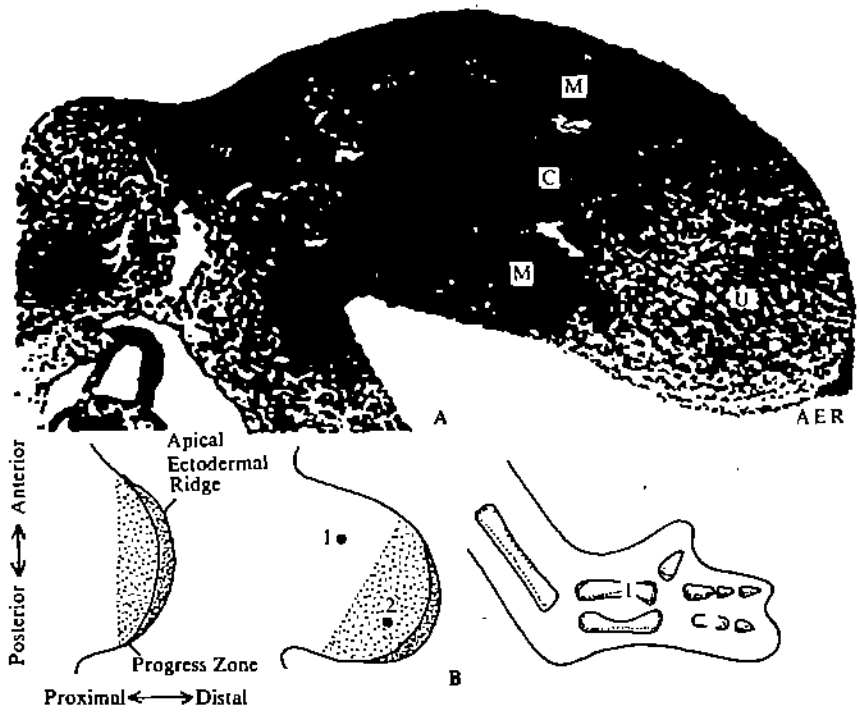


Fig. 17.30: The chick wing bud (A) Sectioned wing bud. The AER is seen at the tip as thickened ectoderm. A region of undifferentiated mesenchyme (U) is present below the AER. In the proximal portion of the limb, muscle (M) and cartilage (C) are beginning to differentiate. (B) Diagram of the wing bud at progressive stages of development.

17.3.5 Role of Mesoderm and Ectoderm in Limb Morphogenesis

A series of very interesting experiments on wing and leg buds of chick embryos have clarified the respective roles of mesoderm and ectoderm and interactions between them in limb development in vertebrates. Many of these experiments have involved separating the two components of early and later embryonic stages of the same or different limb types or even species and studying the development after recombining them with each other or with cells from non-limb regions in various ways (Figs. 17.31, 17.32).

It is established that the presumptive limb mesoderm which is localized very early in the presumptive limb area is essential for limb development. This is demonstrated by the following experiments:

1) When limb mesoderm is removed from under the ectoderm of limb area or replaced by mesoderm from some non-limb region, no limb forms.

2) The type of limb (wing or leg) that develops is determined by the mesoderm of limb site. Thus, when leg bud mesoderm is combined with wing bud ectoderm a leg is formed and vice versa. It means the mesoderm of each limb field is already determined as to what limb type it will form and determination is species specific (Fig. 17.33).

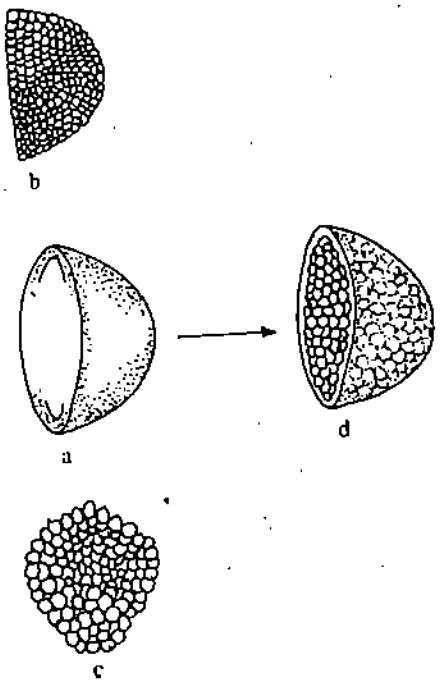


Fig 17.31 : Method of ectoderm-mesoderm recombination. (a) Ectoderm can be removed intact and clearly after trypsin treatment of limb bud; it leaves mesoderm slightly pulpy. (b) Healthy looking mesoderm can be separated from the ectoderm after the EDTA treatment; ectoderm is removed in flakes. (c) Dissociated and reaggregated mesoderm cells can be obtained after trypsin treatment also. (d) Ectoderm-mesoderm combination. It can grow as a graft on chorioallantois or flank.

3) When wing mesoderm is grafted under the ectoderm at a non-wing site in early embryo, a wing is formed at that site.

(4) When limb bud ectoderm is grafted at a non-limb site, no limb is formed; but if limb bud ectoderm is replaced by non-limb ectoderm on the limb mesoderm, a normal limb forms. The ectoderm of any region upto a certain stage chick embryo (and also amphibians) is able to participate in limb development if associated with limb mesoderm.

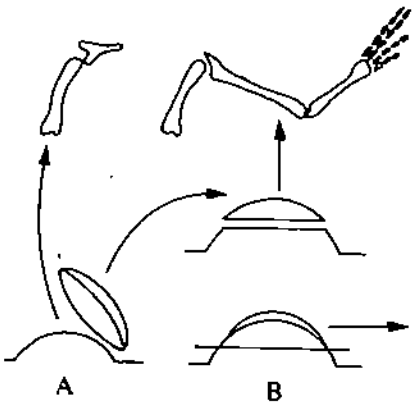


Fig. 17.32: (1) Differentiation of a limb bud after the removal of the apical-ectodermal cap. Only the femur and part of the tibia are differentiating. (2) Graft of an apical-ectodermal cap onto the basal part of the leg after the distal half had been severed. The basal mesenchyme is induced by the apical cap to form the distal components.

The results of some of the experiments are summarized in Table 17.1.

Table 17.1

Chick wing mesoderm	+	leg ectoderm	⟶	Chick wing
Chick leg mesoderm	+	wing ectoderm	⟶	Chick leg
Duck leg mesoderm	+	chick leg ectoderm	⟶	Duck leg (webbed feet)
Chick leg mesoderm	+	Duck leg ectoderm	⟶	Chick leg (unwebbed feet)
Chick wing mesoderm	+	non-limb ectoderm	⟶	Wing
Non-limb mesoderm	+	wing ectoderm	⟶	No limb.

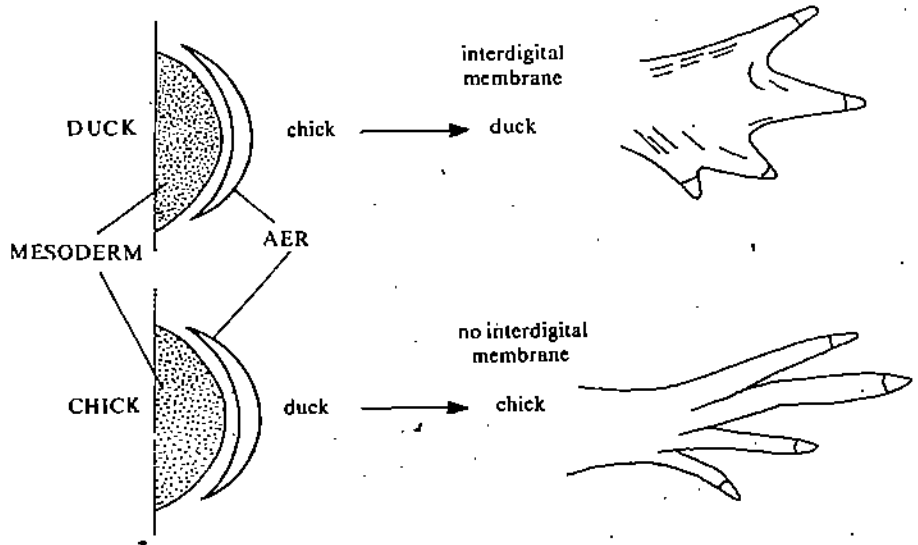


Fig. 17.33: Specific induction of the mesodermal component, demonstrated by changing the apical caps between chick and duck embryos. The mesoderm of the duck induces an interdigital membrane, whereas none is induced by the mesoderm of the chick embryo.

17.3.6 Apical Ectodermal Ridge (AER)

We have described earlier that the AER persists at the tip until the last phalangeal cartilage begins differentiation. The following experiments have demonstrated that the AER is formed and then maintained under the influence of limb mesoderm:

1) When limb mesoderm is grafted under the ectoderm of any region of an early embryo it induces the formation of an AER in the overlying ectoderm (Fig. 17.34).

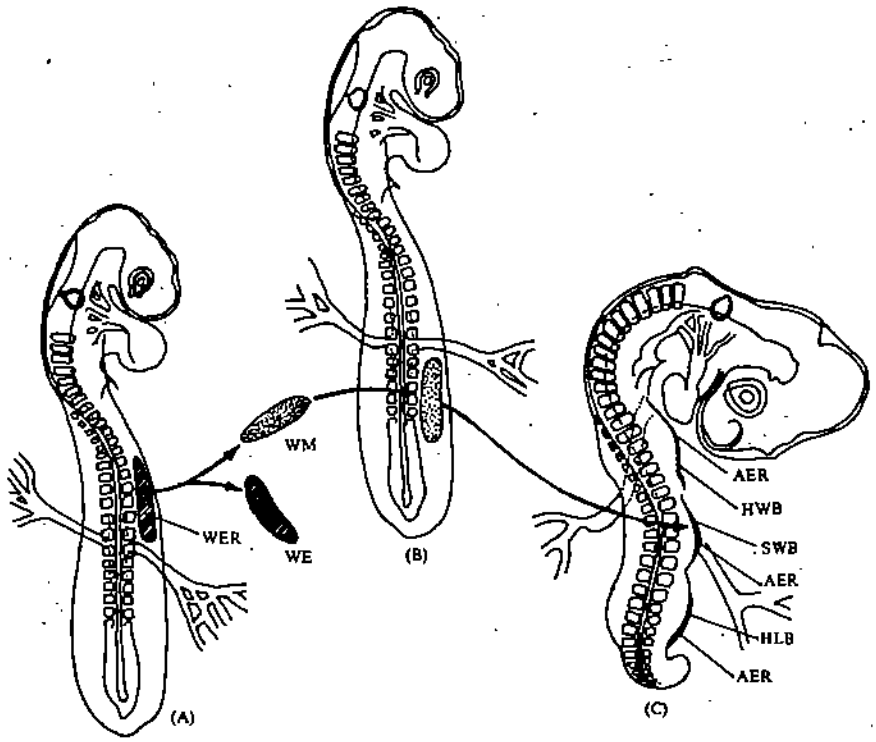


Fig. 17.34: Induction of apical ectodermal ridge by prospective wing mesoderm. A : prospective wing-forming region (WFR) of the stage 15 chick embryo is excised and the ectoderm (WE) is removed and discarded. B : the wing mesoderm (WM) is then grafted to a wound site made by removing a patch of prospective flank ectoderm from a similar host embryo. Host ectoderm then regenerates over the graft. C : the host embryo after twenty hours, showing a supernumerary wing bud (SWB) capped by an apical ectodermal ridge (AER) similar to that on the host wing bud (HWB) and leg bud (HLB).

2) When limb bud mesoderm is removed or separated from the AER by a barrier (e.g. a thin sheet of mica) or is replaced by non-limb mesoderm the AER flattens and

disappears. It is believed that limb mesoderm secretes some substance called *AER Maintenance Factor (AER MF)* that sustains the integrity of the AER.

We have already mentioned that the AER is responsible for maintenance of the progress zone in the growing limb bud by stimulating cell division in the subjacent mesoderm and delaying its differentiation. The AER is also a most important element in determining the proximo-distal progress of limb development as shown by several experiments (Fig. 17.32; 17.35).

1) When AER is removed at any stage further growth of the limb ceases and the distal parts, not yet formed, are not developed (Fig. 17.35)
2) After removal of the AER further distal growth may begin again if and when a new AER is regenerated.
3) When an additional AER is grafted on to the limb bud, the distal parts, not yet formed prior to graft, are developed in duplicate indicating that the AER induces the formation of distal structures (Fig. 17.35).

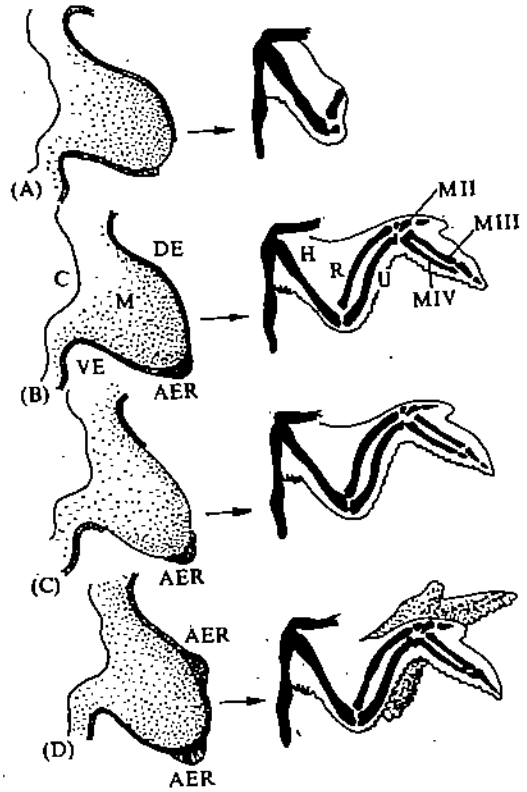


Fig. 17.35 : Induction of limb outgrowth by the apical ectodermal ridge in the chick embryo. On the left, schematic cross sections of the wing bud depicting various operations. On the right, the results obtained. A : excision of the ridge results in deficiencies of terminal limb parts. B : in the presence of a normal ectodermal covering, the wing parts appear in their normal proximodistal sequence and pattern. C : removal of the dorsal and ventral ectoderm permits outgrowth to continue as long as an apical ridge is present. D : grafting an extra ridge to the dorsal surface of the wing bud results in formation of two wings. AER, apical ectodermal ridge; AER', grafted apical ectodermal ridge; DE, ectoderm of the dorsal face of the wing bud; H, humerus; M, mesenchymal core; R, radius; U, ulna; VE, ectoderm of the ventral face of the wing bud. M II, M III and M IV, metacarpals II, III, and IV, respectively.

Thus while the AER is induced and sustained by the mesoderm, the latter forms all the proximal-distal parts of the limb under the influence of the former. The interaction between the mesoderm and AER is therefore of a *reciprocal* nature. However, the inductive influence of the AER is of *permissive* and not of *instructive* type as shown by the following experiments:

1) At an intermediate stage of wing bud development in chick embryo, the leg mesoderm was placed under wing bud AER. A leg not a distal wing part was formed.

2) When leg mesoderm of duck was placed beneath the AER of chick leg bud webbed foot like that of the duck was formed (Fig. 17.33).

The relationship between the limb mesoderm and ectoderm has helped to determine which of these components of the legs of two chick mutants has been affected by mutation. In the *polydactylous* mutant there develop some extra digits on the each foot and in the *Eudiplodia* mutant there are two complete rows of more than normal number of toes. By reciprocal combination according to technique shown in Table 17.2 the two components of mutants with those wild type chicks it could be established that mutation has affected the mesoderm in one and the ectoderm in the other (Table 17.2).

Table 17.2

	Mesoderm		Ectoderm		Resultant type of leg	Conclusion
1.	Polydactylous	+	wild type	⟶	Polydactylous	Mesoderm affected by mutation
2.	Wild type	+	polydactylous	⟶	wild type	
3.	Eudiplodia	+	wild type	⟶	wild type	Ectoderm affected by mutation
4.	Wild type	+	Eudiplodia	⟶	Eudiplodia	

17.3.7 Control of Pattern Formation

Limbs like all other organs have a pattern. What factor (or factors), environmental influences etc. are responsible for specific positioning of its different parts? For example, what determines that there will be one femur in the thigh but two parallel bones in the shank? What controls that the thumb will form on the anterior and the little finger on the posterior side? How is it that the toes of duck are webbed but those of chick are not although both develop from a paddle shaped autopodial segments of the embryonic limb? Numerous such questions have been and are being asked by biologists as well as laymen interested in the phenomenon of development. We still do not have an answer to the basic question "how pattern formation is controlled?" that can be applicable to any one or all organs of any one or all organisms. In this subsection we will discuss some information obtained by experiments related to the problem of control of pattern formation in the vertebrate limbs.

You know that the limb has three polarities or axes, A-P, D-V and P-D which are programmed into the limb mesoderm cells early in the embryo though not simultaneously. Are these programmes rigidly fixed in the cells from the start and their morphological expression in the limb realized by the mesodermal cells themselves? or do the cells remain labile for sometime in these respects and some other influences, say from the ectoderm, also operate in realization of the programmed polarities? Experiments to find answers to these questions have been performed on limb buds of chick embryos in the recent past.

a) Realization of P-D polarity of limb expressed in a definite proximo-distal sequence of differentiation of limb parts is controlled by the AER. Upto quite a late stage of limb bud development in chick embryo the AER can alter the fate of mesodermal cells with respect to their ultimate position along the P-D axis as shown by the following experiments:

   1) When an additional AER is grafted on wing bud a duplicated set of distal wing structures is formed (Fig. 17.33). It shows that under the influence of the additional AER a second P-D axis was created, regulation occurred in the distal mesoderm so that what was normally destined to form one set of structures along one P-D axis now formed two sets, one along each P-D axis.

   2) Distal half of the leg bud of stage 19 chick embryo was cut off and then an AER was grafted on the remaining basal half of exposed mesoderm. A complete normal leg was formed. The basal half of mesoderm would have normally formed only the stylopodial parts, but under the inductive influence of AER regulation occurred in its cells which gave rise to the various parts of all the three segments (Fig. 17.32 B).

b) Realization of D-V axis is also under the control of ectoderm in chick embryos of upto stage 20. This axis is altered when the ectoderm covering the wing bud is

isolated, rotated by 90 and regrafted over the exposed mesoderm. Rotation by 180 results in the formation of an additional autopodium.

c) *Zone of Polarizing Activity (ZPA)* : The A-P axis is the first axis to be fixed in the presumptive limb mesoderm at a very early stage. On the basis of results of a series of recent experiments on chick embryos it has been suggested that determination of the anterior-posterior pattern of wing parts is apparently under the control of a small block of mesodermal cells located at the posterior junction of the wing bud with the body wall. This mesoderm has been termed as the *Zone of Polarizing Activity* (ZPA).

Fig. 17.36 : Duplicated digits emerge as mirror images of normal digits when the zone of polarising activity (ZPA) from posterior side of wing is grafted to anterior region of the bud.

The normal chick wing has three digits numbered II, III and IV in this antero-posterior order (Fig. 17.21 b). In stage 18 or a little older embryos an extra ZPA was transplanted under the ectoderm on the anterior side of the bud near or in contact with the AER. This resulted in the formation of a duplicate set of digits in reverse order on the anterior side so the digital formula antero-posteriorly became IV, II, II-II, III, IV (Fig. 17.36). The second set was a mirror image of the original. The normal polarity was maintained even in this set as the usually the most posterior digit IV was still nearest to the transplanted ZPA.

It was suggested that ZPA secretes some substance, a morphogen, whose diffusion creates a gradient of decreasing concentrations along the A-P axis. The digit IV may be formed in response to the highest concentration and digits III and II in response to successively decreasing levels of this morphogen. A second such gradient would be established in reverse direction by the morphogen produced by the ZPA transplanted on the anterior side and hence the second set of digits in mirror image of the original set.

The above suggested role of ZPA is not generally accepted by most biologists. Similar additional set of digits in chick wing is induced by transplantation of mesoderm from other body regions, several different tissues, transplantation of an additional AER and also by applying retinoic acid (a derivative of Vitamin A) to the anterior side of the bud. Moreover, removal of ZPA from the posterior side of the bud does not prevent the formation of the normal set of digits while in an experimental situation ZPA does produce a polarizing effect, this role of ZPA in the normal development while it is in its usual posterior position in the wing bud is seriously doubled.

d) *Role of cell death*: As development of the limb proceeds waves of death or necrosis of large masses of mesodermal cells occur in certain regions at different stages. This has been observed in lizard, (calotes), chick, duck and mouse, but has not been observed in amphibians. Cell death occurs in four zones : (1) anterior necrotic zone, ANZ and (2) Posterior necrotic zone, PNZ, along the anterior and posterior margins, (3) opaque patch, OP, in deep mesoderm and (4) interdigital necrotic zones, INZ (Figs. 17.37, 17.38).

The death of cells in these is preprogrammed to occur at certain well defined stages. It plays a major role in the separation of radius and ulna in the forelimb, tibia and fibula in the hindlimb as well as in separation of digits from each other at the distal end of both limb types. The tissue between the digits of chick feet and both limbs of calotes is completely destroyed separating all digits from each other. In the duck only some cells of inter-digital tissue die, others remain to form the webs. The morphogenetic significance of cell death in ANZ and PNZ is not clear. It may be helping in sculpturing the contours of the limbs.

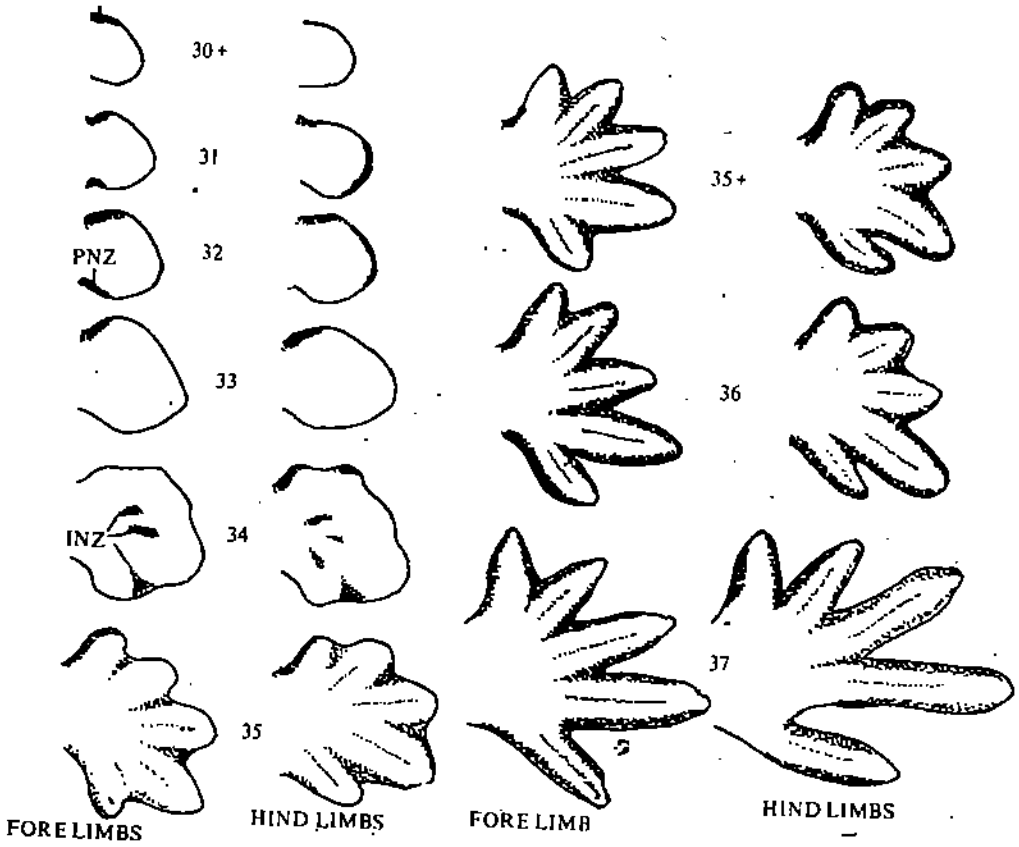


Fig. 17.37 : Cell death as revealed by neutral red vital staining in developing limbs of Calotes. Anterior necrotic zone (ANZ anterior border of stage 30 + forelimbs, posterior necrotic zone (PNZ posterior border of forelimb stages 31 and 32 and interdigital necrotic zone (INZ stage 34 onwards in both limbs).

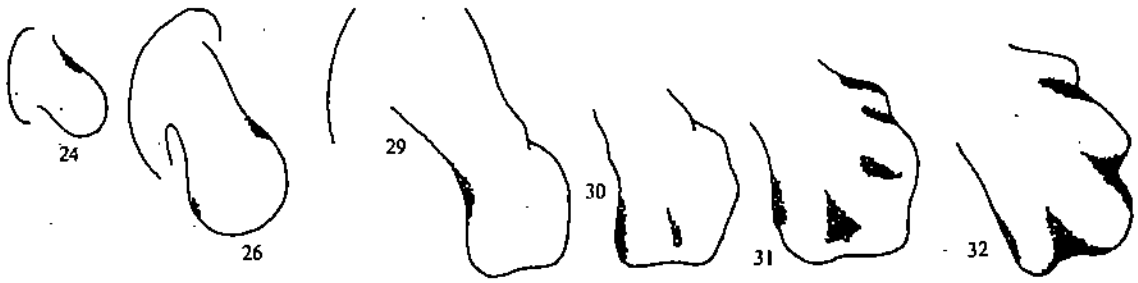


Fig. 17.38 : The topographic distribution of cellular deaths during development of the leg bud in the chick embryo. Numbers refer to developmental stages of the chick in the Hamburger-Hamilton series. Necrotic zones are shaded.

1) Arrange the limb bones in pairs according to their respective serial homologies: *Metatarsals, carpals, ulna, fibula, radius, femur, metacarpals, tarsals, tibia, humerus.*

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2) Which tissue of the limb field is essential for limb development?

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3) In an early amphibian neurula the forelimb area was excised, rotated by 180 and regrafted in the same place. The limb that formed had only one axis (polarity) reversed. Which one of the three limb axes may have been reversed in this case?

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4) What defect in the limb would be expected if the AER is removed after the bend marking stylopodial-zeugopodial Junction has appeared in the growing limb bud?

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5) What is the embryonic origin of cells for (a) limb muscles and (b) limb skeleton?

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6) What would happen to AER of the limb bud if its mesoderm is replaced by non-limb mesoderm?

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7) What effect does AER produce on the mesodermal cells of the Progress Zone of the growing limb bud?

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8) What type of limb, if any, will be expected if a bud formed by following combinations of embryonic cells is grafted in a chick embryo?

a) Chick wing mesoderm + Chick leg ectoderm
b) Duck leg ectoderm + Chick non-limb mesoderm
c) Duck wing mesoderm + Chick flank ectoderm
d) Duck head ectoderm + Chick leg mesoderm

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9) What is the postero - anterior order of the digits of a wing of a normal chick?

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17.4 COMMON FEATURES OF EYE AND LIMB DEVELOPMENT

A comparison of the embryonic development of the vastly different organs, the eyes and the limb, points to several interesting common features of organogenesis.

1) Both the eye and the limb originate in a morphogenetic field, which may be defined as a sum total of properties and commitment of a certain area of embryo in time to form an organ. The concept of a morphogenetic field explains many experimental observations.

2) In both organs the participating cells have diverse origins: surface ectoderm, neural ectoderm, mesenchyme derived from neural crest cells and head mesoderm in the case of eyes; and surface ectoderm, somitic and lateral plate mesoderm in the limbs. In addition cell types like those in the circulating blood and of nervous tissue invade both the organs.

3) Tissue interaction plays a major, in fact decisive role in the proper development of both the organs. The interactions of two types: one way inductions and two way reciprocal interactions. The example of one way induction includes corneal induction by the lens in the eye and the examples of two way tissue interaction include mesoderm-AER interaction in the limbs and optic vesicle-lens ectoderm interaction in the limbs. These ensure the harmonious development of all parts in both the organs.

4) In the organogenesis of both the inductive messages from one tissue to another are often operative across the species and are non-specific.

5) In the development of both the eyes and limbs massive cell death occurs which is genetically programmed and plays a role in pattern formation.

17.5 SUMMARY

Let us summarise what we have learnt so far.

- Both the eye and the limb develop in a morphogenetic field, which produces a normal organ even when some part of it is excised or harmonious addition is made to it.
- Tissue interactions play a major and decisive role in the proper development of both the eyes and the limbs.
- The cells forming the eyes originate from different locations in the embryo: those of the neural retina and pigmented retina from neural ectoderm, of the lens in surface ectoderm, of cornea in the surface ectoderm and mesenchyme of neural crest origin and of the choroid and sclera in the head mesoderm.
- The lens formation appears to be a multistep induction by pharyngeal endoderm, heart mesoderm and the optic vesicle in some species. In some it does occur even in the absence of the optic vesicle.
- Cytodifferentiation of the neural retina is a complex process resulting in a variety of cell types.
- Lens and corneal differentiation are under the control of the neural retina.
- The ectodermal covering of the limb primordium develops an aptical ectodermal ridge (AER) in response to mesoderm which also sustains the AER until all parts of the limb are formed.

- Determination of limb type and species specificity reside in the mesoderm.
- AER controls the expression of the P-D pattern of the limb by controlling distal outgrowth and the formation of distal structures. In the absence of AER further development of the limb bud ceases.
- AER stimulates cell division and delays differentiation in the progress zone of the developing limb.
- Among the three axes (polarities) of the limb the A-P axis is the first to be determined, D-V axis is determined next and P-D axis last.
- The dorso-ventral pattern of the limb appears to be controlled by the limb.
- The hypothesis of the control of A-P pattern of the limb by ZPA is seriously questioned.

17.6 TERMINAL QUESTIONS

1) Briefly describe the inductive interactions that cause the formation of optic cup, lens and cornea during eye development.

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2) Describe the process of lens differentiation.

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3) What are the constituents of cornea and how are they formed?

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4) Which parts of the eye are made from (a) only ectoderm, (b) both ectoderm and mesoderm and (c) only mesoderm?

5) What do you understand by reciprocal inductive interaction. Explain with one example.

6) When thigh mesoderm is transplanted beneath the wing bud AER, toes of feet and not digits of wing are formed. From this result comment on determination of limb pattern.

7) Transplantation of ZPA on the anterior side of a wing bud of chick results in the formation of a duplicate set of digits. What experiment would you design to test whether this effect of ZPA is species specific or not.

8) Suppose in an experiment the head ectoderm of an early embryo of a legless chick mutant is grafted over the exposed leg mesoderm of a normal chick embryo, it is seen that AER is not formed in the grafted ectoderm. What conclusion would you draw from such a result and why?

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9) (a) What is the role of large scale cell death in the interdigital zones of developing feet of chick embryo? (b) What role cell death may have in differentiation of neural retina?

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17.7 ANSWERS

SAQ 1

1) a) Median anterior region of the open neural plate.

   b) Median anterior region of epiblast in front of Hensen's Node.

2) Chordamesoderm underlying the prospective forebrain region of the neural plate.

3) It is formed from the optic vesicle which is an extension of the forebrain and sends messages to the brain via the optic nerve.

4) Both

5) i) Pharyngeal endoderm
   ii) Heart mesoderm and
   iii) optic vesicle

6) a) Yes, b) No

7) Lens will degenerate

8) By lens capsule

9) i) Ganglion layer
   ii) Inner nuclear layer
   iii) outer nuclear layer.

SAQ 2

1) Metatarsals-Metacarpals; carpals-tarsals; radius-tibia; ulna-fibula; humerus-femur.

2) Limb Mesoderm derived from lateral plate mesoderm.

3) A-P axis; because this is the first axis to be determined in the presumptive limb mesoderm.

4) Structures distal to zygopodium will be absent; because when the stylopodial - zeugopodial Junction has appeared the stylopodial and zeugopodial elements are already determined and differentiating and no further growth would occur in the absence of AER.

5) a) Somite mesoderm for limb muscles

   b) Lateral plate mesoderm for limb skeleton.

6) AER will flatten and disappear because non-limb mesoderm does not produce AERMF to sustain AER.

7) AER stimulates cell division and delays differentiation of the mesodermal cells of progress zone.

8) a) Chick wing

   b) No limb will develop

   c) Duck wing

   d) Chick leg.

9) Digits IV, III, II in this postero-anterior order.

Terminal Questions

1) Chordamesoderm induces neural plate during gastrulation. The mesoderm underlyin the prospective forebrain region of the neural plate induces the eye field, then its division into two eye fields and then evagination of the optic vesicles. The optic vesicle induces presumptive lens ectoderm to form lens placode. The lens placode induces optic vesicle to invaginate and form pigmented retina and neural retina. The lens capsule induces overlying epidermal ectoderm to form corneal epithelium and secrete primary stroma.

2) Refer to text in sub-section 17.2.4.

3) Refer to text in sub-section 17.2.4.

4)

a)	From only ectoderm	i)	Retina (pigmented as well as neural retina)
		ii)	Lens
		iii)	Eyelids.
b)	From both mesoderm and ectoderm	i)	Cornea
		ii)	Iris
c)	From only mesoderm:	i)	Sclera
		ii)	Choroid
		iii)	Intraocular muscles

5) Refer to texts in subsections 17.2.5 and 17.3.5.

6) When thigh mesoderm was placed beneath the AER of wing bud it came to occupy a distal position within the bud. In this new location its P-D determination was altered and it formed distal structures (toes) and not proximal parts. That it formed toes and not wing digits shows that its leg type specificity did not change even in the new situation.

7) The ZPA of an early wing bud of some species other than chick having different type of digits, say duck, will be transplanted on the anterior side of the early wing bud of chick. If the duplicated set of digits produced is like that of the chick it would mean that ZPA produced a non-specific effect of permissive nature. If however, these digits are webbed like those of duck it would mean that the effect was specific instructing formation of duck-like structures.

8) The head ectoderm of legless mutant is not competent to respond to the inductive influence of leg mesoderm of the normal chick to form AER. The mutation has affected the ectoderm of the legless chick.

9) a) Cell death in the interdigital zones of developing feet of chick embryos destroys and removes much tissue from between the digits (toes) so that the toes are separated from each other. It helps in patterning and sculpturing the feet.

   b) Cell death in the retina probably serves to separate the cell population of the deeper part of the retina into three zones, ganglion layer, inner nuclear layer and the outer nuclear layer to allow differentiation of the various cell types appropriate for their respective functions in the three zones. Here also cell death seems to play an important role in the development of a pattern.

GLOSSARY

Acrosin : a protease enzyme found on the surface of sperm head that aids the sperm to penetrate its way through zona pellucida.

Acrosome : membrane bound organelle found in the head of the sperm. The organelle contains enzymes and proteins and assists the sperm to penetrate the extracellular membranes of eggs.

Acrosome reaction : response of the sperm when it comes in contact with the egg. The membranes of acrosome are shed and the enzymes are realeased from it.

Animal hemisphere : anterior half of the egg at which nucleus is located. Also this half contains less or no yolk.

Apical ectodermal ridge (AER) : a ridge having a significant role in limb bud margin where dorsal and ventral faces of the bud meet.

Bindin : a protein in the acrosome of sea urchin sperm which promotes species specific attachment of acrosomal process.

Blastula : due to rearrangement of blastomeres to form the blastoderm, a fluid filled space or cavity called blastocoel is formed and this hollow, spherical and single layered embryonic stage is referred to as blastula.

Capacitation : changes that occur in mammalian sperm during its passage in the female reproductive tract that enables it to fertilise the egg.

Cell determination : specification of the developmental pathway of a cell.

Chemotaxis : directed movement of cell along a gradient towards the source of diffusible chemical material.

Cleavage : or segmentation is a series of cell divisions of the fertilized egg through which it is converted into a structure called blastula.

Contact guidance : alignment and migration of cells along discontinuities in the substratum.

Convergence : the migration of cells from the surface of the blastula to the external margin of the blastoporal lip.

Delamination : a mass separation of groups of cells from other cell groups.

Divergence : migration of cells to their future positions within developing embryo.

Embryonic induction : the process by which the eventual fate of a population of cells is altered because of interactions with neighbouring cells.

Gastrulation : a process during which the cavity of blastula (blastocoel) is obliterated and two new cavities

are formed viz. (i) the archenteron of gastrocoel enclosed by the endoderm and (ii) the coelom surrounded by the mesoderm.

Germinal crescent : region in the chick embryo hypoblast at the junction of area pellucida and area opaca where primordial germ cells are found.

Graafian follicle : large follicles in mammalian ovary containing fluid filled spaces called antrum.

Haptotaxis : directed movement of cells along a adhesive gradient.

Hensen's node : the thickened anterior end of the primitive streak in early chick embryo known as the primitive knob or Hensen's node.

Instructive interaction : a type of secondary induction in which the inducing tissue provides instructions to commit cells to a specific pathway development.

Invagination : the process of insinking or infolding of a layer of endodermal cells.

Involution : turning in or rolling under of cells that have to migrate to the blastopore margin.

Lamella : thin fan shaped regions of migratory fibroblast generated to propel the cell forward.

Lens vesicle : an inpocketing from surface ectoderm of the head. It forms the lens.

Limb bud : the limb primordium that projects out of the flank; somewhat like a fin-fish in shape.

Morphogenesis : organisation of differentiated cells into tissues and organs.

Morphogenetic field : a sum total of properties and commitment of a certain area of embryo (larger than the presumptive area) to form a normal organ. Its main feature is regulative ability so that the removal of a part or harmonious addition to it does not affect the normal development of the organ.

Morphogenetic movement : the movement of cells in the embryo from one place to another to establish a particular form or structural arrangement.

Mosaic eggs : eggs in which the fates of blastomeres become restricted during the first few cleavages.

Neurulation : formation and inward displacement of neural tube.

Oogenesis : formation of ova from oogonial cells.

Ooplasmic determinants : intrinsic factors in eggs that control embryonic determination. These factors become distributed unequally during cleavage.

Optic vesicle : an outgrowth from the lateral part of the forebrain (prosencephalon). It forms retina, pigment layer and part of iris.

Palisading : thickening of an epithelial sheet by elongation of cells from a cuboidal to columnar shape.

Permissive interaction	:	a type of secondary induction in which the potential of already determined responding cells is realised on obtaining signals from the inducing tissue.
Pluripotency	:	refers to the potential of a nucleus to support development of a variety of diverse organs and tissues.
Polarity (axis)	:	possession by an organ of opposite ends having contrary qualities, for example, anteroposterior polarity.
Polyinvagination or ingression	:	a process in which individual or small groups of cells in different parts of the external layer of the blastula or blastodisc invaginate or ingress into the segmentation or blastocoelic cavity.
Presumptive area	:	the area which will give rise to a particular organ or part of an organ, as revealed by vital staining or some other labelling technique.
Primordium (anlage)	:	the group of cells that forms an organ during embryonic development.
Primordial germ cells (PGCs)	:	Precursors of vertebrate gametes; these endoderm derived cells migrate from their place of origin to the genital ridge.
Regulative eggs	:	eggs in which cell fates are restricted late in development.
Spermatogenesis	:	formation of sperm from spermatogonia.
Spermiogenesis	:	process of differentiation of sperm from spermatids.
Totipotency	:	refers to the potential of a nucleus to support the development of all cell types of the egg usually laden with yolk.
Vegetal hemisphere	:	posterior half of the egg usually laden with yolk.
Vitellogenin	:	yolk protein precursor.
Zona pellucida	:	non-cellular layer of mammalian oocyte containing three glycoproteins ZP-1 ZP-2 and ZP-3. Assists in species-specific sperm binding process.
Zone of polarising activity (ZPA)	:	The mesoderm which controls the anteroposterior polarity of the limb skeleton and is located at the posterior junction of the limb bud with the body wall.
Zygote	:	fertilised egg.

FURTHER READING

Goel, Suresh C. 1984. Principles of Animal Developmental Biology, Himalaya Publishing House, Bombay.

K.V. Rao 1994 Development Biology — A modern synthesis, Oxford and IBH.

Berrill, N.J. and Karp, G. 1976 Development, McGraw Hill, New York.

Pattern, B.M. and Carson 1977 Foundations of Embryology, TMH Edition.

Dear Student,

While studing these units you may have found certain portions of the text difficult to comprehened. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-06
Block-3

1) How many hours did you need for studying the units?

Unit Number					
No. of hours					

2) How many hours (approximately) did you take to do the assignments pertaining to this block?

Assignment Number		
No. of hours		

3) In the following table we have listed 4 kinds of difficulties that we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

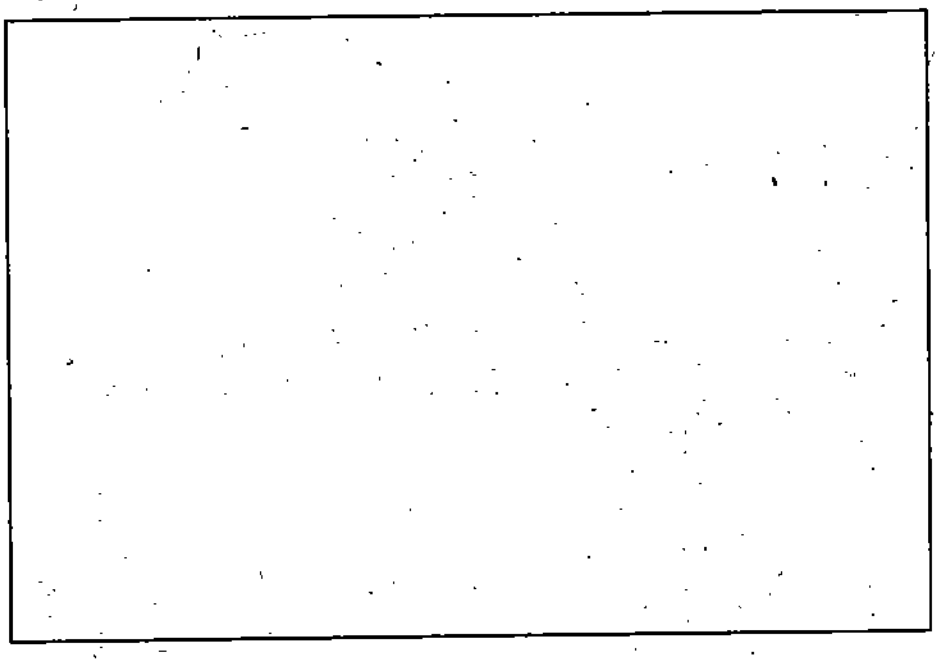
Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

4) It is possible that you could not attempt some SAQs and TQs.
In the following table are listed the possible difficulties. Kindly tick (√) the type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	SAQ No.	TQ No.	Not clearly posed	Type of difficulty		
				Cannot answer on basis of information given	Answer given (at end of Unit) not clear	Answer given in not sufficient

5) Were all the difficult terms included in the glossary. If not, please list in the space given below.

6) Any other suggestion(s)



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UGZY/BY-09
Developmental Biology

Block

4

ANIMAL DEVELOPMENT-II

Unit 18
Metamorphosis 5

Unit 19
Regeneration 43

Unit 20
Growth, Aging, Cancer 82

Unit 21
Human Development 106

BLOCK 4 ANIMAL DEVELOPMENT—II

From Block 3 of this course you learnt that embryonic development is characterized by a series of progressive changes that transform a single cell, the fertilized egg, into a multicellular organism. Development does not cease at birth or hatching. It continues throughout the long post-embryonic life and also involves progressive changes that affect the form, structure, physiology, size, health and longevity etc., of the individual. In the first three units of the present block we will discuss some of the developmental phenomena that occur during post-embryonic life of animals, viz. metamorphosis, regeneration, growth, ageing and cancer. In the last unit we have described human development.

Metamorphosis is the subject matter of **Unit 18.** In many animals the individual at hatching or birth is already a miniature adult in form and structure and its subsequent development involves mainly growth and sexual maturation in addition to functional development of various tissues and organs. Such animals are said to have direct development; but in the great majority of invertebrates and also some chordates development is indirect. In these forms a larval stage intervenes and after a certain period of free existence the larva undergoes changes and is transformed into adult form. The transformation of larva into adult condition is called metamorphosis. In this unit there is a brief general discussion of the significance of the larval phases in the life of animals and enumeration of the common larval forms that occur in different phyla. This is followed by detailed studies of metamorphosis and its hormonal control in amphibians and insects.

Regeneration in animals is the theme of **Unit 19.** Broadly, the term regeneration includes routine replacement of worn out and sloughed off cells of various tissues, healing of wounds and repair of tissues etc., Radical developmental events as restoration of lost part of an organ or restitution of the whole body from a fragment is specifically called physiological regeneration. In this unit we have discussed mainly the physiological or reparative type of regeneration in animals. The regenerative development involves many of the same processes and principles as does the embryonic development but there are also many differences. We have selected regeneration in Hydra and Planaria and regeneration of limb and lens in urodele amphibians for detailed studies. These would help you in gaining an understanding of the processes and problems generally involved in reparative regeneration. The unit also includes a general survey of regenerative ability in the animals of different phyla.

Unit 20 contains discussion of three aspects of post-embryonic development, growth, ageing and cancer.

In the section on growth you will learnt about the mechanisms of growth through increase in the number of cells and in the amount of extracellular materials and their control by intrinsic and extrinsic factors; forms, patterns and rates of growth and the methods of measurement of growth.

The second section of this unit deals with the problems of ageing. We have discussed the deteriorative changes that occur progressively and accumulate with age in the body tissues and cells and in the extra-cellular materials, particularly collagen. The effects of ageing on physiological efficiency of the organism and the various theories about the probable underlying basis and causes of ageing have been explained.

Cancer is considered to be a result of the breakdown of the normal developmental mechanisms that regulate multiplication and differentiation of cells and metastasis, the last fatal stage of cancer, is due to the loss of control on cell migration. The various types of cancer, their origin and comparison of characteristics of normal and cancerous cells have been discussed. The role of cancer causing agents (chemicals, radiations and viruses), oncogenes and tumor suppressor genes are discussed in the last section of Unit 20.

The subject matter of **Unit 21** is human development. This is included in this course to help you acquire knowledge of how we humans arise and develop from a single cell formed by the union of two cells, one derived from the mother and the other from the father, using the same processes as other animals. We have described the processes of gametogenesis in human males and females and then the entire development, beginning from fertilization, through the prenatal period within the mother's womb until birth followed by growth and maturation during post-natal life of adult stage. The unit also includes a discussion of the structure and role of the placenta and the causes of flaws and errors in development that may result in abnormalities and defects in the new born.

Objectives

After studying this block you should be able to:

- enumerate the various larval forms found in animal kingdom and explain the significance of larval phase in the life-history of animals
- describe metamorphosis and its hormonal control in amphibians and insects
- describe the processes, problems and principles of regeneration in hydra, planaria, and of limb and lens in amphibians
- explain how many of the processes and principles that operate during embryonic development also apply to regeneration and metamorphosis
- describe the mechanisms patterns, rates and regulation of growth of animals during post-embryonic life
- define aging and discuss the various theories regarding the probable underlying basis of aging
- explain the characteristics of cancerous cells and the breakdown of developmental control mechanisms that result in transformation of normal cells into cancerous cells
- describe the various embryonic and foetal stages in human development

Acknowledgement: Thanks are due to Prof. A. R. Rao for his constructive criticism and valuable suggestions for Unit 20.

UNIT 18 METAMORPHOSIS

18.1 Introduction
Objectives

18.2 Types of development

18.3 Types of metamorphic changes

18.4 Larval forms in various animal groups

Significance of ecological factors on the evolution, utility and distribution of larval forms

A catalogue of larval forms in various animal groups

18.5 Metamorphosis in Amphibians

The process of metamorphosis in anurans

The process of metamorphosis in urodeles

Hormones in metamorphosis of amphibia

Interaction of amphibian hormones in the process of metamorphosis

Tissue reactivity

Induction in metamorphosis

Molecular response to thyroid hormones during metamorphosis

Neoteny

18.6 Development, growth and metamorphosis in insects

General process of post-hatching growth in insects

Patterns of metamorphosis

Factors controlling metamorphosis in insects

Organs and hormones involved in insect metamorphosis

Interaction of insect hormones in the process of metamorphosis

Effect of metamorphic hormones on gene expression in moulting and metamorphosing insects

18.7 Comparison between metamorphosis in amphibians and insects

18.8 Summary

18.9 Terminal Questions

18.10 Answers

18.1 INTRODUCTION

In the earlier units of this course you have read that most animals develop directly, their zygote undergo multiplication, differentiation and morphogenesis to become a young one, similar to the adult, except in size and sexual maturity. However, in many animals groups ranging from the Porifera to the vertebrates development from zygote to adult is often indirect. These animals have one or several intervening larval stages in their developmental cycle before they develop into an adult by a sudden dramatic transformation known as 'Metamorphosis'.

The phenomenon of **metamorphosis is defined as a 'process during development which involves a dramatic change in morphology and physiology of the larva, so that it is transformed into an adult with completely different morphology and physiology, often for life in a different habitat.**

In many such animals undergoing metamorphosis, the larva is usually very different

from the adult. The best known examples are tadpoles of frogs, caterpillar of butterflies and moths, tadpole larva of ascidians, various larval types of crustaceans, ciliated trochophores of marine annelids and molluscs etc; In fact some times the difference between the larva and adult is so great that without knowing the origin of the egg, or without following the young one through its full development, it would be next to impossible to know that the young and the adult are of the same species. In the past such differences have sometimes led to the larva and adult of the same species being assigned to different taxonomic groups. For example the larva and adult of the axolotl *Ambystoma* (Urodele—amphibia) and *Tribegulians* of blister beetle (Insecta) till quite recently were mistakenly assigned to different species. More than a century ago, only the study of metamorphosis of the tadpole larva of ascidians could decide that the ascidians belong to Phylum Chordata.

The changes that occur due to metamorphosis relate often to a change in habitat with a corresponding change in the organisms' structure and other features. For example, in sea urchins, there occurs a change from planktonic to benthic existence, in frogs and toads from an aquatic to a terrestrial mode of life and in insects from a non-flying to a flying life.

In the present unit, which deals with metamorphosis, you will be studying this phenomenon in two diverse animal groups: namely amphibians among the vertebrates and insects among the invertebrates.

Your study will show that the metamorphic process in animals differ in the nature of transformation and in the mode of causation, making it impossible to describe metamorphosis in a generalized manner; however it must be pointed out that there are certain basic similarities.

In all these animals, development does not stop at hatching but continues during post embryonic life, and the principles that apply to embryonic development also apply to post embryonic development. Furthermore, the post development process in such animals are usually found to be reactivated by specific hormones, which modify and modulate the timing and duration of the process.

The metamorphic changes involve differential destruction of certain tissues accompanied by an increase in growth and differentiation of other tissues.

Objectives

After studying this unit, you will be able to:

- describe the types of developments and metamorphosis in animals
- define the terms metamorphosis, ametabolous, hemimetabolous, holometabolous, moult, instar, nymph, naiad, stadium, pupa, imago and diapause
- enumerate the various larval forms occurring in different animal groups
- explain the process of amphibian and insect metamorphosis and its control by hormones
- explain the interactions of the various hormones which cause metamorphosis in amphibians and insects
- describe the effect of metamorphic hormones on gene expression in amphibians and insects

18.2 TYPES OF DEVELOPMENT

Different animals have evolved different methods of development. These methods can be broadly classified into two categories (i) direct development (ii) indirect development.

Direct development

In some animals whose eggs have little or no yolk, such as the placental mammals, the embryos develop and grow within the womb of the mother from which they also obtain nourishment. Once, the offspring has developed to a stage where it looks like a miniature adult, the mother gives birth to it. This young one achieves its adult size and sexual maturity after birth over a period of time by gradual growth.

In animals whose eggs have a large amount of yolk, such as those of the birds and reptiles, the eggs develop oviparously outside the mother's body. Young ones resembling the miniature adults hatch out. The young individual then attains adult size and sexual maturity by gradual growth over a length of time.

Indirect development

In many animals like frog, *Amphioxus, Herdmania*, insects and many other invertebrates and vertebrates, the offspring which hatches out from the egg looks very different from the adult form, and is called a larva. The larva leads an independent existence for some time that varies from species to species, and then transforms into a miniature adult by the process of metamorphosis.

18.3 TYPES OF METAMORPHIC CHANGES

The process of metamorphosis involves reactivation of the morphogenetic processes. The morphogenetic changes as well as the mode of causation of these changes vary in different animal groups. The degree of changes that occur during metamorphosis depend on the degree of difference between the larva and the adult forms. For example, in urodele amphibians (newts) and hemimetabolous insects (cockroach), larva and adult show a few differences. The metamorphic changes are relatively less and metamorphosis is said to be gradual or incomplete. On the other hand, in anuran amphibians (frogs) and holometabolous insects, where differences between the larva and adult are enormous, the changes during metamorphosis are extensive and drastic. This type of metamorphosis is called radical or complete metamorphosis.

The changes during metamorphosis, include those of structural, physiological and biochemical nature. These are marked by disintegration and atrophy of some structures, cellular death in some tissues, morphogenesis and differentiation of certain new structures and remodelling of some others. These changes of metamorphosis in at least some groups of animals (insects, crustaceans, amphibians), are known to be controlled by hormones which serve as the causative agents of metamorphosis.

SAQ 1

Explain the term (i) direct development (ii) indirect development.

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18.4 LARVAL FORMS IN VARIOUS ANIMAL GROUPS

18.4.1 Significance of ecological factors on the evolution, utility and distribution of larval forms

Environmental factors surround and influence the egg and the young throughout the

animal kingdom. These factors affect such vital activities as the place where the eggs are laid, the amount of food provided for the young during early development, the various devices acquired for their protection, the specialized modifications for accomplishing locomotion and the production of those larvae which change into adult by metamorphosis.

The larvae which undergo metamorphosis present a wide range of variations as compared to the animals which undergo direct development. You are already aware that usually in animals which undergo metamorphosis the larvae and adult live in different habitats and consequently in different environments. Metamorphosis appears essential for those forms in which food and habits of the adult are unsuitable for the developing young.

Among the three habitats, namely saline water, fresh water and land, most of the animals which undergo metamorphosis are found in the marine waters. This seems to be in line with the assumption based on evolutionary evidence that all organisms originated in the sea and that many types of animals develop in a moist atmosphere or in liquid medium. Futhermore, the transition from saline to fresh water, or to terrestrial life present great difficulty to the developing young, and so those animals which live in fresh water or land are more often characterized by direct development.

The sea medium also provides a more constant environment: the temperature rarely fluctuates, the sea water rarely dries up or freezes, food is available aplenty at the surface where most of the metamorphic forms occur.

Metamorphosis is rarely seen in animals occurring in fresh water. This is because the living conditions of the fresh water environment lacks constancy as compared to that of the sea. Except in a few cases the fresh water bodies usually lack sufficient depth and area to maintain even a minimum degree of stability. Fresh water heats and freezes much more rapidly than the sea, and its stream floods and recedes, leaving many forms of life to perish. Furthermore, every stream has depths and narrows where flow is rapid, and flats where flow is slow, as a result of which the vulnerable larvae find it difficult to maintain themselves. These are some of the reasons as to why most animals in fresh water have direct development, where the young and adult are similar and share a similar environment.

The terrestrial environment appears to be the most inhospitable for the developing larva with fluctuations in temperature, glare of the light, lesser moisture and easy exposure to enemies. It is due to these reasons that in land forms development is either direct and fairly simple or greatly specialized with complicated adaptations and structures for protecting the young during the immature period. The latter type of development is exemplified by many forms of insects in which the life cycle is complicated and metamorphosis occurs.

These different environmental factors and habitats are responsible for the lack of uniformity in the occurrence of larval types in the different groups of animal kingdom. The particular forms of larvae within a group are of adaptive rather than taxonomic significance. For example, in those animals whose adults are sessile the young are active. This activity has two obvious reasons: first the species secures its dispersal through migration of the young, and second, it extends the range. This migration is commonly passive but, over a period of time it successfully serves to extend the range of the species, as if the adults themselves were able to move. Furthermore, to ensure disperal large amount of gametes are produced, as the chance method of fertilization results in the loss of many sperms and eggs. Also, all fertilized eggs do not reach maturity as larval mortality is high due to various physical factors, predators etc. Another example of adaption is observed in the marine lobster which lives at the bottom region, is negatively phototropic and feeds on whatever comes its way, unlike its larvae which are positively phototropic and live at the surface of the sea where food is abundantly available. In such a life style it has various structural modifications which change after metamorphosis into an adult. Similarly, other animals have specialized structures for their survival suitable to their environment and needs. You should keep in mind, though, that the larvae would survive only if subsequent changes by metamorphosis allow the organism to undertake a new mode of life. In other words, metamorphosis is a necessity for bringing about those adaptations which

enable the organism to live in its permanent environment and reproduce successfully.

18.4.2 A catalogue of larval forms in various animal groups

The larval stages and types found in different groups are many. Each larval type has a different structure and is known by a different name. Table 18.1 lists out the names of some of the commonly studied larvae which occur in different animal groups.

Table 18.1 : Examples of larval forms in major animal groups.

S.No.	Animal group	Larval form
1.	**Lower Mesozoa**	
	Sponges	amphiblastula (18.1a)
	Coelentrates	planula (18.1b), actinula, syphistoma, ephyra
	Protosomia	
	Flat worms	
	Turbellarians	müller's larva
	Trematodes	miricidium, cercaria, redia, sporocyst
	Cestodes	onchosphere
	Nemertines	pilidium
	Annelids	trochophore (18.2a)
	Molluscs	trochophore, veliger
	Crustaceans	nauplius, metanauplius cypris, zoea, mysis, megalops
	Insects	
	with incomplete metamorphosis	Nymphs (18.2b) naiads
	with complete metamorphosis	maggots, caterpillars, grubs (18.2b) pupae etc.
	Ectoprocts	Cyphonauts
	Phoronids	Actinatroch
	Deurostomia	
	Echinoderms	bipinnaria, pluteus (18.3b), 18.3a) auricula
	Hemichordates	tornaria (18.3c)
	Tunicates	tadpole (18.3d)
	Cyclostomes	ammocoetes (18.3e)
	True eels	leptocephalus
	Salmon	alevin, parr, Smolt
	Amphibians	eft, tadpole

A look at the table will make you aware that metamorphosis is widespread in the animal kingdom. It occurs in many phyla in the invertebrates, ranging from the sponges (porifera) through the echinoderm and in several chordates. Fig. 18.1, 18.2 and 18.3 show some important larval forms which are often the subject of study. However, in this unit too, we are not going to describe all the larval forms or their metamorphosis. Instead we will restrict our study only to those aspects of larval life and metamorphosis, that have been extensively studied by biologists. This unit is devoted to the study of metamorphosis in amphibians and insects and will expose you to some of the recent developments in the area of study.

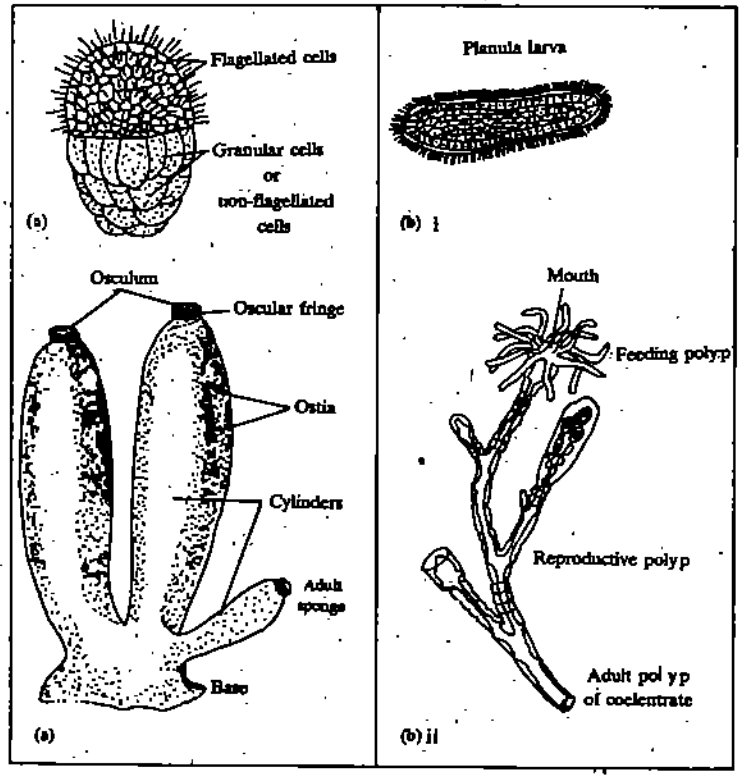


Fig.18.1: Larval forms of lower mesozoa
***a*) (i) Amphiblastula larva of porifera (ii) Adult sponge**
b*) (i) Planula larva of a hydrozoan coelentrate (ii) Adult *Obelia

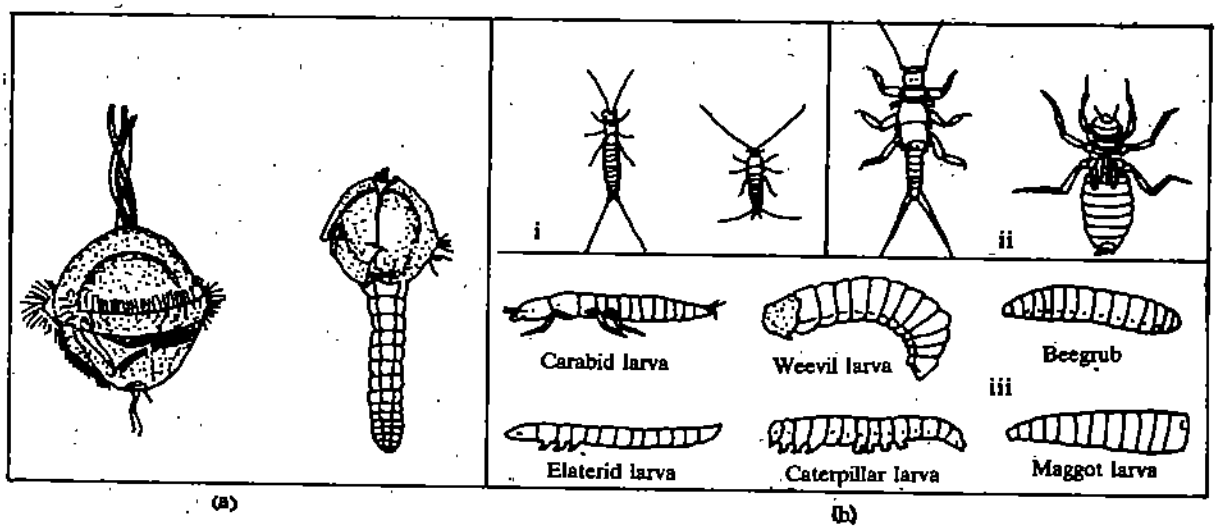


Fig.18.2: Larval forms of representative protostomes
***a*) (i) Trochopore larva of annelid (ii) Advanced stage in metamorphosis**
***b*) (i) Nymphs of ametabolous insects (ii) Nymphs of Hemimetabolous insects**
(iii) Larvae of Holometabolous insects

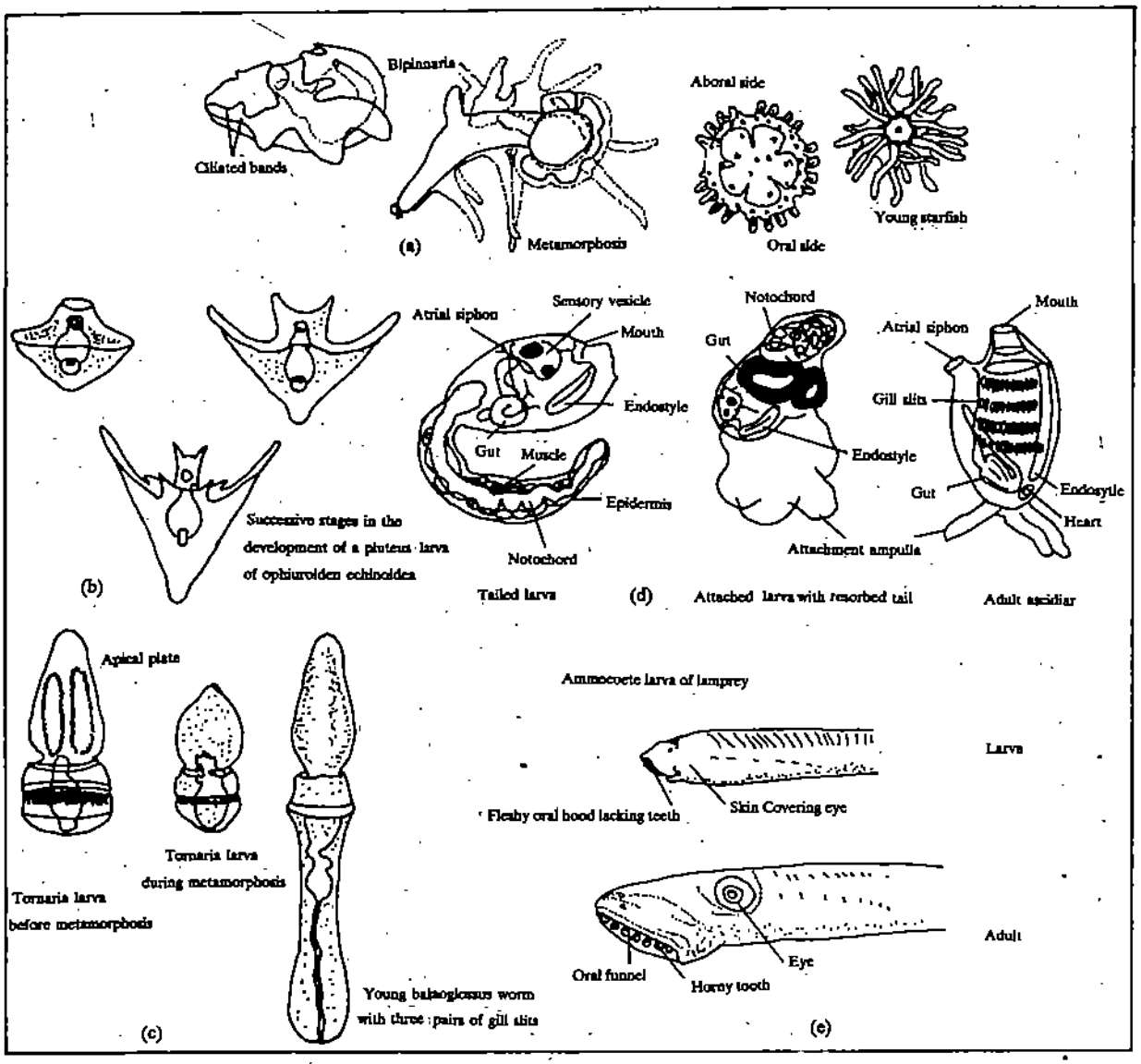


Fig.18.3: Larval forms of Deurostomes at various stages of metamorphosis
***a.* Bipinnaria larva of Asteroidea *b.* Pluteus larva of Echinoidea *c.* Tornaria larva of Balanoglossus**
***d.* Tadpole larva of Ascidia *e.* Ammocoete larva of lamprey**

SAQ 2

i) State whether the following statements are true or false

(a) In animals which undergo metamorphosis the larvae and adult live in same habitats and subsequently in same environments. True/False

(b) Metamorphosis is more commonly observed among the fresh water and terrestrial organisms as compared to marine organisms. True/False

(c) The terrestrial environment is highly inhospitable for the developing larva. True/False

(d) Metamorphosis essentially brings about those adaptations in organisms that enable them to live in their permanent environment and reproduces successfully. True/False

(e) In terrestrial environment insects are the only group that pursue a free larval life. True/False

(f) Among the various animal groups metamorphosis has been studied in detail in insects and amphibians. True/False

ii) Match the following larvae with their adult groups.

a) Trochophore	i) Cyclostomes
b) Tornaria	ii) Crustacea
c) Bippinnaria	iii) Echinoderms
d) Veliger	iv) Balanoglossus
e) Zoea	v) Annelids
f) Ammocoete	vi) Molluscs

18.5 METAMORPHOSIS IN AMPHIBIANS

Metamorphosis is radical in anurans, slight or absent in urodeles. In anuran amphibians like toads and most frogs, metamorphosis is usually associated with a transition from an aquatic to a terrestrial or amphibious mode of life. Occasionally however no transition in mode of life occurs as observed in the larval and adult of the frog *Xenopus laevis* and many primitive anurans which remain aquatic throughout their life. The change in habitat in the frogs and toads also usually results in a change in their feeding habit. In some like *X. laevis* there is no change in food habit since both larvae and adult are carnivorous.

Some anurans undergo an abbreviated type of metamorphosis before hatching, as they pass through a tailed, gilled tadpole-like stage within the jelly membrane of the egg. Others undergo direct development by skipping the larval stages totally.

Metamorphosis in urodele amphibians is usually less striking. Some of them undergo direct development, while others fail to complete their metamorphosis. The latter achieve sexual maturity as larvae, as seen in the axolotl larvae of *Ambystoma*. This phenomenon is called neoteny, and will be discussed in section 18.5.8. Some urodeles like salamanders have been observed to undergo two metamorphosis.

Metamorphosis in both anurans and urodeles essentially involves the activation of the genomic set underlying the adult organization, which requires for its expression a minimum mass of tissue that is greater than that of the egg.

The activation is believed to be due to the secretion of a brain hormone which initiates metamorphosis. The hormone triggers the degeneration of redundant larval organs and growth of hitherto quiescent structures which are needed in the adult.

In amphibians the process of destruction and growth are smoothly coordinated, as a result of which the animal retains its functional integrity throughout metamorphosis instead of lying dormant as in the case of insects.

18.5.1 The process of metamorphosis in anurans

Metamorphosis is most dramatic in the anurans. In them it represents an intensive period of growth and developmental changes during which the structure and function of almost every organ in the body is radically modified for adaptation to a new way of life. Unlike other adaptational changes, however, this process is begun and normally completed in the anticipation of the change of the environment. Some changes seen during this process involve growth and maturation of the tissues concerned. Other changes result in the regression and death of organs; the gills and tails which become redundant in adult life are completely reabsorbed.The most familiar kinds of anurans are frogs whose eggs develop into tadpoles, which are limbless, aquatic creatures with an oval torso and long, finned tail (Fig. 18.4). Metamorphosis transforms these tadpoles into four-legged, hopping carnivorous adults (Fig.18.4). After metamorphosis most types of anurans lead a terrestrial existence, returning to the water only to breed. Toads also exhibit this type of life habit.

Tadpoles have many anatomical and physiological adaptations that distinguish them from the adult. The fish like tadpole is herbivorous, as a result of which its mouth has

two horny beaks with rows of horny teeth (Fig. 18.4) which help in rasping away plant tissues. On each side of the head a fold of skin, the operculum, is present which covers the gill that grow from the lower ends of the visceral arches. Lungs are rudimentary and nonfunctional. The epidermis has large pigment cells of different kinds and is underlaid by a jelly-like dermis rich in hyaluronic acid. Because of its herbivorous diet the intestine of the tadpole is long and coiled. The eyes are recessed in the head, and the visual pigment porphyropsin is present in the retina. Nitrogenous waste products are excreted primarily as ammonia. The red blood cells are produced mainly in the kidney and the haemoglobin (HbF) present in the tadpoles is different from that of adult (HbA).

In order to transform into adults these tadpoles undergo three distinct phases of metamorphic changes which are visible morphologically, and have been described by Etkin as: (i) **Premetamorphosis** is defined as an initial period of extensive growth but little developmental change. This is followed by (ii) **Prometamorphosis** during which growth continues but conspicuous developmental changes such as the growth of hind legs also take place. (Fig. 18.4b) Prometamorphosis ends with the emergence of the forelimbs (Fig. 18.4c) and then the third and final stage of development, (iii) the **Metamorphic climax sets** in. This is a comparative brief period in which profound morphological changes occur very rapidly, the most conspicuous being the complete resorption of the large muscular tail. (Fig. 18.4d) The tadpole is transformed into an immature froglet and metamorphosis is completed. (Fig. 18.4e)

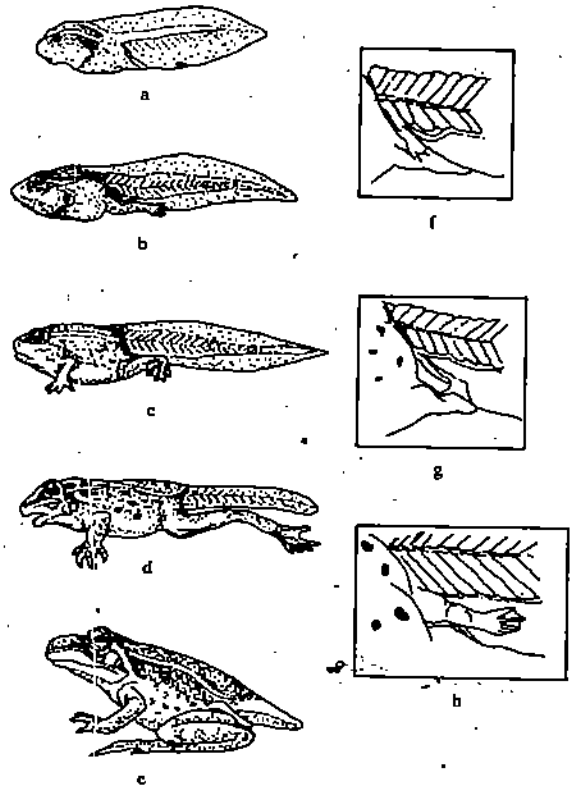


Fig.18.4: Metamorphosis in the frog. *a*, Tadpole with rudiment of hindlimb in the form of a small bud. *b*, Tadpole with fully developed hindlimbs; *c, d*, stages of metamorphosis; *e*, metamorphosed froglet with remains of the tail; *f, g, h*, three stages of hindlimb development (these fall between the stage of *a* and the stage of *b*). (From Witschi, 1956)

The length of the larval period in anurans vary. In some species, tadpoles produced in spring metamorphose during early summer, while in others the tadpole stage may last for a year or more before the premetamorphic transformation begins. The first sign of change occurs in the growth or premetamorphic period by the appearance of swellings on each side at the posterior end of the trunk (Fig. 18.4a). These are the hind limbs which grow slowly during the prometamorphic period.

The growth period is followed by the prometamorphic period during which the length of the hindlimb increases very rapidly relative to the growth of torso (Fig 18.4b, f, g, h). A few days before the end of the prometamorphic period a number of changes begin, notably a shift in the position of the anus and a thinning of the operculum on each side, a process that forms translucent, "skin windows" through which the forelimbs will subsequently erupt.

This is followed by metamorphic climax, as a result of which the the forelimbs grow and erupt through the operculum, the horny beaks of the oral region are lost, the mouth widens and muscular jaws develop. The eyes are repositioned to a higher level and develop lids (Fig. 18. 4c & d). These changes involve a complete remodelling of the head skeleton and are adaptive to a predatory mode of life, requiring an aerial sensory input. The epidermis thickens, hardening the skin and the jelly-like dermis gets replaced by a tougher more fibrous tissue. Within the skin, the pigment cells get arranged in such a manner so as to give the adult pattern of colouration. A muscular tongue used for capturing prey develops, the hyoid cartilages differentiate, the gills are resorbed, as lungs for pumping air into the body which become fully functional. The cells of the larval alimentary tract are almost completely sloughed off and essentially a new and shorter digestive tract develops. Within a week of metamorphic climax the tadpole transforms from a tadpole to a froglet. (Fig. 18.4e)

After metamorphosis some anurans adopt a terrestrial mode of life, returning to the water only to breed. Others spend a considerable amount of time out of water but usually remain in a moist environment. Table 18.2 summarizes the changes in anuran metamorphosis.

Table 18.2 : Summary of Metamorphic Changes in Anurans

1. External Features		
Tissue/Organ	**Changes**	**Function**
Head	Loss of horny beaks to mouth.	
	Widening of mouth. Teeth develop in upper jaw.	Adaptation to new diet.
	Development of tympanum Repositioning of eyes.	Accommodation to aerial sensory input.
Limbs	Complete growth of fore- and hind-limbs.	Locomotion on land.
Tail	Complete resorption.	Loss of swimming as major mode of locomotion.
Skin	Pigmentation changes. Hardening.	Protective colouration. Protection against water loss on land.
2. Internal Features		
Organ/System	**Changes**	**Functions**
Digestive system	Development of long muscular and bifid tongue from the floor of buccal cavity. Major shortening of gut. Repositioning of anus.	Change from herbivorous to carnivorous diet.
Respiratory system	Resorption of gills. Development of lungs. Development of hyoid cartilages and muscles for respiration.	Change from water-to air-based respiratory system.
Reproductive system	Development of gonads.	Sexual maturity only in adult form.
Sense organs		
Eye	Development of nictitating membrane and eye lid.	To protect eyes in the water.
Nervous system	Degeneration of Mauthner cells.	Denervation of degenerating tissue.

	Growth of new neurones and nerves.	Innervation of new structures.
Lateral line organ	Degeneration of lateral line organs	Not required for terrestrial life.
3. Biochemical changes during anuran metamorphosis.		
Eye	Change in visual pigment from porphyropsin to rhodopsin.	
Liver	Synthesis of urea cycle enzymes.	Change in excretory product from ammonia to urea.
	Synthesis of serum albumin.	Maintenance of homeostasis.
	Synthesis of cerulo-plasmin.	Connected with changed iron utilization?
Erythropoietic tissue	Change from synthesis of larval haemoglobin to adult haemoglobin.	Lower affinity oxygen carrier for air-based respiration.
Gut	Synthesis of hydrolytic enzymes.	Resorption of tissue.
	Appearance of peptic activity in foregut.	Change to digestion of animal tissue.
Skin	Melanin synthesis.	Protective colouration
	Induction of Na-K-ATPase.	Maintenance of electrolyte balance.
	Serotonin synthesis.	-
	Changes in collagen deposition and breakdown.	Changes in mechanical properties of skin for terrestrial life.
Tail	Synthesis of hydrolytic enzymes.	Resorption of tissue.

In anurans the metamorphic changes are very striking and almost every organ gets modified as shown in Table 18.2. The changes that occur during metamorphosis can be studied under two categories:

(i) Morphological changes

(ii) Biochemical changes

i) Morphological changes

The changes in the structure of amphibians during metamorphosis are grouped into three categories and are described as (a) regressive changes, (b) progressive change (c) constructive changes or remodelling.

a) **Regressive changes**—These changes involve the gradual reduction and ultimate disappearance of all those larval structures or organs which become redundant in adults. Looking at the table 18.2, the structures which regress can be easily noted. The ventral suckers, external gills, the long tail with fin folds are reabsorbed during early functional life. The gill clafts are closed; the peribranchial cavities, the horny teeth and horny lining of the jaws are lost. The shape of the mouth changes, the cloacal tube shortens and gets reduced. The lateral line organs of the skin of tadpole disappear and some blood vessels are reduced.

b) **Progressive changes**—Some organs and structures become functional during and after metamorphosis. In the anurans these changes are tabulated in Table 18.2 and involve the development of the fore and the hind limbs, the middle ear in connection with the first pharyngeal pouch (the pouch situated between the mandibular and hyoid arches), the tympanic membrane supported by the circular

tympanic cartilage. The eye protrudes on the dorsal surface of the head and develops an upper eyelid. The tongue develops from the floor of the mouth.

c) **Remodelling** —Some structures and organs which occur and function both before and after metamorphoses, get transformed or remodelled during the process in order to meet the requirement of the adult mode of life. These changes, as given in table 18.2, affect primarily the skin, intestine and brain. The skin thickens, and becomes glandular by developing multicellular mucous and serous glands. It also develops an outer keratinized layer as well as characteristic colour and pattern of pigmentation. The brain gets highly differentiated. The intestine which was long and coiled in the herbivorous tadpole shortens and straightens out: Other notable changes which occur are the change in the blood vascular system in order to supply the lungs, the change in the portal system, the change in the heart as it becomes three chambered from being two chambered earlier.

(ii) Biochemical changes

Biochemical changes also accompany morphological changes and are quite striking in anuran metamorphosis. The changes that occur in anurans are summarized in table 18.2 and are given below briefly.

a) The porphyropsin (a complex between the protein opsin and aldehyde of Vitamin A_2) of the eye during metamorphic climax is replaced almost completely by rhodopsin (a complex between the protein opsin and aldehyde of vitamin A_1) as the visual pigment. In the eye the larval form of α crystallin in the lens is replaced by an adult α crystallin that differs markedly in electrophoretic mobility. An adult form of skin keratin replaces the larval form at metamorphosis. During metamorphosis large quantities of hyaluronidase are produced. This enzyme eliminates the hyaluronic acid of the larval skin. This hyaluronic acid is almost replaced by a mixture of other glycosamino glycans. Hyaluronidase is not present in appreciable quantities in the adult skin. Other biochemical changes that occur in the skin as a result of metamorphosis are alteration in the patterns of collagen synthesis and deposition. This results in a tougher skin, more appropriate for a land dwelling existence.

b) After metamorphosis, the frog begins to excrete the bulk of its nitrogenous waste as urea rather than ammonia. Urea like ammonia is very soluble in water but less toxic. As a result after metamorphosis urea can be formed and retained in the blood and then be excreted by the kidneys with less water loss than would be required for elimination of an equivalent quanity of nitrogen in ammonia form. Production of urea requires the activation of the ornithine—urea cycle in the liver Fig. 18.5. In this cycle carbon dioxide and nitrogen are excreted in the form of urea. Metamorphic changes thus involve a reorganization of the metabolic patterns in the liver for production of the appropriate enzymes of the ornithine—urea cycle. This reorganization starts very early in metamorphosis even before there is any apparent change in the body form.

c) At metamorphosis the site of erythropoiesis shifts from the liver to the bone marrow and spleen. This shift is marked by the production of haemoglobin with different physiological and electrophoretic properties. In *Rana catesbeiana* the shift from production of tadpole haemoglobin (HbF) to adult haemoglobin (HbA) is essentially complete just before tail is resorbed. Also during metamorphosis there is considerable synthesis of hydrolytic enzymes involved in the resorption of larval gut and tail.

d) **Degrowth** occurs when feeding by the larva is suspended during metamorphosis. The larva at this stages utilizes the reserve food to produce the energy needed for completing the various metamorphic processes. As a result there is loss of weight, with the consequence that the miniature frog produced at the end of the metamorphosis is smaller and lighter than the mature adult. This reduction of body mass is termed as **'degrowth'**. The reduction in body mass is also partly due to shrinking of some body parts of the larva. For instance during metamorphosis the head and trunk become smaller and some parts like the tail and gills are lost.

e) Autolysis causes the disappearance of larval tail, gills, external gills and fin fold. The process of autolysis is brought about by active movement of amoeboid macrophages which phagocytose the debris of disintegrating cells. The lysosomal enzymes of the phagocytes, in particular the cathepsin, show a high rise in concentration and utilization. The phagocytosed or autolysed material is reabsorbed and is used in the construction of new organs of adult.

f) Enzymes during metamorphosis show a remarkable change. There is a change both in the types and amount of enzymes produced to help in the completion of metamorphosis. Some larval enzymes not needed in adult are no longer synthesized, whereas new enzymes needed in the adult begin to be synthesized. Metabolism of carbohydrates, lipid and nitrogen undergo changes in the adult.

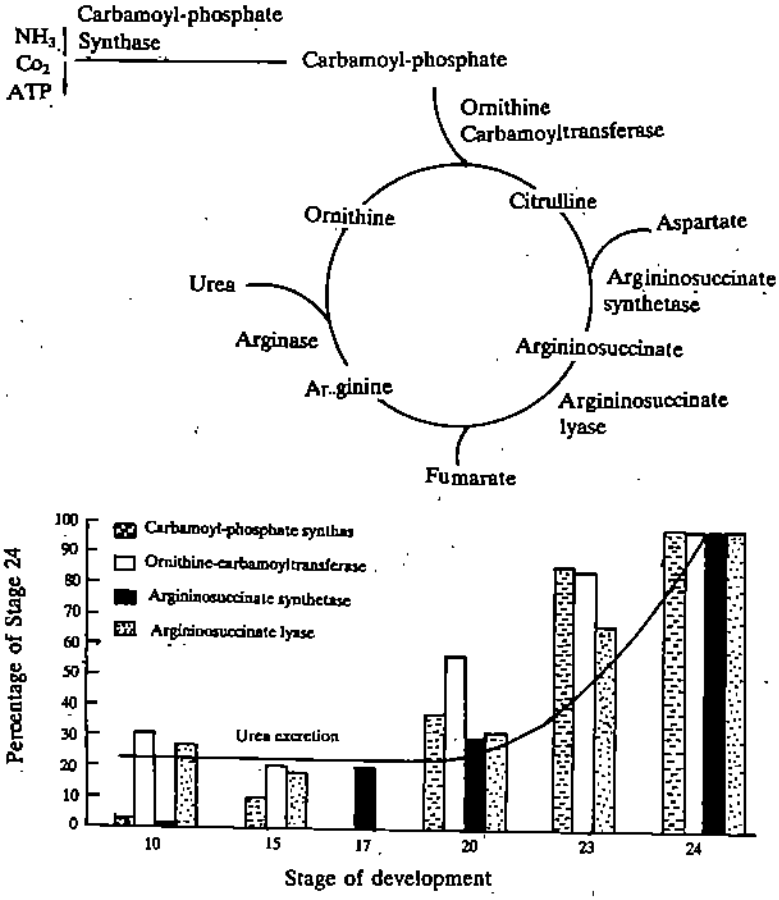


Fig.18.5 : Development of urea cycle during anuran metamorphosis. (A) The major features of the urea cycle, by which nitrogenous wastes can be detoxified and excreted. (B) Emergence of urea cycle enzyme activities correlated with metamorphic changes in the frog *Rana catesbiana*. (After Cohen, 1970)

18.5.2 The process of metamorphosis in urodeles

Metamorphosis in urodele amphibians is considerably less drastic. The larval salamander (a member of genus *Ambystoma*) for instance has external gills and a long tail with dorsal and ventral fins (Fig.18.6). The broad mouth, both of the larva and the adult remains essentially the same. The forelimbs appear earlier than the hind limbs and both pairs of appendages grow gradually, independent of any metamorphic stimulus. Metamorphosis essentially involves loss of external gills and tail fin, development of lungs, closure of gill slits, appearance of eyelids, formation of maxillary bones, ossification of skeleton, cornification of skin and differentiation of skin glands.

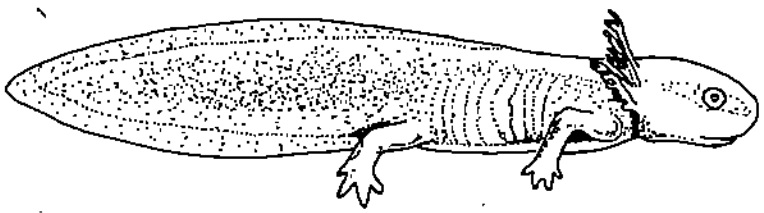


Fig. 18.6: larva of *Ambystoma* , a salamander

In urodeles, metamorphosis is more gradual on the whole and may take several weeks. It involves the following changes:

a) **Regressive changes:** Tail is retained but fin fold disappears. Branchial apparatus gets reduced. The external gills get reabsorbed and gill clefts close. The visceral skeleton becomes reduced.

b) **Progressive changes**

Head shape changes. The eyes develop lid and bulge more on the dorsal sides of the head. Skin becomes multilayered and cornified. Its pigmentation changes and the skin glands also become differentiated. The legs and alimentary canal hardly undergo any change.

After metamorphosis, depending on the species, urodeles may reduce the time spent in water or they may become land dwellers returning to the water only to breed. Newts and Salamanders that become terrestrial undergo a **second metamorphosis** when they return to the water for breeding.

Second metamorphosis—Some urodeles like newts and salamanders show two major changes in form, physiology and life habit between birth (or hatching) and adulthood. For example, the spotted newt *Notophthalmus viridescens*, exhibits two prominent metamorphic changes during its lifecycle. This newt hatches out as an olive green larva with gills, a keeled tail and a well developed lateral line organ system (a system of receptors sensitive to local deplacement of water). After a few months of growth it undergoes metamorphosis, whereby the gills and tail fins are resorbed, the lateral line system becomes nonfunctional and skin becomes rough and dry and orange in colour. The visual pigment which was mainly porphyropsin in the larva changes to a mixture of porphyropsin and rhodopsin. The newt is called an **eft** and it becomes a woodland dweller for 2 or 3 years, remaining on land and growing to full size.

The **eft phase** is terminated when the animal undergoes a second metamorphosis through the action of hormones prolactin and pituitary gonadotropins. These initiate a drive towards water which is accompanied by such physiological changes as the functional reinstatement of the lateral line organ system, the reversion of the skin to a mucous secreting, wet and shiny organ, the restoration of a finned tail, the maturation of the gonads and the adoption of porphyropsin as the main visual pigment. In this manner the newt returns to the water, to spawn and remains there throughout its life which lasts for several breeding seasons.

Some urodeles, like the permanently-gilled salamanders appropriately called 'perinnibranchiate', do not undergo metamorphosis in nature and so grow to sexual maturity as permanently aquatic animals that retain their larval features like external gills. Thus they are neotenic and paedogenic. Neoteny is a condition in which the larval characters are retained for prolonged periods of time. Fig. 18.7 shows some neotenic urodeles. Paedogenesis is the process by which larval individuals reproduce. In subsection 18.5.8 you will read in more detail about neoteny and paedogenesis.

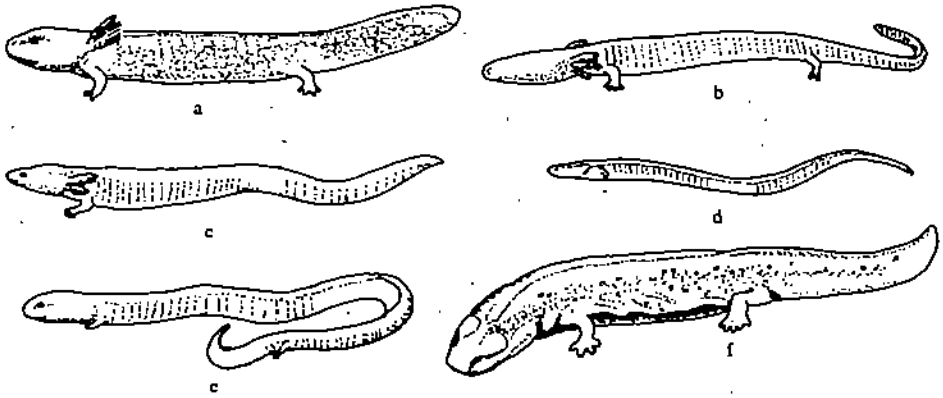


Fig.18.7: Some neotenic urodeles—These retain their larval features but are sexually mature
(a) *Necturus* (b) *Proteus* (c) *Siren* (d) *Pseudobranchus* (e) *Amphiuma* (f) *Crytobranchus*

SAQ 3

i) Fill in the blanks with suitable words

a) In anurans, metamorphosis is usually associated with a transition from .....to .....mode of life.

b) The axolotl larva of *Abystoma* achieves, sexual maturity as a larvae. This phenomenon is known as .....

c) The salamander Notophthalmus viridescens undergoes a ..... metamorphosis before becoming adult.

d) The initiation of metamorphosis is due to the ..... of the genomic set by a ....

e) The three distinct stages of metamorphic changes in anurans are ....., ... and .....

f) The three categories of morphological changes occurring during the metamorphosis of amphibians are ..... , and .....changes.

i) Indicate the following changes that occur during metamorphosis in amphibians either as progressive or regressive or remodelling

a) The development of middle ear in connection with the pharyngeal pouch.

b) The change in the shape of the mouth and the shortening and reduction of the cloacal tube.

c) Disappearance of lateral line organs of skin and reduction of blood vessels.

d) The differentiation of brain.

e) The changes in the portal system and the changes in the vascular system to supply blood to the lungs.

f) The shortening and straightening of intestine.

g) The conversion of the heart into a three chambered one.

h) The development of fore and hind limbs.

i) Closing of gill clefts and loss of horny teeth.

j) Development of tongue from the floor of the mouth.

iii) List any four physiological changes that occur during the transformation of tadpole into adult frog.

.....

.....

iv) List any two regressive and two progressive changes that occur during the metamorphosis of urodeles.

.....

.....

18.5.3 Hormones in metamorphosis of amphibia

In amphibians, the changes that occur during metamorphosis are brought about by hormonal secretions of the thyroid gland. The first indication of this was obtained in 1912 when Gudernatsch reported that frog tadpoles when fed dried and powdered sheep thyroid underwent precocious or early metamorphosis. Similar results however were not observed when tadpoles were fed with preparation of other glands. These experiments made it possible to postulate that thyroid hormones bring about metamorphosis. In 1918 B.M. Allen observed that removal of thyroid rudiment from early frog tadpoles, prevented them from undergoing metamorphosis, and caused them to become giant tadpoles instead. On the other hand, if they were fed with thyroid or immersed in water containing soluble extracts from thyroid glands they then proceeded immediately to metamorphosis. (Fig. 18.8)

Similar experiments in urodele amphibians, in particular in axolotl, *Ambystoma mexicanum*, also indicated the importance of the thyroid gland in urodele metamorphosis.

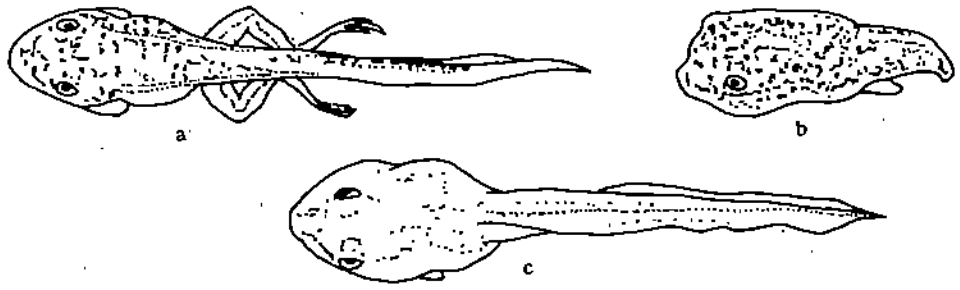


Fig. 18.8: Hormones and metamorphosis (a) Normal metamorphic stage (b) Premature metamorphosis following exposure of young tadpole to thyroxine. (c) Inhibition of metamorphosis following removal of thyroid or the pituitary gland.

Later studies by W. Etkin 1968 have also shown the importance and role of different hormones in metamorphosis. He concluded that development is controlled by a dynamic balance of plus and minus factors or hormones. Furthermore he found that the spacing of metamorphic events depends on the concentration of thyroid hormone, while the sequence of events is inherent in the tissues.

18.5.4 Interaction of amphibian hormones in the process of metamorphosis

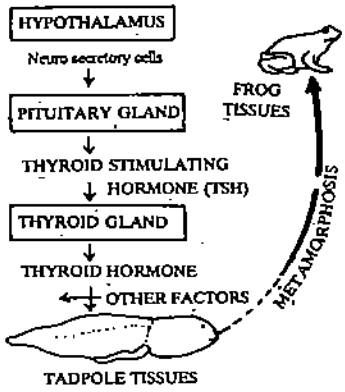


Fig.18.9:Control of release of thyroxin during metamorphosis

All the diverse changes that occur in anuran metamorphosis are brought about by the interactions of the hormones secreted by the thyroid gland, hypothalmus of brain and pituitary.

You now know the principle organs involved in metamorphosis. Let us see how they function and coordinate during metamorphosis. Etkin, 1968 has summarized amphibian metamorphosis as three main events (Fig.18.9) which are as follows:

(1) The pace of metamorphic events depends on the concentration of thyroid hormone. As a result, the level of the thyroid hormone in blood and tissues gradually increases during the last two third of larval life, up to the event of metamorphic climax, after which it drops suddenly.

(2) At the time of metamorphosis the concentration of thyroid hormone in blood and tissues is increased by TSH-RF secreted by the hypothalmus.

(3) The reactivity of different larval tissues to different concentrations of thyroid hormone is inherent (genetically programmed) in the tissue. This means that different tissues have different critical threshold.

The hormonal interactions of metamorphosis are summarized in Fig. 18.10 and are as follows.

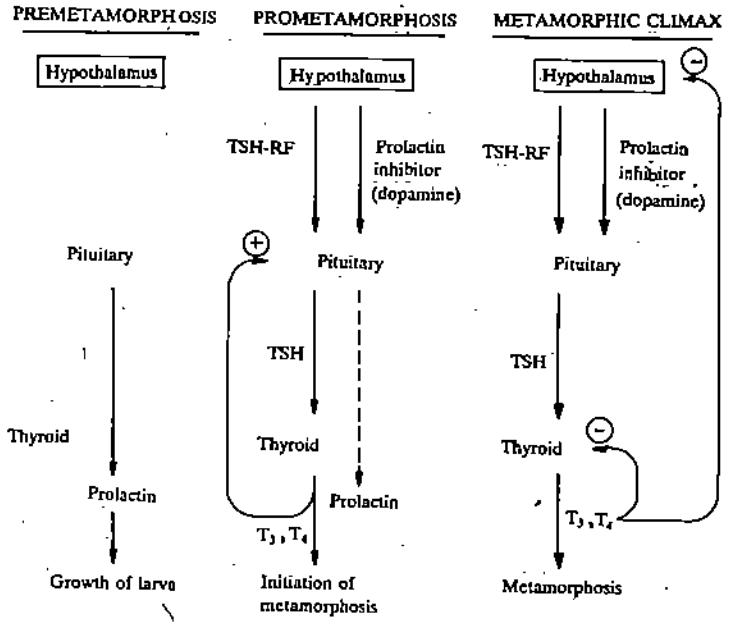


Fig. 18.10: The hypothalamus-pituitary-thyroid axis during different stages of anuran metamorphosis. As hypothalamus develops, it stimulates the pituitary to instruct thyroid hormone secretion and inhibit prolactin secretion. T_3, Triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone; TSH-RF, thyroid stimulating hormone releasing factor.

The thyroid gland secretes a large complex protein thyroglobulin which has a molecular weight of 675000. The thyroglobulin is formed of several molecules of low molecular weight compounds. These componds are tri-iodo-thyronine (T_3) and thyroxine or tetraiodothyronine (T_4). As the figure 18.11 shows in both these compounds 2 residues of the amino acid, tyrosine are joined together . Three atoms of iodine in tri-iodothyronine and four atoms of iodine in thyroxine are attached to the tryosine molecules. During metamorphosis the iodine containing compounds T_3 and T_4 are released from the thyroglobulin.

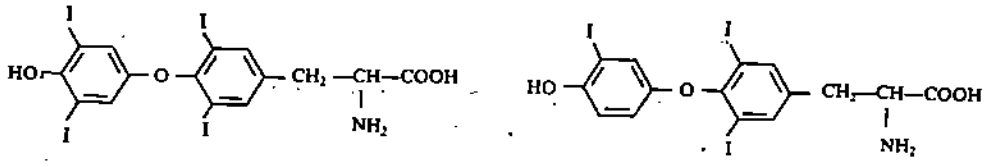


Fig.18.11: Formulae of thyroxine (T_4) and Triiodothyronine (T_3)

Of the two hormones T_3 and T_4, T_4 is the precursor and T_3 is the active hormone and can cause metamorphic changes at a much lower concentration as compared to T_4. In both anurans and urodeles the thyroid in the larva does produce small amounts of T_3 and T_4 hormones. However these amounts are overbalanced; in other words their effect is countermanded by the hormones secreted by the anterior pituitary. The action of the pituitary has been found to be indirect. It secretes two hormones (1) the thyroid stimulating hormone (TSH) which is secreted in larval amphibians only at the onset of the metamorphosis (2) a hormone similar or identical to prolactin whose activity is antagonistic to thyroxine during larval life. This is because prolactin acts as a larval growth hormone and inhibits metamorphosis. However at the onset of metamorphosis the concentrations of T_3 and T_4 hormones increase to such an extent that the action of prolactin becomes ineffective, thereby causing the tadpoles to become frogs and the larval newts to become land dwelling efts.

The release of T_3 is under the control of the hypothalamus. It integrates the chemical information received from the body for determining the time of starting metamorphosis. During the period of larval growth (premetamorphosis) hypothalamus is underdeveloped, so it exerts little control over the anterior pituitary (Fig. 18.10). In the absence of hypothalamic regulation the pituitary secretes high levels of prolactin and little or no thyroid stimulating hormone (TSH). Thus T_3 levels are low and prolactin levels are high. When the hypothalamus develops, its production of **thyroid stimulating hormone releasing factor** (TSH-RF) increases causing an increase in the level of TSH. This causes the thyroid to release more T_3 and T_4. The concentration of T_3 gradually increases until the first changes of metamorphosis (prometamorphosis) appear. During this period the hindlimbs begin to enlarge. The increase in T_3 and T_4 titres as well as hypothalamic secretion, a substance (most possibly dopamine), inhibit the pituitary synthesis of prolactin. The ratio of T_3 to prolactin thus changes and T_3 concentration increases enormously. This leads to *metamorphic climax* in which drastic morphological and biochemical changes associated with metamorphosis occur along with partial regression of thyroid gland. The hypothalamus now diminishes its output of factors, affecting the pituitary hormones, and a hormonal balance appropriate to the life of the growing froglet ensures.

As you know, in certain urodeles the first metamorphosis results in the development of land dwelling eft, which after some time undergo a second metamorphosis in order to be able to return to the water to breed. The second metamorphosis has been shown to be under the influences of prolactin, presumably resulting from a shift in balance between pituitary factors prolactin and TSH rather than an activation of one of them. In other words, the first metamorphosis is induced by a shift in favour of TSH, the second metamorphosis by a return to predominance of prolactin.

18.5.5 Tissue reactivity

The mechanism of metamorphosis in anurans indicates that a single agent, namely thyroxine, evokes multiple responses in different tissues. In other words, the various

organs of the body respond differently to a single hormone agent. In addition, the response of the tissues, though diversified, are specific and can be destructive or constructive depending on the target tissue. Thus the same stimulus will cause certain tissues to degenerate and others to grow and differentiate.

Such diverse reaction of tissues is believed to be due to two reasons:

(1) **Competence of larval tissues or multiple response.** The reactivity of the tissue of the target organ to the thyroid hormone,

(2) **The threshold value of different larval tissues for thyroid hormone concentration.**

(1) Competence of larval tissue or multiple response. The responsiveness of different larval tissues to thyroxine is markedly different. The tissues of the tail becomes necrotic and undergo degeneration due to the action of the thyroid hormone, while the tissues of the limb increase and undergo differentiation. Furthermore thyroxine causes rapid aging and destruction of R.B.C's of the larva undergoing metamorphosis. In addition it stimulates the development of cells that synthesize the haemoglobin of adult frog. The hormone T_3 reduces biosynthesis of nucleic acids in the tail but increases their synthesis in the liver. Furthermore, muscles of the tail undergo degeneration while those of the trunk remain unaffected.

Such examples clearly indicate that the response and reactivity of larval tissue to the same hormone or agent differs greatly. In addition it can also be demonstrated experimentally that the reactivity and response of the target tissues are intrinsic, specific and independent.

This can be demonstrated by transplanting tail tips of the tadpole to the trunk of another tadpole undergoing metamorphosis, or by placing the eye of the tadpole in the tail of a metamorphosing larva (Schwind, 1933, Geigy, 1941). The extra tail transplanted into the tadpole host trunk is not protected from degeneration and undergoes necrosis along with the host tail and gets absorbed. The eye retains its integrity despite the fact that it lies surrounded within the degenerating tail. (Fig. 18.12)

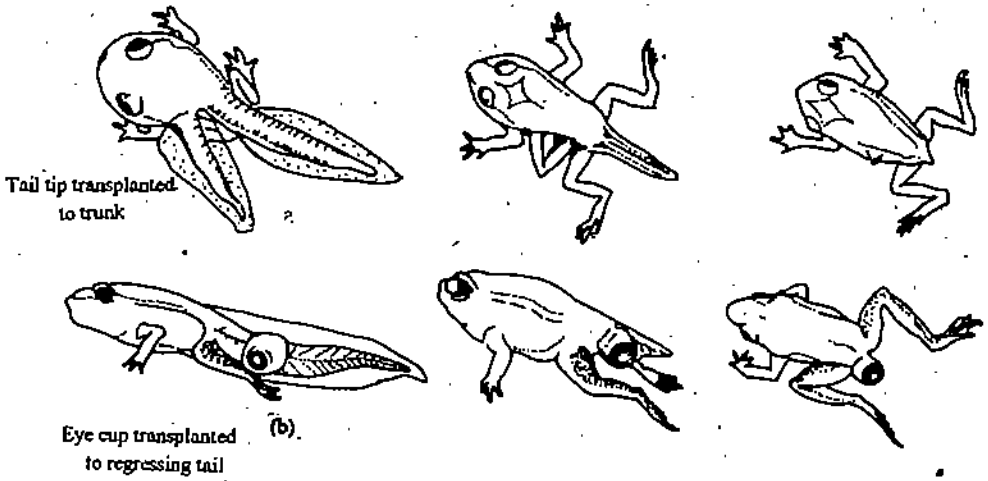


Fig.18.12: Organ specificity during frog metamorphosis. (a) Tail tips regress simultaneously with the hosts tail even when transplanted to the trunk, whereas eye cups (b) remain intact even when transplanted into the regressing tail.

The degeneration of tissues during metamorphosis thus represents **genetically determined cell death.** In humans such programmed degeneration occurs in the tissues between our fingers and toes. The degeneration of the human tail during the week four of development resembles the regression of tadpole tail.

Such observations force us to question as to how does the thyroid hormones bring about diverse metamorphic changes? Do the hormones act directly on all the target tissues or are their actions mediated by some other hormone/hormones? Finally, how is the changing thyroxine titres controlled? Such questions have been answered by experiments which have clearly shown that the hormone acts directly on the target tissue. Weber (1967) demonstrated this fact when he cultured excised tadpole tails in the presence of T_4. (Fig.18.13) While tails treated with thyroxine showed regression similar to that occurring in metamorphosis, the controls (not treated with thyroxine) exhibited no regression and remained healthy.

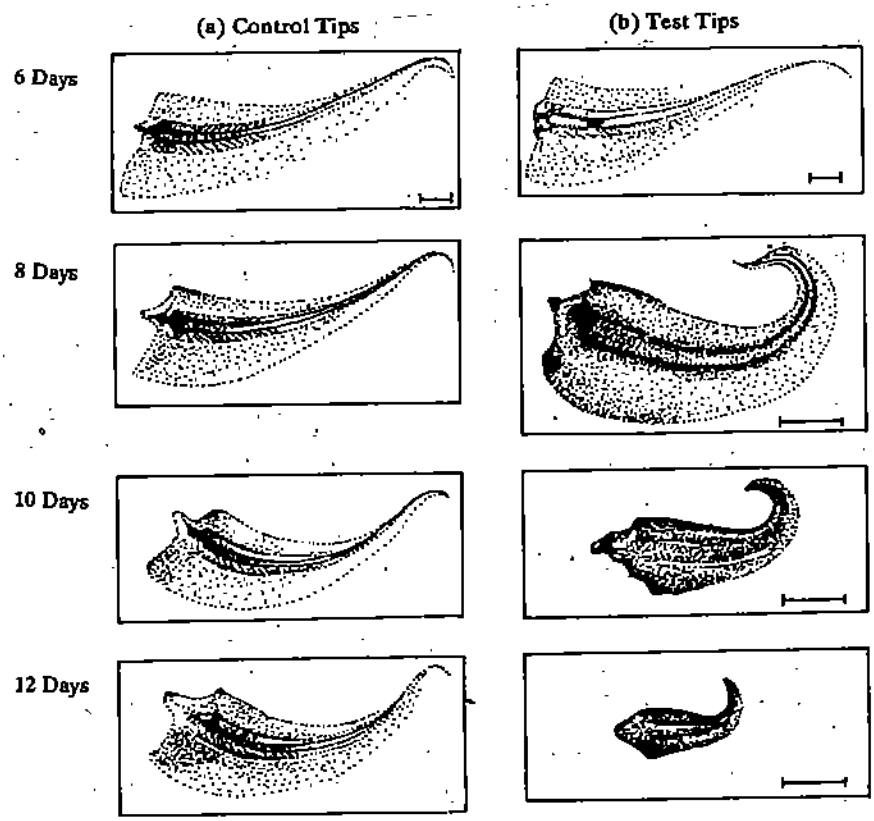


Fig.18.13: Regression of isolated tail ends under the influence of thyroxine (a) control tail tips cultured in Holtfelter's salt solution for 6, 8, 10 and 12 days (b) Treated tail tips at the same ages as controls but having thyroxine added to their solution.

The regression of the tail is believed to occur in four stages. First, protein synthesis decreases in the treated muscle cells of the tail. Then, there is an increase in the lysosomal enzymes. Concentrations of Cathepsin D (a protease), RNase, DNase collagenase, phosphotase, and glycosidases all rise in the epidermis, notochord and nerve cord cells. Cell death is probably caused by the release of these enzymes into the cytoplasm. The epidermis helps to digest the muscle tissue, probably by releasing these digestive enzymes. After this death, macrophages collect in the tail region, digesting the debris with their own proteolytic enzymes. The result is that the tail becomes a large sac of proteoltic enzymes (Fig. 18.14). If the epidermis is removed from the tail tips, the tips will not regress when cultured in thyroxine.

2. Threshold value of different larval tissues for the thyroid hormone concentration

One of the major problems of metamorphosis is the coordination of developmental events. The tail should not degenerate till some other means of locomotion, namely the limbs develop, and the gills should not regress till the animal can use its newly developed lung muscles.

Kollros (1964) demonstrated the possibility of this coordination which shows that different larval tissues have different threshold value for thyroxine. In other words the different larval tissues are sensitive to different concentrations of thyroid hormone. This model is called the **threshold concept.**

It has been observed that structures in the tadpole which change early during metamorphosis are more sensitive to and have low threshold value to thyroxine, than those which undergo transformation at a later stage. As metamorphosis commences the thyroid hormone level gradually builds up and different events occur at different concentrations of the hormone. Experimental studies have revealed that tadpole parts which have a low threshold respond earlier during metamorphoses than those parts having a high threshold response. In other words, the threshold value reflects the order in which metamorphic changes occur in normal development. In urodeles the bulging of eyes react to the weakest dose of thyroid hormone (minimum threshold) and so is the first event in metamorphosis. This is followed by reduction of fin fold and the shortening and disappearance of external gills. After which occurs the closure of gill clefts and transformation of skin.

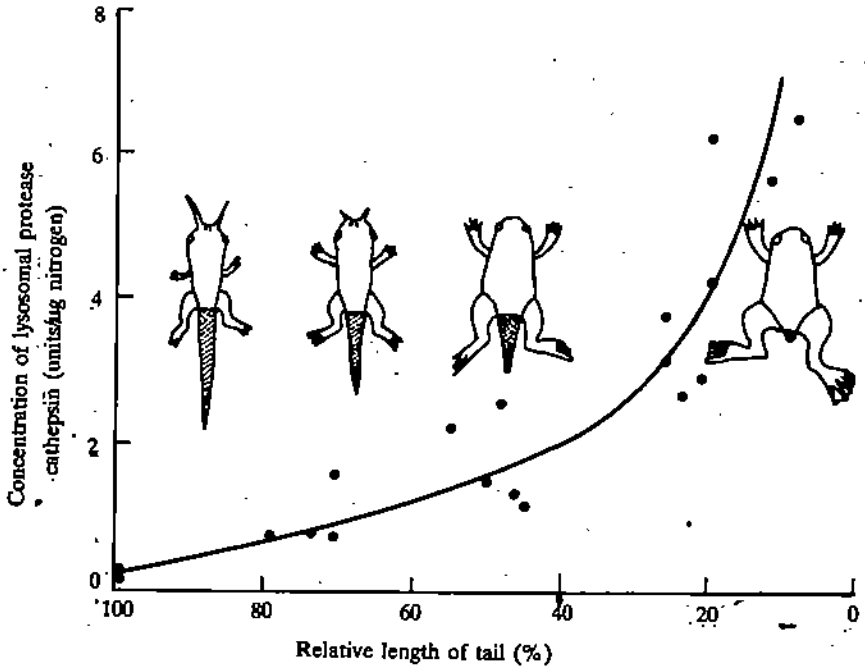


Fig.18.14: Regression of tadpole tail, in metamorphosing of *Xenopus laevis* is accomplished by lysosomal digestion of cells. As metamorphosis proceeds, the enzyme concentration increases (the absolute quantity of enzyme remains constant). Eventually the stub contains almost nothing but lysosomal enzymes and it falls off.

The administration of high amounts of thyroxine at early stages causes precocious but distorted sequence of metamorphic changes resulting in death. Tissues, responsive to low concentrations will not respond, while those responsive to high amount do so precociously. For example, in the frog tadpoles as hormone concentration increases tissue responses become progressively more rapid till maximum rates of change are attained. When the dosage is heavy all metamorphic events start together or crowd together and the normal sequence of events is disturbed. The destructive processes being capable of proceeding faster than the constructive processes result in the forelimb erupting before becoming differentiated; the tail becomes reduced before the legs are sufficiently developed to take over locomotion—resulting in an abnormal animal which dies. There is a progressively increasing threshold of the hormones at different stages of development of a particular organ and during the entire metamorphic process.

18.5.6 Induction in metamorphosis

Some of the morphogenetic changes during metamorphosis are found to be quite independent of hormone action. For example, usually during metamorphosis, the skin of the tail undergoes degeneration under hormonal effect. It is observed that when the skin alone is removed from tail and transplanted onto the tail of another metamorphosing larva, it does not regress despite the presence of the hormone. But if the skin is grafted to the trunk along with its underlying muscle in a developing tadpole, then it regresses. Thus the hormone affects only the muscle directly, which induces regression or progression of the skin depending upon the induction it receives from its underlying muscle of tail or trunk respectively. Similarly tympanum is another example of the induction process and is independent of direct hormone, action as its formation is induced by the tympanic cartilage.

18.5.7 Molecular response to thyroid hormones during metamorphosis

Thyroid hormones can cause existing tissues to break down or can remold the tissues to their adult function. The liver cells of the tadpole for example are not destroyed and replaced during metamorphosis. Instead their structure is remodelled. This change is accompanied by dramatic increase in ribosomal and messenger RNA synthesis. The rate of protein synthesis also increases nearly 100 folds within four hours of thyroid hormone stimulation. Many of the new mRNAs are those which code for new functions of the liver.

The major increase in protein synthesis appears to come from the transcription of new mRNAs. As Fig.18.15 shows, carbamoylphosphate synthase (a urea cycle enzyme) is synthesised after the burst of RNA synthesis. Experiments have demonstrated three

types of molecular response to thyroid hormone. One set of genes increases its low level of transcription in response to either natural or thyroxine induced metamorphosis. Another set of genes decreases its rate of transcription, while a third set of genes remains unaffected by thyroid hormones. Mori and Coworkers (1979) have demonstrated that much of the increase in carbamoylphosphate synthase can be attributed to increased transcription from the gene. Thus metamorphosis appears to some extent to be controlled at the transcriptional level. Other evidences also indicate the ability of thyroid glands to regulate gene activity at the level of transcription. This does not mean that transcription is the only level of gene regulation, operative during metamorphosis, but it is obviously an important one.

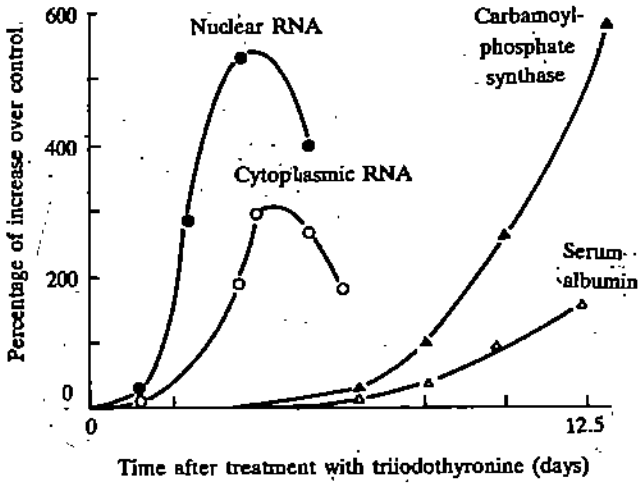


Fig.18.15: Molecular synthesis in *Rana catesbiana* liver cells after treatment of tadpoles with triiodothyronine. First an increase in the nuclear and then in cytoplasmic RNA are observed before an increases in liver-specific proteins, especially those of the urea cycle. (After Graham and Wareing, 1976)

18.5.8 Neoteny

Urodele metamorphoses has shed light on certain aspect of gene expression and tissue response during metamorphosis. A number of urodeles undergo delayed, partial or no metamorphosis. *Trituris vulgaris* has a tendency to retain a few or more larval characters in the adults. This is called neoteny. Many salamanders are able to retain their larval form throughout their lives, becoming sexually mature without undergoing metamorphosis. The degree to which metamorphosis occurs varies from species to species. The Mexican axolotl *Ambystoma mexicanum* does not undergo metamorphosis in nature, remaining a sexually mature animal with larval characters such as external gills and a long tail with dorsal and ventral fins. The animal remains neotonic as it does not release active TSH to stimulate its thyroid glands. However when investigators gave *A mexicanum* either thyroid hormone or TSH they found that it metamorphosed into an adult not usually seen (Huxley 1920). (Fig.18.16)

Other species such as *Ambystoma tigrinum* metamorphose only if given cues from the environment. Otherwise they become neotenic, successfully mating as larvae. Some perinnibranchiates that remain permanently gilled do not even respond to thyroxine or iodine treatment in the laboratory and so remain permanently neotenic. The tissues of these animals consist remarkably of comparatively enormous cells, as though the larva had grown to adult size by the process of cell enlargement instead of cell proliferation. The neotonic species of *Necturus* and *Siren* also remained unchanged despite thyroid hormone treatment, indicating that the larval tissues in these cases have lost their capacity to respond to thyroid hormone. Thus their neoteny is permanent (Frieden 1981). The genetic lesions responsible for neoteny in several species are shown in Fig.18.17.

De Beer, 1940 and Gould, 1977 have speculated that neoteny is a major factor in the evolution of more complex taxa. By retarding development of somatic tissues, natural selection is given a flexible substrate to act upon. According to Gould, neoteny would provide an ecaspe from specialization. Animals can slough off their highly specialized adult forms, return to the lability of youth, and prepare themselves for new evolutionary directions.

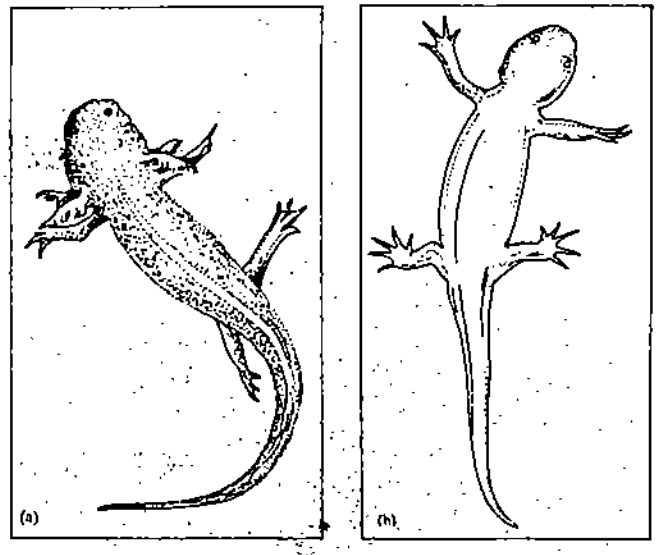


Fig.18.16: Metamorphosis induced in axolotl (a) Normal condition of axolotl (b) Animal treated with thyroxine to induce metamorphosis.

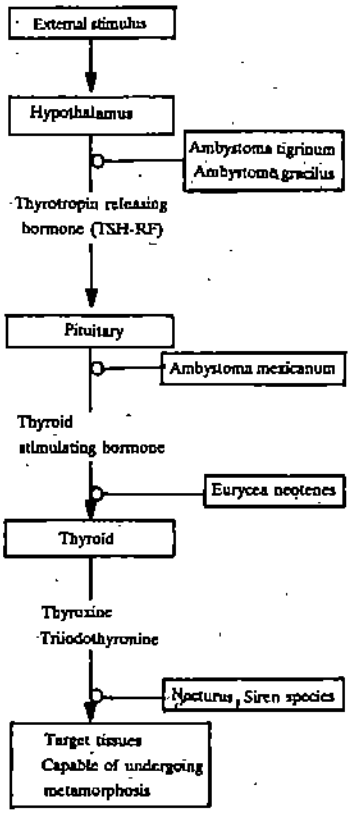


Fig.18.17: Stages along the hypothalamus-pituitary-thyroid axis of salamanders at which various species have blocked metamorphosis. *Eurycea*, *Necturus*, and *Siren* appear to have a receptor defect in the responsive tissues. *Eurycea* will metamorphose when treated with high concentrations of thyroxine. *Necturus* and *Sirindo* not respond to any dose. (After Frieden, 1981).

SAQ 4

1) Indicate whether the following statements are **true** or **false**.

a) Tadpoles when fed with dried and powdered thyroid gland from any other animal will undergo an early and precocious metamorphosis. True/False

b) The sequence of metamorphic events depend on the concentration of thyroid hormone, whereas the spacing of the events is inherent in the tissues. True/False

c) TSH-RF secreted by the pituitary gland activates the thyroid gland to secrete thyroid hormone. True/False

d) Tetraiodothyronine (T_4) is a less potent hormone and is the precursor of triiodothyronine (T_3) True/False

e) Prolactin has an antagonistic action to thyroxine, and so promotes larval growth and inhibits metamorphosis. True/False

f) Thyroxine, the single hormonal agent evokes multiple responses in different tissues and its action whether constructive or degenerative, depends on the target tissues on which it acts. True/False

g) The reactivity and responses of target tissues of the tadpoles to the thyroxine hormone are extrinsic and non-specific. True/False

h) Genetically programmed cell death is responsible for several of the degenerative changes that take place during metamorphosis. True/False

i) According to the threshold concept, different tissues of the tadpole larva are sensitive to different concentrations of the thyroxine. True/False

j) In tadpoles the regression and the progression of all tissues is dependent totally on the action of thyroxine and the induction by underlying tissues do not have any role in the metamorphic process. True/False

ii) Match the statement on the right side (A) with the species on the left (B).

A	B
a) No metamorphosis in nature, retention of all larval characters but sexually mature.	i) *Ambystoma tigrinum* ii) *Necturus* iii) *Ambystoma mexicanum* iv) *Ambystoma mexicanum*

b) Metamorphosis occurs only if environmental cues are provided. Otherwise neotenous larvae successfully reproduce

c) Administration of thyroxine or TSH results in the metamorphosis of the otherwise neotenous larva into an adult

d) Administration of thyroid hormone has no effect on the larva as the larval tissues have lost their capacity to respond to thyroid hormone. These forms show permanent neoteny.

18.6 DEVELOPMENT, GROWTH AND METAMORPHOSIS IN INSECTS

18.6.1 General process of post-hatching growth in insects

When a young insect hatches out from an egg it is covered by a firm, inflexible, sclerotized cuticle, which due to its rigid structure cannot grow along with the increase in length of the hatched larva. Thus during development, for the hatched larva to attain its adult size and form, the old rigid cuticle at every stage of growth has to be replaced by a newer, larger one by the process of moulting. Accordingly development is marked off by a series of moults.

The interval between the two moults is known as **stadium** and the form that an insect assumes as a result of the moults is called **instar**. All insects undergo several moults after hatching from the egg and before emerging as adults which are called **imago**. The number of moults is 4 or 5 in an insect species and is usually fixed or predetermined but it is not absolutely constant. Furthermore, the form of the insect changes with each moult in a precise pattern characteristic of the species. Moulting is not just a mechanical process; instead at each moult, the moulted insect undergoes changes both in its cuticular covering and internal organization. So you can see that development in insects is usually by the process of metamorphosis. The metamorphic changes may be slight and gradual or radical as occurring during the pupal stage. During this stage the larva remains quiescent without feeding or movement, while many larval characters disintegrate and adult structures are formed. Pupal stage follows the last larval molt and from the pupa the imago emerges. Fig. 18.18 shows metamorphosis in the silk moth, *Cecropia*.

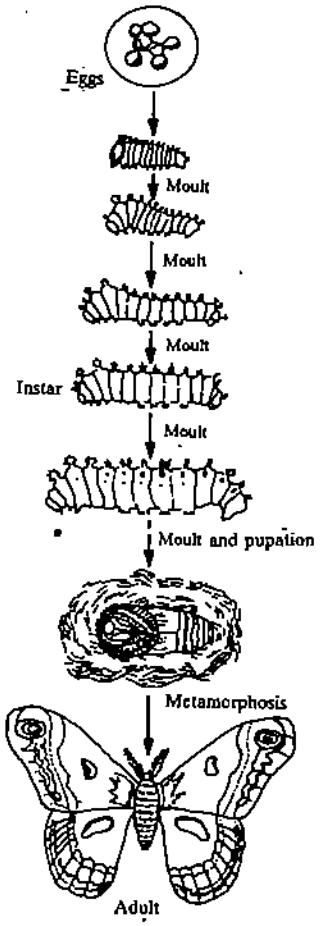


Fig.18.18: Development of an insect (the silk moth *Cecropia*) with complete metamorphosis.

18.6.2 Patterns of metamorphosis

The pattern of metamorphosis is not the same in all insects and so metamorphosis can be broadly classified into 3 types:

(1) Slight or no metamorphosis as observed in Apterygota—ametabolous or direct development.

(2) Incomplete metamorphosis as observed in Exopterygota—Hemimetabolous development.

(3) Complete metamorphosis as observed in Endopterygota—Holometabolous development.

In Table 18.3 you can see how insects are classified on the basis of types of metamorphosis in their life history.

Table 18.3: Classification of insects based on types of metamorphosis

Class	Insecta		
Subclass	Pterygota (Winged, metamorphosis)		Apterygota (wingless, metamorphosis slight or absent; most primitive insects) e.g. silver fish, spring tails.
	present		
Exopterygota		Endopterygota	
Hemimetabolous metamorphosis		Holometabolous metamorphosis	
● Wings develop externally		● Wings develop internally	
● Miniature stages—nymphs, naiads; resemble adults.e.g. Cockroachs, grasshoppers.		● Immature stages—larvae differ in structure and function from adults, e.g. butterflies, moths, ants, bees, beetles.	

(1) Slight or no metamorphosis

In primitive wingless insects (Apterygota) like spring tails, silver fish etc. and in secondarily apterous insects the young ones that hatch from the egg are essentially similar to imago, differing from it only in size, immature reproductive organs and external genitalia. It moults several times leading to a gradual increase in size accompanied by the appearance of the external genitalia and maturation of sex-organs. In apterygotes, growth and moulting are seen to continue even after reproductive maturity. Such a type of development or life cycle is called ametabolous. Fig.18.19 shows such a metamorphosis in silver fish (*Lepisma*)

Egg ——> Young goes through several stadia and moults ——> Imago (Adult)

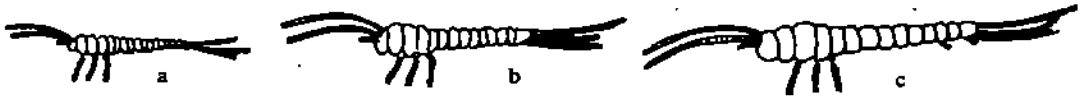


Fig.18.19: Ametabolous type of development in *Lepisma*

(2) Incomplete metamorphosis

In the Exopterygote insects rudiments of wings are present only as buds at hatching and the body form is disproportionate to that of the adult. As the moults occur the form and size of the larva progressively approach that of the adult. The wings become fully developed and sexual maturity is achieved at the last moult. The degree of metamorphosis in the hemimetabolous forms are of two distinct types, namely gradual metamorphosis and extensive metamorphosis.

(a) **Gradual metamorphosis** — In Exopterygote insects like grasshoppers, crickets, cockroaches etc., the nymph is similar in both structure as well as in habit to the adult, but lacks wings, gonads and external genitalia. It undergoes several moults as it grows and develops genitalia, gonads and wing pads in the later larval period. The wing pads get transformed into functional wings at the last moult, after which no more moults occur. This type of metamorphosis has no quiescent stage and there is

no loss or remodelling of larval parts. Fig.18.20.

Egg ——> Nymph ——>several instars and moults——> Imago (Adult)

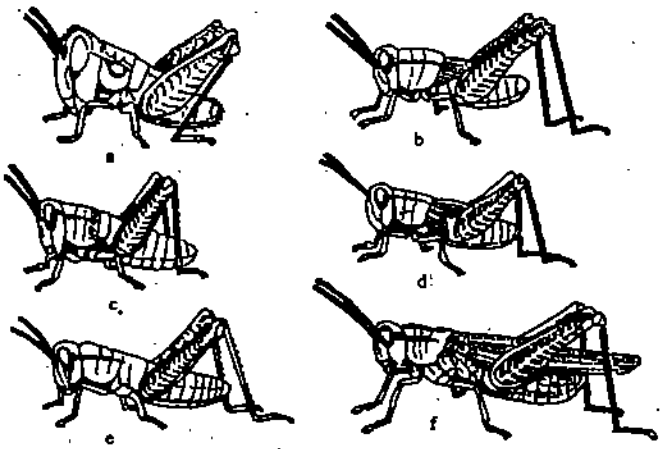
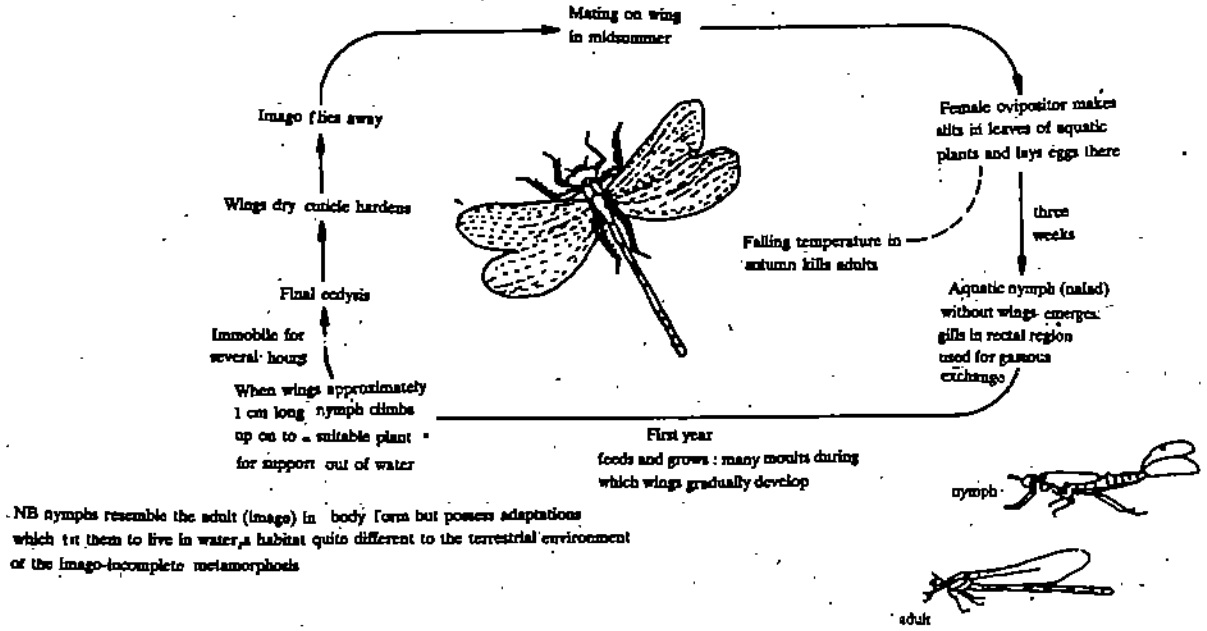


Fig.18.20 shows gradual metamorphosis in grass hopper. There are five nymphal stages before the adult condition is reached.

(b) Extensive remodelling — In insects like damsel flies, mayflies, dragonflies etc., the nymph differs considerably in both external structure as well as in habits from the adult. The nymphs are aquatic and herbivorous and possess some organs for locomotion in water, tracheal gills for respiration and mandibulate mouth parts for cutting vegetation. Such aquatic larval forms are called **naiads.** They undergo extensive remodelling to assume the adult form. They shed gills and modify their mouth parts and develop wings. Moulting may lead to one or more quiescent or semiquiescent larval stages.

This type of metamorphosis is called incomplete extensive metamorphosis Fig.18.21.

Egg ——> Naiad ——> several moults ——> Imago (Adult)



NB nymphs resemble the adult (imago) in body form but possess adaptations which fit them to live in water,a habitat quite different to the terrestrial environment of the imago-incomplete metamorphosis

Fig.18.21: Shows incomplete extensive metamorphosis in dragon fly.

(3) Complete Metamorphosis

In all Endopterygote insects, in which wings and other structures develop internally, (in invaginated imaginal epidermal pockets) like beetles, wasps, bees, butterflies, moths etc., the larva which hatches out of egg is very different from the imago, in habit, appearance and structure. The larva has a worm-like body, biting and chewing mouth parts, simple eyes and weakly developed walking legs. It has quite a different

Pupation is a metamorphic moult and may involve the formation of special pupal cuticle as in butterflies and moths which spin a cocoon and pupate within it. In others the pupa may remain in the old larval skin which forms the puparium.

habit. For example the mosquito larva lives in water and feeds on protozoa and algae, while the adult either sucks blood or fruit and flower juices. A more common example is the butterfly larva which crawls on the ground and feed on leaves and then gets transformed into an aerial organism feeding on nectar from flowers.

The larvae of these types of insects are either swimmers or crawlers, and are voracious eaters. They grow in size and moult several times till they attain a quiescent, non-feeding stage called pupa. The pupa is enclosed in a pupal case or the puparium secreted by the labial glands of the larva. This pupa does not move or feed and its energy must come from the nutrients it ingested while a larva. Externally it appears as an inactive structure. However internally it undergoes basically two types of changes at a rapid pace and moults only after the complex series of internal changes have taken place. These changes involve wholesale destruction of most of the larval tissues (histolysis) and the formation of an entirely new adult body whose organs and systems are developed (histogenesis) from nests of organ specific cells, called the imaginal discs. Histolysis results in systematic destruction of the old body of the larva so that all the organs except for the central nervous system are broken down by special amoebocytic cells called phagocytes. The tissue fluid, which arises due to the destruction is used as raw material in the formation and histogenesis of the adult organs. After these changes are completed as a result of histolysis and histogenesis the pupa undergoes the pupal moult and the imago (adult) emerges fully ready to lead a short or long independent existence and to reproduce. This type of metamorphosis is called complete metamorphosis as shown below and given in Fig.18.22.

Egg ——> Larva ——> several instars and moults ——> Pupa ——> pupal moult—> Adult.

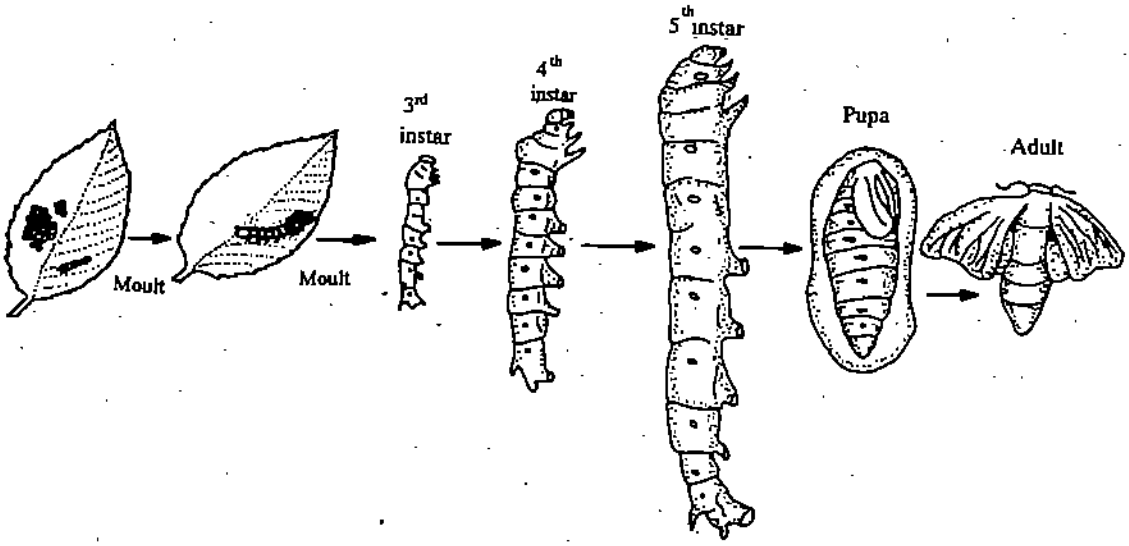


Fig.18.22:shows complete metamorphosis in silk worm moth.

Imaginal Discs: In the holometabolous larva, there are two cell populations: (1) the larval cells which are used for the larval structures and (2) the imaginal disc and the histoblasts which are present in clusters, awaiting the signal to differentiate. Imaginal discs do not occur in the nymphs and larvae of hemimetabolous insects.

The imaginal discs or buds are actually rudiments of future organs of the adult, such as mouth parts, wings, antennae, walking legs, and internal organs etc. Fig.18.23 shows the imaginal discs in the larva and pupa of a fly (*Musca*).

These discs develop directly from the eggs and remain nonfunctional throughout the larval stages. During the pupal stage they grow in size and differentiate to form adult structures which remain collapsed and folded. When the reorganization is completed the pupa moults to set free the adult or imago. Fig 18.19 and 18. 22 show the process of complete metamorphosis. Once the adult or imago blood is pumped into these collapsed structure it causes them to unfold and inflate. Furthermore chitin is deposited on them to harden them.

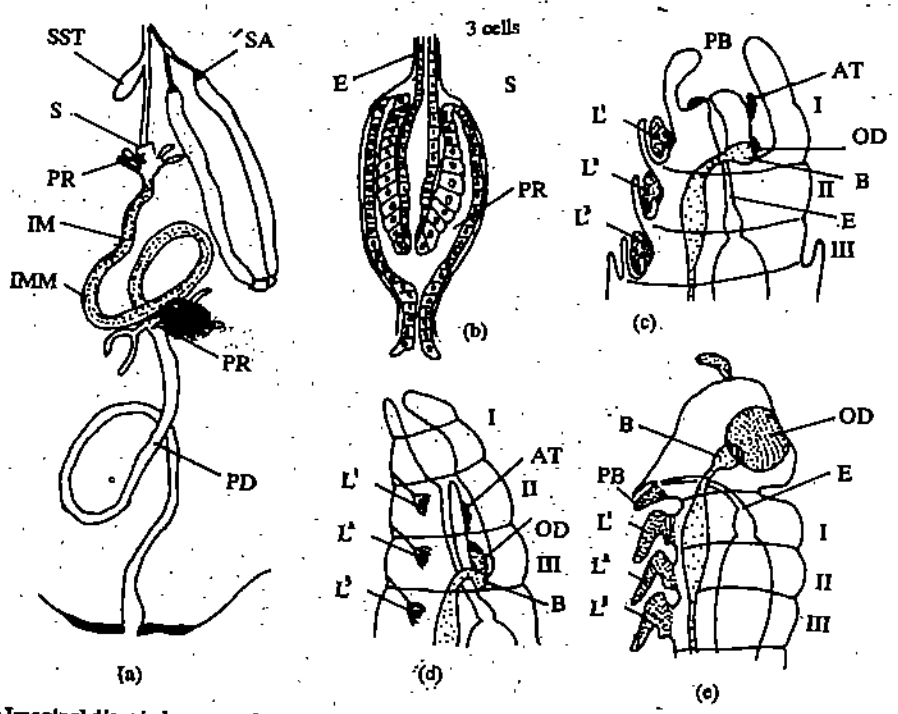


Fig.18.23: Imaginal discs in larvae and pupae of *Musca*, (fly) a: the imaginal discs of the larval digestive tract *b*: close-up of the larval proventriculus showing imaginal cells of the stomodaeum. *c, d, e*: Imaginal discs of the legs, and head parts seen from the left side in *c* for the larva and in *d* and *e* for the pupal stages. (AT) antennal imaginal discs; (B) brain; (E) esophagus; (IM) Imaginal cells of mid gut epithelium; IMU, Imaginal cells of mid-gut muscles; (IPA) posterior abdominal imaginal disc; (PIR) imaginal disc of proctodaeum, L^1, L^2, L^3, discs for the first, second, and third legs; (OD) optic disc; (PB) proboscis; (PD) proctodaeum; (PR) proventriculus; (S) sucking stomach; SG, salivary gland; I, II, III, first, second, and third thoracic segments

The mode of development of imaginal disc varies from species to species and also from organ to organ. Imaginal discs are sometimes formed during late embryonic development and their cells are separate from the prospective larval cells, as for example in *Drosophila* and other Diptera. In some insects the imaginal discs are derived from larval cells during the later phase of larval growth.

18.6.3 Factors controlling metamorphosis in insects

For a larval moult to be successful all parts of the body must take part in the process and carry it out at the same time. This indicates that a common factor acts on all parts of the body. The existence of a common factor or cause is even more apparent in metamorphosis, in which the involvement of both external and internal organs may be more radical and farreaching. This common factor may be external or internal.

External factors

In some cases an external factor may be responsible for initiating moulting, as for instance in the blood sucking *Rhodnius* the intake of food (blood meal) is such a factor. Another example in which external factor is essential to initiate a moult is the case of the pupa moth *Platysamia cecropia*. After pupation the insect falls into a quiescent state with a reduced rate of metabolism — diapause — which continues throughout winter. It is essential that during this time the pupa be exposed to cold, otherwise the diapause is prolonged indefinitely. The diapause may be broken precociously if the pupa is exposed to cold (3° to 5° C) for at least two weeks. The temporary cooling activates the vital processes in the pupa and on return to a warmer environment development is completed, the pupa moults and the imago emerges.

In other insects factors such as humidity, population density etc., appear to initiate metamorphosis. However in the majority of insects no external cause of any moult has been detected and moults follow one another at intervals which appear to be determined entirely by the internal processes in the animal.

Internal factors

Similar to the amphibian metamorphosis, moulting and metamorphosis in insects has been found to be initiated internally by hormones.

Hormonal control involves at least three organs of endocrine secretions . They are (1) brain (protocerebrum) (2) corpora allata (3) prothoracic gland. Fig 18.24 shows the endocrine glands associated with the brain in moths namely protecerebrum and corpora allata.

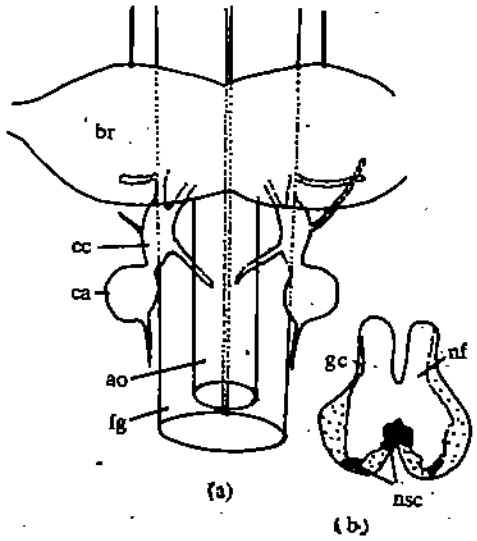


Fig.18.24: The endocrine glands associated with the brain in meal moths. a) Dorsal view of the brain in a meals moth pupa, b) Transverse section of the protecerebrum in a caterpillar of the meal moth. Ao, Aorta; br, brain; ca, corpora allata; cc, corpora cardiaca; fg., foregut; nsc, neurosecretory cells.

18.6.4 Organs and hormones involved in insect metamorphosis

(1) Brain neurosectory cells and their hormones

Kopec was the first to suggest the role of hormones in controlling metamorphosis. On the basis of his experiments on the larval gypsy moth (*Portheria dispar*) he observed that at a particular period the brain releases a substance into the blood which is essential for pupation and hence for metamorphosis. His findings on the role of brain hormones in metamorphosis of insects was supported by subsequent workers who found similar mechanisms in different insect groups. Large secretory nerve cells in the brain of the insects called neurosecretory nerves were also identified as being responsible for affecting pupation.

The secretion of these cells is called activation hormone (AH) or brain hormone (BH). The activation hormone is comprised of proteins or Lippoproteins. The AH or BH after synthesis pass along the axons of these cells and end blindly into a pair of storage and release organs called the **corpora cardiaca** (CC), located in the posterior brain. The CC releases the active hormone material called **prothoracotropic hormone** (PTTH) which is a small polypeptide. This prothoracotropic hormone acts upon the **prothoracic gland**, causing it to secrete the moulting hormone called 'ecdysone'.

Corpus allatum (Singular) and Juvenile hormone.—The corpora allata are rounded glands attached to the posterior side of each corpus cardiacum forming a compact body just behind the brain. In some (Hemiptera, higher Diptera) they are fused to form a single median structure. Experiments by Wiggleworth have shown that the CA secretes a hormone which determines the character of each larval instar by limiting the degree of differentiation towards the development of the adult. He named this hormone as **juvenile hormone** (JH). This is also known as neotenin (youth substance), (Wigglesworth 1954) and gonadotrophic hormone (Englemann 1957). The juvenile hormone is similar in structure to terpenes. A large number of compounds with juvenile hormone activity have been isolated and many have been synthesized. Some synthetic ones appear more potent than the naturally occurring ones. The juvenile hormones ensures the occurrence of larval moults and inhibits metamorphosis. Its presence or absence determines whether the larva will moult into a larval/pupal stage or into adult stage. The major natural hormone isolated from the adult male *Hyalophora cecropia* moth has the following structure (Roller at al 1967) Fig.18.25.

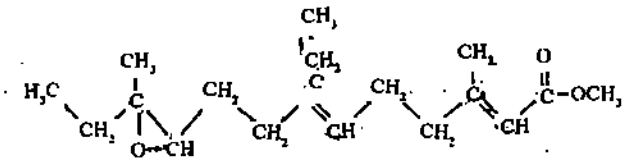


Fig.18.25: Juvenile hormone isolated from *Cecropia* moth

The role of the corpus allatum and the juvenile hormones in metamorphosis has been proved by several experiments (Fig. 18.26). It has been observed that if an extra corpora allata from a second stage larva is transplanted to the fourth instar last larval instar then the moult does not produce a pupa or adult, instead an oversized larva develops. Removal of corpora allata early in larval life results in premature metamorphosis .

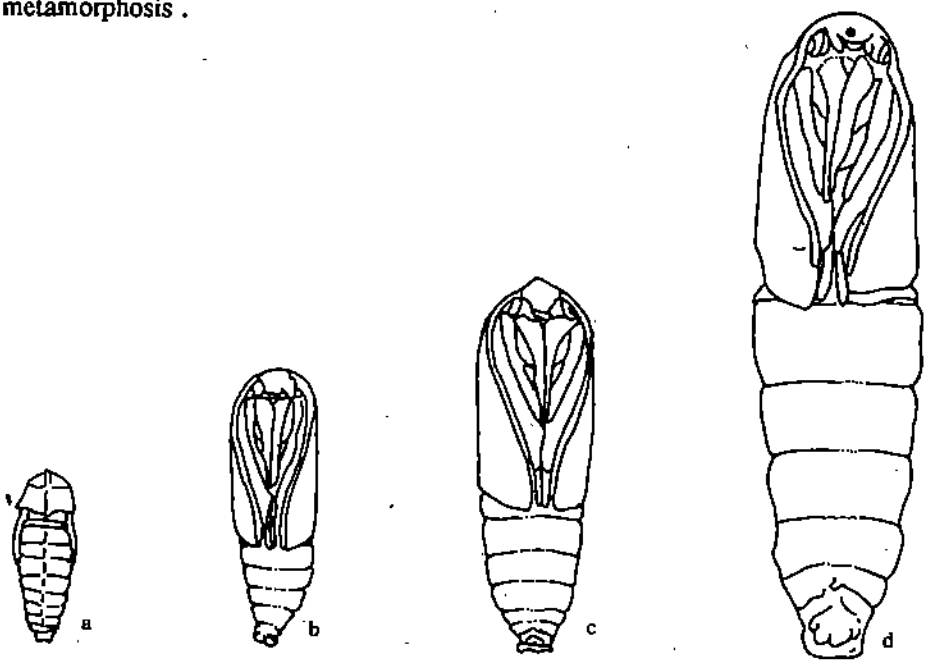


Fig.18.26: Experiments on effect of moulting hormone on pupa size a, b, pupae of moth, resulting from the removal of the corpus allatum (source of the moulting hormones from third-and fourth-instar larva) c, A normal pupa. d, A giant pupa produced by implanting an extra corpus allatum from a young larva into one that had already reached the stage at which it would normally pupate.

Prothoracic gland (PTG) or moulting gland and ecdysone or moulting hormone

The third endocrine gland—prothoracic gland— is an irregular branching mass of glandular cells located in the prothorax (segment of the thorax that bears the anterior most of its three pairs of legs), in close association with the tracheal tubes and secretes the hormone ecdysone which has been isolated and crystallized in pure form from *Calliphora* and from pupa of a silkworm. It is a unique water soluble steroid and is related closely to cholesterol (Fig.18.27). Recent studies however show that all α ecdysone is converted into β ecdysone (ecdysterone) after liberation into the haemolymph and that ecdysterone is the active moulting hormone.

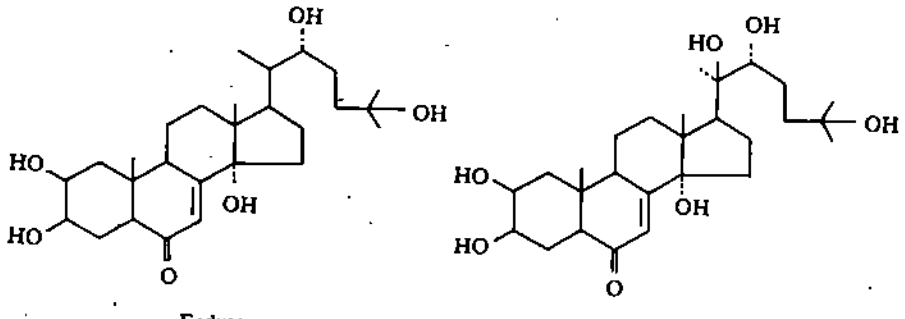


Fig.18.27: Commonly occurring moulting hormones (a) (ecdysone) (b) ecdysterone, in insects undergoing metamorphosis

Ecdysterone exerts its effect directly upon those cells concerned in growth and moulting; it activates them and stimulates them to renew protein synthesis. It is believed that it acts directly upon those loci in the chromosomes which are concerned with growth. It induces characteristic puffing in the giant polytene chromosomes of Diptera. These puffs are considered to be the sites for the formation of the messenger R N A needed for protein synthesis.

18.6.5 Interaction of insect hormones in the process of metamorphosis

We have discussed the organs and the hormones generally involved in metamorphosis of insects. This is because despite the fact that there may be around 1 million different insect species, there is a striking similarity in the endocrine function of different hemimetabolous and holometabolous insects. Fig.18.28 shows a schematic outline of the interaction of hormones in the metamorphosis of insects.

On the basis of figure 18.28 let us study how the actions of the various hormones bring about metamorphosis in insects.

The moulting process, the start of metamorphosis, is initiated in the brain. The stimulus (as you already know) may be neural, hormonal or environmental, and causes the neurosecretory cells of the brain to release the activation hormone which after synthesis changes into the active hormone called prothoracicotropic hormone (PTTH). The PTTH stimulates the prothoracic gland to produce ecdysone. Ecdysone after being converted into its active form, the ecdysterone, stimulates growth and causes the epidermis to secrete a new cuticle, initiating the moulting process. The ecdysterone further stimulates the epidermal cells to synthesize enzymes which digest and recycle the components of the cuticle. As long as the juvenile hormone is present, the ecdysone-stimulated moult results in a new larval instar. In the last larval instar stage, the synthesis of juvenile hormone is reduced, causing its levels to drop below a critical threshold value. This again

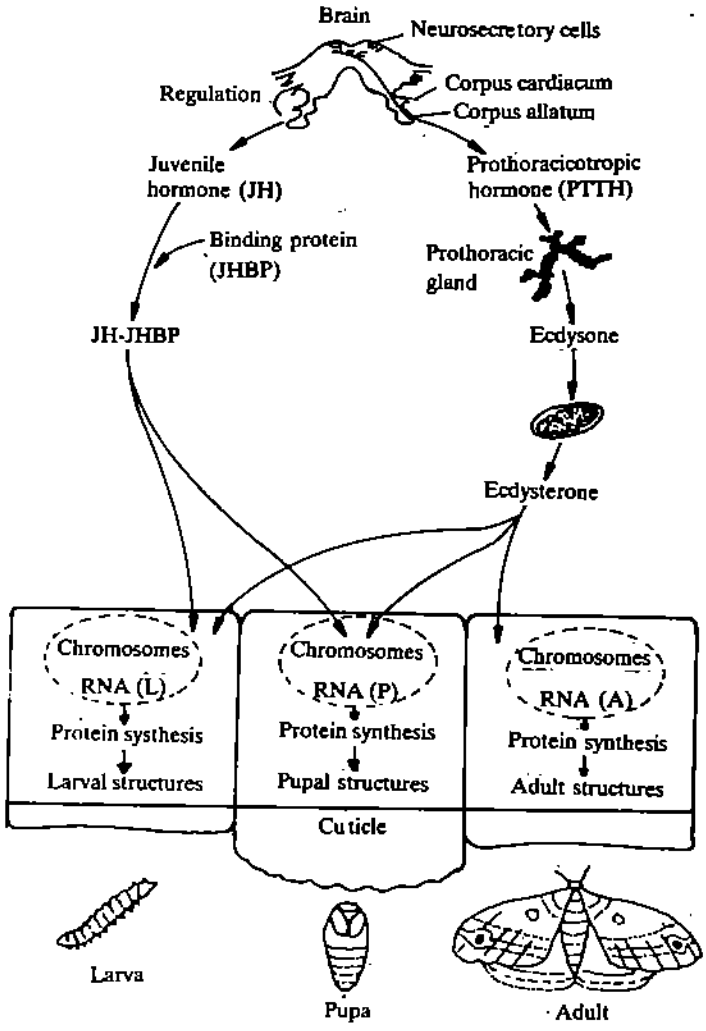


Fig.18.28: Schematic diagram illustrating the control of moulting and metamorphosis in the tobacco hornworm moth. (After Gilbert and Goodman, 1981).

triggers the release of PTTH from the brain. The PTTH in turn, stimulates the prothoracic gland to secrete an unusally large quantity of ecdysone. The resulting ecdysterone, in the relative scarcity of JH, causes the instar to pupate. In other words the occurrence of the subsequent moult in the larva in the relative scarcity of JH and abundance of ecdysone, shifts the organism from larva to pupa. During the period of pupation the corpora allata do not release any juvenile hormone and the ecdysterone stimulates the pupa to metamorphoses into the adult insect.

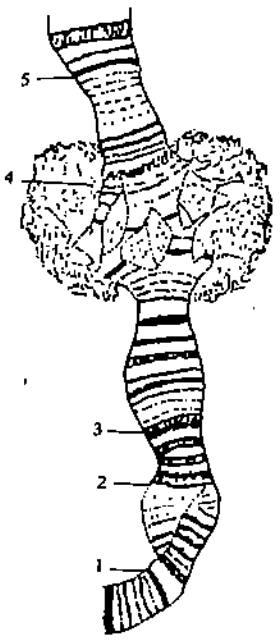


Fig. 18.29: Puffing in insect chromosome—five prominent bands (numbered) can be seen in each chromosome.

18.6.6 Effect of metamorphic hormones on gene expression in moulting and metamorphosing insects

The first report of a specific hormone being able to influence specific gene loci, control their transcription and finally influence cell differentiation was reported in the 1960s by Clever and Karlson on the basis of their studies on Dipteran larva *Chironomus*. These findings were based on experiments in which Clever injected minute quantities of ecdysone into the larva and observed within fifteen minutes, hormone induced puffings (swellings) at specific regions of a particular chromosome.

Clever also studied patterns of puffing in polytene chromosones at various larval developmental stages. He further established that puffing of specific chromosomal bands takes place at specific times in normal course of development and follows a fixed sequence whereby certain bands puff at one stage to synthesize RNA and regress at a later stage. For example he injected ecdysone into an intermoult larva in which puffing of a band 1-19A was known to be particularly active, and observed that in 10-15 minutes, puff 1-19A regressed, band 1-18C started to puff, followed by puffing of band IV-2B. This puffing sequence was exactly similar to the sequence that occurs just before moulting, a time at which the insect spontaneously releases ecdysone into the haemolyph Fig.18.30 show similar findings in *Drosophila* as observed by Becker, 1959.

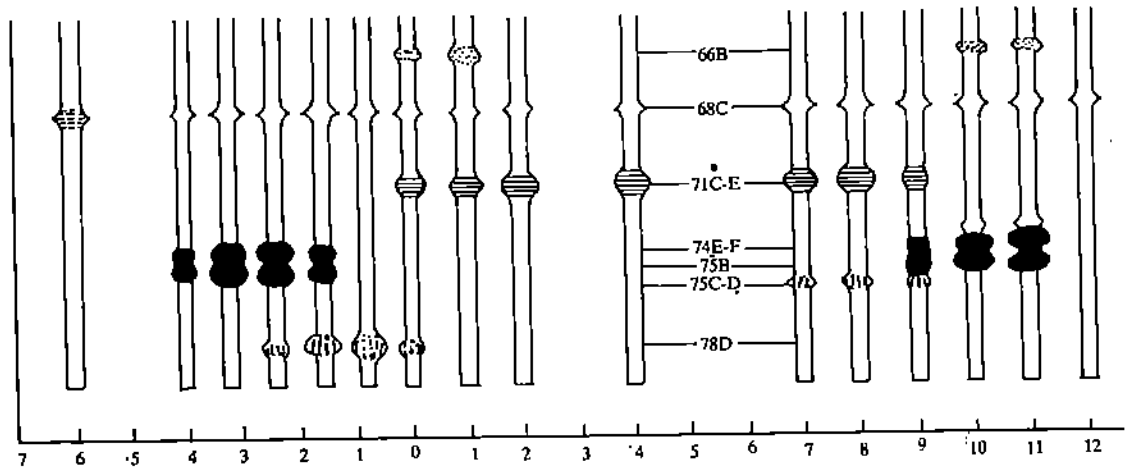


Fig.18.30: Puffs appearing and dissappearing during the third larval instar and the prepupal stage at the base of chromosome arm 111 L of *Drosophila melanogaster* salivary glands. Numbers indicate hours before or after pupanum formation. After H. Becker, Chromosome, 10-654 (1959).

On the basis of such experiments it appears reasonable to assume that the hormone ecdysone somehow turns on the transcription of one or several genes in the larva (which is apparent due to the occurrence of puffs)

The action of the ecdysone, as to whether it acts as such on the gene or changes the internal nuclear environment is still not well understood and so several theories have been advanced. Kroeger (1968) found that ecdysone causes the cell to accumulate potassium ions (K^+), since the primary sets of puffs can also be induced without ecdysone by increasing the intracellular or intranuclear concentrations of K^+. Thus the stimulation of K^+ uptake by cell and by nucleus may be the primary effect of ecdysone.

Goodenough and Levene (1974) have suggested that ecdysone might control puffing and hence transcription of the genes.

18.7 COMPARISION BETWEEN METAMORPHOSIS IN AMPHIBIANS AND INSECTS

You may have realized that the metamorphic process in amphibians and insects show certain fundamental similarity. Perhaps you have been able to identify them. They are given below:

(1) In both insects and amphibians the stimulus necessary for the secretion of hormone responsible for initiation of metamorphosis is provided by secretory organs associated with the brain, namely hypothalamus in amphibians and protocerebrum in insects.

(2) In both cases the hormones influence differentiation of cells and morphogenetic processes during metamorphosis.

(3) In both amphibians and insects the brain and its secretion does not act directly on the larval tissue, instead they stimulate the secretion of another endocrine gland, the thyroid in amphibians and the prothoracic gland in insects.

(4) Metamorphosis in both involves some destructive activities (histolysis) and some constructive activities (histogenesis).

(5) The juvenile hormone of insects however has no counterpart in amphibians and so in amphibians there is no hormone which can check or prevent precocious development.

(6) Larval growths in amphibians is not divisible into instars or inter stages. Instead, it is continuous development. In insects, growth occurs periodically marked by moults. There seems to be no growth in the intermoult period.

You can thus see that a comparison of metamorphosis between two groups which are so widely separated phylogenetically as insects and amphibians and which have a certain number of differences also reveal a striking similarity. The onset of puberty in humans as you can read in Box. 18.1 also exhibit a striking similarity to metamorphosis.

Box 18.1

Metamorphosis and Puberty in Humans

The hormonal basis of puberty in humans is very striking, and research suggests that the process of puberty may in fact be quite similar to metamorphosis. As you have read, the metamorphoses of both amphibians and insects were both seen to be regulated by hormonal changes that were initiated by the brain or neurohormones (TSHRF and PTTH, respectively). Similarly in humans the changes accompanying puberty in both sexes are initiated by luteinizing hormone releasing factor (LHRF) from the hypothalamus of the brain (Fig.18.31). Like TSH-RF, this factor is released from the hypothalamic neurons to the pituitary gland. Likewise the LHRF is then transported by the blood vessels of the pituitary to the anterior lobes of the gland. Once in the anterior pituitary, the releasing factor causes the release of a tropic hormone. In human puberty, the LHRF releases the luteinizing hormone (LH) and the follicle stimulating hormone (FSH). These two hormones are collectively called gonadotropins as they stimulate the development of ovaries in females and testes in males. Due to this stimulation, the gonads secrete the sex hormones estrogen from the ovaries and testoterone from the testes. The numerous morphological and behavioural changes of puberty are thus due to the activity of these hormones on the various target tissues.

As in metamorphosis, there appears to be in puberty a maturation inhibiting hormone, whose activity decreases in order to permit the reactivation of development. In humans this particular hormone is most probably **melatonin**, whose serum concentration is found to decrease with the increase of LH concentration. So you can see that puberty has many parallels to metamorphic changes as it is a hormonally controlled reactivation of development, leading to maturity and several changes in bodily form and physiology.

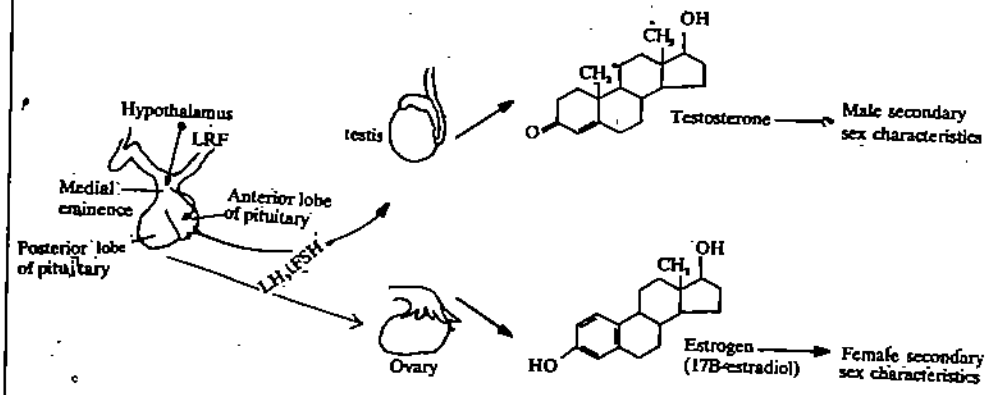


Fig. 18.31: Hypothalamus—pituitary-gonadal axis in mammalian sexual development

SAQ 5

1) In one or two sentences, define the following terms:

   a) stadium b) instar c) imago d) Exopterygota

   e) holometabolous metamorphosis f) imaginal discs

ii) Fill in the blanks with suitable words

   a) A ..... is necessary for initiating moulting in *Rhodinus*.

   b) Exposure to .....breaks the diapause in pupae of *Platysomia cecropia* .

   c) Activation hormone secreted from the ..... of the brain and stored in .... acts on prothoracic gland which releases .....

   d) The ..... hormone released from corpora allata is a ..... compound.

   e) ..... hormone is responsible for retention of larval characters and ecdysone promotes the ..... process.

   f) Removal of ..... may result in precocious metamorphosis.

   g) Ecdysone is converted into ....., an active moulting hormone.

18.8 SUMMARY

- After birth or hatching the young of an animal may develop directly into the adult form or the newly hatched individual may show indirect development, appearing first as a larva morphologically and functionally and then transforming into an adult by metamorphosis.
- Larva occur in a number of most animal phyla ranging from porifora to the vertebrates (anurans and some fishes).
- Metamorphosis is one of the many ways in which an animal overcomes the problem of growth, owing to the egg being essentially smaller than the adult.
- In animals undergoing metamorphosis the larva which emerges after development from the egg is smaller than the adult but may be (i) similar to the adult, differing only in size and sexual maturity or (ii) greatly different from the adult in habitat, form, organization, physiology as well as in size and sexual maturity. In either case it transforms into an adult by the process of metamorphosis.
- Metamorphosis is a phenomenon in which the larva changes in morphology, organization and physiology before it transforms into an adult.

- Different animals show different degrees of changes during metamorphosis. Thus (i) in urodeles and **hemimetabolous insects** where larva and adults are largely similar, differing only in size and sexual maturity and where other changes are few of metamorphosis is **gradual and incomplete and is called incomplete metamorphosis.** In animals like **anuran amphibians** and **holometabolous insects** where the larva and adult show enormous differences, metamorphosis is more radical and is called **complete metamorphosis.**

- Metamorphosis is accomplished by cell death, cell proliferation and cell differentiation. Many genes active in larva switch off while many genes inactive in the larva become active.

- Metamorphosis is essentially controlled by hormones. But many extrinsic and intrinsic factors influence metamorphosis, by intiating hormonal action.

- In the amphibians the pituitary-thyroid hypothalamus axis control metamorphosis. The main effecter hormone is thyroxin T_4 a precursor of T_3. Removal of the thyroid prevents metamorphosis and addition of iodine or powdered thyroid gland in water of the tadpole accelerate it. The thyroxine is secreted by the thyroid as T_3. This secretion is triggered by the production of thyroid stimulating hormone (TSH) from the pituitary. The increased production of TSH causes metamorphosis. Before the onset of metamorphosis high levels of prolactin (a hormone which promotes growth and inhibits metamorphosis when level of thyroxin is low) and low level of thyroxin do not allow metamorphosis to take place. The increasing levels of thyroid secretion cause the tadpole to undergo (i) premetamorphosis (ii) prometamorphosis and finally when levels are high (iii) metamorphosis climax. The high levels of thyroxin make the prolactin action ineffective.

- The response of the cells to any given hormone is inherent in them and it is independent of the position of the cell in the body. Some cells undergo necrosis while others proliferate under the action of the same hormone. Also cells of the same organ but at different stages of development respond differently to the same hormone.

- During metamorphosis, in insects the neurosecretory cells of the brain secrete a brain hormone (BH) or Activation hormone (AH). When BH which is stored in corpora cardiaca is released as the active hormone prothoracicotropic hormone PTTH it stimulates the prothoracic gland to produce the moulting prohormone ecdysone which changes into the active ecdysterone hormone. This stimulates growth and moulting. During the larval life of insects the corpora allata of the brain secretes juvenile hormone (JH) which suppresses metamorphosis at each moult. The quantity of JH decreases with successive moults and when its concentration becomes low the larva develops into a pupa or into the last larva stage (where pupa formation does not occur). When the JH is absent the pupa or the last larva stage changes into the adult. The ecdysone appears to affect mRNA synthesis (transcription) as is experimentally shown by changes in the puffing patterns of polytene chromosome of insect. Moulting hormone also alters K^+ content in cell and nucleus.

18.9 TERMINAL QUESTIONS

1) Why is the marine environment more conducive for the developing larvae than fresh water or terrestrial environment?

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2) Briefly describe the process of metamorphosis in anurans.

3) List the regressive/progressive and constructive changes that occur during amphibian metamorphosis.

4) Briefly discuss the hormonal control of metamorphosis in amphibians.

5) What are the molecular responses that the thyroid hormone evokes during metamorphosis?

6) What is neoteny? Briefly discuss neoteny in different species of urodeles. What is the significance of neoteny in evolutionary process?

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7) Distinguish the terms gradual and extensive metamorphosis with suitable examples.

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8) How do different hormones interact to bring about metamorphosis in insects?

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18.10 ANSWERS

Self Assessment Questions

1 i) Direct development refers to the type of development in which the new born at the time of birth resembles the parent except for the size and sexual maturity. Direct development is observed in reptiles, birds and mammals.

ii) Indirect development is observed in many invertebrates, as well as in protochordates like amphioxus of and also in vertebrates such as amphibians. The young ones that hatches out of the eggs do not resemble the adults, and infact appear very different from the parent. Such young ones are called larvae which lead an independent life, subsequently undergo transformation in their structure by the process called metamorphosis and then resemble the adults.

2 i) (a) False (b) False (c) True (d) True (e) True (f) True

ii) a) v; b) iv; c) iii; d) vi; e) ii; f) i

3 i) a) aquatic, terrestrial b) neoteny c) second d) activation, brain hormone e) premetamorphosis, prometamorphosis, metamorphic climax f) regressive, progressive, constructive

ii) a) progressive b) regressive c) regressive

d) constructive e) constructive f) constructive

g) constructive h) progressive i) regressive j) progressive

iii) 1) Switch over from ammonia excretion to urea excretion

2) Ability of the liver to synthesise enzymes of urea cycle.

3) Ability of kidneys to maintain water balance by increased water absorption.

4) Replacement of larval haemoglobin by adult haemoglobin.

iv) **Progressive Changes:**

1) Development of eye lid

2) Development of a multilayered and cornified skin

Regressive changes

1) Resorption of external gills and closure of gill clefts

2) Reduction of visceral skeleton

4 i)

a) true	b) false
c) false	d) true
e) true	f) true
g) false	h) true
i) true	j) false

ii)

a) iii	b) i
c) iv	d) ii

5.i a) **Stadium:** The interval between two moults during the larval period of an insect is known as stadium.

b) **Instar:** Instar refers to the form an insect assumes as a result of moulting.

c) **Imago:** The final adult form that emerges at the end of metamorphosis in an insect

d) **Exopterygota:** A division of subclass Pterygota of class insecta in which the wings of the insects arise as a result of invagination of the integument.

e) **Holometabolous metamorphosis:** Metamorphosis of insects which produce young ones that are completely different from their parents both in form and habits.

f) **Imaginal discs:** Imaginal discs are the rudiments of future organs of the adult such as wings antennae, and internal organs.

2 ii. (a) blood meal (b) cold (c) protocereberum, corpora cardiaca, ecdysone (d) juvenile, terpenoid (e) juvenile, moulting (f) corpora allata (g) ecdysterone.

Terminal Questions

1) See subsection 18.4.1
2) See subsection 18.5.1
3) See subsection 18.5.1
4) See subsection 18.5.4
5) See subsection 18.5.7
6) See subsection 18.5.8 and 18.5.2
7) See subsection 18.6.2
8) See subsection 18.6.5.

UNIT 19 REGENERATION

Structure

19.1 Introduction
Objectives

19.2 Types of regeneration
Physiological regeneration
Reparative regeneration
Compensatory hypertrophy

19.3 Patterns of reparative regeneration
Epimorphic regeneration
Morphallactic regeneration

19.4 Limb regeneration in amphibians
Sequence of events in urodele limb regeneration
The origin of regeneration cells of blastema
The problem of potency of blastema cells
Role of wound epidermis and apical epidermal cap
Role of Nerves
Role of hormones in regeneration
Rule of Distal Transformation of Blastema
Limb regeneration in anuran amphibians

19.5 Lens regeneration in amphibians
The process of lens regeneration from dorsal iris in *Notophthalmus viridescens*

19.6 Regeneration in Hydra
.Process of regeneration
.Source of cells for regeneration
.Problem of polarity

19.7 Regeneration in planarians
Process of regeneration
Origin of blastemal cells or regenerative cells
Problem of polarity
Induction phenomenon

19.8 Comparison between regeneration and embryonic development

19.9 A survey of regenerative capacity in the Animal Kingdom
.Regeneration in invertebrates
.Regeneration in vertebrates

19.10 Atypical form of reparative regeneration: Hetromorphosis

19.11 Summary

19.12 Terminal Questions

19.13 Answers

19.1 INTRODUCTION

You may have observed that when a predator grabs a lizard by its tail, the latter evades capture by simply leaving the distal part of its tail in the grip of the former. The tail which moves for a while, apart from being unnerving for the predator also allows the lizard to escape. The tailless lizard is not unduly worried about the loss of

parts of its tail, as it has the ability to develop the lost part, though imperfectly, by the mechanism of **regeneration.**

Regeneration has intrigued scientists for several generations and has resulted in voluminous literature on the subject starting from the eighteenth century. T. H. Morgan 1901 has been primarily responsible for formulating the principles which form the basis of the recent studies of regeneration. The major problems of regeneration identified by Morgan are being investigated even to this day. These involve the origin and developmental potential of cells that are responsible for the production of the regenerate, the role of the adjacent tissues in determining the structure of the regenerated limb and the reasons for the enormous variation in the regenerative capabilities of various animals.

What does regeneration mean in biological terms? Regeneration is a fascinating phenomenon. It involves continuity of the developmental processes or reawaking of the process of morphogenesis and differentiation in post-embryonic life in an already formed and functional organism. Regeneration occurs at various level of organization. At the sub-cellular and molecular level it is manifested in the continuous synthesis to replenish used up substances in the cells. At the sub-cellular and tissue levels it involves replacement of worn out cells, repair of damaged tissues and healing of wounds. At these levels the ability to regenerate is a universal characteristic of all animals without which life of any individual would be impossible. At the organismic level regeneration consists of de novo (afresh) development to restore the lost part of an organ or the reconstitution of the whole body from the residual part of the organ concerned. This involves retracing many of the complex steps of the original ontogenetic development in a functional body under quite different physiological and environmental conditions. The capacity for this type of regeneration is referred to as reparative or restitutive regeneration and is unevenly distributed in the animal kingdom. Some have great powers to restore lost parts, or even to form a whole body from a small piece. Others have variously restricted and limited abilities of such regeneration, and still others have no power of reparative regeneration at all. The reasons for such inequality of regenerative power among animals are not clear. In many groups, the animals exhibit the phenomenon of autotomy, by which they themselves cast off or lose one or more parts of the body when disturbed or threatened by an enemy or a predator. The autotomized (self-amputated) parts are subsequently regenerated. Different animals employ diverse method for the regeneration of lost parts. The study and investigation of the phenomena of regeneration are of great help in the efforts to understand the basic processes and mechanisms of development as such.

Regenerative ability has been examined in a large number of animals belonging to almost every phylum since this phenomenon was discovered more than 250 hundred years ago. The most favourite animals for analytical studies to understand the various developmental aspects of regeneration have been the amphibians (limb, tail, eye and lens regeneration) among vertebrates and hydra (coelentrata) and planarians (flat worms) among the invertebrates. Annelids, arthropods and some others also have received a fair amount of attention. The core of this unit consists of studies on regeneration in hydra and planaria and of limbs and lens in urodele amphibians, to make you familiar with the basic principles of regeneration. Brief information on various aspects investigated in some species of polychaetes and oligochaetes among annelids, insects among arthropods and on tail regeneration in cyclostomes, amphibians and reptiles is also included in the section dealing with the general survey of regenerative ability in different animal phyla.

Objectives

After studying this unit you will be able to:

- explain the phenomenon of regeneration in animals and also explain how the study of regeneration helps in understanding embryonic development,
- distinguish between physiological regeneration, reparative regeneration and compensatory hypertrophy,

- define and distinguish between the processes of regeneration by epimorphosis and morphollaxis,
- explain the terms autotomy, metaplasia, dedifferentiation, blastema, polarity and gradients,
- describe briefly the regenerative abilities among invertebrates and vertebrates,
- describe the processes of regeneration of limb and lens in urodele amphibians,
- describe regeneration in *Hydra* and *Planaria*.

19.2 TYPES OF REGENERATION

The three basic types of regeneration that occur in animal are:

(*i*) Physiological regeneration. (*ii*) Reparative regeneration. (*iii*) Compensatory hypertrophy.

19.2.1 Physiological regeneration

This type of regeneration is a regular physiological function involving the continuous replacements of cells and tissues, and so is indispensible for the maintenance of life in all animals. It is a primary attribute of all living systems. Without such regeneration there would be no life, as the very maintenance of an organism depends upon the incessant turnover by which all tissues and organs renew themselves. For example, regular replacement of epidermal skin layers and RBC etc., in our body is a must. In some instances quite substantial quantities of tissues are replaced periodically, as in the successive production of follicles in the ovary or the moulting and the replacement of feathers and hairs. In our body one per cent of the total $25X10^{12}$ RBCs present in active circulation, die everyday and so are replaced daily.

The time span of regeneration varies; as for example, mammalian skin epidermal cells, produced at the basal level may take several weeks to reach the outer surface and be sloughed off, while the life span of an individual epithilial cell in the intestine may be limited to a few days. The motile hairlike flagella and cilia of single-celled organisms have the ability to regenerate within an hour or two after amputation.

19.2.2 Reparative regeneration

This kind of regeneration, as the name suggests, involves repair of a wound or replacement of a body part removed intentionally or due to injury . This type of regeneration may include restoration of parts of an organ or an organ as in regeneration of eye and lens in amphibians or parts of the whole organism as in limbs of urodeles, or it may be the regeneration of an entire organism from a part detached from the parent body as you will see in hydra. The power of this type of regeneration is not found uniformly in all animals. Some have great powers of such regeneration; in others it is limited to varying degrees and in yet others it is not found at all. This unit deals primarily with the phenomenon of reparative regeneration, generally referred to simply as regeneration as found in various invertebrates and vertebrates.

19.2.3 Compensatory hypertrophy

It has been observed that the exact replacement of a part or organ or tissue is not the only way to regenerate in animals; many of the body's internal organs compensate for their loss by enlarging what remains, instead of regrowing the missing part Fig.19.1. This process, called compensatory hypertrophy, is possible as the remaining mass is usually as good as that which was lost. Liver regeneration in mammals is a well documented example of this process, where the size of the residual lobes expand, thus restoring the original mass of hepatic tissues as well as its function.

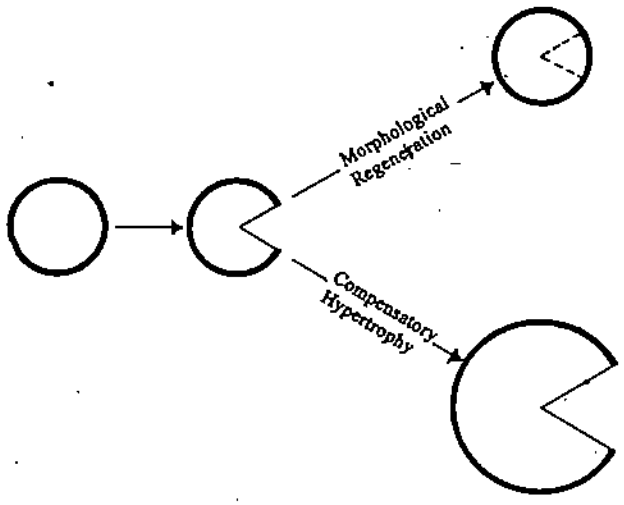

Fig.19.1: Comparison between qualitative and quantitative modes of regeneration. Some structures are replaced in situ by morphological regeneration. Others are not regrown but their residual portion enlarge by compensatory hypertrophy.

Compensatory hypertrophy in liver is accompanied by **hyperplasia** of its cells and of the histological functional units into which they are organized. Similar mechanisms have been noted in many endocrine and exocrine glands following surgery or physiological insufficiency. Pancreas , thyroid, adrenals and ovaries are the other organs which regenerate by compensatory hypertrophy. The way they compensate for such loss is the same way they grow during ontogeny.

However, not all organs are able to multiply their functional units in such a way. Nerves, muscles, lungs and kidney are unable to do so.

SAQ 1

Distinguish between physiological and reparative regeneration and give two examples for each.

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19.3 PATTERNS OF REPARATIVE REGENERATION

Two different patterns or modes of regeneration can be distinguished. These two patterns were described by Morgan in 1901 as: a) Epimorphic Regeneration—Epimorphosis b) Morphallactic Regeneration or morphallaxis.

19.3.1 Epimorphic regeneration

In this type of regeneration the lost part is reformed and restored by the growth of a bud or blastema from the remaining part of the organism followed by the blastema's redifferentiation and morphogenesis. A much studied example is salamander limb regeneration. You will study this type of regeneration in greater detail in section 19.4 of this unit. In brief, in this type of regeneration, a bud or blastema is formed at the site of amputation. The blastema contains the cells that will form the regenerated part, and is encountered in the regenerative processes of all animals in which epimorphic regeneration occurs. The blastema is made up of cells that look very much alike despite their diverse origins from different tissues of the residual stu

19.3.2 Morphallaxis

This type of regeneration occurs in plants, sponges and coelenterates such as jelly fishes and hydra. The missing parts are replaced by reorganization or remodelling of the pre-existing ones. The wound is healed and the neighbouring tissues reorganise themselves into whatever parts may have been lost or removed. Thus in this type of regeneration the residual part of the animal is capable of restoring the lost part or giving rise to the entire organism just by remodelling or reorganising the entire available mass of cells into a new whole. The process does not involve growth until the lost part or the whole body is regenerated, which is necessarily small at first. Growth to attain normal size occurs later. Morphallactic regeneration can occur in complete absence of cell division, as is seen in the case of regeneration in *Hydra.* A part of Hydra as small as 1/200th of the original individual can form a complete animal without cell proliferation being involved. Thus even a few cells are capable of forming a new organism; Similarly in sponges a few archaeocytes are capable of regenerating a whole sponge body. The morphallactic process of regeneration is observed only in lower groups of animals. Animals with more complex organisation regenerate their parts differently, usually by the production of a specialized bud or blastema, as you will read in subsection 19.3.1

SAQ 2

The sea anemone *Metreidium*, frequently sheds fragments of its foot as it glides across a rock. These fragments reorganise into new, miniature anemones that feed and grow. What kind of regeneration is this?

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19.4 LIMB REGENERATION IN AMPHIBIANS

In vertebrates, the amphibians, in particular the urodeles have spectacular power of regeneration. This power of regeneration has made them a favourite subject of research and so these animals have been exhaustively studied. Much information about the processes, mechanisms and systemic factors involved in regeneration in these vertebrates is now available. We have picked two well studied models of regeneration in vertebrates namely: (*i*) regeneration of limb in amphibians particularly in the anurans and (*ii*) regeneration of lens in the urodele

19.4.1 Sequence of events in urodele limb regeneration

Unlike reptiles, birds and mammals the urodele amphibians possess unusual ability to regenerate amputated limbs throughout life. The anuran amphibians (frogs and toads) can also replace the lost part of a limb but only during the larval or tadpole stage. Virtually all phenomena associated with development, especially those associated with vertebrate embryos and particularly with the ontogenetic development of limbs in vertebrates, are inherent in regeneration of limb in amphibians. In addition, the principles that regulate patterning of the regenerating limb may be the same as those involved in pattern formation during the initial development of the limb.

Limb regeneration in the larval and adult newts has been extensively studied by many investigators, and is by all accounts the most exhaustively studied example of epimorphosis. Let us see how the regeneration of limb occurs. Figs. 19.2 and 19.3 show the regeneration of fore and hind limbs as observed in newts.

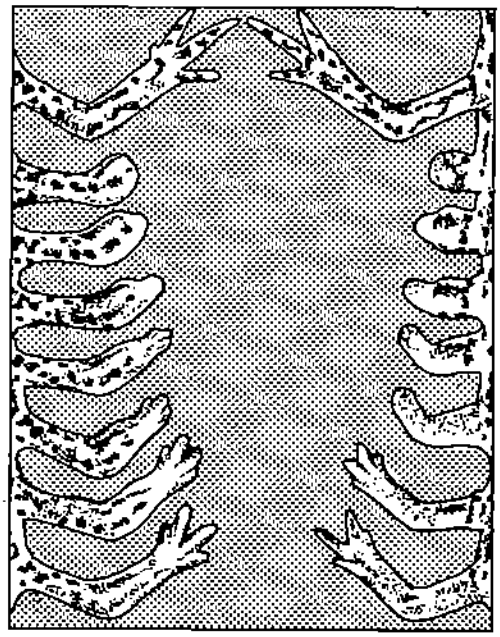

Fig 19.2: Adult salamander forelimbs (top) were amputated across the middle of the lower arm (left) and the middle of the upper arm (right). In both cases a normal limb regenerated (bottom) with the appropriate parts of the limb distal to the respective level of amputation.

After the limb is cut in urodeles, the healing of the wound begins. The epidermis spreads from edges of the wound to cover the open wound surface (Fig. 19.3 a & b). The closure of the wound is a fairly rapid process and is accomplished in a day or two, depending on the size of the animal. Once this wound is closed the epidermal cells proliferate and produce a multilayered mass of cells which form a conical bulge at the tip of the limb stump. This structure is called the **apical epidermal cap (AEC)** which you can see in Fig 19.3 .

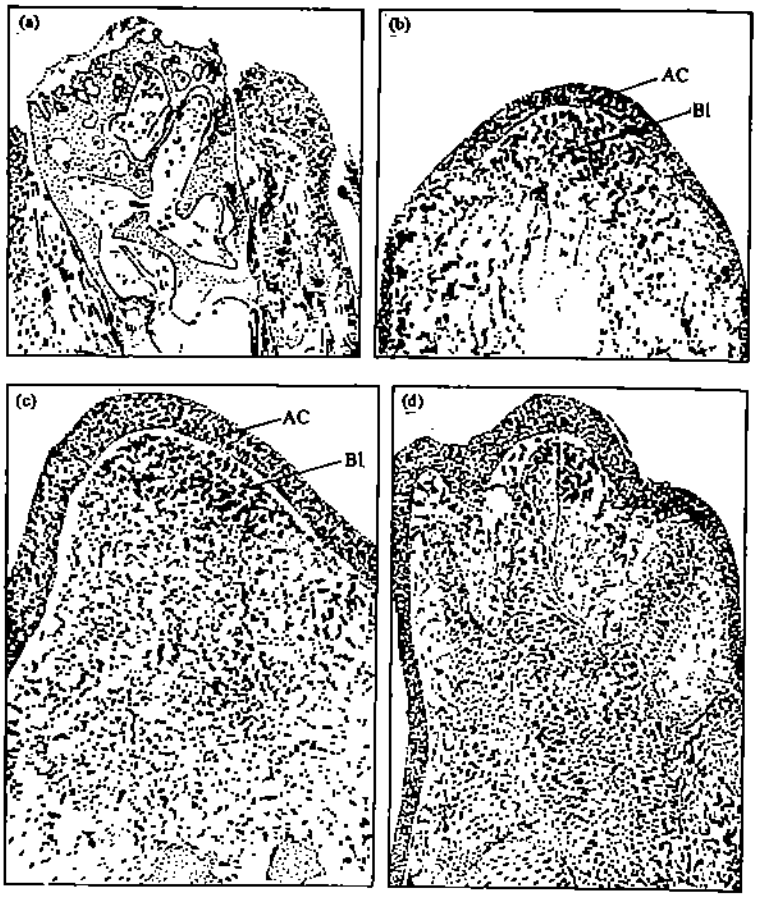

Fig. 19.3: Histologic changes during regeneration showing differentiation and redifferentiation of cartilage and muscle cells in the limb of the adult of *Triturus viridescens*. a: freshly amputated limb, observe retraction of skin and muscles from skeleton. b: sixteen day regenerate; showing thickening of ectoderm and accumulation of blastema cells, c: a twenty-one-day regenerate showing a large blastema that is beginning to redifferentiate d: twenty eight days after amputation a well-developed regenerate showing regenerated skeletal rudiments. AC, apical cap; BL, blastema. (Thomas B.Connelly, T.B. Sprague, and Dr.C.S.Thornton.)

Wound healing is accompanied by removal of the debris of damaged and dying cells of the stump tissues injured by amputation. This debris is removed by phagocytes that accumulate under the wound epithelium and cause some inflammation for some time. Concurrently, many undifferentiated and morphologically similar mesenchymal cells with large nuclei and basoplilic cytoplasm accumulate beneath the AEC and give rise to the regeneration bud or blastema (Fig. 19.3b).

The blastema grows by rapid mitotic divisions of its cells and assumes a more or less conical shape with the apical epidermal cap at its apex (Fig. 19.3 c) and then enters the phase of redifferentiation. Redifferentiation to form the missing parts begins with the blastema first becoming spatula shaped. This is followed by gradual morphogenesis so that the blastema assumes the shape of the lost parts of the limb (Fig.19.2). The small regenerated portion then undergoes rapid growth and in course of time becomes indistinguishable in form and function from the original part that was removed by amputation as the internal tissues (skeletal elements, muscles, connective tissues, blood vessels etc.) differentiate from blastema cells. Rudiments of the cartilaginous skeletal elements are the first tissue to appear as the blastema enters the phase of redifferentiation (Fig. 19.3 d). The various skeletal elements appear proximo distally just as they do in normal embryonic development of the limb. The cartiligenous skeletal elements undergo ossification later. Muscles are reformed both by de novo from blastema cells and by repair of the persisting muscles in the region of injury (Fig. 19.4).

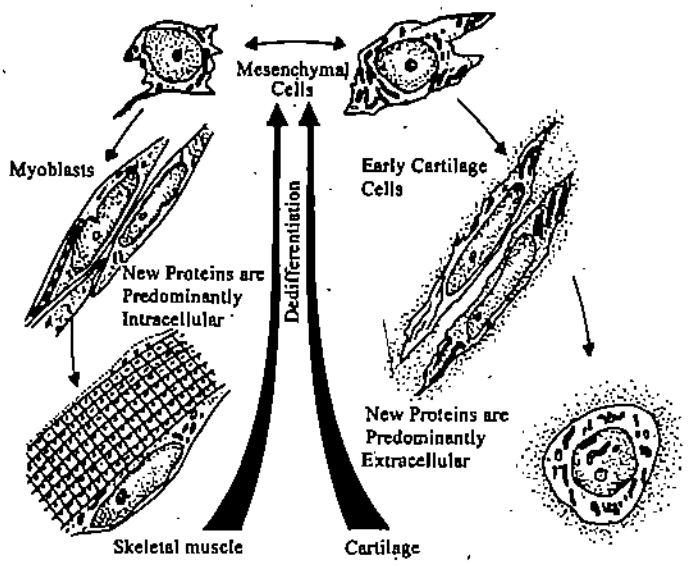

Fig. 19.4: Diagram summarizing the changes in fine structure which occur in the mesenchymal blastemal cells as they differentiate into myoblasts and muscle cells, early cartilage cells and cartilage. The formed cells dedifferentiate (arrows, center of figure) into mesenchymal cells when the limb is amputated.

Blood vessels are not apparent in the very early stages of regeneration but they soon extend into the blastema from the stump, and in the final regenerated limb the original pattern of vascularisation gets replicated. Several nerves are also cut when the limb is severed by amputation. However, soon after amputation their axons grow into the wound and reform the original nerve pattern. As you will learn later, the nerves play a very important role in limb regeneration. The process of differentiation, tissue organization and morphogenesis during regeneration are similar to those during embryonic development. Limb regeneration occurs according to the same sequence of stages and events (as described above) irrespective of the level of amputation or whether it is the forelimb or hind limb in the larvae or adult urodeles as also in the tadpoles of anuran amphibians.

SAQ 3

What do you understand by dedifferentiation?

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19.4.2 Origin of regeneration cells of blastema

The origin of the cells forming the blastema in vertebrates has been investigated by several workers in the case of regenerating legs of newts and salamandars. Their studies have shown that cells which form the blastema are of local rather than of systemic origin. To come to this conclusion they have performed several irradiation experiments on urodeles. It has been observed that appropriate dose of x-rays supress the ability of urodeles to regenerate. Using this knowledge scientists found that those urodeles whose bodies were irradiated failed to regenerate their limbs after amputation. Likewise, irradiated limbs also failed to regenerate upon subsequent amputation. However if the limb was shielded from the x-rays while the rest of the body was irradiated then regeneration proceeded normally in the limb when the limb was amputated. If only a portion of limb, for example the knee joint, was irradiated, then regeneration of the limb took place only if the amputation was done above or below the irradiated segment and not through it. Fig. 19.5 shows some of the irradiation experiments performed on the urodeles. These experiments clearly showed the local origin of the cells of the blastema.

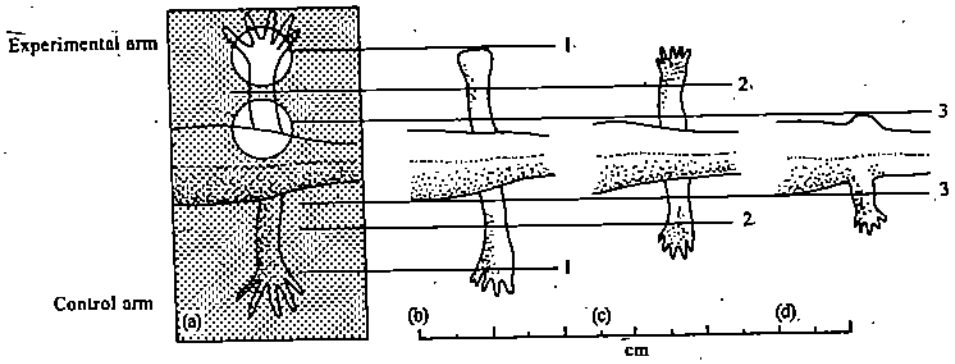

Fig. 19.5: (a,b,c) Histological sections of regenerating newt limbs. From top to bottom. Wound healing, blastema formation, and early differentiation. (a) Local irradiation of parts of the limb in a newt, and results of amputation at different levels in the irradiated limb (right) and in control limb (left). Drawing at left (a) shows shielding plate covering the whole animal except for two circular openings over right forelimb through which the x-rays could pass. The limb fails to regenerate when amputated inside irradiated areas (lines 1 and 3). Control (left) side regenerates at all levels. (From V.V. Brunst. Quarterly Review of Biology 1950 by Williams and Wilkins Inc. Blatimore.)

19.4.3 The problem of potency of blastema cells

You now know that local cells are responsible for regeneration. Now you could ask which of the blastema cells differentiated into which kind of limb tissues? Do differentiated cells such as muscles, cartilage etc., lose their specialised properties, proliferate and subsequently redifferentiate solely in accordance with their previous differentiated state i.e. into muscle, cartilage etc respectively? Or, do the dedifferentiated cells truly become pluripotent cells capable of forming a variety of differentiated cells. To find an answer scientists performed an experiment in which the skeleton was removed from the limb segment of an adult newt. Fig. 19.6. This was done by extirpating the bone so that the limb was permanently without a skeleton. Then the boneless segment was amputated. It was observed that the limb which regenerated also had its skeletal component. This clearly indicated that skeletal elements can differentiate from a blastema that received no contribution from preexisting skeleton. Thus tissues, in particular skeletal ones, do not need to be formed by cells from their specific tissue counterparts in the stump.

This experiment also shows that regeneration blastema arise by the dedifferentiation of stump tissues. Studies with the help of radioactive tracers have also positively proved that dedifferentiating tissues are actually involved in the formation of the blastema and not in one particular cell type. Tissues of amputated limb of newts were supplied with tritiated thymidine (a DNA precursor) in one experiment and tritiated leucine in another. In both experiments it was seen that the radioactively tagged precursors were taken up by cells adjoining the cut surface. The intake started while the tissue had not yet lost their histological characteristics and it was possible to ascertain that all kinds of tissues particularly the muscles, fibroblasts, periosteum, endosteum, Schwann cells of the nerves and the epidermis participated in the upsurge of synthetic activity. The

blastema was later found to consist of labelled cells of all these tissues. It follows thus that none of the tissues of the amputation stump are excluded from participation in regeneration. But that all cells respond to the wounding by growth (synthesis) and later by proliferation. However, it has been experimentally observed that epidermis does not contribute to the internal blastema.

Finally, biologists alternatively also wonder if there exist local population of reserve cells that retain the embryonic property of pluripotency and so are capable of forming the various differentiated tissues in the limb regeneration. They have found it extremely difficult to test this alterrative hypothesis experimentally, as the limb tissues are not made of pure populations of a single cells type. Furthermore, in addition to specialised cells most tissues have connective tissue cells some of which may be pluripotent.

The standard procedure used by investigators for examining or finding out the differentiation potential of tissues is to irradiate a diploid host animal. Irradiation prevents proliferation of the host cells, thus limiting their ability to take part in regeneration. After irradiation a tissue implant is made in the host from a triploid donor. The limb is then severed in order to allow it to regenerate. After regeneration the regenerate is subjected to histological analysis. The triploid nuclei in the tissues indicate that they are from the donor tissue, and if found in the regenerate as they usually are, they indicate that they have participated in the regeneration of new tissues. Often in some experiments the donor triploid cells are labelled with 3H-thymidine. The donor cells due to the presence of the triploid radioactive nuclei, are easily identified.

It has experimentally been observed that when donor tissue is cartilage from which the muscle and connective tissues have been carefully cleaned, then the regenerate has cells of donor type in the regenerated cartilage, namely perichondrium (the connective tissue layer surrounding the cartilage) connective tissue of joints and fibroblasts; but, no donor cells are found in the epidermis or muscle. However, it is reported that the cartilage taken from limb of young axolotl larvae but not the adult implanted into irradiated limb of host axolotl contributed cells to some muscle tissue in the regenerate which developed after amputation of host limb through the implant. On the other hand several investigators have found that if muscle is the donor tissue then the donor cells of muscle origin are found in all regenerated mesodermal derivatives including that cartilage. This result naturally suggests that the dedifferentiated cells originating in muscle tissue have greater potency than the cells derived from cartilage tissue. However, you should know that muscle is intimately associated with perimuscular fibroblasts which cannot be rigorously excluded, and could be the source for differentiation of tissues other than muscle in the regenerate in which muscle from a donor was implanted.

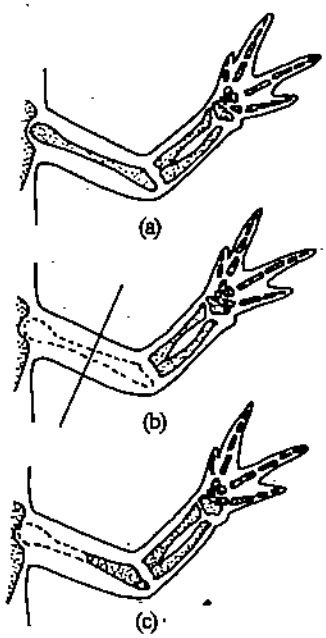

Fig. 19.6: Stump counterparts not needed for regeneration of the distal parts of the limb skeleton. a: forelimb skeleton of a newt. b: the humerus is excised and then the limb is amputated through the upper arm. c: regeneration of distal parts, including skeleton, proceeds from the cut surface distally.

However in one series of such experiments on larval Xenopus, cells bearing a nucleolar marker cloned from single myoblasts or fibroblasts were implanted into limb of a different host *Xenopus*, which were later amputated. It was found that cells of both myoblast and fibroblast origin can form all the various mesodermal tissues in the regenerate.

Epidermis is incapable of producing mesodermal tissues and it definitely does not contribute any cells to the blastema. However, skin dermis contributes cells of fibroblast nature which can differentiate into several mesodermal tissues. Transplantation studies on the axolotl. (*Ambystoma americanum*) using diploid and triploid cell markings have revealed that a very large proportion (43%) of the blastemal cells are of dermal fibroblasts origin. Both dermis and muscle tissue contain a larger number of fibroblasts. Therefore, there does exist the likelihood that many of the mesodermal derivatives of the regenerate are produced by fibroblast cells.

19.4.5 Role of wound epidermis and 'Apical Epidermal Cap'

You already know, that following amputation, the stump epidermal cells at the wound edge migrate over and rapidly cover the wound to from a multilayered apical epidermal cap. The formation of this cap depends on the early innervation of the wound epidermis by nerve fibres. The cap fails to form if the limb is denervated

before or immediately after amputation. If this cap is removed regeneration fails to occur. If an additional cap is grafted on the developing blastema it induces regeneration of supernumerary limb. Thus the apical epidermal cap formed by wound epidermis is necessary for permitting regeneration. Any other epidermis grafted on the wound surface does not form the apical cap and does not support regeneration. This shows that only the apical cap which developed from the wound epithelium could promote and support regeneration of the limb. In the non-regenerating amputated limb-stumps of older tadpoles and adults of frogs, the characteristic apical epidermal cap does not develop and so regeneration does not occur.

Experimental results indicate that wound epidermis stimulates dedifferentiation and mitosis in the underlying cells arising from stump tissue, thus inducing the formation and growth of the blastema and the subsequent regeneration of the limb. The blastema cells in the zone immediately adjacent to the apical cap remain in an undifferentiated and proliferating state, while those proximal to this zone begin to differentiate. In this way proximal distal pattern of redifferentiation of blastema cells is controlled by the epidermal cap.

You may recall that the apical ectodermal ridge (AER) of the embryonic limb bud also plays a similar role in allowing distal outgrowth of the limb. Both the AER in embryonic limb development and the apical cap of the regenerating limb stimulate cellular proliferation in cells located beneath them and keep them from differentiating, which enables differentiation of limb tissues in a proximo-distal sequence.

19.4.5 Role of Nerves

It has been observed that soon after amputation nerves invade the regeneration blastema. If the stump is denervated by cutting the nerves supplying the limb as they emerge from the spinal cord, and are prevented from regrowing into the site of amputation them regeneration fails to occur. The apical cap is not formed. In larval urodeles, denervation results in large scale regression of stump tissues and the entire limb stump may disappear. If the nerve fibers are allowed to regrow into the stump before a thick skin forms on the wound surface regeneration may be reintiated. These studies have shown with certainty that the presence of nerves is essential for regeneration of the limb. A number of experimental studies have been made to understand the nature and mechanism of nerve influence on limb regeneration in urodeles. The nerves are believed to produce a "trophic influence" on the blastema cells of the regenerating limb.

This neurotrophic effect has been found to be produced by all nerves, whether motor or sensory or central, irrespective of whether they have functional association with the central nervous system or not. Grafting of spinal ganglia in the regenerating area of limbs, whose own nerves have been cut and severed, also promote regeneration. However, it has been found that the presence of a minimum number of nerve fibers is necessary for regeneration. Below this threshold regeneration does not take place.

Experiments have shown that the neurotrophic influence is essential for initiating regeneration and for the growth of the blastema. But once the blastema begins redifferentiation and morphogenesis the subsequent course of regeneration is independent of the nerves. Denervation at this stage does not prevent completion of regeneration process.

It has been observed that vertebrate species (such as advanced tadpoles and adults of anurans), in which limb regeneration does not occur, contain fewer nerve fibres per unit area of amputation wound as compared to the urodele *Triturus*. It is possible that inability of these species to regenerate a limb may at least be partially due to an inadequate nerve supply. This hypothesis finds support from the results of experiments in which growth of regeneration blastema was induced in the amputated limbs of lizards, frogs and the marsupial opposum by increasing the nerve supply at the site of amputation.

Experiments have demonstrated that nerves exert their effect on regeneration of limbs by promoting mitosis of blastema cells and synthesis of DNA and proteins Recent studies have shown that the neurotrophic factor is probably a **fibroblast growth factor** (FGF), a protein with a molecular weight of about 13,000. It is released from myelin, the chief component of nerve sheaths. FGF is present in the nerve extracts and is also present in the blastema. It has been found to stimulate mitotic activity in the blastema of denervated limbs.

19.4.6 Role of Hormones in Regeneration

Most neurosecretory effects in regeneration are integrated into a neuroendocrine feed back system. It is often difficult to distinguish hormonal from neural influences during regeneration, particularly in invertebrates, although the two influences are easily distinguished in vertebrates.

In amphibians, the hormones of the three glands, namely pituitary, adrenal and thyroid appear to affect limb regeneration. These hormones control different phases of the regeneration process though the mechanism of their action is till not very clear.

Furthermore the hormonal response varies with the age of animal. For example, larval urodeles regenerate limb in total absence of pituitary hormone, whereas the adult is completely dependent on the pituitary gland for limb regeneration, a dependence acquired during metamorphosis. The role of the pituitary gland and its hormones in regulating regeneration has been worked out with great difficulty by means of several experiments.

In some experiments the pituitary gland of the newt was removed (hypophysectomy) at the time of limb amputation while in others it was removed later. Results showed that hypophysectomy at the time of amputation inhibited regeneration while delayed hypophysectomy until several days after amputation allowed some regeneration; to occur. The extent of regeneration was found to be dependent on the delay between amputation and operation. Hyposectomy performed three days after amputation allowed some limb regeneration, while that performed after 13 days or more allowed the entire limb to regenerate. These findings indicated that pituitary hormones are needed only during the very early stages of regeneration (like wound healing).

Other studies indicate that pituitary glands exert only an indirect effect on regeneration by stimulating the adrenal gland to produce cortisone. This observation is based on two experiments. In one experiment the regenerative capacities of the hypophysectomized newts were restored by replacement therapy of either cortisone (secreted by adrenal gland) or adrenocorticotropic hormones (ACTH) (secreted by pituitary). In the other experiment it was found that inhibition of cortisone secretion by administration of drugs, inhibited regeneration, which could again be relieved by cortisone but not by ACTH of the pituitary. These experiments thus indicate that the pituitary gland is regulated by the adrenals. The role of cortisone has also been found to be apparently limited to early phase of wound healing as the formation of blastema appears to be independent of the presence of cortisone which only promotes wound healing. In the absence of cortisone, In the absence, of cortisone wound healing fails and a thick dermal pad forms at the stump, preventing regeneration.

Thyroxine is produced by thyroid gland. It affects regeneration and also controls metamorphosis in amphibians particularly in anurans where the larval adult transformation is most dramatic as you will read in unit 18 of this block The effect of thyroxine in regeneration is however poorly understood as it inhibits tadpole limb regeneration if administered before amputation, but accelerates morphogenesis if given at the blastemal stages. Thyroxine inhibition is consistent with loss of regenerative capacity after a larva transforms into an adult. Thyroxine stimulates limb morphogenesis in the anuran tadpole but once formed, a limb loses thyroxin dependency and its regeneration is inhibited by thyroxin itself.

19.4.7 Rule of distal Transformation of Blastema

An intriguing phenomenon characteristic of limb regeneration is, that only the part of the limb removed distal to the level of amputation is regenerated. For example, suppose a fore limb is cut through the middle of upper arm, removing the distal half of the upper arm, wrist and hand leaving behind a stump consisting of only the proximal half of the upper arm. In this case the blastema formed at the cut end of the stump produces a regenerate consisting of the distal part of upper arm, lower arm, wrist and hand in this order; the proximal part of the upper arm is not duplicated in the regenerate (Fig.19.2). Thus a complete fore limb is regenerated without any duplication. The blastema always forms structures or parts distal to its own level of origin, along the proximo-distal axis of the limb. Furthermore, regardless of the level of amputation the blastema formed always regenerates only the distal structures, even if the polarity of stump is reversed as demonstrated in an elegant experiment on axolotl larva (Fig. 19.7). This is known as the **Rule of Distal Transformation of Blastema** and applies equally to limb regeneration in urodeles and anurans amphibians as well as to leg regeneration in insects.

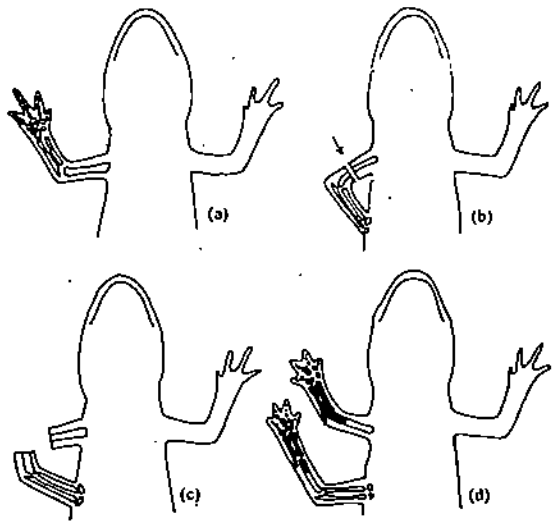

Fig. 19.7: Reversal of polarity. The distal end of a limb is grafted to the flank, allowed to establish vascular connections and then severed (arrow) from the rest of the arm; when properly innervated, such a backward limb may regenerate in a proximal direction but will always form distal structure in doing so.

According to the recently propounded theory of positional information based on the concept of gradients, it is believed that cells at various levels along the proximo-distal (P-D) axis of the limb have the information specifying their position at that level. These have been called positional values. For example, suppose the cells at various levels of a forelimb along P-D axis are considered to have positional values, say from 1 to 10 with 1 specifying the position at the base near the girdle and 10 the position at the top of the digits and position 5 falling below the elbow in the lower arm. If amputation is made across the level 5, the cells of blastema formed at this level are able to generate the missing positional values 6 to 10 i.e. distal to the level of amputation but not the values 1-5 proximal to that level. Only the missing positional values are supposed to be regenerated in the blastema. The formation of parts only distal to the level of amputation is thus sought to be explained in terms of positional values along the proximo distal axis of the limb.

Experiments have also indicated that the mechanisms which regulate regeneration in tetrapod limbs may also, be the same which regulate pattern formation during development. It has been experimentally seen that not only does regeneration mimic embryonic limb development, but both regenerating and developing limb tissues can also interact with each other to form a normal limb. Muneska and Bryant 1982 (Fig.19.8 a) obtained a normal limb in salamander when they grafted limb buds from a salamander tadpole onto a regenerating blastema stump so that their axis were properly aligned. However, supernumerary digits resulted if the axis of the regenerating blastema and stump were misaligned (Fig. 19.8 b). It is hoped that studies on developing and regeneration systems may explain the control of pattern in both cases, and furthermore outline, common principles in pattern formation in other developing systems.

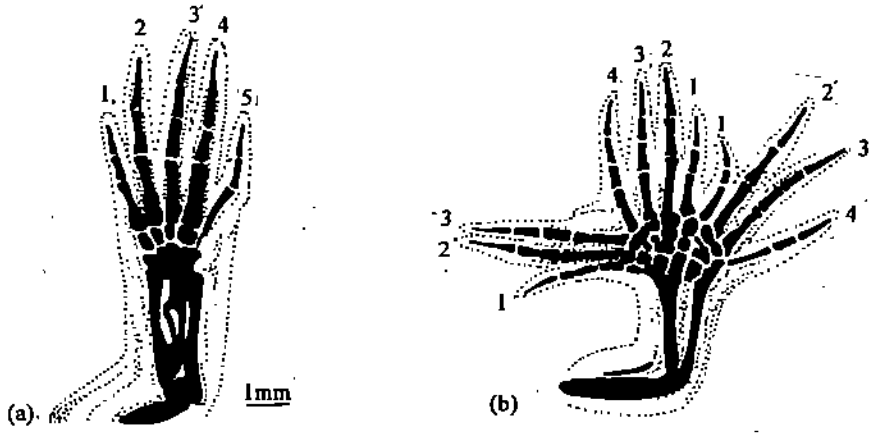

Fig. 19.8: Regenerating and developing limbs may follow the same positional cues (a). Grafting a limb bud onto a regenerating limb stump results in a normal limb (b). If the limb bud is rotated and reattached to a regenerating limb stump so that the anteroposterior axes are reversed, supernumerary digits form as they do when the same experiment is performed with regenerating blastemas and stumps only. (Muneska and Bryant, 1982. from Nature)

It has been found, however, that the rule of distal transformation of blastema can be subverted if the amputated limbs of urodeles and anuran tadpoles are treated with a suitable amount of any of the derivatives of vitamin A, collectively known as retinoids, including vitamin A palmitate, Vitamin A alcohol, retinoic acid etc. During 1970s while studying regeneration of amputated limbs in anuran tadpoles I.A. Niazi and his coworkers at the Zoology Department of Rajasthan University, Jaipur, found that when vitamin A palmitate (a retinoid) was added to the water in which the tadpoles were kept, the regenerated part formed was not only the distal part that had been removed by amputation; instead, in many cases complete limbs regenerated consisting of parts both distal as well as proximal to the level of amputation. This showed that the restriction on the blastema to form only distal structures was removed by the retinoid. In many cases more than one such limb (mirror images of each other) regenerated from the same stump. This was the case at whatever level the tadpole limb was amputated (Fig. 19.9).

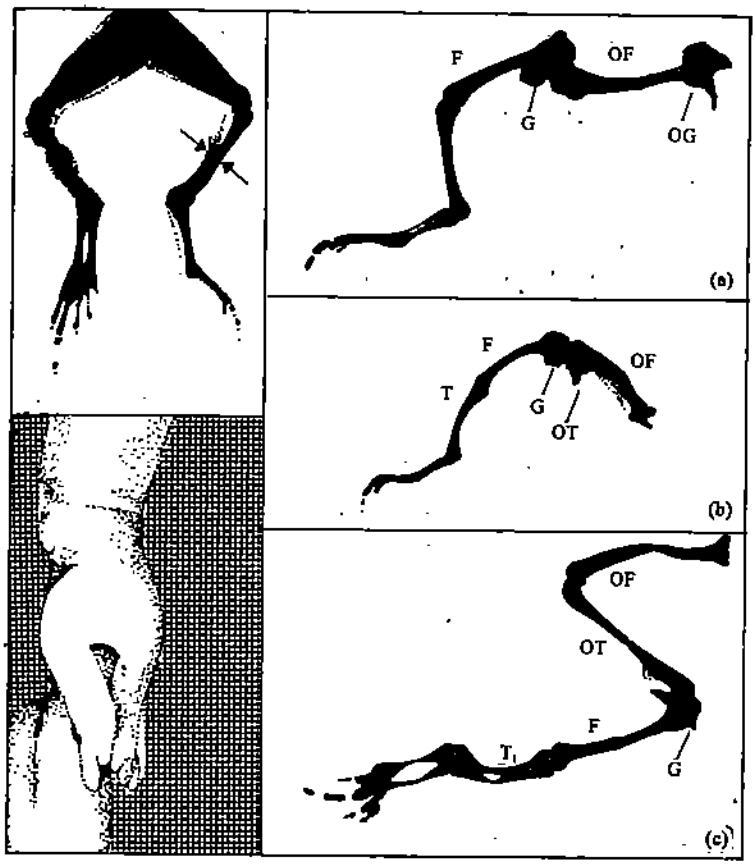

Fig. 19.9 : Vitamin A (Retinoid) breaks the rule of distal transformation in limb regenerates of *Bufo* tadpoles. Effect of retinoid on pattern of limb regeneration. After amputation of hindlimbs just below the knee (a), across midshank (b) and proximal ankle region (c) the *Bufo* tadpoles were placed in vitamin A palmitate solution for 3 days. In all cases regenerates had duplicated proximal structures: an additional girdle (G), an additional femur (F) and an additional tibiofibula (T) in b and c. (OF. OT, OG are original femur. I. A Niazi: Regeneration studies in India in Developmental Biology: An Afro-Asian perspective (1983). Eds. S.C. Goel and Ruth Bellairs.

Subsequently Maden (1982) and others used this path-breaking research on limb regeneration in the axolotl and other urodele as well as in anuran species and also obtained similar results They found that if the limb of adult urodeles was amputated and then the animals were treated with retinoic acid or other retinoids the regenerated limb had a duplication of stump elements. No matter at what level the limb was cut, a complete limb would grow out from the stump. For instance, if the limb was severed through the wrist, a full limb with humerus, radius, ulna, wrist, and hand would grow out distal to the amputation plane (Fig. 19.10). How much of the structures proximal to amputation level could be regenerated by the blastema of the retinoid treated amphibians depended on the type and level of concentration of the retinoid used and on the duration of treatment (Fig. 19.10). Retinoic acid is found to be so far the most potent among the retinoids in producing this effect on amphibians limb regeneration.

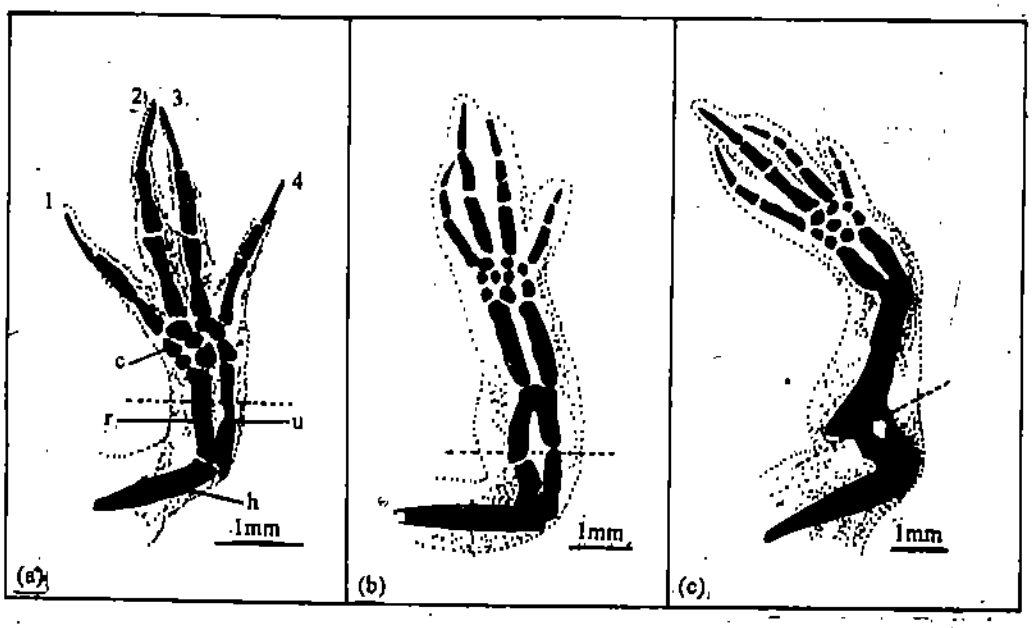

Fig. 19.10: Regeneration studies of axolotl limbs amputated through the radius and ulna and treated with increasing concentrations of retionol palmitate, (a derivative of vitamin A). The dotted lines indicate the amputation plane. (a) Control showing the replacement of the radius (r), ulna (u), carpals at (c) and 4 digits, h: humerus. (b) A medium dose that results in the radius and ulna being replaced, followed by a duplicated radius and ulna then wrist and digits. (c) At high doses, a complete limb beginning, at the humerus emerges from the amputation plane.

This dramatic effect of retinoids on the pattern of limb regeneration indicates that the retinoids apparently resets the positional information of the cells in the blastema to a more proximal value so that the cells that would form distal structures are reprogrammed to form proximal structures as well. In other words, the genetic programme or the developmental potential of the blastema cells involved in regeneration is changed by the retinoids. This effect is now referred to as **proximalization of the blastema.** See box 19.1

Box 19.1: tadpole cells forget to grow into a tail

More recently in 1990s P Mohanty-Hejmadi and her co-researchers at Zoology Department of Utkal University, Bhubaneswar obtained very surprising results when they placed young tadpoles of a frog species after tail amputation in Vitamin A palmitate solution for one to six days. In several cases complete multiple hind limbs regenerated at the cut surface of the tail, far away from the site of the hind limbs which themselves were not amputated at all.

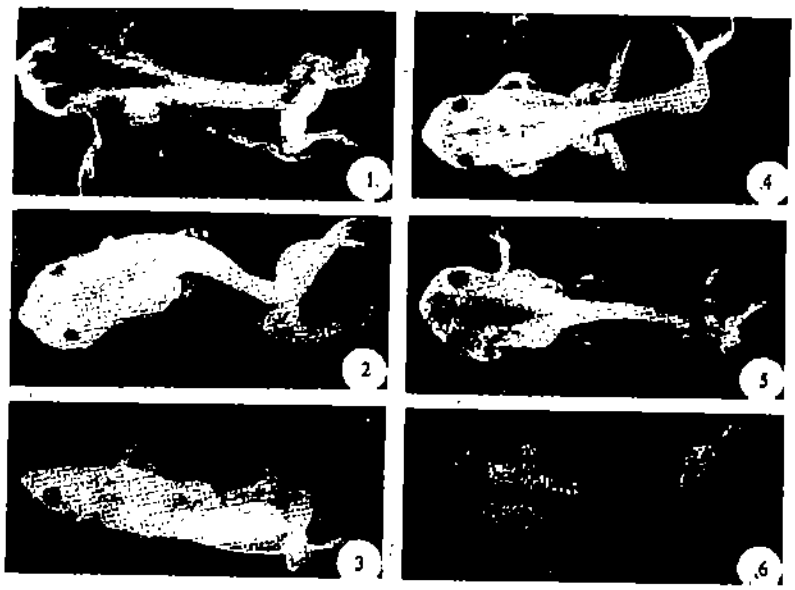

Fig. 19.11 : Experiments show that when tadpole tails were amputated and bathed in Vitamin A, they grew into a variety of hind limbs. Scientists are trying to unravel this mysterious cell amnesia (Experiments by Utkal university scientists.).

So far retinoids are the only known external agent that can reset the pattern of development during embryogenesis and regeneration and change the fate and developmental potential of the cells. The effect of retinoic acid (or any other retinoid) at the cellular level has so far not been understood. However, its importance in understanding pattern formation is very apparent, since it produces somewhat similar response in developing wings of chick embryos and regenerating limbs of amhibians. It is interesting to realise that if the effect of the retinoic acid are determined at the cellular level, then embryologists will have an important lead into what mechanism normally regulates the pattern of limbs in both development as well as regeneration.

19.4.8 Limb regeneration in anuran amphibians

You are already aware that the limb of adult frogs do not regenerate after amputation. However some regeneration has been found to occur in experimental animals. Recovery of regeneration ability in most metamorphic frogs is made possible by tissue trauma and prevention of wound healing. This is achieved by extensive piercing of the amphibian amputaton site with a needle as well as traumatization by using hypertonic solutions. Normally wound healing in adult anurans is brought about by the movement of the epidermis and the dermis over the wound surface. In contrast only the epidermis covers the wound in the urodeles. Normally when a part of the adult anuran is amputated the wound heals by the production of connective tissue from dermal elements(dermalization) and the scarring of injured tissues. Continued injury in some way interferes with dermalization and allows a variable amount of regeneration to occur. Similar results of regeneration have been obtained by implanting batteries and applying a continuous direct current through fine wire to the amputation area. Superficially this appears to stimulate action of the nerves. Regeneration also sometimes results from irritation. Implanting of adrenal glands also appears to prevent dermalization. It has been observed that if larval skin is applied over adult frogs then regeneration becomes possible. Other studies have implicated the wound epithelium as an important factor in initiating regeneration.

SAQ 4

What will happen if

a) the nerve supplying the leg of the newt is destroyed at the time of amputation?

b) the blastema of regenerating leg of newt is exposed to x-rays?

19.5 LENS REGENERATION IN AMPHIBIANS

It is an amazing fact that among all vertebrates only certain amphibians have the unique ability to regenerate lens, iris and retina in adult life. The amphibians also vary in their ability to regenerate a lens and in the source of cells from which a new lens is formed. Members of sub-order salamandroidae (*salamander, Triturus*) have the remarkable ability of growing a new lens from the dorsal iris epithelium (neural ectoderm). This is a unique example of metaplasia, as the regenerating iris is derived from neural ectoderm and may be regarded as an extension of the brain, while the original lens is derived from the epidermal ectoderm. This type of regeneration has demonstrated that even adult differentiated cells can retain their potential to produce other cell types. In the larvae of the South African frog, *Xenopus laevis* an invagination of corneal epithelium gives rise to a ball of cells which transforms into the new lens. The process of the new lens regeneration in *X.* laevis more closely duplicates the embryonic process, as both the first lens and the regenerate lens develop from cells of epidermal ectodermal origin, However , even so it is a case of metaplasia, since differentiated cells of corneal epithelium give rise to lens cells which are morphologically and biochemically very different from those of the corneal epithelium. The regenerating capacity in this anuran however species disappears at metamorphosis.

19.5.1 The process of lens regeneration from dorsal iris in *Notophthalmus viridescens*

Wolffian regeneration, that is, regeneration of the lens from the dorsal iris has been more extensively studied than any other type of lens regeneration. Let us briefly study how this type of regeneration occurs in *Notophthalmus virridescence* (*Triturus*) when the lens is removed. For understanding the process of this regeneration refer to the process of eye development given in Unit 17 of this course).

When the eye is lensectomized (lens is removed) in *Triturus viridescens* now called *Noto phthalmus viridescens* regeneration of lens occurs in 13 recognizable stages which, for convenience, can be grouped into 4 periods listed below (Fig. 19.12):

Period - I days 0-5; Latent period; stages 1, 2.

Period - II days 6-9; Initiation of regeneration (dedifferentiation); stages 3-6.

Period - III days 10-18; Primary and secondary lens fibre formation ; stages 7-11.

Period- IV days 20-30; Growth of regenerated lens; stages 12-13.

The first five days after lens removal constitute the latent period. Around the 4th day the palillary margin of the dorsal iris swells, and so a slight thickening of the dorsal iris becomes visible; and by the 5th day, a cleft appears between the two epithelial layers of the iris (Fig. 19.12 a). The period from 6th to 9th day is marked by the process of dedifferentiation of the cells of the margin of the dorsal iris. During this period pigment disappears from these cells and their nuclei enlarge. Around the 7th day the dorsal margin of the iris assumes the shape of a small hollow vesicle lined by cuboidal cells (Fig. 19.12 b v). On about 8-9 days, the cells of the posterior wall of the vesicle elongate. By the 9th day the growing cells cause the posterior layer to bulge into the vitreous chamber of the eye and so for the first time the acidophiljc lens fibrils become detectable in these cells (Fig. 19.2 b VI).

Some of the changes observed histologically in the cells during the process of dedifferentiation are notable. Electron microscopy has clarified the process of depigmentation showing, that the pigment granules are extruded from the cells and are taken up by macrophages. The cytoplasmic basophilia increases and RNA synthesis also increases and reaches a peak at about 5-7 days. Furthermore, a marked increase in new protein synthesis is noted by the 8th day. The cells begin to divide, and studies have also shown that the proliferating cells are relatively undifferentiated in appearance. During the days 10-18 of period III redifferentiation and morphogenesis of the new lens occurs. Around the 10th and 11th day, the posterior cells enlarge to form a definite primary lens nucleus. (Fig. 19.12 c VII,VII, X), and soon thereafter the primary lens nucleus obliterates the lumen of the vesicle. On the 12th day, the secondary lens fibers begin to develop from the equatorial zone of the vesicle. At about 18th day the new lens detaches from the iris.

Between stages 9-11 (day 12-18) there is a rapid increase in leucine uptake and the bulk of lens proteins are laid down in the cytoplasm of cells of the regenerating lens. During days 20-30 (stages 12, 13) the regenerated lens continues to grow and by the 30th day it has grown to almost full size. The nuclei of the lens fibers lose their basophilic staining characteristics and become filled with lens protein.

Interaction of Tissues during Lens Regeneration:

It has been experimentally proved that the presence of a lens in the eye inhibits the formation of another lens. For the lens to regenerate from the dorsal iris the immediate stimulus is provided by the removal of the lens.

Several experiments have also indicated that the neural retina is the source of a stimulus (retinal factor) essential for lens regeneration. It has been observed that when the iris is transplanted alone in a foreign site, then no lens regeneration occurs. However, if it is transplanted along with the retina then a lens is regenerated.

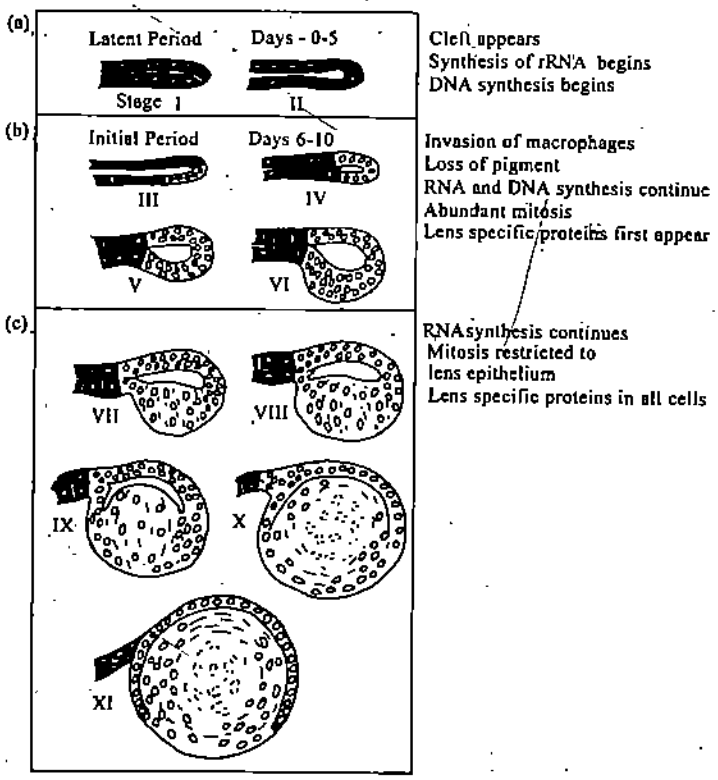

Fig 19.12: Morphological changes and changing patterns of macromolecular synthesis during regeneration of the lens from the dorsal margin of the iris in urodeles.

SAQ 5

What stimulates regeneration of lens in *Notophthalmus viridescens*

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Regeneration in Hydra

You already know that the Hydra has spectacular regenerative ability. The hydra is a small tubular, two layered fresh water animal measuring 20mm in length (Fig. 19.13). Structurally the *Hydra* has a head or hypostome at the top end, consisting of a mouth, surrounded by a ring of about six large tentacles which bear a number of stinging cells called cnidoblasts. Towards the posterior end, the hydra has a stalk or penduncle ending in a broad base called pedal or basal disc or foot. The creature attaches itself to the substratum by means of the pedal disc. Close to the base is the budding region from where the asexual buds arise. The stomach or gastric region where most of the digestion occurs is located between the hypostome and the budding region. The body wall of the *Hydra* consists of two concentric epithelial layers a) epidermis derived from *the ectoderm* (external layer), and b) gastrodermis derived from the endoderm (internal layer), surrounding a central gastric cavity. The epidermis and gastrodermis are separated by an acellular matrix called mesogle

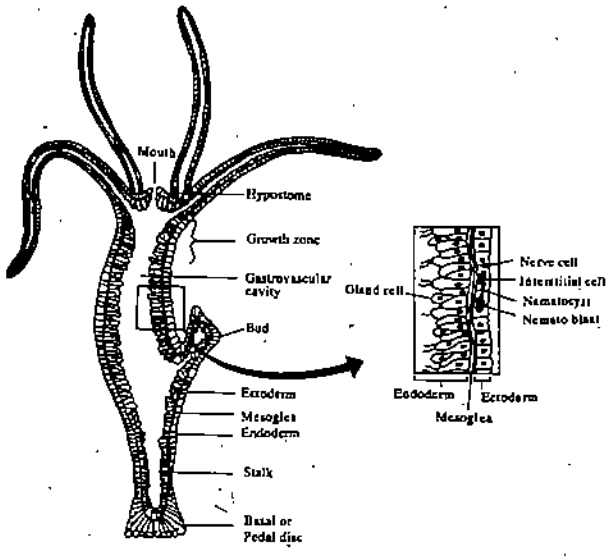

Fig. 19.13: Anatomy of Hydra

19.6.1 Process of regeneration

It has been observed that when the hydra is cut transversely through the gastric region, the proximal part gives rise from its distal cut surface to a new hypostome with mouth and tentacles, and the distal portion regenerates the basal end from its proximal cut surface. Furthermore, it is also observed that if the hydra is transversely sliced into annular segments, then each segment gives rise to a hypostome distally (anteriorly) and a basal part proximally (posteriorly). (Fig.19.14)

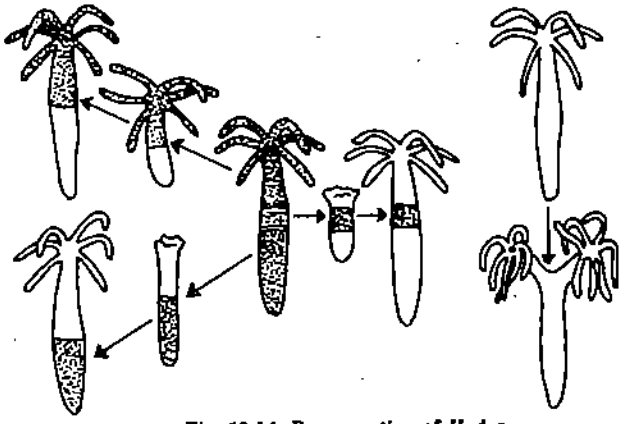

Fig. 19.14: Regeneration of *Hydra*

Sometimes however, it has been noticed that a short segment, cut just below the hypostome, will regenerate a hypostome with mouth and ring of tentacles at both ends, giving rise to a 'bipolar hydra' (Fig.19.15)

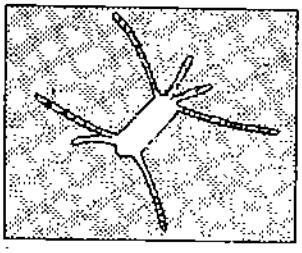

Fig. 19.15: A *Hydra* showing bipolar regeneration. Regenerate formed from a small central segment or annule, shows hypostome and tentacles at each end.

Even in the normal course of life hydroid polyps are in a `continuous state of regeneration'. The cells comprising the tentacles and hypostome and the basal pedal disc get continuously worn out, discarded and replaced by cells shifting distally and proximally from an area called the growth zone, located below the ring of tentacles. The growth zone is easily identified by the presence of large number of interstitial cells (Fig.19.13). In *Hydra*, thus there is a steady migration of cells from the middle of the body in both direction to the hypostome and tentacles and to the penduncle and foot.

The interstitial cells are small undifferentiated cells with basophilic cytoplasm and relatively large nucleus. These interstitial cells act as a pool of undifferentiated cells and are capable of being transformed into all the various cell types (epidermal, endodermal, nerve cells, germ cells, cnidoblasts etc). Maintenance of life of hydra is effected by a continuous supply of undifferentiated interstitial cells from the growth zone to replace the worn out and discarded cells at the basal as well as the hypostomal and tentacular ends of the body, where they differentiate into appropriate cell types.

Let us study how exactly does the Hydra regenerate when it is cut transversely. The process of regeneration is 'morphallactic' and blastema formation does not occur.

Regeneration process begins with the closure of the wound by contraction of the epithelio-muscular cells of the epidermis. After this the damaged surface is covered by ectoderm which forms a sheet of epithelial cells. In the next few hours more than the usual number of cells are given off from the growth zone towards the cut surface of the damaged area, which may be towards the oral or the aboral side of the body. Regeneration of each piece will occur to form complete miniature hydras even in complete absence of cell division in either piece.

19.6.2 Source of cells for regeneration

It had been thought earlier that interstitial cells constitute a reserve of undifferentiated cells which provide the cellular material for regeneration in hydra by mitotic divisions. However recent studies have shown that cell division in the interstitial cells can be prevented by nitrogen mustard or irradiations, and still regeneration occurs. The cells then taking part are the differentiated cells of epidermis and gastrodermis which, following amputational injury lose their specialized features, dedifferentiate and are then reorganized or moulded to from the missing structures, the hypostome, mouth, tentacles or the pedal disc as the case may be. Interstitial cells are not essential for regeneration, which occurs even when they are put out of action. However, normally, the interstitial cells also participate in morphallactic regeneration in Hydra. The ectodermal and endodermal cells belong to two other self-renewing lineages. Studies done by Haynes and Burnett (1963) have shown that in hydras like *Pelmatohydra oligactis* and *Hydra viridis*, the endoderm alone can regenerate an entire animal. In this type of regeneration, the piece of endodermal cells of hydra first lose their differentiated features and then redifferentiate into ectoderm, insterstitial cells

and endodermal components of the regenerated part.

19.6.3 Problem of Polarity

The control of polarity in regenerating coelentrates such as hydra has received much attention for many years. You are already aware that when a hydra is cut in half, the half with the basal disc will form a new hypostome and the half containing the hypostome will generate a new basal disc. Furthermore, if a hydra is cut into several segments perpendicular to the body axis, then each middle segment will regenerate both a basal disc and a hypostome. Thus every region of the hydra can give rise to a new organism (Fig. 19.14). Yet hypostomes, as well foot do not form anywhere or at all levels along the longitudinal axis of the animal; they form only at the distal end. This indicates that a series of gradients arise from the two poles in *Hydra* which is rigidly polarized along the distal proximal axis. Grafting experiments have provided further evidence for the existence of gradients in the hydra. (Fig. 19.16) When the hypostome tissue was added to the middle region of another hydra it formed a new bud with hypostome extending outward. When the basal disc cells were similarly grafted the new bud extended to form a new pedal disc and foot. Furthermore if grafts of both basal disc and hypostome cells were grafted together to the gastric region no supernumerary parts formed. This observation suggests that signals from opposite ends of the hydra tend to counteract each other, causing the hydra to lose its polarity. Other experiments have also shown that normal regeneration of the hypostome can be inhibited when an intact hypostome is grafted into the body of the hydra.

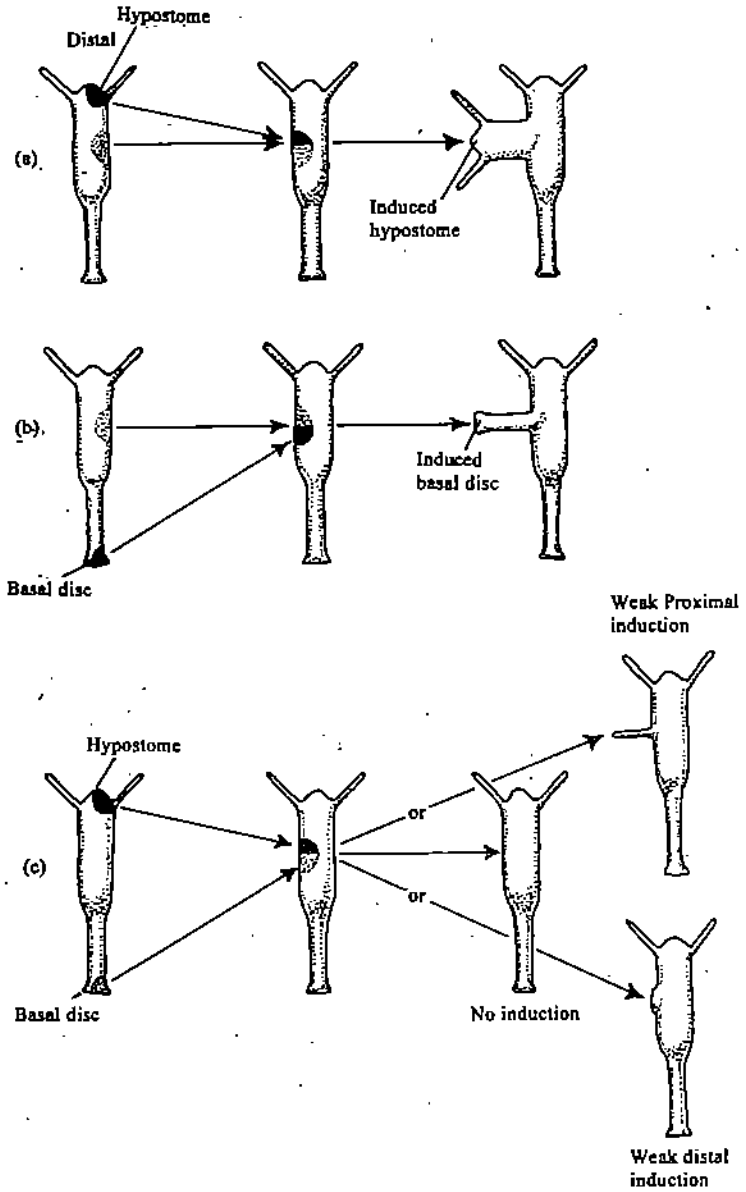

Fig. 19.16: **Evidence for gradients in *Hydra*. (a) Hypostome plus midhydra regions grafted laterally to the host's middle region cause distal (hypostome) induction. (b) Basal disc-plus midhydra regions grafted laterally to the host's middle region cause proximal (basal disc) induction. (c) Hypostome plus basal disc region grafted laterally cause little or no induction with no distinct polarity. (After Newman,1974.)**

These experiments and other studies have resulted in the identification of two opposing sets of gradients arising from the two opposing poles of Hydra—(1) Head activating and head inhibiting gradients (2) Foot activating and foot inhibiting gradients. Morphogenetic signalling in hydra is apparently the function of substances of relatively small molecular weights that are produced at the opposite ends of the animals.

So far four distinct morphogenetic substances of relatively small molecular weight have been isolated from hydra, which affect regenerative gradient when added to hydra culture medium in micromolar quantities. The first and the best known head activator is a peptide which accelerates regeneration of hypostome and tentacles and promotes budding. It has been observed that annular segments of hydra placed in culture medium containing this activator give rise to bipolar regenerates, and other strange forms. This is because the polarity is disturbed by the activator. The distribution of the activator in the hydra body paralleles the distribution of nerve cells which are more abundant in the hypostome and less proximally, except for a slight increase in the basal region.

The head inhibitor was described by Berking in 1977. Its distribution in the animal also parallels that of the head activator but it has an opposite effect. It diminishes the rate of regeneration of the hypostome and tentacles and retards bud formation. In an intact head or hypostome of hydra the presence of this inhibitor prevents formation of additional heads.

A foot activator and a foot inhibitor accelerate and retard respectively, The rate of regeneration of the basal region of the hydra . The concentration of both the substances diminishes sharply distally to the base. The foot activator like the head activator is a peptide. However the foot inhibitor is not a peptide and has not been further characterized, except that it appears to have approximately the same molecular weight as the activator. O The fact that these head and foot activators and inhibitors are small molecules and their distribution as shown for the head factors, parallels the distribution of nerves suggests that the activating and inhibitory factors are neurohormones. If this is so then it would be reasonable to presume that elimination of nerve cells could affect maintenance of polarity and polarized regeneration. Experiments have shown, however, that nerve-free animals maintain their polarity, grow and produce buds at a normal rate. They also regenerate in a normally polarized manner. The question as to whether nerve-free hydrs contain the head and foot activators was examined by Schaller et al 1980. Their experiments have shown that in nerve-free hydra the specific activity of head activator is 8.3 times as great as that of normal hydra. Specific activities of head inhibitor and foot activator of the nerve-free animals were respectively 3.7 and 1.8 times greater in the non innervated animals, but the foot inhibitor showed only 80% of the specific activity of the normal hydra. These results thus show that nerve-free hydra contain the same morphogenetic substances as the normal hydra. In nerve free hydra these substances obviously must be made in the epithelial cells. Yet in normal hydra it is quite certain that essentially all of the head activators are in the nerve cells. These findings raise a number of so far unanswered questions. (1) Do nerve cells in the normed animal inhibit the production of morphogens by epithelial cells? (2) Do nerve cells in normal animals store morphogenetic substances produced by epithelial cell?

SAQ 6

Describe an experiment which reveals that interstitial cells in Hydra are not essential for regeneration.

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19.7 REGENERATION IN PLANARIANS

Among flatworms, planarians have unique regenerative powers. Planarians may be cut across or lengthwise and each part will regenerate the missing part. An entire planarian can be regenerated from a small transversely sectioned part of the body. If the anterior end of a planarian is cut along the length into two or more parts each part develops into a new head giving rise to a many headed animal (Fig. 19.17).

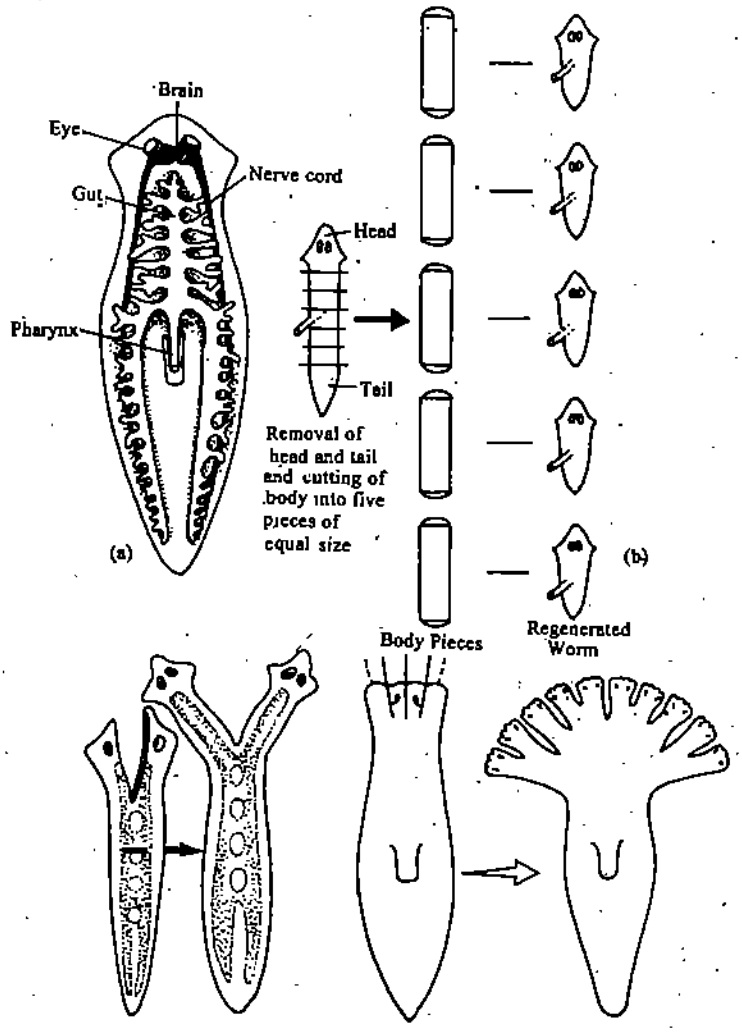

Fig. 19.17: A Planaria and its regeneration (a) generalized body plan of Planaria (b) various regenerating possibilities in Planaria.

19.7.1 Process of regeneration

Regeneration in planarians has been extensively studied and considerable information regarding this phenomenon is now available. Let us see what this tells us about how planarians regenerate (Fig. 19.18). Consider a planaria whose head is cut. The wound formed is initially covered by the epidermis of the skin. The cells move tangentially over the wound surface without proliferating. This process occurs in the first twenty four hours after the cut. Next a blastema is formed below the epidermis.

Regeneration involves a combination of morphallactic and epimorphic events as it occurs at either cut end, not only by migration of coherent sheets as in coelentrates, but also by proliferation of undifferentiated cells of the blastema. The head and tail blastema are both determined from the start. The head blastema forms the head and the tail blastema the tail. The blastemal cells also give rise to most of the regenerating organs: the connective tissues, the pharynx, the nervous system, the muscles and even the reproductive organs. The skin epithelium also arises from the skin at the edge of the wound. It is believed that the cut edge of the old intestine gives rise to the intestine in the regenerate.

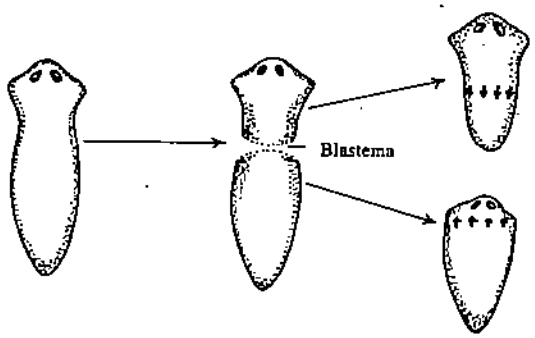

Fig. 19.18: Blastema formation in Planaria.

19.7.2 Origin of Blastemal Cells or Regenerative Cells

For many years regeneration of the missing parts in planarians was believed to be due to neoblast cells. These cells, which are distributed throughout the body, are distinguished mainly by an abundance of cytoplasmic RNA in them. These cells seem to accumulate at the wound surface after an amputation and appear to form a regenerating blastema which proliferates and then forms the missing part.

The nature and source of the neoblast has aroused some controversy, which has not been resolved so far. Muscle dedifferentiation during regeneration has also been observed in planarian regeneration by some workers. While Hay and Coward (1975) on the basis of electron microscopic studies on neoblasts in planarians have concluded that the neoblasts are in reality gland cells and not undifferentiated cells, as earlier studies with light microscope indicated. These cells have a prominent juxta nuclear Golgi zone and an elaborate endoplasmic reticulum as expected in gland cells. Hay's and Howard's further electron microscopic studies have also shown the presence of very small cells called beta cells which earlier under the light microscope were poorly visible and resolved. These cells occur in the parenchyma surrounding the various glandular, muscular and digestive tissues, and have the characteristic of undifferentiated cells.

The Beta cells have a nucles with small chromatic clump and no nucleolus, while their cytoplasm has ribosomes but lacks endoplasmic reticulum. Transitional stages between beta and various differentiated cellular types have also been found. These findings have indicated that the beta cells constitute progenitor cells for various tissue types with which they are associated, in the same way as cells of the germinal layer of the skin are progenitor cells for the keratinizing cells or cells of the intestinal crypts are the progenitors of mucosal cells. Thus it appears that beta cells would not necessarily be totipoent. A number of studies of planarian regeneration have however still not fully solved the origin of the regenerative cells which may to the neoblasts or

the B cells however and the mechanism of regeneration in these animals. some graft experiments have been conducted to see whether the regenerative cells are of local origin or capable of migration. Such studies show that if an animal is irradiated by x-rays prior to wounding then its regenerating ability is inhibited. However this can be restored by a graft of healthy (Fig. 19.19). It has also been observed that if in the irradiated planaria with a healthy graft an amputation is made at a site remote from the graft, then regeneration occurs, usually after a period of delay which is apparently needed for migration and proliferation of the regenerative cells from the graft to the site of the wound. This indicates that the regeneative cells are capable of long distance migration and the formation of blastema on the cut surface.

19.7.3 Problem of Polarity

As in Hydra, flatworm regeneration also appears to occur in a polar fashion. There seems to be an anterior-posterior gradient system as the cut anterior surface will form a head and the cut posterior surface will form a head. As in *Hydra*, middle pieces are capable of regenerating both a head and a tail. Similar to the Hydra there is an exception to this rule, for if the middle piece is too thin then the planarian segment will grow two heads. It is believed that the anterior-posterior gradient is not sufficiently delineated in such a small segment and that the piece cannot determine "Which end is up", as neither end of the segment has an advantage over the other. Evidence exists that the polarity gradient is due to metabolic activity and that the more metabolically active end forms the head. Two-headed flatworms (Fig. 19.20) are thus produced by removing anterior posterior metabolic gradient. It has also been observed (Fig. 19.17) that flatworms in addition to retaining anterior-posterior polarity

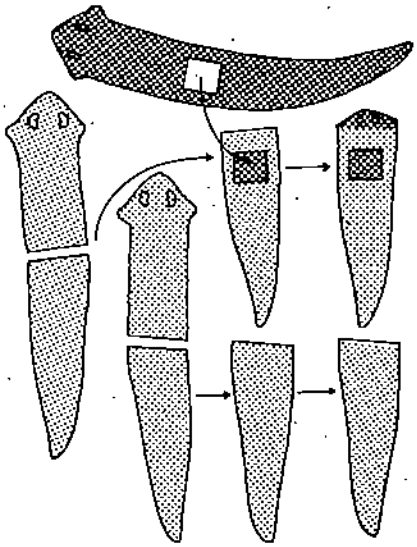

Fig 19.19: Restoration of regenerative power to an x-irradiated flatworm. An irradiated flatworm that receives a tissues graft from a non-irradiated animal can regenerate even if the graft is far from the wound site. Irradiated planarian without the graft of a non irradiated animal are unable to regenerate.

can also regenerate the missing half if split longitudinally. It is believed that the pattern of Planaria regeneration is similar to hydra as it also controlled by diffusible gradients.

19.7.4 Induction phenomenon

The presence of the old tissue in the planarians makes regeneration a more complicated process than embryogenesis. The remaining tissues may influence regeneration in an inductive or inhibitory manner. Their influence may be specific upon one tissue or may be general Fig. 19.21

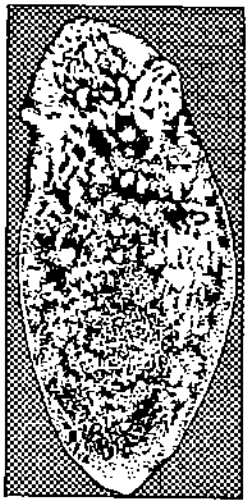

Fig. 19.20: Two headed planaria

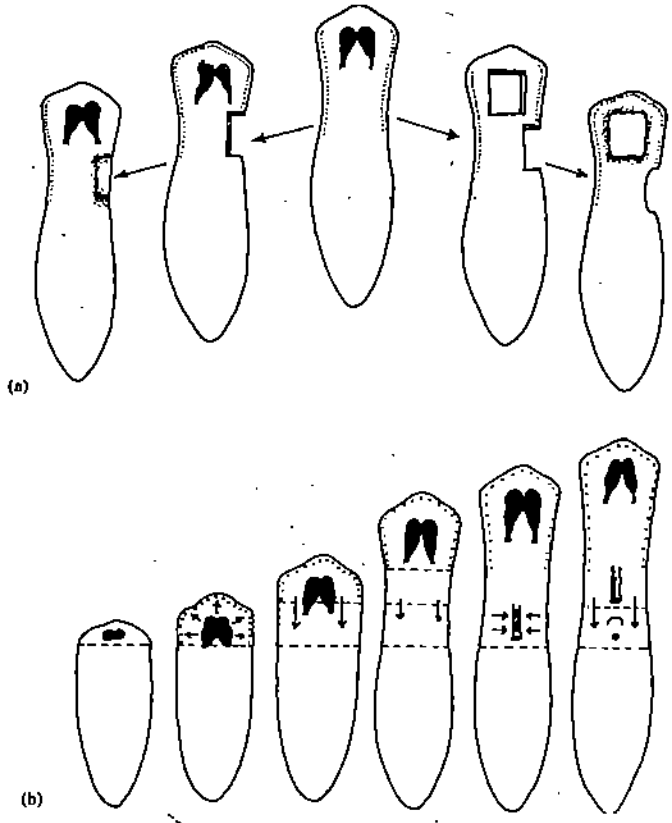

Fig. 19.21: Inductive phenoenon in Planarians (a) The numerous eyes of *Polycelis nigra* will regenerate following their excision provided the brain is intact (left); the eyes do not regenerate in the brain (right) (b) Sequence of inductive relationship (arrow) in regenerating flatworm *(Polycelis nigra)*. From left to right, the brain is the first organ to develop, which is in turn responsible for eye differentiation. The head thus formed then induces a prepharyngeal region behind it and this brings about the development of the pharyngeal region farther back. The pharyngeal region induces the formation of a pharynx medially and reproductive organs posteriorly.

Studies have shown that old tissues do influence the formation of new tissues, particularly in planaria. You have already read in detail about the phenomenon of induction in Block 3 unit 13 of this course. Several experiments have shown that the brain appears to have the capacity to induce the formation of the eye from a distance by means of chemical substances. If the eyes are removed they will regenerate again. However if the eyes and brain are removed simultaneously, the eyes will not reappear in the absence of the brain (Fig. 19.21a). The head thus formed induces the formation of the pharyngeal region, which then induces the formation of the pharyngeal zone which produces the pharynx medially and the reproductive organs posteriorly. The formation of additional brains and pharynx in planarians are prevented by inhibitory substances produced from the brain.

In brief the brain appears to act as initiator which subsequently influences the development of other parts of the blastema into various organs in a stepwise fashion during regeneration. This stepwise determination also includes a negative influence on developmental potential in the various regions of the blastema fig. 19.21b.

SAQ 7

Describe an experiment which shows that in planaria blastema is formed by migratory cells from distant parts of the body.

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19.8 COMPARISON BETWEEN REGENERATION AND EMBRYONIC DEVELOPMENT

By now you must have realised that the processes of regeneration and embryonic development have many fundamental similarities. Both require an external stimulus to begin; it is the penetration of the sperm into the egg (fertilization) in the case of embryogenesis and injury (accidental or intentional) in the case of regeneration. Both phenomena involve basically similar cellular activities including cell division, cell movements, tissue interactions, induction, progressive determination, morphogenesis, histogenesis, cytodifferentiation and growth.

However, there are important differences between regeneration and embryonic development. Regeneration in multicellular animals does not begin from a totipotent single egg cell but from a group of cells recruited from the tissues of the functioning body of an organism. These cells may be derived by dedifferentiation of already differentiated cells of various tissues of the residual part of the body or organ, or mobilized from some reserve of undifferentiated cells, if any such reserve is maintained by the organism for use in emergency. Cells participating in regeneration may not be even pluripotent if no reserve of undifferentiated cells is available. Regeneration may involve initial growth by proliferation of these cells to form a bud or blastema, or it may involve remodelling of the lost parts by cells of existing tissues or reserves without involving proliferation. Moreover, the process of regeneration is influenced by the systemic factors (nerves, hormones) and physiological conditions of the organism, whereas embryonic development is free from such influences.

At a fundamental level both regeneration and embryonic development are epigenetic processes, involving gene expression and programming. The knowledge of regenerative processes can, therefore, be very rewarding for a better understanding of the developmental processes during embryogenesis.

Regeneration studies help in answering the following questions among others. How stable is the differentiated state of a cell? Are any or all differentiated cells capable of dedifferentiating and then redifferentiating in a new pathway *(metaplasia)*? Or do differentiated cells fluctuate only between an overtly differentiated phenotype and a dedifferentiated but determined state (modulation)? Do cells dureing embryonic development get irreversibly committed into germinal or somatic cell lines? Is there any interaction between various tissues or organs of an adult leading to maintenance of their integrity or size.

19.9 A SURVEY OF REGENERATIVE CAPACITY IN THE ANIMAL KINGDOM

The ability to regenerate lost structures is found in essentially all living things, at least to some extent or degree. This process, however, is developed to a greater degree in invertebrate animals such as sponges, coelenterates, flat-worms, annelids and tunicates. Many of them routinely practice asexual reproduction from fragments of the body. Among vertebrates in the larval and adult stages with the exception of urodele amphibians, whose regenerative capacity is extraordinary only a limited ability to regenerate major body parts is present in cylcostomes, fishes, anuran amphibians and reptiles. Very little regenerative capacity is found in birds and mammals.

Some animals have attracted specially keen interest of investigator, for they are easy to work with and provide good and suitable material for investigations on many different problems and mechanisms of development during regeneration. These include some ciliates among protozoans; sponges among porifera; hydra, tubularia and Obelia among coelentrates; flatworms (Planaria) among Platyhelminthes; Nemerteans; polychaetes and some oligochaets among annelids (*Clymenella, Sabella,* earthworm); insects (cockroaches), crustraceans (lobster, crabs); among arthropodes, colonial tunicates (*Clavelina, Perophora*) larval lampreys, fishes, amphibians and lizards among vertebrates.

Let us see in greater detail the regenerative ability in various animal groups.

19.9.1 Regeneration in Invertebrates

(i) **Protozoans:** Most single celled animals like protists, i.e. protozoans, regenerate very well. If part of the cytoplasm is removed from the amoeba it is readily replaced. A similar process occurs in flagellates and ciliates. In each instance, however, regeneration occurs only from that fragment of the animal (cell) which contains the nucleus.

A protozoan in which regeneration has been extensively studied is *Stentor*. It is a large ciliate organism in which regeneration occurs only if both the cortical cytoplasm and nuclear genome (which is a large beaded macronucleus) are present in the part which is to regenerate. It regenerates in the same way as it reproduces (Fig. 19.22).

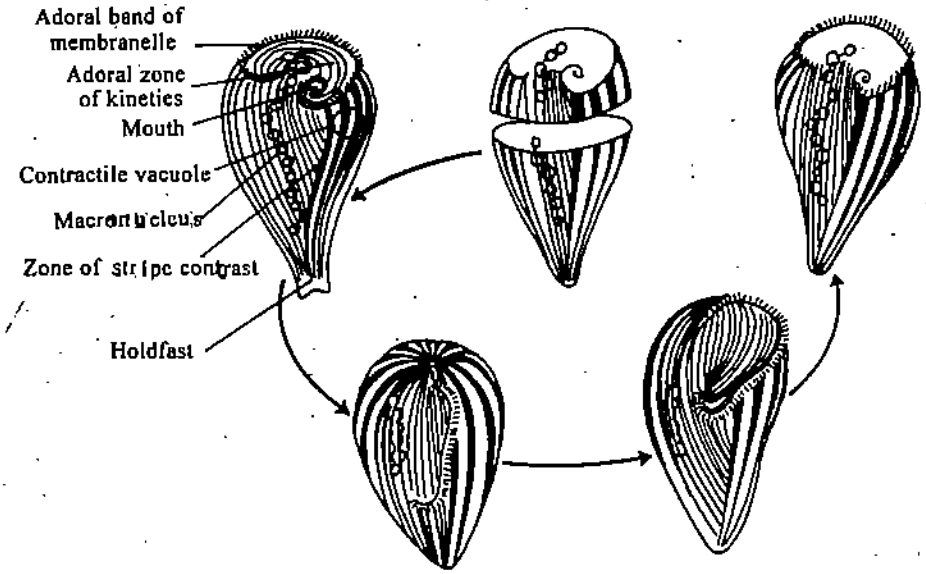

Fig. 19.22: Anterior regeneration by the posterior portion of a bisected stentor. Following closure of the wound, a new oral primordium appears along the zone of stripe contrast. Segments of many thin pigment stripes are subsequently incorported into the new adoral zone. Meanwhile, the macronuclear remnant condenses and renodulates, augmenting its number of lobes as it does so.

For reproduction it first divides transversely and then each half reforms an entire animal. Similarly when *Stentor* is cut transversely into two halves, then the wound at each cut end heals. The anterior part reconstitutes first by regenerating its posterior end. After this the posterior cut end regenerates its anterior portion. Thus two animals are formed each complete with cortical cytoplasm and nuclear genome.

ii) **Porifera:** Sponges have great regenerating ability and show two ways of doing so as shown below. :

(a) Regeneration from small segments—Small fragments of the body parts containing both layers can regenerate into a new sponge. Some sponge use fragmentation, as a regular method of asexual reproduction. In other words, these sponges normally break off their branches to from new and independent sponges.

(b) Reconstitution from isolated cells—The entire sponge organism can also be reconstituted from a few undifferentiated cells (archeocytes). For example, if a mature sponge is squeezed through silk bolting cloth, its cells are separated as though the sponge has been put through a sieve. Furthermore the separated cells are gently stirred in a dish of water to mix them thoroughly, completely disrupting any organization the cells may have. When the stirring is stopped the sponge cells gradually move and form a complete sponge similar to the original one.

Cells of sponges have been shown experimentally to exhibit cell recognition. The cells of sponges of three different species were disaggregated by passing them through bolting silk and then thoroughly mixed into a single mass of cells. Instead of forming a single large mass of cells, each species sorted itself out of the mass of cells and reaggregated with their own sort to form the new sponges, similar to the original ones.

Reaggregation of isolated cells is termed reconstitution and is related to regeneration. It had been first observed in the sponges by V. H. Wilson, 1907 (Fig. 19.23). About 2000 cells are needed to produce a new individual.

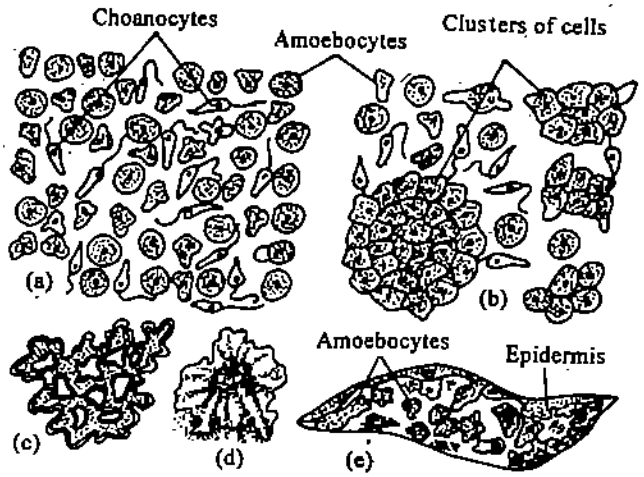

Fig. 19.23: Wilson's experiment of regeneration in sponges. (a) Cells of *Microciona* separated by squeezing living sponge through bolting cloth. (b) Cells aggregating into small masses. (c) A reticulate reunion mass. (d) Later stage forming a young sponge or Spongelet. (e) Section through a stage like d.

iii) **Coelenterates:** The regenerative ability in coelenterates is high in polyoid forms but much reduced in medusoid forms. Hydra, Tubularia and Obelia have been favourite animals for the study of regeneration in coelenterates In hydra, as you know, even a 1/200th part of the body can regenerate to form a complete through miniature individual. The posterior cut end of the hydra regenerates the mouth and tentacles; while the anterior end regenerates the posterior end with foot and adhesive disc, as you have read earlier in section 19.6.

In colonial hydroids such as *Tubularia*, there is a series of branching stems each of which bears a hydranth at its end. If a hydranth is amputated it grows back within a few days (Fig 19.24). In the normal course too, the organisms naturally shed their hydranths from time to time and regenerate new ones. To a great extent the regenerative faculty is similar in both *Hydra* and *Tubularia*. In both cases it involves morphallaxis without the formation of a blastema.

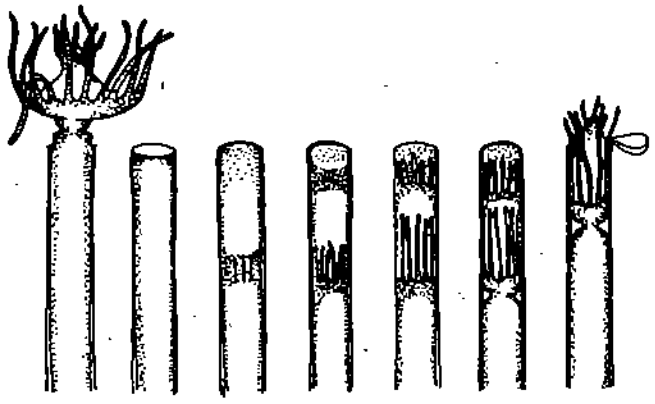

Fig. 19.24: Successive steps in regeneration of *Tubularia*. Following the amputation of the hydranth, new rings of tentacles develop. When fully formed the regenerated hydranth unfolds and pushes out.

iv) **Platyhelminthes (Flatworms):** Among flatworms the turbellarians, (mostly fresh water species and fresh water and terrestrial triclads) routinely reproduce asexually by fragmentation; and most of them have considerable power of regeneration. Their regenerative ability has been investigated by numerous workers, particularly in several species of the planarians (fresh water triclads) such as *Dugesia*. According to some recent studies dedifferentiated cells are apparently the chief source for regeneration of new tissues. But it has been held from the past that there is a reserve of undifferentiated cells, neoblasts, which are the main source of cells for regeneration. The process of regeneration seems to be a combination of morphallaxis and epimorphosis. A distinct physiological gradient exists in the flat worms so that the body is polarized, the anterior (head end) representing one pole and the posterior (tail end) representing the other. Regeneration is correlated with this polarity. Thus in an excized piece the anterior cut surface regenerates the head and the posterior cut surface regenerates a new tail. (Refer section 19.7)

v) **Nemerteans:** Nemerteans also possess remarkable regenerative ability, as even a small fragment can regenerate into a complete worm. Nemerteans; especially the larger species, have a marked tendency to fragment when irritated. Very frequently the proboscis becomes detached when irritaled. The proboscis soon regenerates. Some species usually reproduce by fragmentation, and even posterior sections of the body are capable of regeneration.

vi) **Annelids:** Among the segmented worms both the polychaetes and oligochaetes have considerable powers of regeneration. Leeches totally lack this ability. In polychaetes tentacles, palps, and even heads ripped off by predators are soon replaced. This is of common occurrence in burrowers and tube dwellers. Some worms also display self-amputation or autotomy. For example, the posterior segments are detached when the worm is disturbed and they are regenerated soon thereafter.

Earthworms (oligochaete) can also regenerate both anterior and posterior segments. If an earthworm is cut into two halves, the posterior half regenerates the anterior segments including the mouth, and the anterior half regenerates from its posterior cut end, the new posterior segments. Two new individuals thus rise from the original one.

The number of segments that can be regenerated varies in different annelids. Some species of worms, replace the same number of segments as were lost. *Clymenella*, a

polychaete has twenty two segments and replaces the same amount of segments (Fig. 19.25). Anteriorly it can regenerate up to a maximum of nine segments, replacing the exact number of segments removed. Amputation of more segments is however followed only by the production of short, segmented outgrowths. Posteriorly too it regenerates the exact number of segments amputated, though the maximum number of segments it can regenerate is limited to fourteen. Amputation in front of the eight segment or behind the tenth results in little or no regeneration and ultimate death.

In the majority of annelids regeneration is somewhat restricted at the anterior cut end, and only limited number segments, depending upon the species involved, are formed. In earthworm Allolobophora foetida only four or five anterior segments regenerate. *Chaetopterus* will regenerate a complete animal from only one of its first fourteen segments. There is no restriction on the other hand in the regeneration of the posterior segments. The earthworm *Eisenia foetida* (Fig. 19.26) has approximately one hundred

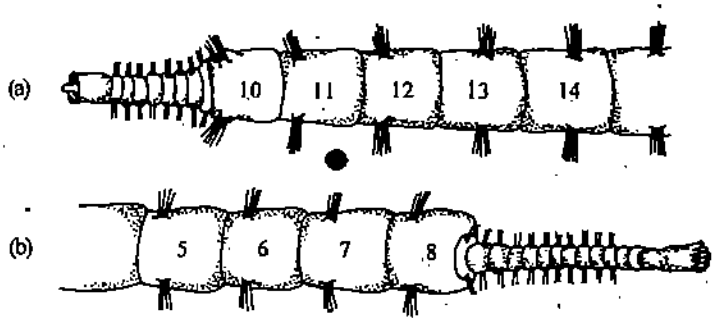

Fig. 19.25: Regeneration in *Clymenella* which has exactly twenty two segments (a) Anterior regeneration may restore upto nine of the segments following amputation (b) daily however, as many as fourteen may be regenerated. Amputation behind tenth or in front of 8th segment results in little or no regeneration and eventual death.

segments and regenerates posteriorly approximately the same number of segments that may have been amputated; if for example ten segments are cut off then ten are replaced. This is not the case if some of the anterior segments are removed as only a few of them regenerate which is typical in earthworms.

You can thus see that the expression of regenerative capacities in annelids depends greatly on the level of amputation.

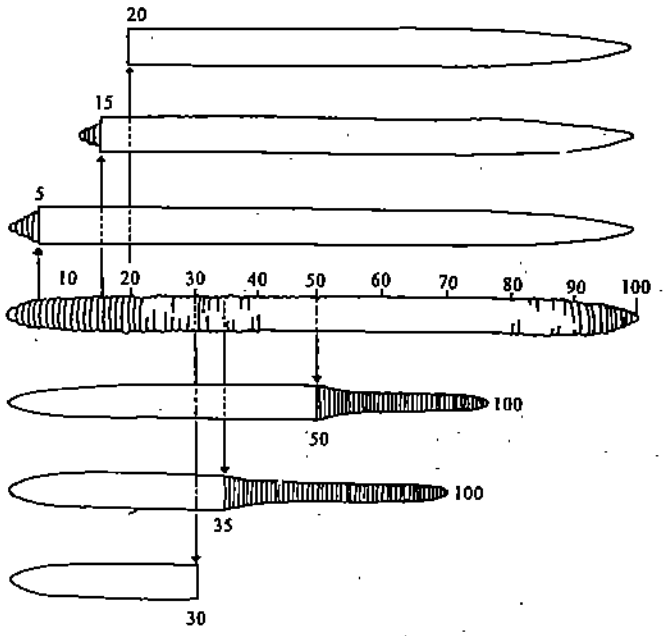

Fig. 19.26: Anterior (above) versus posterior (below) regeneration. In the regenerating earthworm *Eisenia foetida* (an olig ochaete) If the cut is made up to as far back as the 15th segment, a maximum of 5 anterior segments are regenerated; less then 5 if amputation is made between the 15th and 20th segment. If the cut is made at any level posterior to 20th segment no anterior segments regenerate. All the posterior segments removed are regenerated if the cut is made up to as far as 35th segment. Regeneration of neither anterior or posterior segment occurs if the cut is made anywhere between the 20th and 35th segments.

Cells for regeneration in annelids are supplied by the remains of whatever tissues have been lost, i.e. new epidermis is derived from cells of old epidermis, and mesodermal tissues are regenerated from cells derived from dedifferentiation of muscle cells, coelomocytes, and other mesodermal tissues. Intestine regenerates from cells of intestinal origin. (Fig 19.27). The nervous system has an important inductive role in regeneration; cutting the nerve cord alone can cause regeneration of an additional head. Anterior regeneration depends on the presenc of the verve cord. If this is cut or deflected from the wound surface little or no forward regeneration ocurs. (Fig. 19.27) The presence of the brain is necessary for caudal regeneration also. Posterior regeneration also requires the presence of the intestine. If the intestine is also removed then no regeneration of posterior segments take place. The process of regeneration is epimorphic. A regeneration blastema is formed.

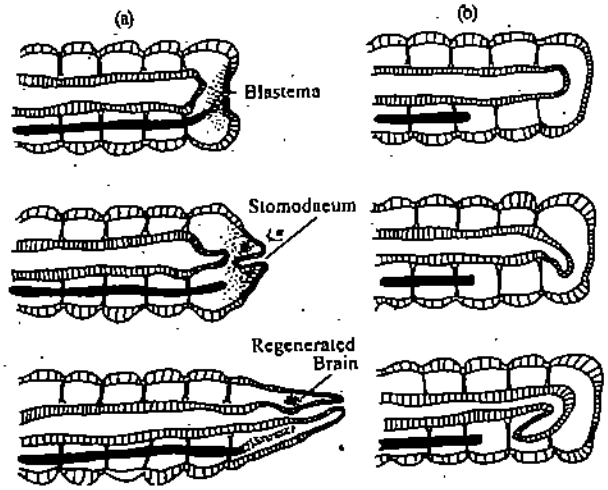

Fig. 19.27: a) Normal stages of head regeneration in *Eisenia foetida*. Ectoderm and endoderm maintain their identities. The new brain and ventral nerve cord differentiate from blastema cells. (b) Results of experimental resection of ventral nerve cord several segments back from level of amputation. Stomodaeum fails to develop from the ectoderm, but intestine regenerates anyway.

Segmentation in regenerating annelids:

Most worms as they grow continue to add new segments at the posterior end. In these, segmentation occurs in a growth zone located immediately anterior to the terminal pygidium. It is in this region that extra segments are partitioned off from time to time by the development of new septa. Caudal regeneration recapitulates this normal mode of growth in annelids. There is no growth zone at the anterior end to provide for increase in segments anteriorly during normal ontogeny. So, in the regenerating head, certain number of intersegmental septa are formed simultaneously, delimiting certain number of segments. This is why the power of regeneration of anterior segments is generally less than that of the posterior segments. Fig 19.28.

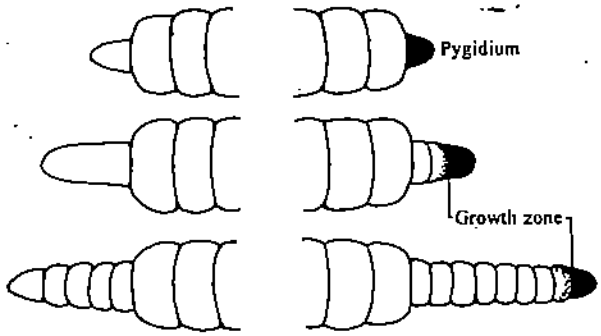

Fig. 19.28: Segmentation in regenerating annelids usually occurs all at once in an anterior outgrowth (left) with no provision for the addition of more segments. In caudal regeneration (right) the pygidium forms first, followed by a series of segments partitioned off sequentially in the growth zone immediately proximal to the pygidium.

vii) **Mollusc:** Mollusc (e.g. snails and *Sepia*) have poor regenerative ability. Among gastropods, (e.g. *Helix*) eye stalks with eyes, and extensive part of head are regenerated. The whole head does not regenerate, and if the central ganglia are removed along with a part of the head then the remaining part of the head which is otherwise potentially capable of regenerating will not regenerate. In some gastropods (e.g. *Nassa*, *Harpa*) the foot can be replaced. In *Harpa* large portions of the foot are autotomized and then regenerated. Gills are regenerated in some nudibranch molluscs. The males of some cephalopods lose an arm by autotomy during sperm transfer and later regenerate it.

viii) **Aschelminthes**: This superphylum includes nematodes and several other phyla: Gastrotricha, Rotifera, Kinorhyncha, Nematimorpha, Acanthocephala, Gnathostomulid. The animals of these phyla lack the regenerative capacity completely, except for some capacity to close superficial wounds. In all these animals cell division ceases early during embryonic development, in all parts of the body and from then on the number

of cells in the body remains constant. This characteristic feature of nematodes and other aschelminthes must be the cause of the lack of regenerative ability in these animals.

ix) **Arthropods:** Among arthropods such as insects, crustaceans, centipedes, scorpions and spiders, the ability of regeneration is low or absent. They do not have the capacity to regenerate head or tail. In most arthropods, only some or all appendages can be regenerated; regeneration is correlated, with moulting or ecdysis and can occur only as long as the animals growth is not complete. Crustaceans are capable of regenerating appendages throughout life because they do not stop growing at any stage of development including the adult as periodic multing keeps occurring throughtout adult life. Crabs and spiders exhibit autotomy'. On being caught by their enemy they shed their limb at a pre-fixed breaking point across the second joint by violent contraction of extensor muscle (Fig. 19.29). The part of the appendage lost by autotomy is then regenerated.

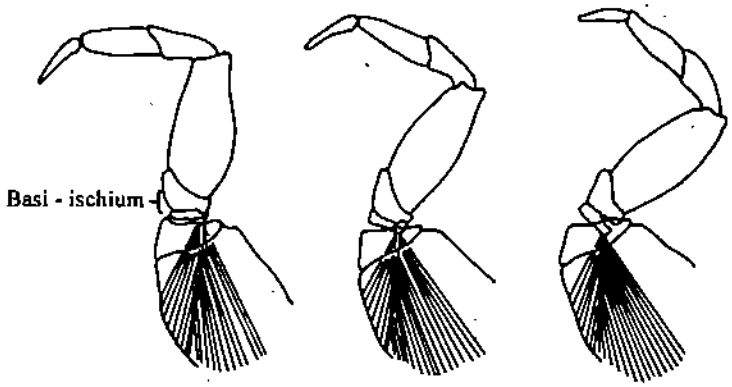

Fig. 19.29: Successive stages in autotomy of the crab leg. (a) normal leg in resting position (b) contraction of autotomizer muscle forcibly elevates leg, pressing basi-schium. The pressure exerted on the basi-ischium splits it in two across the preformed breakage plane.

Insects can replace lost parts of legs only during the larval period during which moulting occurs. Growth stops after the last moult (ecdysis) when adult stage is reached. Therefore, regeneration of any part of any leg is not possible during adult life. Leg regeneration has been studied in the larvae of several orthopteran insects' including grasshoppers, stick-insects, preying mantids and particularly in the nymphal stages of cockroach (Fig.19.30).

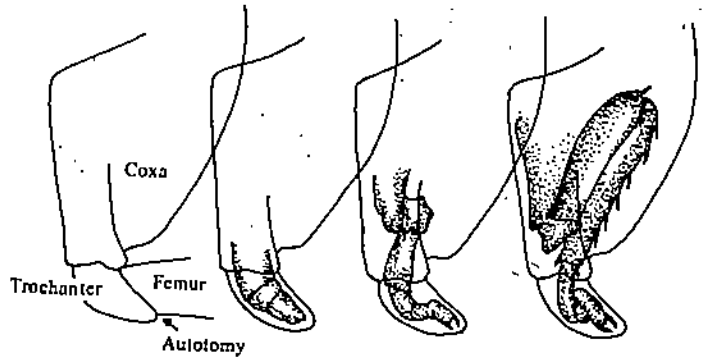

Fig. 19.30: Insects regenerate appendages inside the next proximal segments. Here a metathoracic leg of an eight instar nymphal cockroach (*Periplaneta americana*)) is regenerating within the coxa and trochanter. After about a week following autotomy the slightly flexed leg lies partly within the old coxa, where space is made available for it by muscle degeneration. In two weeks regeneration is essentially complete, by which time the folded limb nestles in the coxal stump ready to be extended at the next moult.

The blood sucking bugd, *Rhodnius* of the order Hemiptera, is also a suitable species for study of leg regeneration. Fig. 19.31.

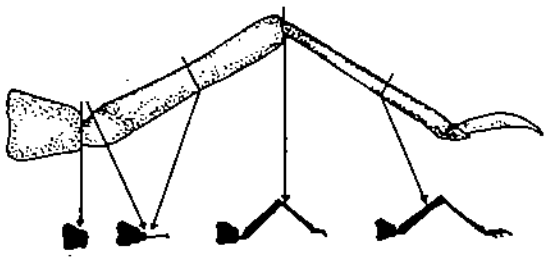

Fig. 19.31: Maximum extents of regeneration in *Rhodnius* leg amputated at different levels along its length.

In an arthropod, after amputation of an appendage the wound gets covered by a chitinous plug. Beneath this a regeneration bud forms which later replaces the limb by epimorphic regeneration. Initially the regenerated limb is small. It attains normal proportion as a result of accelerated growth in the course of several moults. (Fig. 19.30).

x) **Echinodermata:** Asteroids (starfishes), ophiuroids (brittle stars) and crinoids (sea lilies) can regenerate their lost arms and even parts of their disc. The arms of starfishes exhibit autotomy. When disturbed or caught by a predator they cast off one or more arms near the base and regenerate them again. In *Linckia* species an arm totally devoid of any part of disc regenerates the entire body. Such regenerating sea stars are called comets (Fig. 19.32). Brittle stars and sea lilies also practice autotomy in distress conditions and can regenerate their arms from the remaining part of the arm. Autotomy is particularly striking in sea cucumbers (Holothuroidae) which, when startled or disturbed, can eject through their anus all their internal organs, particularly the respiratory tree and alimentary canal. This spontaneous 'self-evisceration' is followed by complete regeneration of the viscera. If transversely bisected each half of the holothurian, regenerates the missing half to complete the body.

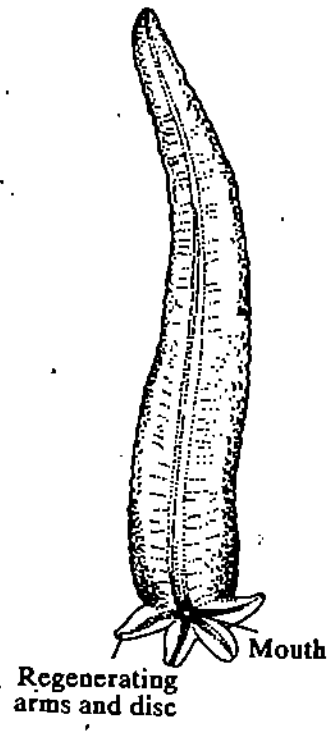

Fig. 19.32: Comet stage of regenerating Sea star (Linckia) from an arm

xi) **Lophophorates:** These include the phyla Phoronida, Bryozoa, Ectoprocta and Brachiopoda. Most animals of these groups practise asexual reproduction by budding and/or fragmentation. The fragments regenerates the missing structures or the whole animal.

SAQ 8

Arrange into two columns the animals listed below into regenerating and non regenerating groups :

leeches, sea lilies, sea cucumbers, adult scorpions, nematodes, snails, earthworm, planarians, sponge.

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19.9.2 Regeneration in Vertebrates

Among vertebrates, the regenerative power is most spectacular in amphibians, particularly the urodeles. Let us, however, briefly survey the regenerative ability of some animals in various vertebrate groups

i) **Hemichordates;**: Asexual reproduction by fragmentation has been reported for several species including those of *Balanoglossuss* and *Glossobalanus*. Most species can regenerate at least the posterior missing part of the trunk.

ii) **Urochordates:** Among the lower chordates extensive power of regeneration as also asexual reproduction by budding or fragmentation are found in several colonial tunicates such as *Clavelina, Perophora* etc; but the solitary ascidians are unable to regenerate lost parts.

(iii) **Cyclostomes:** The larvae of the lampreys among the jawless primitive fishes are able to regenerate their amputated tail. Blastema is formed at the wound surface. It contains dedifferentiated cells derived from mesodermal tissues of stump and is covered by the epidermis with an apical epidermal cap at the apex. Regeneration is epimorphic. The presence of spinal cord at amputation surface is essential for blastema to form and regeneration to proceed. The regenerated tail is anatomically complete with notochord, spinal cord, segmental somice muscles and fin tissue. Even a few new spinal ganglia are regenerated . Fig 19.33 shows a regenerated larval lampray tail and its comparison with regenerated tails of other vertebrates in the larval or adult stage or both.

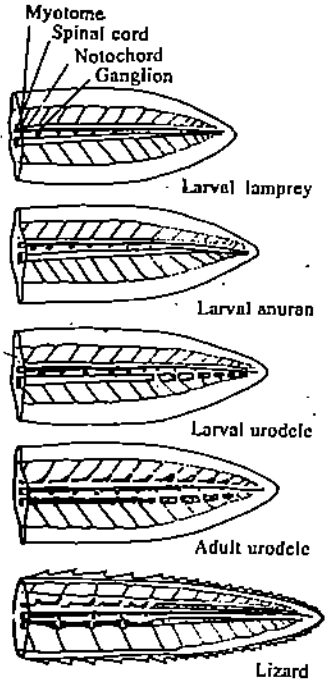


Fig 19.33: Comparative anatomy of tail regeneration. The regenerates of larval lamprey and tadpole tails are morphologially similar, but the latter are in complete in that the new spinal ganglia are not produced. Urodole tails regenerate completely, including ganglia. Those of larval urodeles, however form cartilaginous vertebrae in place of the noto-chord. Lizard tails regenerate an unsegmented cartilaginous tube instead of vertebrate, and give rise to segmentally arranged myotomes.

(iv) **Fishes:** Many different parts of the fish body will regrow. Plucked scales are promptly replaced by new ones and amputated gill filaments can regenerate easily. The taste barbels of the catfish are regenerated easily. Fins are the most conspicuous structures in fishes capable of regeneration. Following amputation through the fin, lost the part grows out from the stump and everything that was missing is restored. Even coloured stripes or spots that adorn some fins are reconstituted by new pigment cells that repopulate the regenerated part (Fig. 19.34). Fin regeneration depends on an adequate nerve supply. If the nerve leading into the fin are cut, regeneration of neither the amputated fin nor incised pieces of bony fin rays can take place.

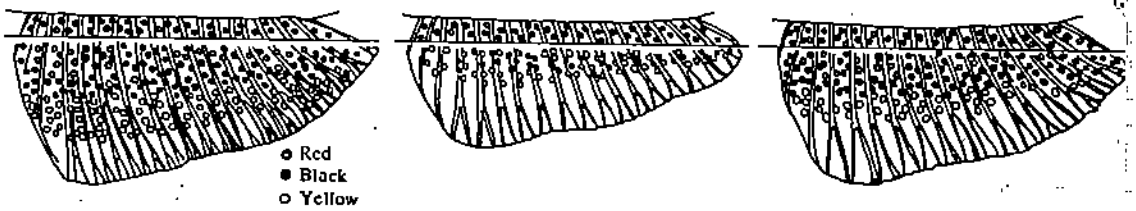


Fig 19.34: Regenerating fish tail of zebra fish, showing the restitution of colour pattern. Three horizontal stripes are normally present on the fin due to the presence of red ,black yellow pigments. When these are lost by amputation, the new fin becomes repopulated by pigment cells deployed in a stripe pattern that is identical to the original colour.

v) **Amphibians:** Newts and salamander as you have read in section 19.4 exhibit remarkable regenerative ability in larval as well as adult stages. In larval stages apart from limbs and tail, external gills, upper and lower jaws, parts of intestine, lens and retina can regenerate. Tadpoles of anuran amphibians (frogs and toads) also possess the ability to regenerate limbs and tail. (Fig 19.33). In the anuran tadpoles the ability to regenerate amputated limbs is gradually lost proximod-distally along the long axis of the limbs as they grow and approach metamorphosis. With few exceptions such as *Xenopus,* adult anurans are unable to regenerate limbs at all. Fig. 19.35 shows the difference in the regenerative ability of urodele (salamander) and anurans (frog) in regenerating an amputated limb.

vi) **Reptiles:** Among reptiles, the lizards such as the *Gecko*, *Hemidactylus flaviverdis* and *Anolis carolineusis* can regenerate their tail, following autotomy (self amputation). When in a lizard the tail is shed by autotomy, a regeneration blastema forms at the wound surface which gives rise to a new tail. Regeneration occurs by epimorphosis. The regenerated tail of lizard however differs from the original tail as the normal segmentation of the vertebral column is not resorted instead' a long tapering cartilaginous tube develops with in which the spinal cord gets located. Furthermore the scales covering the regenerated tail also differ from the scales in the original tail. The regenerated spinal cord is only an ependymal tube without neurons. Fig. 19.33.

Lizards however, are unable to regenerate amputated limbs. In an experiment, only early stages of regeneration up to blastema formation in amputated legs could be induced in the lizard, *Anolis* by augmentation of nerve supply.

vii) **Birds:** Birds replace parts of their beaks and also their feathers. While most species shed and regenerate feathers one at a time so as not to be grounded, flightless birds such as penquins may shed them all at once.

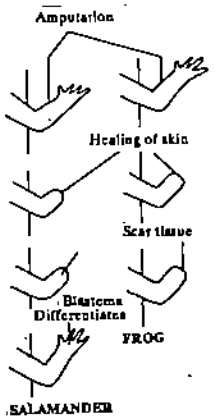


Fig. 19.35: Limb regenerative ability of urodele (salamander) and anuran (frog) after amputation

viii) Mammals: Mammals are incapable of regenerating limb or tails. However, there are a few exceptions in which lost tissues are regenerated. The annual replacement of antlers in deers is ones such example. The old antlers are shed, and new ones grow to replace them.

It has been observed that in the infant opossums (marsupial) which are rather incompletely differentiated at birth, amputated hind limbs possess considerable ability for regeneration. A cut hind limb regenerates when stimulated by implantation of a fragment of brain into it. Generally, however, regenerative ability in mammals is limited only to tissue regeneration, such as wound healing, repair of bone fractures and damaged muscles. Mammals possess extensive power to regenerate liver after more than 75% of it is removed. However, the lost part of the liver are not restored. The residual part of liver increases in size by cell division to restore the original size of the organ. This is an example of compensatory hypertrophy. So far there is no evidence to show that major limb parts in mammals regenerate, though amputation through the terminal phalanges of hands in human children can be followed by complete regeneration of the finger tip. The full length of the finger may be restored and the nail and finger print whoole appear normal. There is usually no loss of mobility or sensation. Regeneration in these instances however occur only in absence of surgical intervention. Some researches have indicated electrical currents as being helpful in regenerating finger tips in humans. (see Box 19.2).

Box 19.2

A few years ago, in the Children's Hospital in Sheffield, England, it was surprisingly found that an accidently cut finger of a boy regenerated completely when the stub was simply bandaged over. Nowadays this technique is routinely employed in many hospitals for young children and there have been several cases of regrown fingers, complete with nails and finger prints. How does this happen as it is contrary to all medical expectations? This phenomenon of reparative regeneration is apparently associated with youth. It has not been found in adults Subsequent research has indicated that this type of regeneration of fingers has some relationship to age. It has been observed that children under the age of eleven have marked powers of regeneration which decrease rapidly beyond this age. It was demonstrated during the 1970s that electrical charges surrounding the tissues in young people reversed about the time that regenerative powers dissipated. Researchers now focus on maintaining particular (young) electrical fields around those areas in which regeneration is being attempted. The time when humans can regenerate lost limbs is probably far but scientists have new reason for optimism (Fig.19.36)

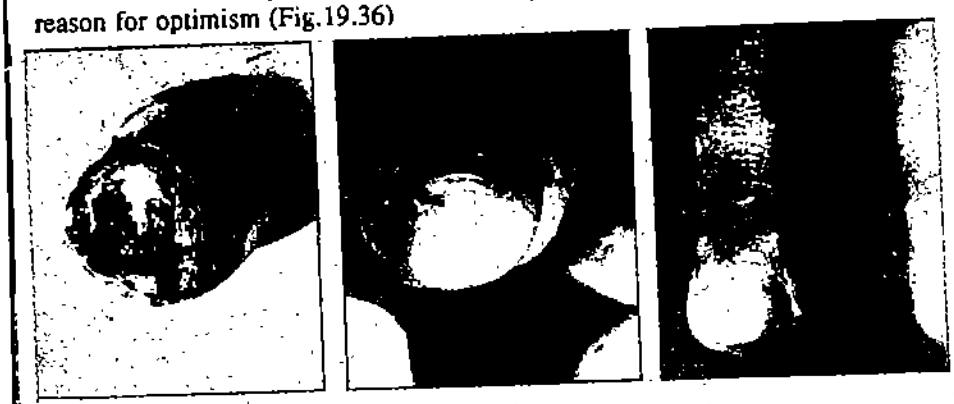


Fig. 19.36: Regeneration of a child's finger tip.

The reasons for uneven distribution of the power of regeneration among animals are not clearly known. From a survey of the regeneration in these groups it appears that lower or simpler organized forms of animal life have greater regenerative power than the more complex, higher evolved forms. That is, they are able to restore normal structure from small parts of the original individual which generally is not possible for more highly in animals. However, this cannot be regarded as a rule. Many phylogenetically lower animals with simple organization such as nematodes and related forms are unable to regenerate at all, whereas more highly evolved forms like echinoderms and many annelids have substantial powers of regeneration of lost parts. Within the same phylogenetic group, the urochordates, the colonial species *(Clavelina, Perophora)* are good at regeneration whereas this ability is absent in the solitary forms such as *Ciona* and *Herdmania*. Similarly, within amphibia, urodeles can regenerate many structures throughout life but most anurans can regenerate limbs (and also tails) during larval period only. Similarly, adult insects cannot regenerate their leg though nymphs or larvae can.

SAQ 9

(i) What parts can be regenerated in cyclostomes and at what stages of life?

.....

(ii) Enumerate the different parts that are regenerated in fishes.

.....

(iii) In what way is the regenerated tail of lizard deficient?

.....

(iv) During what stage of life cycle can ffogs regenerate limbs?

.....

(v) Is tail regeneration in cyclostomes, amphibians and lizards epimorphic or morphallactic ?

.....

19.10 A TYPICAL FORM OR REPARATIVE REGENERATION: HETEROMORPHOSIS

Sometimes the part that grows back is not the same as that which was lost. The phenomenon is called **hetromorphosis**. In hetromorphic regeneration or hetromorphosis, the part which is regenerated is very different from the one which is lost. In such cases the tissues or organs are not replaced by a corresponding structure. Instead another organ is regenerated to replace it. For example, in the shrimp *Palinuras* if the base of the eye stalk and ganglion are removed along with the eye, then the eye fails to regenerate. Instead, regeneration results in the formation of an antennae like structure. The eye regenerates only if the eye stalk is cut proximal to the eye and distal to the nerve ganglion (Fig.19.37). Similarly in stick insects if the antenna is cut off at the end joint, then the antenna is not regenerated and a minute leg comprising a tibia and tarsus with four joints is formed instead of the antenna.

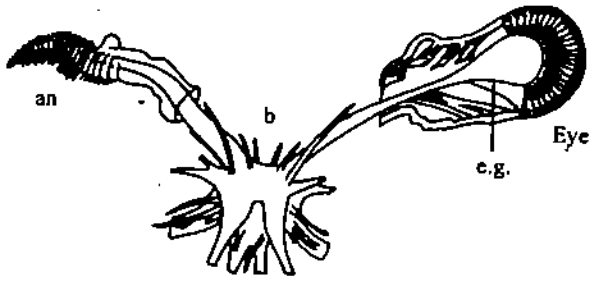


Fig 19.37: Heteromorphic regeneration occurs when an antenna in *Palinurus* regenerates in place of an amputated eye. (an-antenna regenerated; b- brain; e.g. eye ganglion.

In certain crabs, for example in the pistol crab, heteromorphosis involves reversal of symmetry (Fig.19.38). The normal crab has an enlarged right chela and a small left chela. Amputation of the large chela causes the small one on the left side to enlarge while a small new chela regenerates on the right side from the amputation site.

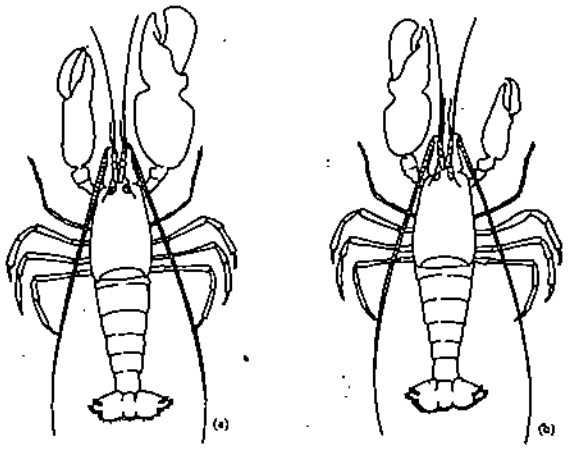


Fig. 19.38: An example of homorphic regeneration which show reversal of symmetry in pistol crab. (a) Normal crab has enlarged right chela. If the large chela is amputated, the smaller one then enlarges at the next moult (b) and takes on the morphology of the lost chela. The latter is replaced by a small chela.

19.11 SUMMARY

- The ability to replace or to compensate for physiologic loss is found in all animals.
- The ability to restore a whole organism from a part, sometimes only a very small part, is widespread in the animal kingdom.
- There are three types of regeneration,
  - (a) Physiological—the regular replacement of cells and tissues, e.g., skin epithelium.
  - (b) Reparative—the replacement of an amputated or autotomized body part e.g., regeneration of urodele limb.
  - (c) Compensatory hypertrophy— the phenomenon where the cut portion of a tissue or organ just increases in size e.g. liver segment of animal.
- There are two patterns or mechanisms of reparative regeneration:
  - (a) Morphallaxis—A minute fragment of the body can completely rearrange itself to form a new animal as in hydra.
  - (b) Epimorphosis—An amputated or autotomized part is reformed by blastema formation, e.g, urodele limb.
- Amphibians, particularly the urodeles, have the ability to regenerate complete limbs. Epidermis heals over the cut stump, beneath which blastema cells arise by dedifferentiation and the proliferation of cells at the cut end. All types of cells of the stump except for the epidermis contribute to the regenerating blastema, though even now it is not clear as to what extent the morphologically dedifferentiated cells form cells other than those of their type of origin during regeneration. X-irradiated limbs are unable to regenerate. If however at the amputation site the limb is provided with unirradiated limb skin, muscle or a segment of nerve, then a blastema will form and all tissue types of a normal limb will regenerate. The apical cap or wound epithelium is essential for formation of regeneration blastema. The intervention of skin dermis between the epithelium and the amputation plane prevents regeneration. In non-regenerating limbs, as in adult anuran, dermis normally covers the wound surface quickly and so regeneration fails.

- A minimum number of nerves, sensory or motor, are necessary for regeneration of limbs in amphibians. Nerves provide a trophic factor (Fibroblast Growth Factor FGF) which promotes DNA synthesis, protein synthesis and mitosis in blastema cells. Once blastema has entered redifferentiation phase it becomes independent of nerves.

- When a limb regenerates, the blastema gives rise to those parts that normally lie distal to the cut surface, regardless of the level of amputation. This is called the *rule of distal transformation*. Also, the anteroposterior and proximodistal axis of the regenerated parts correspond to those of the stump. Therefore, there must be present at the amputation site positional information that determines the polarity of the regenerated part.

- A group of chemicals called retinoids (vitamin A and its derivatives) in amphibians have the capacity to reset the positional information and to generate more than one limb (supernumerary) at an amputation site.

- Wolffian lens regeneration in urodeles is a good example of epimorphic regeneration and is a result of metaplasia. The removal of lens in larval or adult urodele initiates activity in the adjacent pigment iris epithelium. The melanic secreting pigment cells of iris epithelium undergo dedifferentiation by sloughing off their pigment granules. The dediferentiated cells next proliferation and redifferentiation to form lens forming cells.

- Among invertebrates, hydra regenerates by means of morphallaxis. Interstitial cells (a source of regeneration) of the Hydra are proginitor cells of the nerve cells, germ cells, gland cells of the endoderm and nematocyts. The hydra is rigidly polarized along the distal-proximal axis. Two sets of morphogenetic substances help in maintaining this polarity namely: (1) head activator and head inhibitor and (2) foot activator and foot inhibitor.

- Planarians regenerate by a mix of both epimorphosis and morphallaxis. A regeneration blastema formed of cells of local origin is clearly observed. The initial hypothesis that neoblasts or reserve cells of the planarians are used for regeneration has become questionable as these appear to be gland cells under electron microscope. Instead recent EM studies have indicated, though not definitely, that Beta cells occuring in parenchyma surrounding various glandular, muscular and digestive cells, are the proginator cells during regeneration for various tissue types with which they are associated. Similar to Hydra, planarians maintain their original polarity during regeneration, which also like hydra may be experimentally altered. Induction phenomenon in planarians cause regeneration to proceed in a stepwise fashion brain ->eye—> pharynx etc.

- The ability of reparative regeneration is unevenly distributed among animals of different phyla. It is generally greater in phylogenetically lower than higher animals. However, there are numerous exceptions. For example lower animals such as nematodes do not regenerate at all, while higher animals such as annelids urodeles etc and arthropods have a much greater ability to restore their lost parts. Regenerative ability within the same animals group is also not uniform, e.g., in annelids, leeches do not regenerate while earthworms do.

- Among invertebrates regeneration of the whole animal from fragments, that is by morphallaxis, is commonly seen as a means of a asexual reproduction, and it occurs readily under experimental conditions. Epimorphic type of regeneration also occurs in some invertebrates (planarians, insects etc.).

- Heteromorphosis is a phenoenon in which the amputated part is replaced by a different part as in shrimp, where an antenna instead of an eye regenerates on the removal of the eye.

19.12 TERMINAL QUESTIONS

1. Dedifferentiate between epimorphosis and morphallaxis
2. Define the following terms:

   (a) Heteromorphosis, (b) Metaplasia,
3. Show diagrammatically :

   (a) The difference in the regenerating ability of salamander and frog.

   (b) What happens when the anterior end of the planaria is cut along its length into two or more parts.

   (c) The sequence of induction in the regenerating planaria.
4. Fill in the blanks:

   (a) In earthworms the anterior regeneration depends on the presence of .....and the posterior regeneration on the presence of.....

   (b) The rule of..... states that regardless of the level of amputation, the limb always regenerates in the distal direction, irrespective of the polarity of the stump.

   (c) The regular replacement of RBCs is an example of..... regeneration.
5. Which of the cells in the regenerated urodele limb are believed to have formed from the dedifferentiation of the muscle cells?
6. Write short notes on:

   (a) Interstitial cells of *Hydra*,

   (b) Neoblasts of *Planaria*.

   (c) Role of nerves in urodele limb regeneration.

   (d) Histological changes in the cells during the process of dedifferentiation in lens regeneration of *Notophthalmus virridescence*

19.13 ANSWERS

Self Assessment Questions:

1. Physiological regeneration is the regular replacement of cells and tissues that are lost due to normal wear and tear; for example replacement of RBCs and hair in animals Reparative regeneration is the repair or replacement of lost part due to injury as in the case of tail regeneration in lizard or limb in salamander.
2. This type of regeneration is reparative morphollactic.
3. The process in which differentiated cells lose their distinct morphological characters, becoming morphological indistinguishable or undifferentiated is termed dedifferentiation.
4. No regeneration will take place in either case .
5. The removal of the original lens and the presence of the neural retina are the necessary pre-requisites for lens regeneration in *Triturus*.
6. The fact that in *Hydra* interstitial cells are not necessary for regeneration is experimentally proved by first destroying the interstitial cells in Hydra by treating it with x-rays and then observing whether it regenerates or not. Since the Hydra was found to regenerate despite the destruction of its interstitial cells,

it was safely concluded that interstitial cells are not absolutely necessary for regeneration.

7. The fact that blastema in Planarians can be formed by cells migrating from distant parts of the body was demonstrated by means of an experiment. A planaria was x-irradiated which inhibited its regenerative ability. After this a graft from a non irradiated planaria was transplanted into it. When a part of the body of the planaria with the graft was amputated then it was seen that the planaria regenerated. It was observed that the lost part regenerated even if it was a distance from the graft.

8.

Regenerating animals	Non-regenerating animals
Sea lilies	leeches
Sea cucumbers	adult scorpion
Earthworm	nematodes
Planaria	
Sponges	

9. (i) In cyclostomes only the tail can be regenerated by the larval lamprey

   (ii) Fishes can regenerate scales, fins, tail and barbels (catfishes)

   (iii) The regenerated lizard tail differs from the original tail in a number of ways. It lacks the normal segmentation of the vertebral column, which in the regenerate is only a long tapering cartilaginous tube. The scales covering the tail are also different and the spinal cord, called the epindymal tube unlike the original tail lacks neurons.

   (iv) Frogs can regenerate their limbs only in the larval stages

   (v) Tail regeneration in these animals is epimorphic.

Terminal Questions

1. Refer sections 19.3.1 and 19.3.2

2. a). **Heteromorphosis**— In this type of regeneration the part which regenerates is different from the part which was lost. It is thus also appropriately called faulty regeneration.

   b) **Metaplasia** is a process in which first the fully differentiated cells lose their differentiation, becoming de-differentiated and then again redifferentiate either into cells of different types.

3. See

   (a) Fig. 19.35

   (b) Fig. 19.17

   (c) Fig. 19.21

4. a) nerve cord, intestine

   b) distal transformation/regeneration

   c) physiological

5. In urodele limb regeneration the muscle cells probably give rise to the following cells in the regenerate: muscle, connectiver tissue, cartilage cells and bone cells

6. a) The interstitial cells of *Hydra* are distributed throughout the body. Some of them, however, also occur in large numbers in the growth zone of the *Hydra* which is easily distinguishable due to their presence. The interstitial cells are small in the gut epithilium. These cells occur in size with large nuclei and basophilic cytoplasm. Studies on these cells reveal that they act as a pool of undifferentiated cells for the replacement of cnidoblasts and can also be transformed into nerve cells. They also serve as a source of gonocytes during sexual reproduction. However, interstial cells are not essential for regeneration.

   b) See section sub section 19.4.5 -Role of Neurons.

   c) Notable histological changes that occur during dedifferentiation of the cells of the dorsal iris. First, the cells divide and RNA synthesis increases and reaches a peak at about 5-7 days. Furthermore, the cytoplasmic basophilia increases and there is a marked increase in the protein synthesis. The cells get depigmented by extruding their pigment granules which are taken up by the macrophages.

UNIT 20 GROWTH, AGING, CANCER

Structure

20.1 Introduction
Objectives

20.2 Growth—a Biological Phenomenon
Allometric and Isometric Growth
Measurement of Growth
Factors Governing Growth

20.3 Aging—An Aspect of Development
Consequences of Aging
Theories of Cellular Aging
Extracellular Aging

20.4 Cancer—Result of Developmental Error
Characteristics of Malignant Cells
What Causes Cancer?
Stages of Carcinogenesis
Mechanism of Carcinogenesis
Multicausal Nature of Cancer

20.5 Summary

20.6 Terminal Questions

20.7 Answers

20.1 INTRODUCTION

While studying the earlier units (13 to 19) of blocks 3 and 4 you would have come to realise that development of animals is a highly integrated process. It is not limited to morphogenesis and differentiation but also includes an increase in size and weight, that is, growth of the animal. The limits to growth in virtually all organisms are predetermined at the outset of development and after the organism reaches sexual maturity, aging process begins. Deviation from the normal process of growth and aging may result in the formation of neoplasms or new growth of abnormal tissue. These interrelated aspects of development, that is, growth both of cells and organisms, their aging and what happens if normal developmental process go awry form the theme of this unit.

In this unit you will first learn that the term growth has several meanings. Growth can mean an increase in the number of cells or it can also be described as an increase in protoplasmic material. You will also learn how growth of cells or an organism is measured and depicted in the form of growth curves.

Aging is an important part of development after birth and you will learn that aging involves progressive and irreversible loss of functions that increase the likelihood of death. Numerous theories have been put forward to explain the phenomenon of aging. We have discussed some of the important ones that try to explain cellular and extra cellular aging.

Studies of embryonic development and cancer formation have been closely linked, because the development of cancer in many ways is the opposite of differentiation. Understanding either process would help explain the other. Therefore, in this unit we have explained briefly how normal cells are transformed to become malignant cells. Since cancer is the result of abnormal and uncontrolled cell proliferations you will learn how proto-oncogenes and oncogenes (genes with the potential to cause cancers) that apparently carry out normal cell functions are activated to transcribe abnormal proteins or abnormal amounts of proteins that can disrupt the growth signalling sequence in cells. This leads to uncontrolled cell proliferation.

Before you start studying this unit we advise you to revise Unit-16 of Cell Biology course (LSE-01) and Unit-17 of the Genetics course (LSE-03). These two units have been referred to quite extensively and will help you to understand the concepts explained in this unit.

Objectives

After studying this unit you will be able to:

- define growth with reference to individual cells in multicellular organisms and their parts
- interpret growth curves
- discuss the factors that influence growth
- list out the consequences of aging
- identify the factors that contribute to aging from the theories put forward to explain the process of aging
- enumerate various causes of cancerous growth and changes associated with origin and progression of cancer
- explain on the basis of available information how proto-oncogenes and oncogenes direct the abnormal growth signalling sequence in transformed cells, and
- explain that cancer is a multicausal and multistep process.

20.2 GROWTH—A BIOLOGICAL PHENOMENON

Growth is a universal attribute of organisms. Animals are small when they are born. They grow by consuming food and assimilating it into their living mass. Plants assimilate atmospheric carbon dioxide using solar radiation and produce carbohydrates. They also absorb various mineral salts and synthesise the living material. This results in their growth. Therefore, we can say that an overall increase in size of an animal or plant is a measure of its growth. It can be expressed in terms of its weight. Increase in the number of animals or plants is also a form of growth, but this represents the growth of populations rather than individuals. In this unit we are concerned only with the growth of individual organisms. For population growth refer to the Ecology course LSE-02, Unit-12.

The adult human being is made up of 6×10^{13} cells while the newborn has only 2×10^{12} cells.

To explain the growth of an individual let us take the most familiar example, that of a baby. At birth an average baby weighs around 3 kg but over the next 20 years it matures into a 65 kg individual. Growth in this individual's body involves an increase in the number of cells of its organs while the size of individual cells remains more or less the same. We can assume, therefore, **that the fundamental aspect of growth in multicellular organisms is an increase in the number of cells.** Though this is the general rule in case of most multicellular organisms including human beings not all growth is due to increase in cell numbers. Cells produce some extracellular materials which add to the general mass of the body without adding to the number of cells. The bone is a tissue in which most of the 'growth' is due to deposition of extracellular matrix rather than increase in the number of living cells.

On the other hand in certain animals, for instance nematods, rotifers and some tunicates, the increase in size of the individual is due to the increase in size of cells. In nematods, cell division stops rather early during organogenesis, therefore, the number of cells of a fully grown nematod is the same as in the young that emerged from the egg. The excretory system in a nematod, for example, consists of only three cells as the number of cells in organ rudiments is fixed early during embryogenesis.

In other multicellular animals, growth of the organs by proliferation of cells usually stops once the cells become differentiated except in some cases where certain special cells continue to divide, like the epidermal cells of the skin, the proliferating cells at the bottom of the intestinal glands, blood forming stem cells in the bone marrow and other tissues. But these are all reserve cells that proliferate to supply new cells to replace and add to the functionally differentiated cells when the need arises.

However, in each case a well organised control of division exists in all organisms and if certain cells escape from this control due to mutation, they proliferate into a neoplastic growth which may invade and destroy healthy tissues eventually killing the organism. Such cells may persist for long periods without detection but can develop into slow benign tumors or fast growing malignant tumors once they are stimulated or promoted. We shall learn how this happens in a later section of this unit.

20.2.1 Allometric and Isometric Growth

In multicellular animals you would have observed that most of the changes in the body form are due to two factors:

1. Various organs start growing at different times in life.
2. Individual organs may also grow at different rates as compared to each other and the rates of growth of the whole body.

This type of growth where the different parts of the body grow at different rates is known as **allometric growth.** In contrast if an organ grows at the same rate as the rest of the body it shows **isometric growth.**

A good example to illustrate allometric growth is our own body. Our arms and legs grow at a faster rate than our torso or head. Fig. 20.1 shows human allometric growth. You would note that proportions of different parts of the adult body differ markedly from those of the infant.

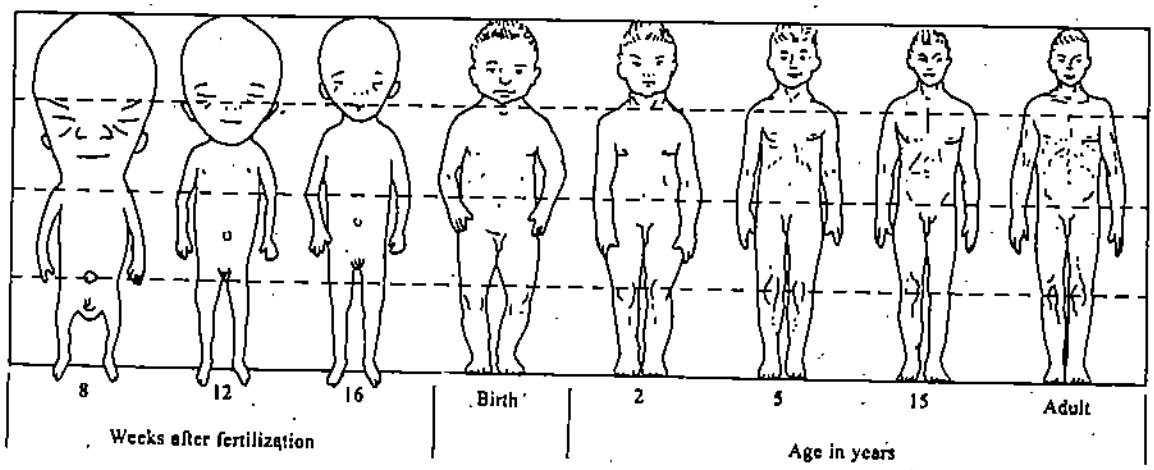


Fig. 20.1: Allometric growth in human beings. The head is exceedingly large in the fetus in proportion to the rest of the body. After birth the growth of the head slows down.

Another vivid example of allometric growth is seen in the male fidler crab *Uea pugnax*. In small males the two claws are of equal size making 8% of the total weight of the crab. As the crab grows the size of its chela (the large claw) enlarges till it constitutes 38% of the total body weight! (See Fig. 20.2) However, in the female both claws grow at the same rate as the rest of the body *i.e.*, isometrically. Allometry is seen only in the males.

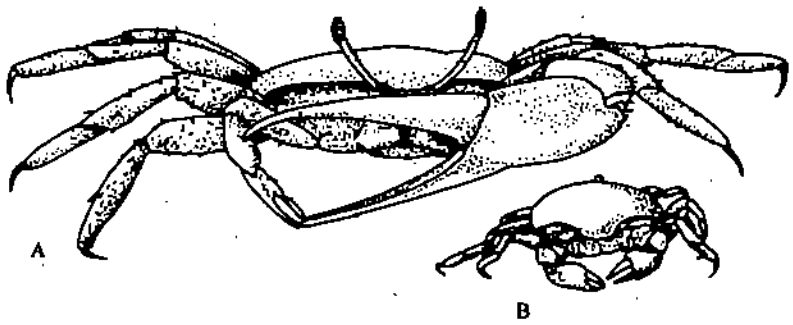


Fig. 20.2: Allometric growth of left chela in fidler crab.
A) Adult male B) young male crab with equal sized chelae.

Most organisms, however, show isometric growth, when they add new material into the existing tissues of the body. The organism increases its volume and retains the same proportion among its different parts (there is a rule that when an animal increases its weight two fold its length increases only 1.26 times).

SAQ 1

After having read the description of growth can you formulate a definition of growth?

.....

.....

SAQ 2

In the statements given below indicate the type of growth responsible for the structures seen:

(i) In baboons the jaw and other facial structures have a growth rate 4.25 times that of the skull

(ii) An animal in a shell is seen to grow by widening and lengthening in the same proportion

(iii) Workers and soldiers in ants have different sizes, soldiers have larger jaw structure than other workers.

20.2.2 Measurement of Growth

Growth of multicellular organisms in terms of increase in their cell numbers is difficult to measure, neither can the amount of basic cell substance be measured. Hence we must measure some quantity which is, as nearly as possible, directly proportional to its true growth. We generally say that an organism has grown if we can discern any increase in size. Since increase in size is accompanied by an increase in body substance, we measure the increase in weight, and this may be measured at different intervals on the same organism. Usually the dry weight is taken, as the water content of the body keeps fluctuating. This is done by weighing groups of individuals from a large population and average weight of one individual is then calculated.

This data is then plotted against time to give us a **growth curve.** Growth curves give us the **growth pattern** as a whole and the **rate of growth** at any time during the investigation. The increase in weight or size of an organism taken as a difference between the initial and final weight or size for any period of time is known as **absolute increase. Absolute increase does not give us the rate of growth.** Therefore, it is not useful if growth at different periods of life or in different animals is to be compared. For example, if a large animal and a small animal show the same absolute increase in a given time, it does not indicate that their growth rates are the same. The smaller animal would have to grow at a faster rate to achieve the same absolute increase as the larger animal. Therefore, the rate of growth is calculated. The rate of growth is indicated by the rate of change in weight or size with respect to time. If you have studied Calculus (ref. our course MTE-03, Block-2) then you know that the rate of change is given by dw/dt where $w(t)$ denotes the weight or size of the organism at any time (t).

Types of Growth Curves

Growth curves are plotted using the absolute increase against time and according to the type of growth seen in organisms: If the growth of an animal is exponential then the growth curve will be a hyperbola *i.e.*, it will begin at zero and continue to rise with increasing steepness. Such exponential growth is not seen in organisms. In most multicellular animals the rate of growth does not remain the same but varies at different times. Therefore, their growth curves are 'S' shaped or **sigmoid** as seen in Fig. 20.3. In a sigmoid curve there is an initial period where the curve rises very gradually. This is the **lag period** characterised by little or no growth. The lag period is followed by the period of active growth or the grand period of growth where the curve rises steeply. This is the **exponential period.** The exponential period does not go on indefinitely and the last period where the rise of the curve slows down and approaches to be almost a horizontal line means that the growth rate is reducing and ultimately becomes constant.

This steady state merges with a condition where the body begins to lose some of its substance as it ages. This is the period of senescence which ends in death.

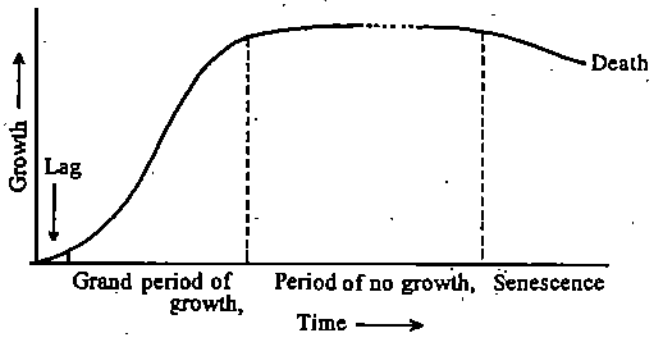


Fig. 20.3: A typical sigmoid growth curve showing the lag period, exponential and declerating growth period, and senescence leading to death.

Measurement of the total growth of multicellular animals and their organs show that some of them never stop growing. This is especially true for invertebrates and their growth patterns are represented by part of parabola rather than a sigmoid curve. Fig. 20.4 shows the growth curve of the lobster. This curve shows unlimited growth. However, this does not mean that such animals can live for ever. The growth rate declines with age and slows down to zero, metabolic rate also slows down and so, in a natural population the aged are eliminated.

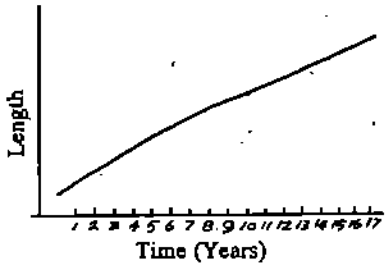


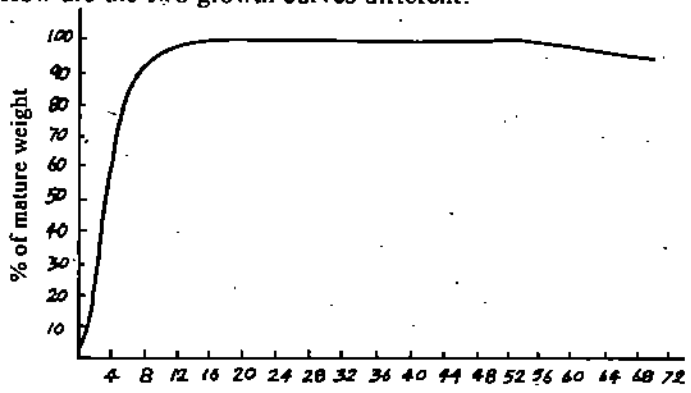
Fig. 20.4: Growth of lobster

In case of higher animals like certain fishes and reptiles, growth slows down but does not stop altogether. A good example is the giant tortoise, that continues to grow slowly until it dies.

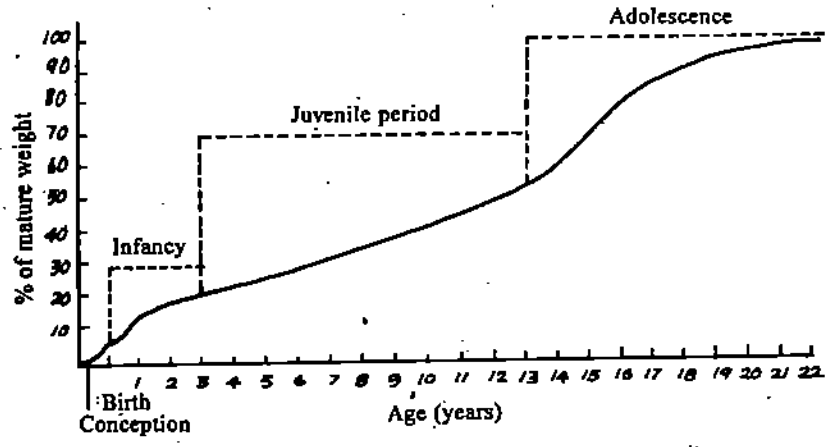
In case of birds and mammals, growth ceases after a particular size is attained. The same is true of insects. In the majority of animals growth slows down and gradually the curve becomes flat as cell replacement balances cell disintegration. Finally, the downward slope of the sigmoid curve indicates that disintegration of cells predominates and there is negative growth. This is the period of aging or senescence, which continues until death.

SAQ 3

In mammals the exact shape of the growth curve depends on the speed at which sexual maturity is attained. Figure (a) shows the growth of rat and Figure (b) of humans up to maturity. How are the two growth curves different?



a) Growth of rat over a life span



(b) Growth of human being up to maturity

20.2.3 Factors Governing Growth

In the earlier sub-sections you learnt that growth of organisms takes place by two means 1) by cell proliferation and 2) by increase in cell size. You also learnt that all growth eventually declines with time and may finally stop altogether. What is it that controls the rate of division of cells? Why is it that some cells such as neurons, fat cells and skeletal muscle cells hardly divide during growth while some other cells like fibroblasts, bone cells and kidney cells have limited proliferation; and still others like stem cells for blood and sperm formation divide continuously during an individual's life time?

Cell division seems to be under two levels of control.

1) Extrinsic control: wherein the growth of the organ depends on factors derived from other tissues.

2) Intrinsic control: wherein the tissue or organ regulates its own growth.

Extrinsic Control

Extrinsic control is seen when one organ influences the growth of another organ. In vertebrates the growth hormone or **somatotropin** is the extrinsic factor that regulates the growth of the entire body. Its effect is seen most obviously on the long bones of the limbs of humans. Lack of growth hormone produces proportional dwarfism in humans. However, its effects are seen in all other body organs. Foetal tissue appears to be more sensitive to somatotropin than adult tissue and it is produced in the foetus as early as 70 days after fertilisation in humans. Growth hormone acts directly (as in the case of long bones) or indirectly. The indirect effects are thought to be brought about by **somatomedins.** These compounds are secreted by the liver cells in response to growth hormone.

People who do not have enough growth hormone, have low somatomedin levels and when these individuals are given growth hormone, their somatomedin level also increases. It has been found that adolescents undergoing their growth spurt and people with growth hormone secreting tumors have higher levels of somatomedins.

Somatomedins include insulin like Growth Factor I (IGF-1) and Growth Factor II (IGF-II). These growth factors work at specific stages of the cell cycle (recall cell cycle from LSE-01, Unit-16). Fig. 20.5 summarises the somatic cell cycle of normal mammalian cells. The cell cycle is divided into mitosis (M), a gap (G1), a period of DNA synthesis (S) and another gap (G2) between S and M. Cells no longer dividing are said to be in Go state. Cells starting in Go are stimulated by growth factors to a state where they become competent to enter the cell cycle. Halfway through G1 they make a decision to divide. Before this stage, inhibitors could have worked but after this point S and M proceed independent of external stimulation.

IGF-1 works by allowing cells to pass from G1 phase of the cell cycle to S phase. A combination of growth hormone and IGF-1 may provide a strong mitotic signal. Growth hormone may stimulate the division of stem cells into cells that are responsive to IGF-1 and the new cells would undergo further replication in response to somatomedin. The pygmies of the Ituri Forest of Zaire have normal growth hormone and IGF-1 until puberty. However, at puberty, the IGF-1 levels of pygmies fall to about one-third that of other adolescents.

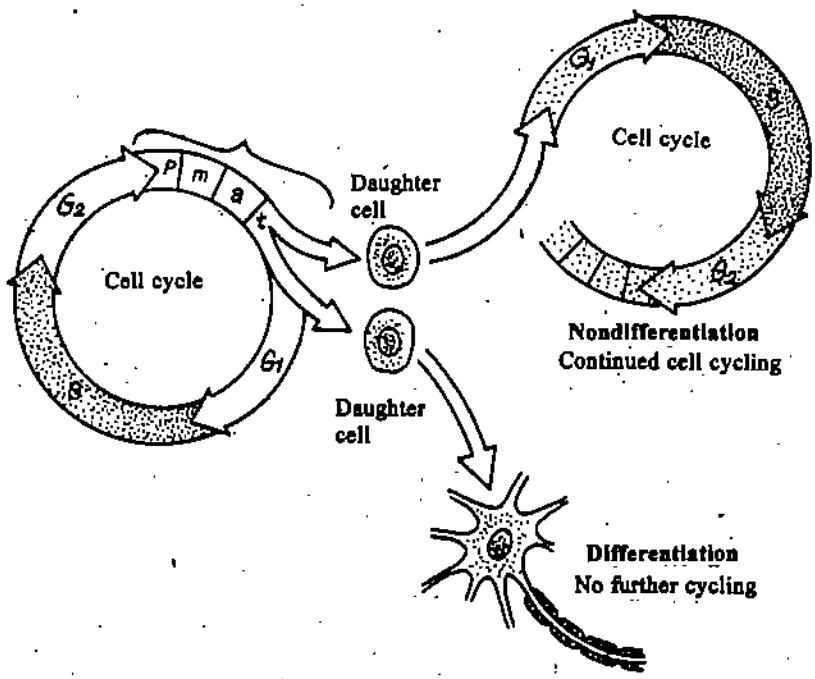


Fig. 20.5: Cell cycle in brief. Cells that are actively dividing pass through the 4 stages ending with mitosis. Some specialised cells do not divide and remain in Go stage permanently but growth factors can stimulate cells to divide from Go. Inhibitors can stop division before cell enters S phase.

The receptor for these growth factors is a protein that spans the cell membrane and can be divided into three functional parts. 1) an outer domain that binds to the specific growth factor, 2) a domain of hydrophobic residues that span the membrane, 3) a domain within the cytoplasm that can be activated into an ATP-tyrosine phosphokinase. This enzyme is relatively inactive unless the receptor is bound to growth factor.

Insulin, growth hormone, somatomedins, placental lactogen and nerve growth factor comprise a family of related growth factors. These growth factors also include platelet derived growth factor PDGF which stimulates smooth muscle cells, fibroblasts and glial cells to divide. Along with epidermal growth factor (EGF) and at least one haematopoietic growth factor CSF-1 (colony stimulating factor-1), all work by a similar general mechanism *i.e.*, phosphorylation of tyrosine residues. You would recall the mechanism from LSE-01, Block 4, Unit-16.

Intrinsic Growth Factors

While extrinsic growth factors usually promote growth, the intrinsic growth factors generally inhibit cell growth (cell growth here and subsequently means cell proliferation or mitosis). These intrinsic factors may affect the growth of many cell types or they may be very specific to their target cells. The intrinsic growth factors that are very specific for a particular cell type are called chalones (pronounced Kay-lones). Studies have shown that chalones control the mitotic rate of epidermal cells. (see Box 20.1)

Two epidermal chalones have been isolated that act at different stages of the cell cycle. The G1 Epidermal Growth Inhibitor stops the cells from entering the S phase and the G2 Epidermal Growth Inhibitor prevents division of the cells whose DNA has already duplicated. These chalones are tissue specific and do not inhibit mitosis in the cells of other tissue.

Box 20.1

The effect of chalones was shown by a very interesting experiment. A small incision was made in the epidermis of mouse. It was observed that 1 mm away from the tear cells began to divide. This division took place only if the epidermis was injured and not if even adjacent tissue like dermis was injured. If the wound was enlarged a wider area of cells was not stimulated as might be expected if the cells were stimulated because of the injury. The investigators concluded that division of epidermal cells is normally suppressed because of some inhibiting factor and when the cells are injured less of the inhibitor would be produced in the area of the wound and therefore neighbouring cells could resume mitosis.

In addition to specific chalones there exist certain proteins that inhibit the growth of a wide variety of cells. Two of these are B-Interferon (BIFN) and Transforming Growth Factor-B (TGFB). The effect of TGFB depends on the target cells and presence of other growth factors. TGFB has a dual nature. It can enhance the growth of fibroblasts in culture but is generally inhibitory to growth of other cells. Interestingly it is also able to inhibit the growth of various cancer cells in culture. It is now known that cells become predisposed to malignancy if they lack receptors for TGFB or if they are not able to synthesise this inhibitor.

B-Interferon a member of the interferon family of proteins makes the target cells stop dividing and makes them unable to respond to mitotic stimuli. This condition is reversible once the B-interferon is removed.

Thus we see that action of growth promoting factors and growth inhibiting factors together appear to regulate cell division in such a way that cell division does not cross the genetically defined limits.

SAQ 4

Fill in the blanks with appropriate words from the text:

i) ..... are growth factors secreted by liver cells in response to growth hormone.

ii) Cells are stopped from entering the S phase by .....

iii) ..... inhibits cell divisions by making them unable to respond to mitotic stimuli.

iv) Extrinsic Growth factor works by ..... of tyrosine residues.

20.3 AGING—AN ASPECT OF DEVELOPMENT

In the earlier section we analysed growth in terms of cell multiplication, cell enlargement and accumulation of extracellular matrix. We saw from growth curves of various animals that over the years, growth stabilises, gradually declines and ultimately stops all together.

The biological science that deals with aging is called gerontology. Geriatrics is the branch of medical sciences specialising in the treatment of debilities and disease characteristic of the aged.

In this section we discuss the process of aging or senescence. The aging process begins after the organism has attained sexual maturity and ends in the death of the organism. It involves progressive and irreversible loss of functions.

The process of aging is not recognised in bacteria. After a single cell has grown fully it divides into two cells which then repeat the process. However, most multicellular animals do not live long enough to undergo the complete process of aging. For example, field mice can live for 3-4 years but 99 per cent of them hardly reach their first birthday!

The timing of aging appears to be a developmentally regulated event *i.e.*, each species has a characteristic life span. For example, house flies do not live longer than 70 days. Dogs live for 10-15 years (see Table 20.1 for data on the life spans of different animals.) Human life expectancy is variable. Though the ideal is said to be 100 years, the actual average lies between 60 and 70 years in different developed countries. In underdeveloped countries with problems of poverty, malnutrition and poor public health measures, it is substantially lower than this. However, there are some individuals who live even longer than a hundred years. There are three regions in the world, Vilkabamba in Ecuador, Hunza in Pakistan occupied Kashmir and Caucasus in a region between the Black Sea and Caspian Sea — where many people live longer than a hundred years.

The genetic control over life span can be seen in the case of certain mutations in humans. In progera (Hutchinson-Gelford Syndrome), an illness in children, many symptoms of extreme old age are seen by the age of 6 years and usually the child dies of heart failure by the age of 12.

Table 20.1: Maximum recorded longevity for some mammals

Animal	Maximum life span (y - years; mo - months)
Wild goat	18 y
Indian rhinoceros	40 y
Indian elephant	70 y
Dog	20 y
Domestic cat	28 y
Guinea pig	7 y 6 mo
House mouse	3 y 6 mo
Gorilla	39 y 4 mo
Chimpanzee	45 y
Human	90 to 100 y

20.3.1 Consequences of Aging

It is difficult to enumerate the general consequences of aging in different animals. Gradual loss of reproductive ability, neuromuscular coordination and inadequate assimilation to compensate for wear and tear are probably the most general features correlated with aging. Human aging is characterized by a number of additional features such as greying hair, loss of permanent teeth, changes (mainly loss of elasticity) in the dermis leading to the development of wrinkles, and diminished acuity of sense organs. A general loss of strength as indicated by the hand grip is also correlated with aging. The vital capacity of lungs decreases and blood pressure increases with age. Bones are markedly affected by aging. The total loss of skeletal weight amounts to about 15% between youth and old age. In post-menopausal women the loss of skeletal weight is more rapid than in men of comparable age. Beyond the age of 65 both sexes experience increased bone loss. This is due to a progressive malabsorption of calcium. This is why aged persons are prone to sustain fractures easily.

Physiological Changes

Several physiological regulatory mechanisms show decreased efficiency due to aging. For example, normally the glucose level in the blood shows very little change with age, but if glucose is injected into the blood the rate of restoration to normal level shows a marked dependence on age: it is far slower in old people than in young. It has been concluded from many physiological studies that the greatest change is found in functions which involve the coordinated activity of a number of organ systems. Those which involve only a single organ or system show little change with age.

Biochemical Changes

Many biochemical changes are correlated with aging. Detailed biochemical studies in rats have been carried out by Prof. M.S. Kanungo of the Banaras Hindu University, Varanasi, revealing that many enzymes and their sub-types undergo sequential changes during aging. The change of pattern is not uniform in all organs. An important enzyme that shows age-related changes is acetyl cholinesterase. Its level in the cerebral hemispheres is high in immature rats (9 weeks old). The level of this enzyme decreases progressively thereafter. Several brain functions like learning, storage and retrieval of information are known to decrease with age. The decrease in the activity of acetyl cholinesterase may be correlated with this. However, one ought to be cautious in extrapolating the data from rat to humans. It is known that many men and women remain mentally alert and creative in spite of exhibiting many other physical features of old age. It is possible that the different physical and physiological features characterizing old age set in at different times and progress at different paces, depending on the individual's constitution and life style. Similarly the changes may set in at different times in the various organs.

Aging is a complex subject and many changes are seen during senescence, but it is

difficult to establish which phenomena are causes of aging and which are its effects. However, there is no doubt that progressive changes take place both within the cells and in the environment surrounding the cell.

SAQ 5

List out the changes generally associated with senescence in human beings.

.....

.....

.....

There are several theories, put forward to explain the process of aging. Let us examine some of the important ones, that try to explain the process of aging at the cellular level.

20.3.2 Theories of Cellular Aging

In 1950 Leonard Hayflick of Stanford University (U.S.A.) suggested that death of cells resulting in the malfunctioning of organs is a normal programmed event in the development of organisms. Each cell has a finite life time determined by the number of times it can divide. Thus collectively the cells set a limit on the life of an organism.

Hayflick and his-co-workers found that fibroblasts from embryonic human lungs instead of growing in a culture medium indefinitely, could proliferate only for about 50 times after which the cells stop dividing and degenerate even though favourable culture conditions are maintained. Moreover, fibroblasts seem to 'remember' how many divisions they have undergone. If human fibroblasts are frozen after the 20th division, they will still divide 30 more times. The 'clock' for this seems to be present in the nucleus. In another experiment nuclei from young fibroblasts (about 10 division old) were transplanted into old cells (which had already gone through 30 divisions) from which the nucleus had been removed and vice versa. It was found that in the former case the cells divided 40 more times and in the reverse case the cells divided only 20 more times. This showed that further cell division in each case was controlled by the nuclei of these hybrid cells. Interestingly tumor cells do not show this limitation; they reproduce indefinitely under favourable conditions.

But can this model of aging *in vitro* be applied to cells *in vivo?* To find an answer to this question further experiments were conducted on laboratory mice using different types of cells. Cells from old mice were transferred to young mice of the same inbred strain. The remarkable finding was that **cells in vivo too had a limited life span.** This argues well for the concept of programmed cell death. What causes this lack of cell division? It was demonstrated that aged fibroblasts fail to respond to normal physiological stimulators of mitosis. Therefore, much of the senescence may involve the **inability to respond to extrinsic growth factors.**

The **concept of programmed death of cells** is supported by other investigators. It appears that the cell synthesises an enzyme, a nuclease that cuts the cell's DNA into fragments. Interestingly, cells that meet an untimely death such as those that are poisoned or are deprived of oxygen, do not show precise fragmentation of their DNA. Another example of programmed cell death is the steady decline of the thymus gland with age. In this case the glucocorticoids from the adrenal glands trigger the built-in-death mechanisms of the thymic cells. This built in mechanism is associated with the activation of a gene in the cell's DNA.

Another theory that attempts to explain cellular senescence is **the somatic mutation theory** which attributes cellular aging to gradual accumulation of mutations during cell divisions. Presumably as errors occur during cell division, cell function is adversely affected and the additive effect of these errors results in tissue aging. Certain types of errors are likely to produce a great number of subsequent errors. For example, an error in the DNA polymerase gene will make further mistakes during replication. The cause of such mutations may be free radicals or other molecules that can damage DNA.

The concept that free radicals play a role in aging is not new. It was introduced in the 1930s by Denham Harman of the University of Nebraska (USA). Cellular damage occurs as a result of highly reactive free radicals. The superoxide anion (O_2^-), Hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\cdot}OH$) are all very reactive. These free radicals are generated by many normal biological reactions in the body; and are highly toxic to the cell membranes, DNA bases and proteins. It has been calculated that free radicals modify approximately 10,000 DNA bases per cell per day.

Ordinarily free radicals are destroyed in the cells by protective enzyme systems. The enzyme **superoxide dismutase** is present in all cells. It catalyses the conversion of superoxide anion to H_2O_2 which is converted to water and oxygen by the enzyme **catalase. Glutathione peroxidase** also destroys H_2O_2 (This enzyme contains selenium, which is required in trace amounts in our food). Production of free radicals increases with age. Oxidised proteins in the cell have attached carbonyl groups that make them markers for the aging process. In fibroblasts of the young humans 10% of the total cellular protein is oxidised while with age this increases to 40%. This has been confirmed with fibroblasts obtained from children suffering from progeria disease, whose fibroblasts cultures exhibit carbonyl levels comparable to 80 year olds. Age related increase in oxidised proteins is accompanied by decrease in neutral alkaline protease activity. Cells with damaged proteins are more likely to exhibit metabolic disorders.

Theories on cellular aging must explain the entire age related spectrum of changes. One such theory is the **immunological theory of aging.** Elderly people and aged mice are both prone to infections and autoimmune diseases. Both these conditions are linked to the deterioration of the thymus. The thymus reaches its maximum weight in humans by ages 7 to 11 and as the body ages more and more adipose tissue replaces thymic tissue. The levels of thymic hormones decrease and by age 60 are undetectable. This change is compatible with the decline in number of T cells and B cells in the elderly. There are several ways in which a less effective immune system is deleterious to the body. For instance, the ability to mount an antibody response against infections decreases. Immune systems must also be able to recognise and eliminate abnormal cells. With decreasing thymus function there would be an expected curtailment of the ability of the immune system to destroy newly formed tumor cells. With decreasing thymus functions increase in appearance of autoantibody is well documented.

20.3.3 Extracellular Aging

The primary components of extracellular space are mucopolysaccharides and fibrous proteins, particularly collagen and elastin. These proteins are synthesised in connective tissue and secreted out of the tissue. Collagen is estimated to form up to 40% of the body protein and fills the extracellular spaces. It has been suggested as the primary site of age related changes. You would recall from LSE-01, Unit-16, that collagen molecules are polymers of tropocollagen monomers. Newly polymerised molecules of collagen have noncovalent bonds and with age covalent cross-linking takes place between the monomers. As the age of the organism increases cross-linking becomes more difficult to dissociate and by the time of maturity in mammals collagen becomes insoluble. From maturity to old age, cross-linking is strengthened and its effect can be measured.

The age related changes in collagen are indisputable. Age related changes in the structure of collagen affect the functioning and structure of bones, blood vessels, joints, tendon, ligament etc. The inflexibility of collagen fibrils may be a contributory factor for the onset of atherosclerosis and hypertension.

Role of Hormones

Aging also reflects the inefficiency of the endocrine system. A change in the hormonal secretions may contribute to the development of physical ailments of old age. For example, decrease in secretion of estrogen from the ovaries causes oesteoporosis in women. Hypothalamus appears to be an important agent in aging. The production of gonadotropin releasing hormones from the hypothalamus is influenced by drop of catecholamine (chemical neurotransmitter) in the nerve endings of the hypothalamus.

Experiments were done using the drug L-dopa to reinitiate the release of catecholamines from the nerve endings of the hypothalamus of rats in which ovaries had stopped discharging eggs. L-dopa also promotes estrous in old rats. Accordingly, an age-related deficiency in hypothalamic nerve endings or receptors of catecholamine may be responsible for the loss of ovarian function in elderly women.

Patterns of growth in organisms and the aging processes that we have learnt about in this unit show that the two phenomena, growth and aging, can be studied at any level from molecular to organismal. We see that all aspects of the organism are clearly involved in the general process of aging and no single one can be assigned the responsibility for the overall phenomena.

SAQ 6

Indicate which of the statements given below are true or false:

i) Aging may be due to deficiency of hypothalamic functioning or disfunctioning of catecholamine receptors in the hypothalamus.

ii) Extracellular collagen looses its elasticity with age and aging can be reversed if collagen is denatured.

iii) The steady decrease in thymus gland size is related to loss of immunological function with age.

iv) Cells *in vitro* have a limited life span and *in vivo* the same cells can divide indefinitely due to favourable conditions.

v) Decrease in estrogen level is totally unrelated to osteoporosis seen in post-menopausal women.

vi) Free radicals are generated in the body only in the old individuals.

vii) Aging as the result of gradual accumulation of mutations during repeated cell divisions is the main theme of the somatic mutation theory of aging.

viii) When young nuclei are transferred to old denucleated cells in culture, the hybrid recipient cells divided according to the instructions of the new nucleus..

20.4 CANCER—RESULT OF DEVELOPMENTAL ERROR

In Section 20.2 you studied processes involved in cell growth. We know that in normally growing embryonic organs there is a control over the growth process. Divisions of cells ceases once the definitive size of the organ (appropriate to the species and overall size of the adult individual) is reached. However, cell division continues in most organs to compensate for loss due to injury, wear and tear etc. This too is subject to processes of growth control. Thus a fully grown adult individual is formed and maintained. When a cell escapes this control and proliferates unchecked, and the descendents of such a cell inherit this quality to grow without responding to regulation, the result is a **clone** of cells able to expand indefinitely. Ultimately, a mass of **neoplastic tissue** or **tumor** arises. When the tumor is localised and confined to the organ of origin it is known as **benign** but when the tumor cells spread to other regions of the body, the tumor is **malignant** and known as **cancer.**

Most of us are aquainted with benign tumors such as polyps and warts. Malignant tumors escape the rules of differentiation. The major feature by which we distinguish malignant tumors from benign tumors is their ability to invade and spread in tissues away from the site of their origin. This spread of tumor cells and establishment of secondary areas of growth is called **metastasis.** We will learn more about this in the next subsection, when we deal with characteristics of malignant cells.

Malignant tumors are by no means found only in human beings, but throughout the animal kingdom, from ants to whales. Corresponding phenomenon also occurs in plants. The crown gall, for example that affects a wide variety of plant species, resembles neoplasia.

The induction of crown gall requires that a wound in a suitable host plant be innoculated with *Agrobacterium tumefaciens*. After enough cells have been transformed into crown gall cells, the tumor continues to grow even after the bacteria are killed. The transformation occurs when a segment of a plastid transferred from the inducing bacterium is incorporated in the host cell DNA.

Cancer can afflict at any age but its prevalence is seen more during childhood and old

age. Increased incidence of certain cancers with increasing age in humans can be related to the increased longevity of modern times. Earlier people did not live long enough to show cancers peculiar to middle and old ages.

Cancer is usually derived from cells that normally maintain a proliferative capacity. Thus we see that, mature neurons and cardiac muscle cells do not give rise to tumors. Malignant tumors are classified according to the tissue from which they originate (see Table 20.2) and a tumor express varying degree of differentiation from relatively mature structures that mimic normal structures to cells so primitive that it is difficult to describe their origin.

Table 20.2: Major types of Cancers

	Cancers		Site of Origin
1.	Carcinoma	Solid tissue	Epithelial tissue (skin, glands, nerves, breast, lining of respiratory, gastrointestinal, urinary and genital systems)
2.	Sarcoma	Solid tissues	Embryonic mesoderm (connective tissue, bone, cartilage, muscle, fat)
3.	Leukemia	Abnormal number of leucocytes	Bone marrow
4.	Lymphoma	Abnormal number of lymphocytes	Lymph nodes

It is not even known whether cancer is many diseases exhibiting a common pattern of general symptoms or one disease that is manifested in many forms depending on the organ from which it evolves. More than 200 clinically distinct types of cancers each having a unique set of symptoms and requiring specific therapy are known.

Before we discuss the possible causes and mechanism of development of cancer. Let us see how malignant cells differ from normal cells.

20.4.1 Characteristics of Malignant Cells

Three major characteristics define cancer:

i) hyperplasia

ii) anaplasia

iii) metastasis.

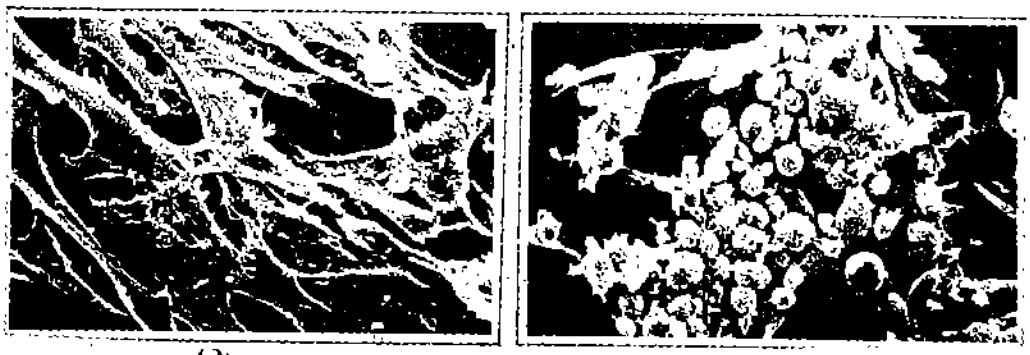
i) **Hyperplasia** is the excessive proliferation of cells which can be seen in normal as well as cancerous tissues. In normal tissue, as a result of interaction with physical, chemical or biological agents, cells may proliferate excessively for a while. However, this state of affairs eventually comes back to normalcy. In case of neoplastic tissue, cells proliferate excessively resulting in more and more abnormal cells. Contrary to popular belief, hyperplasia does not mean an increased rate of proliferation. Malignant cells show the same variation in rates of division as do healthy cells. However malignant cells simply do not respond to regulatory processes as do healthy cells. Tissue cultures of malignant cells are immortal, that is, they continue to divide infinitely as long as nutrition is provided, whereas normal cells stop dividing after a limited number of divisions.

Normally cells in tissue culture stop growing when they come in contact with other cells to ensure that cells do not overlap but cancer cells lack this ability and grow to form heaps.

ii) **Anaplasia** is a structural abnormality in which cells resemble primitive or embryonic tissue in which adult functions are diminished or totally lost. For example, malignant lymphocytes do not have the ability to fight infections. Advanced cancers show a varied cytological picture.

1) Cancer cells have a high nucleus to cytoplasm ratio. The nucleus may be large, often giant sized, more than one prominent nucleoli may be present.

2) Cancer cells show many abnormal mitoses in each cell. This phenomenon indicates that the mitotic processes usually so orderly in normal cells have been dramatically disrupted. Fig. 20.6 shows a normal tissue and cancer tissue. Note the structural differences.



(a) (b)

Fig. 20.6: (a) Electron micrograph of normal connective tissue cells that adhere to the surface of the laboratory dish in which they are cultured. They have a flat extended shape. (b) On infection with Rous sarcoma virus they get transformed into rounded cells that cluster together in piles.

3) Cancer cells and embryonic tissue resemble in several aspects of function and structure. Therefore, it is often said that cancer cells are **dedifferentiated** *i.e.*, they seem to have reverted to an earlier embryonic stage of development. Table 20.3 compares several features of malignant and embryonic cells.

Table 20.3: Embryonic cells and cancer cells — a comparison.

	Embryo	Cancer
i)	originates from a single cell.	clonal origin
ii)	high mitotic activity and initially exponential growth.	high mitotic activity and exponential growth in malignant state.
iii)	specific cell surface antigens found. E.g. Carcinoembryonic Antigen (CEA) found in foetal gut tissue.	foetal antigen in certain tumors E.g., CEA found in blood of patients with advanced colon cancers.
	Alfafoetoprotein found in foetal liver.	Alfafoetoprotein found in liver cancers and some other tumors.
iv)	cell migration from site of origin and then differentiation into various tissues takes places.	metastasis also a form of cellular migration. Partially differentiated cells are formed.

iii) **Metastasis** is the ability of a malignant cell to detach itself from a tumor and establish a tumor in another site. This ability reflects the lack of contact inhibition seen in cancer cells in tissue culture. It also shows that malignant cells have the capability to sustain themselves while circulating freely in the blood stream or lymph ducts. Metastasis as we have mentioned earlier is the diagnosistic feature of cancer cells and distinguishes malignant growth from benign growth.

The mechanism by which a malignant tumor initially penetrates and invades the normal surroundings tissue is thought to have three steps as shown in Fig. 20.7.

1) The cancer cell first aquires the ability to bind a component of the basement membrane of the tissue of origin *i.e.*, receptors bind to tissue matrix components.
2) The basement membrane is then lysed by proteases found in cancer cells.
3) The tumor cells then move through the defect in the membrane and the process is repeated till they reach the blood stream or lymph vessels.

The anatomy and vascular system in a specific organ influence the pattern of metastatic spread but it is also true that certain tumors prefer some organs over others because of interaction between tumor cells and host organs. However, it is to be noted that despite the rich vascularisation, skeletal muscles and spleen are not common sites

of secondary tumor formation.

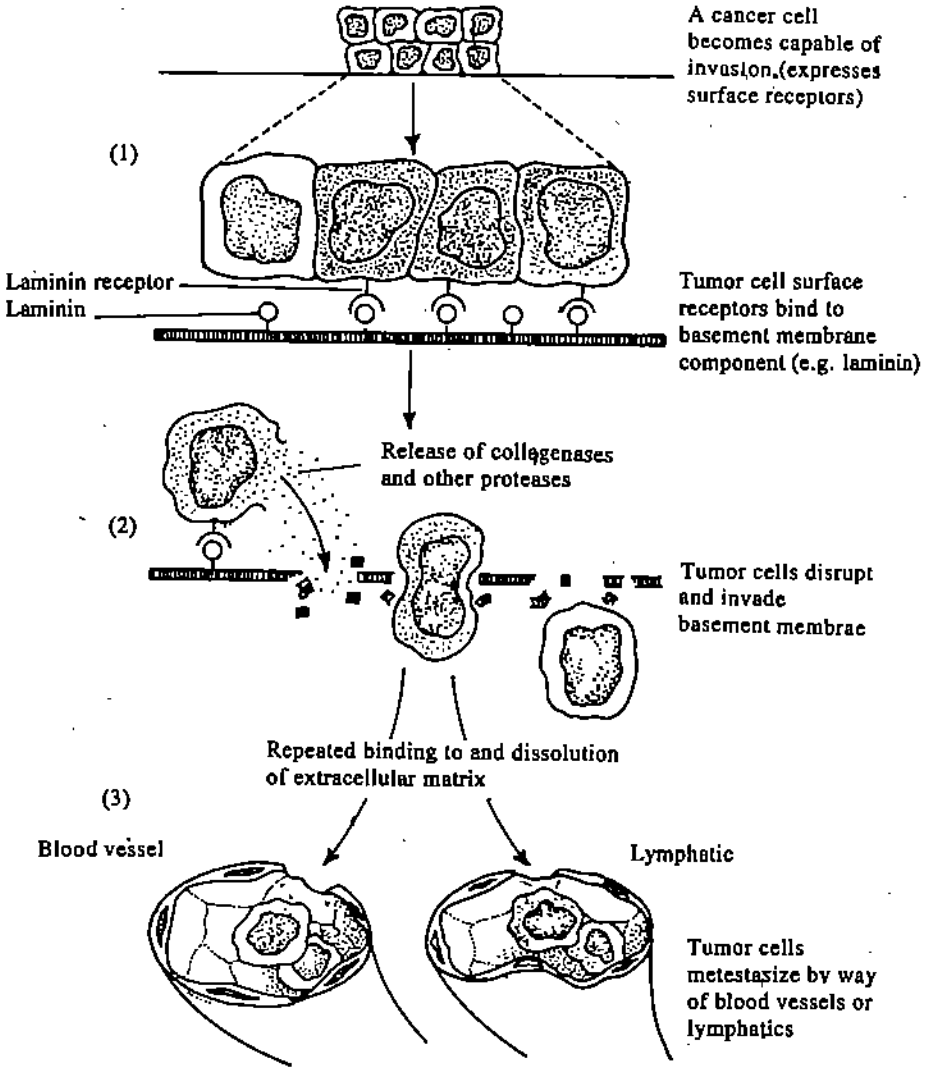


Fig. 20.7; Mechanism of metastasis

SAQ 7

Make a table to show how properties of cancer cells differ from normal cells of the body.

20.4.2 What Causes Cancer?

We know that a malignant tumor is a large aggregation of cancer cells, all of them descended from a single founder cell which was once a normal cell with normal functions in a particular tissue. What happens to the cell to free it from the normal

constraints on cellular growth? We can examine the cause of cancer mainly at two levels: (1) the agents that trigger the change from normal to cancerous stage and (2) the change that converts a normal cell to a cancer cell. In Unit-17 of LSE-03 we examined that radiation and certain chemicals cause certain changes that eventually lead to cancers. These are called **carcinogens**. For example X-rays and ultraviolet rays in sunlight are both physical agents that cause cancer. Exposure to X-rays is a major cause of leukemia and brain cancer, while UV is known to cause skin cancer including the deadly type—**melanoma**. Similarly various chemicals are known carcinogens. For example tobacco is one of the most common agents linked to cancer of the oral cavity. We also learnt that certain viruses can cause cancers in animals such as cats, laboratory mice, birds and frogs. Certain viruses have also been associated with human cancers. In all these cases we should realise that some change occurs at the level of genes which can be passed on at each mitosis, thus giving a clone of cancer cells.

20.4.3 Stages of carcinogenesis

How do carcinogens cause cancer? Cancer biologists have realised now that the processes of carcinogenesis is very complex, involving many events but to simplify the process it can be divided into at least three stages initiation, promotion and progression.

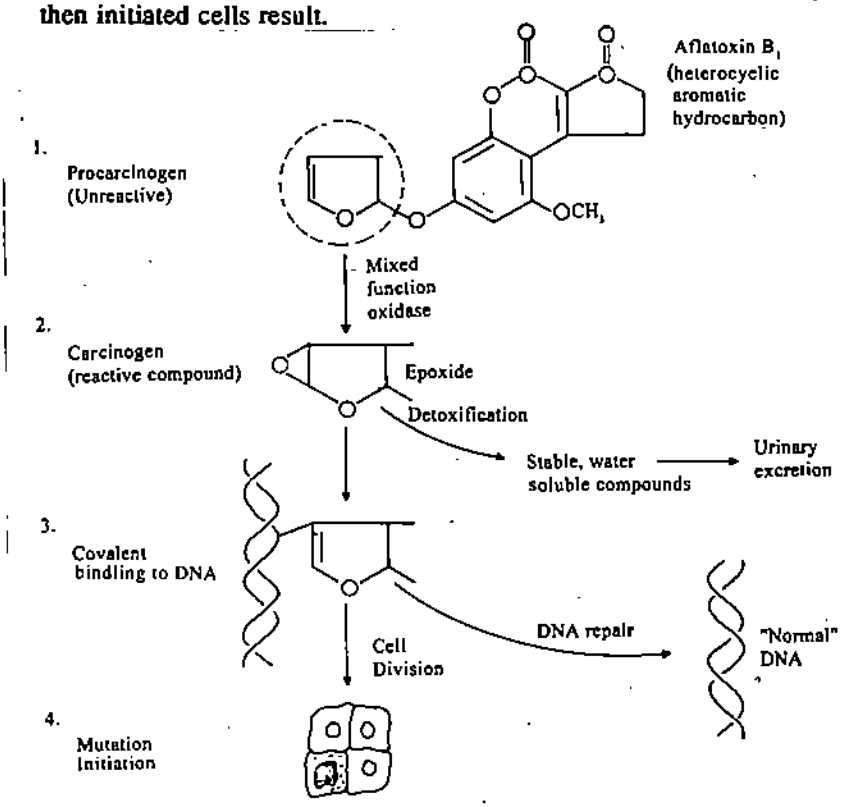
1) Initiation

The first stage **initiation** results from an irreversible genetic alteration, most likely

Box 20.2

Many chemicals are now proven carcinogens. They cause cancer directly or more often after being metabolically activated into more reactive compounds in the cells of the body. This activation is enzymatic. One potent liver carcinogen is aflatoxin a natural product of the fungus *Aspergillus flavus*. It produces tumors in fish, birds, rodents and primates. Since aspergillus species are found everywhere, contamination of vegetable foods, particularly peanuts and grain exposed to warm moist conditions may result in production of significant amounts of Aflatoxin. The figure given below shows the steps in the activation of the compound and initiation of cancer cells.

1) Aflatoxin is metabolised to form an epoxide by enzymes in liver cells.
2) The epoxide is either detoxified and excreted or can bind to DNA in liver cells.
3) The resulting DNA is either repaired or if liver cells divide before repair then initiated cells result.



one or more mutations. It is difficult to identify and isolate initiated cells, except in some experimental conditions. One such example is the initiation of liver cancer in rats by a known chemical carcinogen **aflatoxin** (see Box 20.2). Most initiated cells never proceed to the next stage of cancer formation and scientists fear that adult organism contain many initiated cells in most organs. Therefore, the first step of cancer is a very common event that often occurs spontaneously and may be induced readily in animals and humans.

2) Promotion

The second stage or **promotion** is reversible and does not involve changes in structure of DNA but rather in the **expression of the genome** of the initiated cell. This results from an interaction between the changed DNA structure and some environmental factors termed 'promoting agents'. The stage of promotion requires extended interaction with the promoting agent, if the third stage of progression is to be reached. Fig. 20.8 explains the process of promotion in a simplified manner.

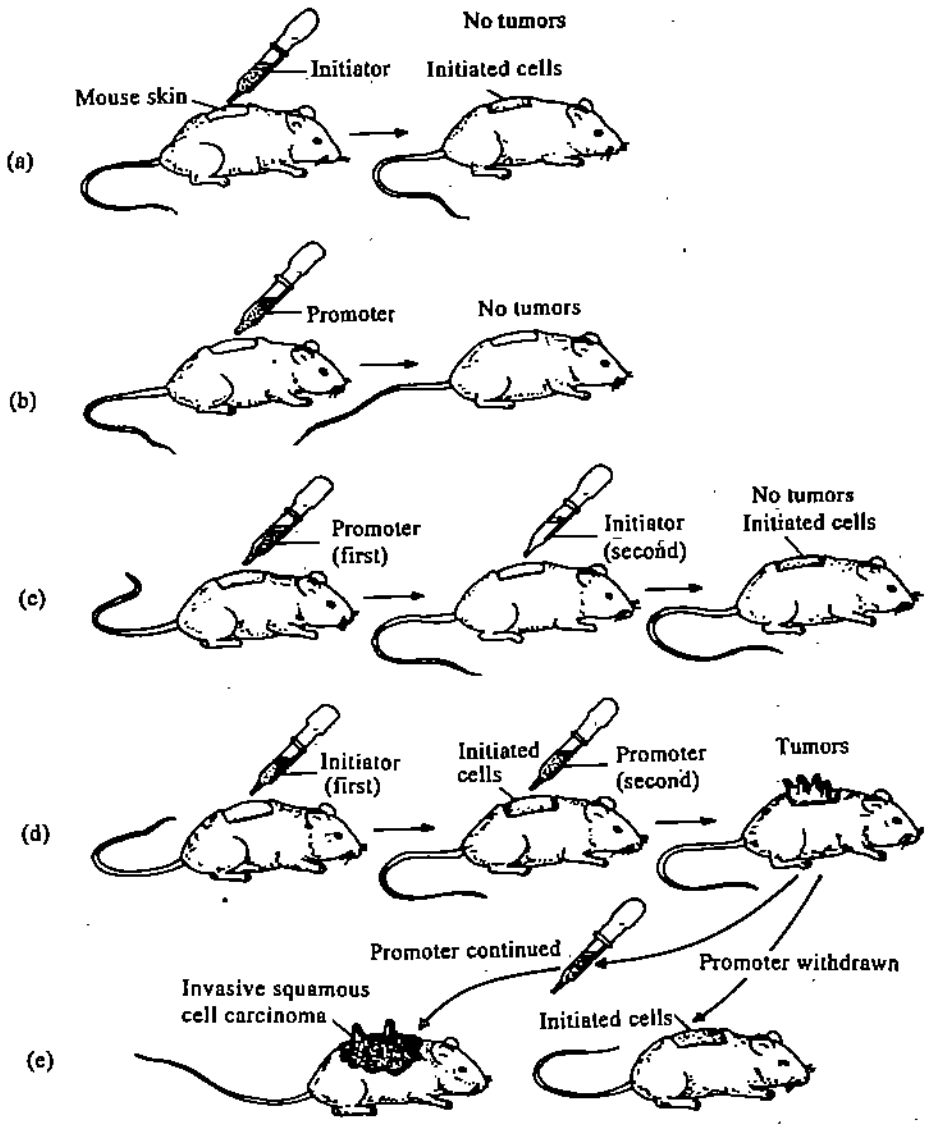


Fig. 20.8: The concept of initiation and promotion

A single application of an initiator to the skin of a mouse produces initiated cells but no tumor (a) The application of a promoter alone to the skin also does not produce tumor (b) If the promoter is applied before the initiator is applied, even then no tumor is formed (c). However, if the initiator is applied to the skin first then subsequent application of the promotor results in tumor (d). If the promoter is withdrawn the tumor regresses leaving behind initiated cells. and continued application of promoter on tumor bearing skin leads to invasive carcinoma (e).

3) Progression

The final irreversible stage is known as **progression** and is characterised by karyotipic instability, *i.e.*, the chromosomal number keeps on changing and there is a continued evolution of chromosomal abnormalities. As a result of this different cell sub-populations with varied chromosomal patterns are seen. Malignant growth is a part of this stage. Whereas the normal dividing cell is able to regulate the structure of its genome and karyotype after multiple cell division, the malignant cell is unable to do so.

20.4.4 Mechanism of Carcinogenesis

In the previous sub-section you learnt that carcinogenesis is a multistep process. Although cancer may be caused by many agents—chemical, physical and viral, they all have a common pathway, namely, damage to DNA. This damage or alteration of the normal DNA of the cell can be affected mainly in two ways. Either the normal genes are mutated or as in the case of viral agents, abnormal genes are inserted in the host cell which encode abnormal proteins (Box 20.3)

The question may arise, which of the genes of the normal cell on mutation, give rise to neoplastic cells?

We said earlier that neoplastic cells arise when normal cells somehow escape the clutches of cell growth regulatory mechanisms. Therefore, genes that are responsible for regulating cell growth and differentiation would be the obvious targets of mutation.

Research over the years has revealed that two main types of genes play a role in cancer induction. The first is a set of genes that regulate cell growth and differentiation. These genes are called **proto-oncogenes** and their mutated versions that can cause cancer are called **oncogenes**. (Refer to Unit-17 LSE-03). The second is a set of **tumor-suppressor genes** also called **anti-oncogenes** whose normal function is to prevent overgrowth of cells.

Proto-oncogenes

Proto-oncogenes are normal genes present in all organisms and appear to be highly conserved in the course of evolution. They have been isolated in species ranging from yeast to highly evolved vertebrates including human being. This high degree of conservation implies their crucial role in the cell. Genes homologous to cellular proto-oncogenes are found in retroviruses known to cause cancer in various animal species. These viruses transform cells either by inserting their oncogene in the host genome or by being present in multiple copies in the host cell. It is thought that the retroviruses picked up these oncogenes from metazoan cells they infected (see Box. 20.3)

Since their discovery more than 60 proto-oncogenes have been identified. Each has a role in mitosis. Some proto-oncogene encode **growth factors**, proteins that stimulate cell proliferation. Many proto-oncogenes encode **receptors for growth factors**. A large number of them encode **intracellular transducers**, proteins that bring signals from cell receptors to nucleus, and **transcription factors** that turn on gene transcription in the nucleus.

The products of all proto-oncogenes work harmoniously at the right time in right amounts to induce cell division and differentiation.

Let us now examine some of the possible ways in which proto-oncogenes are converted into oncogenes. Fig. 20.9 illustrates alternate ways to make oncogenes from proto-oncogenes.

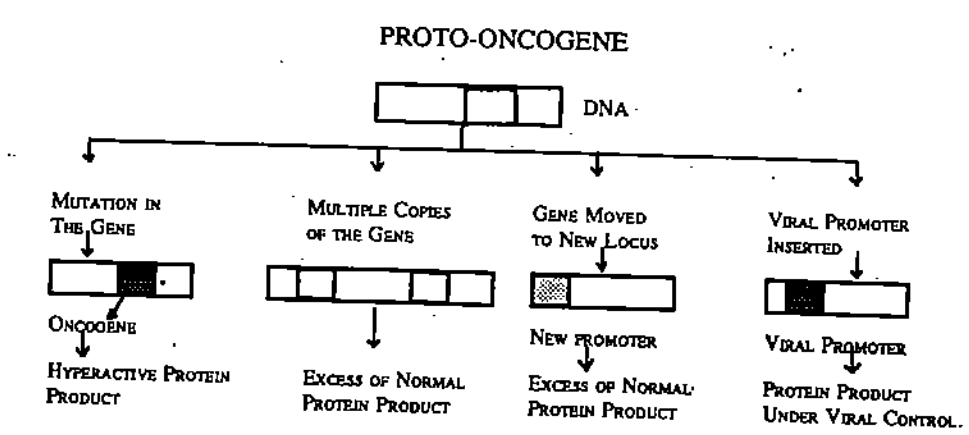


Fig. 20.9: Alternate ways of converting proto-oncogenes to oncogenes.

You can see in the figure that:

a) a mutation may occur in the gene which becomes an oncogene. This oncogene encodes for a hyperactive protein different from normal protein,

b) an error in DNA replication or recombination can make several copies of the proto-oncogene. In which case much more than normal amounts of the protein may be produced,

c) the proto-oncogene may be moved from its original position to a new position in the cell's DNA. At the new site it would be under the control of a new promoter or some other genetic control. For example in Burkett's lymphoma a tumor of B-cells, a small portion of the long arm of chromosome 8 is translocated to the region of the immunoglobin genes on any of the chromosomes 14, 22 or 2. (See Fig. 20.10). The proto-oncogene *c-myc* comes under the influence of immunoglobin gene promotor.

d) A new promoter can be inserted into the cell by a viral infection. This could deregulate the proto-oncogene message. Placing it under viral control.

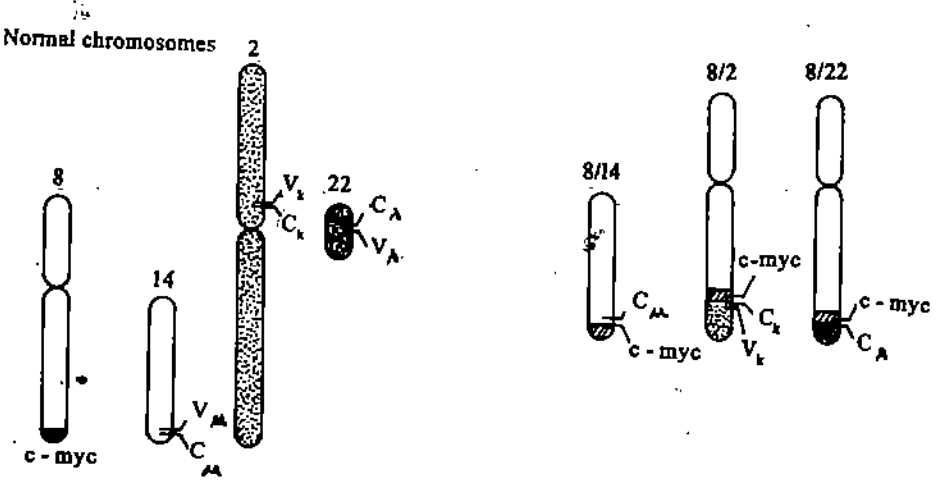


Fig. 20.10: The chromosomal translocation that gives rise to Burkitis lymphoma. The *c-myc* gene of chromosome 8 is transferred to one of the three immunoglobin gene region on chromosomes 14, 22, 2.

Products of oncogenes mimic the normal proto-oncogene proteins and are thereby, able to disturb the normal regulatory processes involved in cell proliferation and differentiation. The discovery of newer oncogenes is leading towards a better understanding of normal cell growth pathways and also the role of proto-oncogenes in developmental processes.

Tumor suppressor genes

Tumor suppressor genes or **anti-oncogenes** normally inhibit cell division in cooperation with proto-oncogenes. The very presence of these genes prevent cells

from undergoing neoplastic transformation. When tumor suppressor genes are inactivated by mutations or are deleted, the absence of their protein products allows malignant growth to occur. The most extensively studied disease caused by suppressor genes is the childhood tumor retinoblastoma (cancer of the retina). The gene involved is Rb and the genotype of normal individuals is Rb/Rb. A prerequisite of tumor formation is the development of homozygosity or heterozygosity of the rb allele. The possible genetypes for retinoblastoma are, therefore, rb/rb; rb/- or -/-

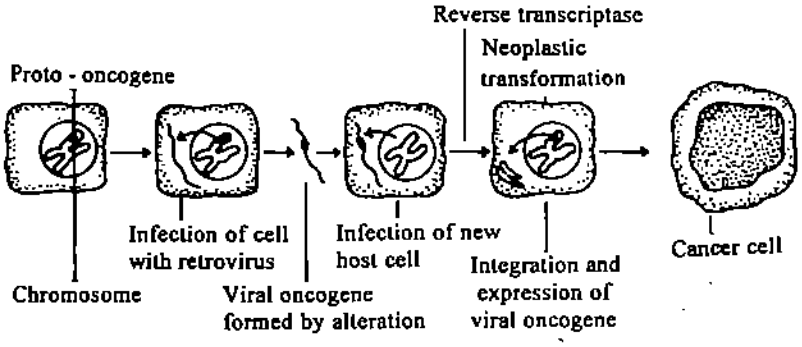
Box 20.3 Rous Sarcoma Virus and Cancer

Viruses for a long time have been known to cause cancer in animals and crucial advances in understanding cancer research have come from the study of viruses that induce tumors. In 1910 Peyton Rous discovered that cancer could be induced in healthy chicken by injecting them with cell free extracts of tumors from sick chicken. This was the first demonstration of an oncogenic virus. The Rous Sarcoma Virus (RSV) is a member of the family of retroviruses. It has only four genes in its RNA molecule (1) *gag* which encodes the capsid protein (2) *pol* which encodes reverse transcriptase (3) *env* which encodes envelope protein and (4) *src*, which encodes an enzyme that attaches phosphate groups to a variety of host proteins. By a simple experiment, it was found that *src* gene was the oncogene that caused transformation of host cells when inserted into the host cell DNA.Cells were infected with a strain of RSV that was normal in all respects except that the *src* gene carried a mutation making it ***temperature sensitive***. Actually the protein product of the gene was temperature sensitive. At 35°C the protein behaves normally and cells are transformed but at 41°C the protein looses its activity and the cells revert to normal. Either step is reversible. The cells can be switched from one state to another by changing the temperature, showing that the correct expression of *src* is all that is required to transform normal cells.

In 1976 American molecular biologists J. Micheal Bishop and Harold Varmus and their colleagues discovered that *src* of RSV was actually an altered version of a gene found in normal chicken cells. Apparently the virus picked it up from the host cell during a previous infection (see accompanying figure). This cellular gene was called proto-oncogene and designated *c-src*. It later turned out that *src* like genes are found throughout the animal kingdom among vertebrates and invertebrates alike. Humans have a *c-src* gene that occupies a locus on chromosome 20.

In 1966, 55 years after the discovery of the RSV Peyton Rous was awarded the Nobel Prize in recognition of the key role that the virus had played in cancer research.

In 1989, Bishop and Varmus received the Nobel Prize for their pioneering work that led to the understanding that organisms have genes with the potential to cause cancer.



SAQ 8

List out the mechanisms that are known to convert proto-oncogenes to oncogenes.

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20.4.5 Multicausal Nature of Cancer

With so many dividing cells in the organism and the various proto-oncogenes and tumor suppressor genes that can be altered to cause cancer, one might expect cancer to be a more common event than it is. Many biologists believe that probably our/body has many initiated cells aquired throughout our life time but the various defence mechanisms of the body destroy them before they can cause cancer.

Increasing evidence now suggests that any single mutation in a cell is never by itself enough to cause cancer. Only when a single cell suffers several sequential mutations does it finally loose its regulation over its cell cycle and a full fledged cancer results. One of the diseases that explains this concept is colorectal cancer. Initially, in normal epithelium a suppressor gene on chromosome 5 is inactivated or lost. Next comes hypomethylation of DNA, which is followed by mutation of *ras* proto-oncogene.The losses of chromosomes 18 and 17 complete the transformation into a cancer cell. Further chromosome losses provide the tumor the ability to form metastases.

More and more research is being done on what actually activates or turns on the genes in tumor cells and the role that individual oncogenes play in making the cells cancerous. By comparing DNA sequences of known oncogenes with genes for cell growth and growth receptors, investigators have begun to identify key genes. For example, an oncogene has been found to correspond to the gene that functions as a growth factor in the healing of wounds. The discovery of newer oncogenes is leading towards a better understanding of normal cell growth pathways and also the role of proto-oncogenes in development processes.

The current efforts in prevention and treatment of cancers are characterized by a multidisciplinary approach. Early detection and characterization of the tumor is an important prerequisite for effective treatment. Understanding the cellular and molecular aspects of growth, differentiation and neoplastic change will give clues to development of effective drugs for treatment. The combined efforts of developmental biologists and medical scientists can eventually lead to alleviation of human suffering from the disease.

20.5 SUMMARY

In this unit you have studied that:

- Growth in multicellular organisms is fundamentally an increase in the number of cells, and cell proliferation in most tissues is stopped once they differentiate. Most of the changes in body form are due to allometric growth. Growth is isometric when new material is added to existing material..
- Growth is measured as an increase in body weight and depicted in the form of growth curves. Most animals show a typically sigmoid growth curve. However, growth curves in some fishes and reptiles are parabolic.
- Growth is regulated by extrinsic and intrinsic growth factors that act on the various stages of the cell cycle.
- Aging in animals is inevitable and usually starts after sexual maturity is attained. It is a genetically programmed process as shown by the different life spans of organisms. The general consequences of aging in man and animals are a gradual loss of reproductive ability, neuromuscular coordination and inadequate assimilation to compensate for wear and tear. Several theories have been postulated to explain the cause of aging. Most of them attribute aging of cells to accumulation of mutations and error in the genome; loss of immunological functioning; a finite lifetime determined by the number of times a cell can divide; insufficiency in endocrine system.
- Uncontrolled proliferation of cells leads to neoplastic growths or cancer. Cancer cells differ from normal cells in morphology. They appear dedifferentiated and are able to metastasize. All cancer cells are clones of a single transformed cell. Carcinogenesis is a multistep process involving initiation, promotion and progression. Two sets of genes are involved in cancer induction, proto-oncogenes and tumor suppressor genes. Proto-oncogenes are vital genes in normal cells

involved in growth regulation and differentiation. Proto-oncogenes can get converted to oncogenes as a result of accumulation of mutations with age, or viral infections or translocation in the chromosomes of the cells, Oncogene products misdirect the cell growth stimulating signals at various stages by mimicking growth factors, or growth factor receptors or by overstimulating the transcriptional activity in the nucleus.

20.6 TERMINAL QUESTIONS

1) What is the role of free radicels in aging?

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2) Define the terms neoplasm, sarcoma, carcinoma, oncogenes& proto-oncogene.

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3) From what you have read in this unit and previous knowledge of retroviruses, describe how viral transforamation of cells takes places.

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4) List out the various extrinsic and intrinsic factors that affect growth.

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20.7 ANSWERS

Self Assessment Questions

1) Growth essentially means any type of increase in size, volume or bulk of an individual. Thus growth is the result of far more anabolic than the catabolic activities in the organism. If anabolism and catabolism go on at the same rate then there would be no growth.

2) i) allometric growth

   ii) isometric growth

   iii) allometric growth

3) In the case of the rat the curve is a single continuous sigmoid curve with the growth rate increasing before sexual maturity. While the growth curve of a human being after birth has two distinct phases of increasing growth rates (infancy and adolescence) and stabilises only after adolescence

4 i) somatomedins ii) G1 epidermal growth inhibitor

iii) B-Interferon iv) phosphorylation

5) Changes as a consequence of aging in humans are:

1) changes in physical appearance e.g. skin becomes wrinkled, less elastic producing a typically 'old' look

2) changes in physiological ability—lowered energy reserves, lowered immune response, decrease in perceptual acuity

3) general atrophy of body organs leading to decline in muscular strength, coordination, softening of bones leading to easy fracture, loss of flexibility of joints, senile brain diseases

4) often decline in mental abilities, loss of memory, reduced rate of fresh learning etc.

6) i) true ii) false iii) true iv) false v) false vi) false vii) false viii) true

7)

	Normal Cells	Malignant Cells
a)	Growth is restricted to regeneration/wound healing; forms monolayers *in vitro* i.e. contact inhibited	Unrestricted growth in the body and in culture medium. Growth beyond monolayer formation *in vitro*, forms clumps
b)	karyotype diploid	various chromosomal alterations found
c)	stable differentiation in the body	loss of differentiation; often resembles embryonic tissue
d)	will not migrate and lodge in other parts of the body	decreased adhesiveness to other cells and extracellular matrix; invades other parts of the body to start new clones of cells

8) a) mutations

b) gene amplification

c) Translocation and transposition of regulatory genes

d) insertion of viral oncogenes & viral promoters

Terminal Questions

1) Refer to sections 20.3.2

2)

Neoplasms	—	'New growths' or abnormal mass of cells forming tumor
Sarcoma	—	malignant growth originating from connecting tissue and their derivatives
Carcinoma	—	malignant growths arising from epithelial tissue e.g. breast cancer
Oncogenes	—	genes that cause transformation of their target cells, just discovered in retroviruses
proto-oncogenes	—	cellular counterparts of oncogenes involved in the normal growth and DNA synthesis in cells

3) 1) RNA virus infects cells

2) RNA released into host cytoplasm. Reverse transcriptase produces, double strand of provirus using viral RNA as template.

3) Provirus integrated into host DNA

4) Copies of viral RNA and proteins formed

5) Provirus may influence part of host cell gene for abnormal expression

6) Newly formed enzymes and proteins change properties of cells

7) New viral particles are formed.

4) Extrinsic factors promote growth. They are growth hormones, somatomedins, insulin like growth factors, placental lactogen, PDGF, EGF etc.

Intrinsic growth factors are usually tissue specific and inhibitory in action. For example Chalones, Interferons, Transforming growth factor B (TGF B).

UNIT 21 HUMAN DEVELOPMENT

Structure

21.1 Introduction
Objectives
21.2 Gametogenesis
Spermatogenesis
Oogenesis
21.3 Female Reproductive Tract
21.4 Pre-Embryonic Development
Fertilization
The Pre-embryo
21.5 Embryonic Development
Third Week
Fourth Week to Eighth Week
21.6 Foetal Development
21.7 Developmental Changes After Birth
21.8 Extra Embryonic Membranes and Placenta
21.9 Flaws in Development
21.10 Summary
21.11 Terminal Questions
22.12 Answers

21.1 INTRODUCTION

In the earlier units of this block you studied the process of regeneration and metamorphosis in animals and the several mechanisms involved in the process of growth, aging and cancer formation. In this last unit of the block we will study human development. All the general principles involved in animal development that you had studied in Block 3, with the help of examples taken from invertebrate, and vertebrates, hold good for human beings. We study human development, for it is a subject that is of interest to all of us. A knowledge of human development is of practical value in helping us to understand the normal relationships of body structures and the causes of congenital malformations. This knowledge is of significant value in various branches of medicine and it also helps in the understanding of healing of wounds, for tissues are restored to normal by processes that characterize embryonic differentiation.

Human development is a continuous process that begins when the ovum from a female is fertilised by sperm from a male to form the zygote. Growth and differentiation transform the zygote into a multicellular adult human being. However, it is important to realise that development does not stop at birth. It is a continuous process. It is usual to divide human development into **prenatal** and **postnatal** periods. Prenatal period refers to the period before birth. During this period for the first eight weeks the developing human being is called an **embryo** because the organ systems are forming. From the 9th week onwards the term **foetus** is used. The foetal period (9 weeks to birth) is characterised by growth and elaboration of structures. The postnatal period begins at birth and ends at death. Important developmental changes in addition to growth occur after birth, for example, the development of teeth and the changes during puberty. The brain triples in weight between birth and 16 years of age. However, most developmental changes are completed by the age of 25.

The 266 days between conception and birth are traditionally divided into about three month periods, each called a **trimester.** We deal with each trimester but more emphasis is given to the first trimester as more dramatic changes occur during this period. But before we discuss the development of the human embryo it is important to recapitulate the process of gametogenesis and the general structure of the female reproductive tract as the entire prenatal period is spent inside the mother's womb.

We would advise you to pay attention to the diagrams and tables given in the unit as these would help you to understand the subject better. We also suggest that you read Unit 8 of LSE-05 'Reproduction' once again before you start reading this unit, as we assume certain knowledge of reproductive biology.

Objectives

After studying this unit you would be able to:

- describe the major events in the first two weeks of development
- outline the various stages in human development
- differentiate between embryonic and foetal periods of development
- describe the formation and function of human placenta
- outline the general path of foetal circulation
- describe the major developmental changes in respiratory and circulatory systems in the newborn and
- elaborate some of the causes of common defects and abnormalities that may occur during development before birth.

21.2 GAMETOGENESIS

Gametogenesis as you are aware is the process of formation and development of specialized reproductive cells, ova in females and sperms in males. (Refer to Unit-13 of this course and Unit-8 of LSE-05.) In this process the chromosome number is reduced by half in both sperms and ova. Any disturbance during gametogenesis for example, non-disjunction, results in abnormal development as seen in trisomy-21 and Down's syndrome. You should remember, that in humans,gametogenesis is a continuous process that starts at puberty. It is not a seasonal or periodic phenomenon as in many other animals including mammals.

21.2.1 Spermatogenesis

The process of maturation of spermatogonia into sperms begins at puberty (about 14 years) and continues into old age. The spermatogonia that lie dormant in the testis since the foetal period begin to increase in number at puberty. After several mitotic divisions the spermatogonia transform into **primary spermatocytes** which are one of the largest germ cells in the tubules. Each of these primary spermatocytes undergo reduction division to form 2 haploid **secondary spermatocytes.** Subsequently, these undergo a 2nd reduction division forming four haploid **spermatids.** The spermatids gradually transform into four mature **sperms** by an extensive processes of differentiation known as **spermiogenesis.** This whole process of spermatogenesis takes about 64 days. The mature sperm (Fig 21.1A) is a free swimming actively mobile cell consisting of head and a tail. The anterior two thirds of the nucleus in the head is covered by **acrosome,** an organelle which contains enzymes that help the sperms to penetrate the outer covering and membranes of the ovum.

One human ejaculate contains 200 to 300 million sperms and out of these, only 50 to 100 sperms reach the ovum and only one will fertilize it.

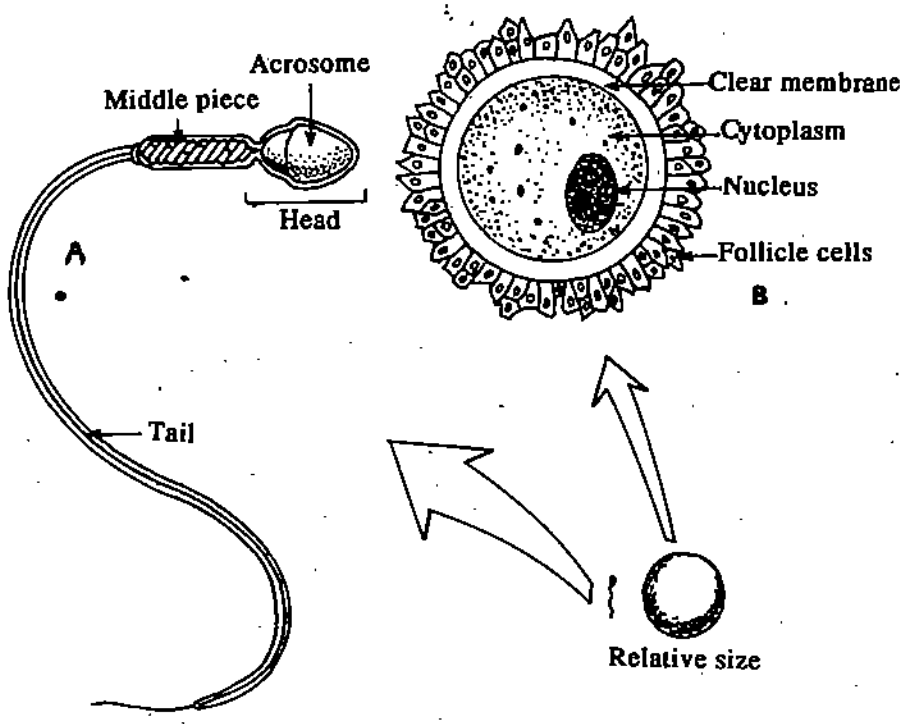


Fig. 21.1 : Human Sperm and egg. Note the relative sizes. The human sperm-head contains chromosomes and hardly anything else. Almost the entire of zygote comes from the egg.

21.2.2 Oogenesis

The maturing process in oogenesis leading to the formation of ovum begins before birth but is not completed until after puberty. **The primary oocytes** form before birth; these get surrounded by a single layer of flattened follicular cells forming the primordial follicle. When these primordial follicles enlarge at puberty the flattened follicular cells become cuboidal and then columnar forming the primary follicle. The primary oocytes begin the process of first meiotic division before birth and remain in suspended prophase till the reproductive cycle begins at puberty.

The follicular cells surrounding the primary oocyte secrete an inhibiting substance called **oocyte maturation inhibitor (OMI)** which keeps the meiotic division at a standstill. This suspended meiotic division may account to some extent for the large number of errors like nondisjunction, that occur as the mother's age increases.

Ovaries of new born female infants contain approximately 2 million primary oocytes but by puberty only about 30 to 40 thousand remain. Of these only about 200 are believed to develop into secondary oocytes and are released during ovulation during the reproductive period of life of normal females which is from 12 to 45 or 50 years. Normally, beyond 50 years ovulation does not occur as menopause sets in.

It is important to know that new primary oocytes are not formed after birth. In contrast primary spermatocytes keep forming in the male. After puberty 36 to 48 hours before each ovulation the primary oocyte completes the first meiotic division producing the **secondary oocyte** and the **first polar body.** The division of cytoplasm is unequal, the secondary oocyte receives all the cytoplasm while the first polar body receives hardly any and soon degenerates. At ovulation the 2nd meiotic division starts in the secondary oocyte and progresses up to metaphase; it is completed only if fertilization occurs. Again most of the cytoplasm is retained by only one of the two resultant cells the ovum, the other cell or the **2nd polar body** degenerates.

The secondary oocyte that is released at ovulation is surrounded by the thin non-cellular envelope, the **zona pellucida** and a layer of follicular cells called **corona radiata** (Figs. 21.1 and 21.5). In comparison to other body cells, it is a large cell but to the unaided eye, it appears only as a tiny speck.

SAQ 1

Why is it that women over the age of 35 have greater chances of bearing a child with Down's Syndrome?

.....

.....

21.3 FEMALE REPRODUCTIVE TRACT

The female reproductive tract includes the fallopian tubes, uterus and vagina. Look at Fig. 21.2. You would notice that the ovaries lie one on each side of the pelvic cavity. The fallopian tubes extend from the uterus and are not attached to the ovaries but have fingerlike projections called **fimbria** that sweep over the ovaries. During the time of ovulation, when the oocyte is released from the ovary it is usually swept into the fallopian tubes by the action of the fimbria and beating of the cilia that line the tubes.

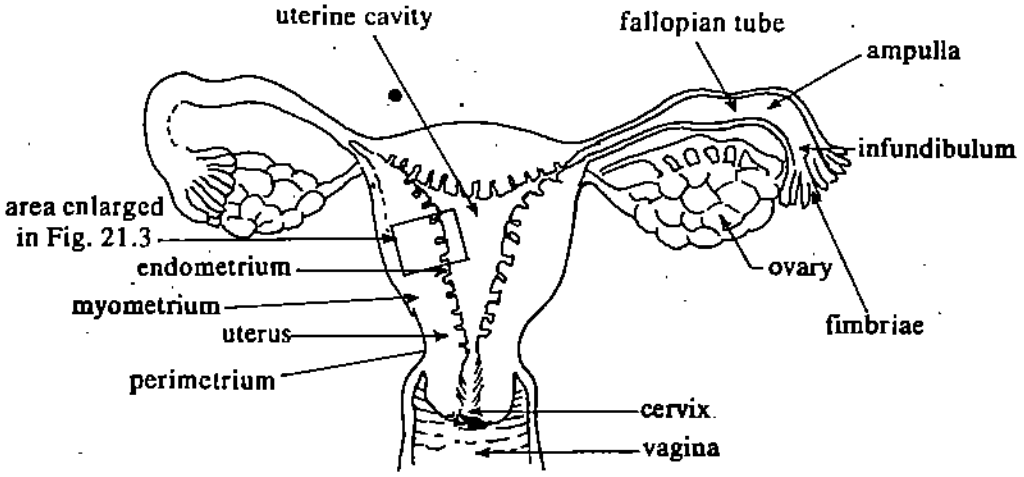


Fig. 21.2: Female reproductive tract along with the ovaries

The wall of the uterus is relatively thick and consists of three layers—the endometrium, myometrium and perimetrium (Figs 21.2 and 21.3). The **endometrium** forms the inner mucosal layer lining the uterine cavity. This lining is covered with columnar epithelium and contains numerous glands. The **myometrium** is thick and muscular. The **perimetrium** consists of the outermost thin layer covering the body of the uterus.

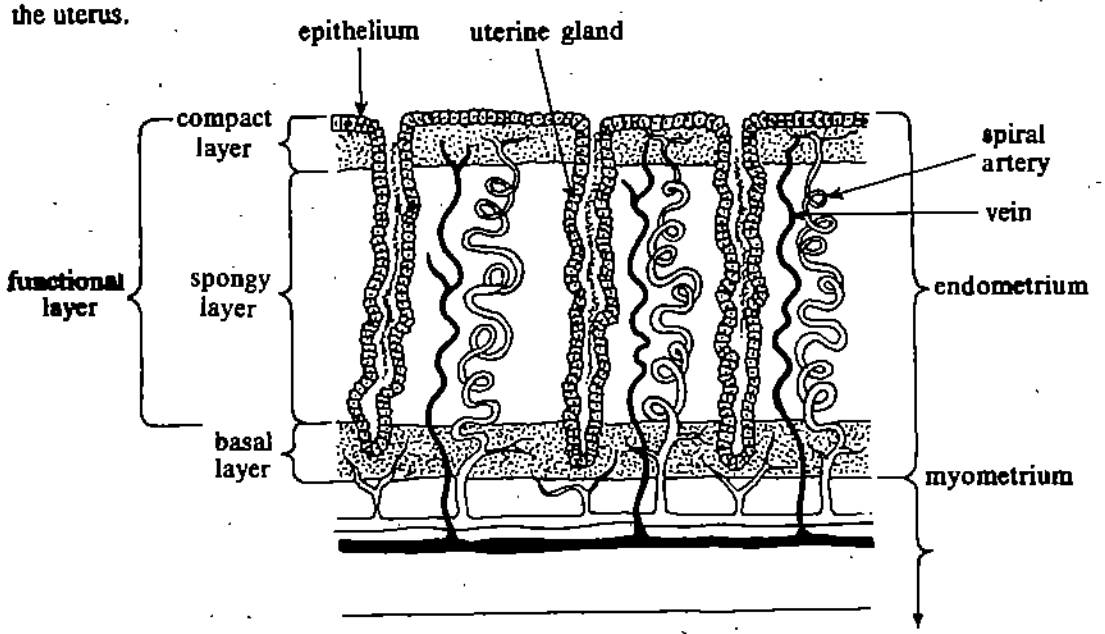


Fig. 21.3: Structure of uterine wall

Out of the three layers of the uterus only the endometrium participates in the formation of the placenta. In a non-pregnant female the endometrium varies in its thickness according to the monthly reproductive cycle or the menstrual cycle. The term menstrual cycle as you know refers specifically to changes in the uterus. During the monthly reproductive cycle in non-pregnant females only the functional layer of the endometrium (See Fig. 21.3) undergoes cyclic changes and varies in thickness.

The entire reproductive cycle consists of an ovarian cycle and an uterine cycle. Fig. 21.4 illustrates one complete reproductive cycle. You can see in the figure the interrelationship between the hypothalamus, pituitary, ovaries and uterus. The average reproductive cycle is spread over 28 days. It commences at puberty and normally

continues throughout the reproductive years. The cycle prepares the reproductive system for pregnancy. Refer to Fig. 21.4 frequently as you read the following discussion. It will help you to understand the close relationship between the two components of the entire reproductive cycle.

The reproductive cycle in human females is known as the menstrual cycle which has a duration of 28 days. It is characterised by regular recurring changes in the uterine lining which prepare the uterus for pregnancy. In case pregnancy does not occur then the uterine endometrium is shed from the uterus through the cervix and vagina in a bleeding known as menstruation. The menstrual cycle is accompanied by a follicular growth phase in the ovary or the ovarian cycle.

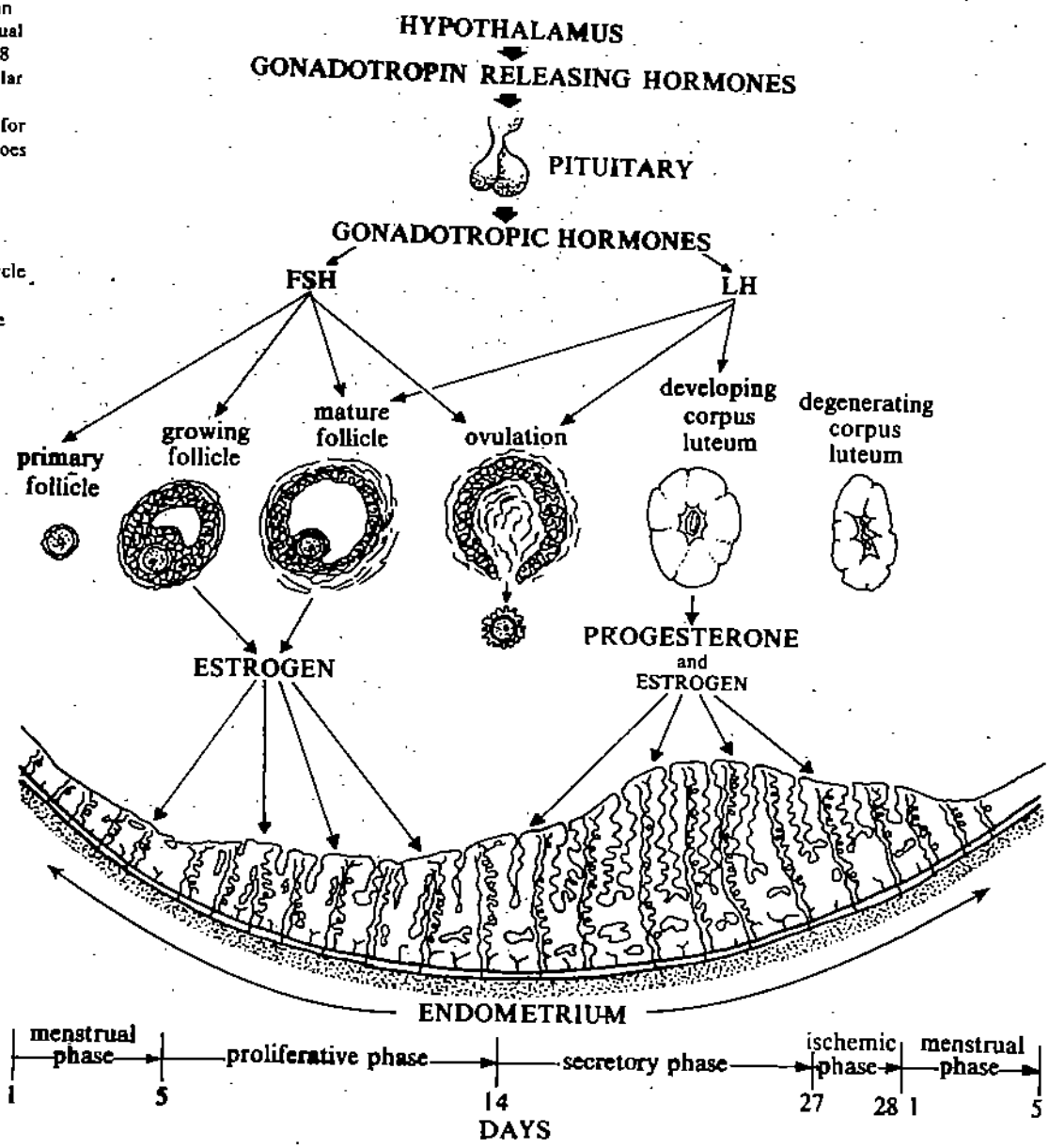


Fig. 21.4: Interrelationship of the hypothalamus, pituitary, ovaries and endometrium. One complete menstrual cycle shown. Note that the cyclic activity of the ovary is intimately linked with the changes in the endometrium.

The hypothalamus synthesises the gonadotropin releasing hormone (GnRH) that stimulates the anterior pituitary to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). The ovarian cycle begins with the phase of follicular growth in the ovary after the end of preceding menstrual cycle and lasts for about 14 days. In the first half of this period FSH stimulates the growth of 5-12 primary follicles out of which usually only one matures at a time. The maturing follicle secretes estrogen which exerts a feedback control on anterior pituitary inhibiting further FSH production hence preventing maturation of more follicles. The follicular phase comes to an end with the maturation of one follicle and its release from the ovary leading to ovulation, on day 14. At the time of ovulation the ovum is in the secondary oocyte stage surrounded by the non-cellular zona pellucida and follicle cells that form the zona radiata (or corona radiata). Following ovulation, from day 15 to 28 production of LH from anterior pituitary stimulates the formation of corpus luteum from the follicle cells left behind in the ovary. The corpus luteum secretes the hormone progesterone which prepares the endometrium for implantation of embryo

and later maintenance of pregnancy during early stages. If the ovum is not fertilized, the corpus luteum degenerates and menstruation begins again.

The cyclic changes in the ovary are matched by cyclic changes in the uterine wall as you can see from Fig. 21.4. From day 1 to 5 the level of sex hormones in the body is low hence uterine wall breaks down and menstruation begins. This is externally manifested by bleeding. This is the **menstrual phase.** As the level of estrogen produced by the growing follicle increases from day 5-14 the endometrium gradually thickens and becomes glandular. This is the **proliferative phase.** From day 15 to 28 the endometrium doubles in thickness and is richly vascularized preparing to receive the embryo for implantation, this is the **secretory phase.** If pregnancy does not occur the uterine wall breaks down leading to bleeding into the uterine cavity and the menstrual cycle begins all over again.

Now that you know how the uterus prepares itself for pregnancy we proceed to study the events in early development after fertilization takes place.

SAQ 2

Match the following stages with their characteristic features:

1)	Ovulation	a)	Corpus luteum and uterine wall breaks down, sex hormones low
2)	Menstrual phase	b)	Under influence of FSH estrogen secreted, uterine wall becomes thick and vascularised
3)	Proliferative Phase	c)	Post ovulation estrogen and progesterone secretion, corpus luteum enlarges, functional layer of uterus thickens
4	Secretory phase	d	Ovum released under the influence of LH and FSH

21.4 PRE-EMBRYONIC DEVELOPMENT

We had said in the beginning of the unit that prenatal development can be divided into two phases, embryonic and foetal. The embryonic phase of development is said to really start from the second week after fertilization; and subsequently lasts up to 8 weeks of the 1st trimester. In this section we describe the **pre-embryo** stage starting with the events that take place at fertilization and then proceed to describe the development of the pre-embryo up to two weeks of development.

21.4.1 Fertilization

Ovulation usually takes place between 9th to 14th day after the end of last menstrual period. Fertilization of oocyte initiates development and should occur within about 24 hours after ovulation; otherwise the oocyte degenerates. The female reproductive tract plays a very active role in the fertilization process. Newly ejaculated mammalian sperms are unable to undergo acrosomal reaction unless they reside in the female reproductive tract for some time. This requirement is known as **capacitation.** During this process the sperm membrane cholesterol is thought to be reduced as some of it is taken up by albumin molecules present in the female reproductive tract. Without these changes in the membrane the acrosomal reaction can not occur. Apart from this, there are coating factors or inhibitors that are to be removed which would otherwise prevent fertilization.

To reach the ovum the sperm has to move upwards through the uterus aided by its tail as well as the secretions of the cervix which contains a protein *mucin*. This forms threadlike highways along which the sperm travels.

When the sperm reaches the ovum (secondary oocyte) it invades the follicular cells (Fig 21.5). An enzyme **hyaluronidase** is released from the acrosome of the sperm head. This enzyme removes the extracellular matrix and disperses the corona radiata cells. Another enzyme **acrosin** digests a path for the sperm through the zona pellucida. In the acrosome of the sperm this trypsin like enzyme is inactive and needs to be activated by a glycoprotein in the female reproductive tract.

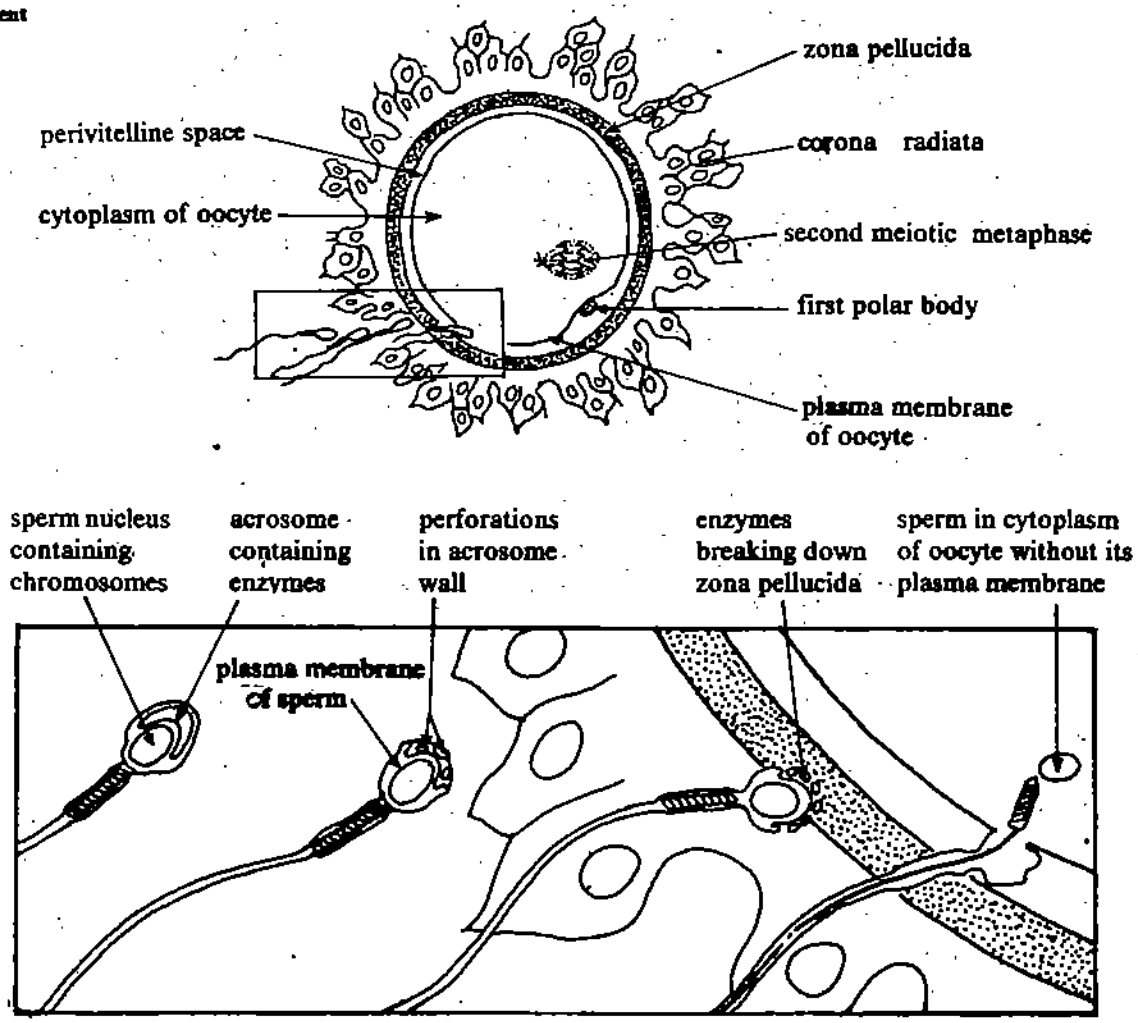


Fig 21.5: Steps in fertilization

Once a sperm passes through the zona pellucida, this covering becomes impenetrable by other sperms. This is known as the **zona reaction.** The structure of the membrane changes and lysosomal enzymes are released from the secondary oocyte that prevent other sperms from attaching to the membrane.

Usually only one sperm enters the ovum. Two sperms may participate in fertilization under an abnormal process known as **dispermy.** The resulting embryo contains 69 chromosomes and may appear normal, but it is always aborted. Sometimes a triploid infant may be born but it dies shortly. Thus polyspermy does not produce viable embryos.

As the sperm head enters the egg it loses its tail. The secondary oocyte completes its second meiotic division forming a mature ovum and a second polar body. The nuclei of the sperm and ovum fuse to form the zygote completing the process of fertilization.

The offspring's sex is determined at fertilization by the kind of sperm that enters the ovum. You would recall that all female gametes bear X chromosome while the male gametes may bear either the X or the Y chromosome. If the ovum is fertilized by a sperm bearing X chromosome it will develop into a female; and into a male child if it is fertilized by a chromosome bearing the Y chromosome. Which kind of sperm may fertilize the ovum depends entirely on chance. Therefore, whether the offspring will be a boy or a girl is the genetic responsibility of the father rather than of the mother.

SAQ 3

Mark the statements given below true or false:

1) The sperm cell penetrates the zona pellucida of the ovum using an enzyme hyaluronidase.

2) The sperm swims to reach the ovum only due to the lashing of its whip like tail.

3) After penetration of the ovum by the sperm head polyspermy is prevented due to structural changes in the cell membrane of the ovum.

4) The second polar body and the sperm nucleus fuse to form the zygote.

Box 21.1

In Vitro Fertilization

In case a woman cannot conceive because her uterine tubes are blocked she can become pregnant by means of *in vitro* fertilization.

In this process, oocytes are removed from the women's ovary and mixed with sperm cells in a laboratory dish for fertilization. After checking that development has begun one or more morulas are returned to the uterus of the women for implantation and subsequent development.

To increase the chances of success, the woman is usually treated with fertility drugs (clomiphene or gonadotropin). Growth of follicles is monitored by ultrasonography and when follicles have reached a certain size she is given HCG to induce ovulation.

Oocytes released from the ovary are collected by laproscope and incubated at 37°C in a buffered medium with pH 7.4 and when they are mature, they are mixed with sperms that have been washed to remove inhibitory factors.

Fertilized eggs are then incubated in a special culture medium and normal embryos at 8-16 cell stage are transferred to the uterus through the cervix. The woman is then treated with progesterone to promote a favourable uterine environment for implantation. Successful implantation occurs in 20% to 30% of the cases.

21.4.2 The Pre-Embryo

About thirty hours after fertilization the zygote undergoes mitosis giving rise to 2 blastomeres (Fig. 21.6). These blastomeres undergo further cleavages and by the 3rd

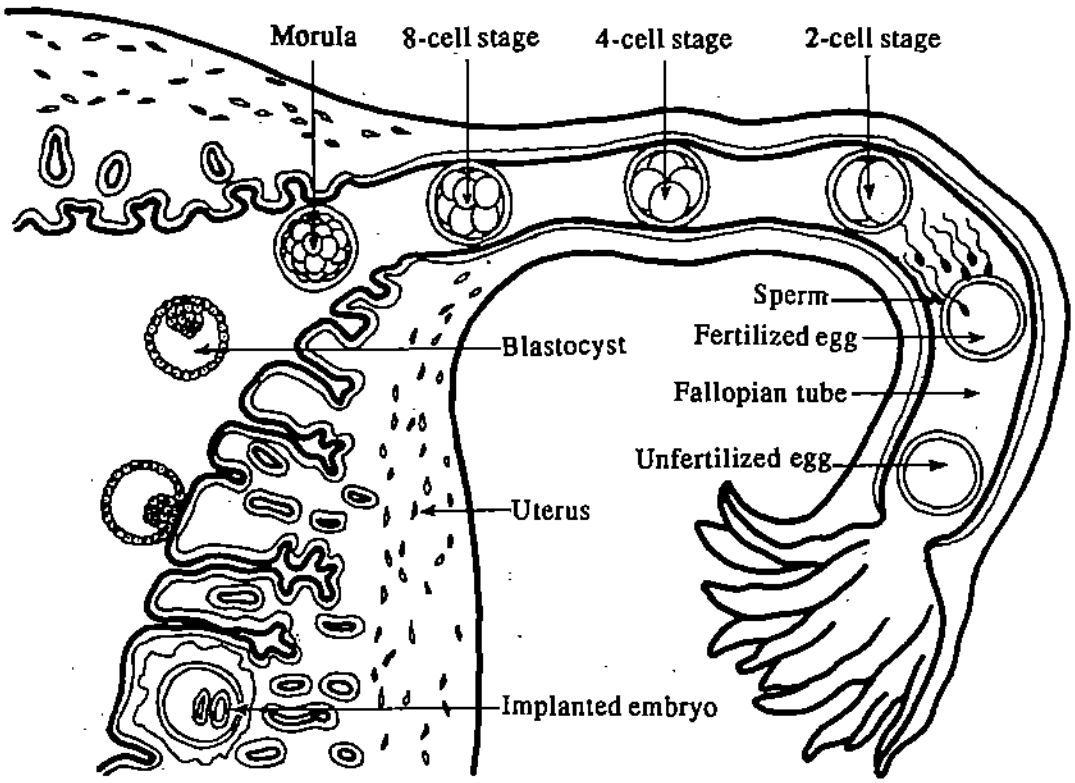


Fig 21.6: Stages in Early Development

day after fertilization, the tiny mass of cells reaches the uterus. By this time it is a solid ball of cells called **morula**. In the next three days the morula is transformed into

a hollow ball of cells called **blastocyst**. The cavity of blastocyst is filled with fluid secreted by the cells. One portion of the blastocyst contains a concentrated mass of cells that will give rise to the entire body of the new individual. This is known as the **inner cell mass** (ICM). Interestingly, each cell of this mass has the ability to develop into a complete individual. Identical twins are formed if the ICM splits (See Box 21.2). The outer ring of cells of the blastocyst surrounding its cavity is known as **trophoblast** and gives rise to all the extra-embryonic membranes including much of the placenta. This stage of development is important as the cells begin to move for the first time and become rearranged to shape the new individual. This process you might recall is the beginning of morphogenesis. However the major morphogenetic events take place during the third to eighth week.

Implantation

After entering the uterus and formation of ICM, the blastocyst begins to embed in the endometrium of the uterine wall. By one week after fertilization the trophoblast secretes enzymes that digest the tissues and blood vessels of the uterine wall. The invading trophoblast differentiates into two layers, the outer **syncytiotrophoblast** and the inner **cellular layer**. As the syncytiotrophoblast swallows more blood vessels in the uterine wall lacunae develop in the syncytiotrophoblast which get filled up with blood from the mother and exchange of gases takes place here. Thus a primitive utero-placental circulation is established (Fig. 21.7). This nourishes the embryo till the placenta is formed.

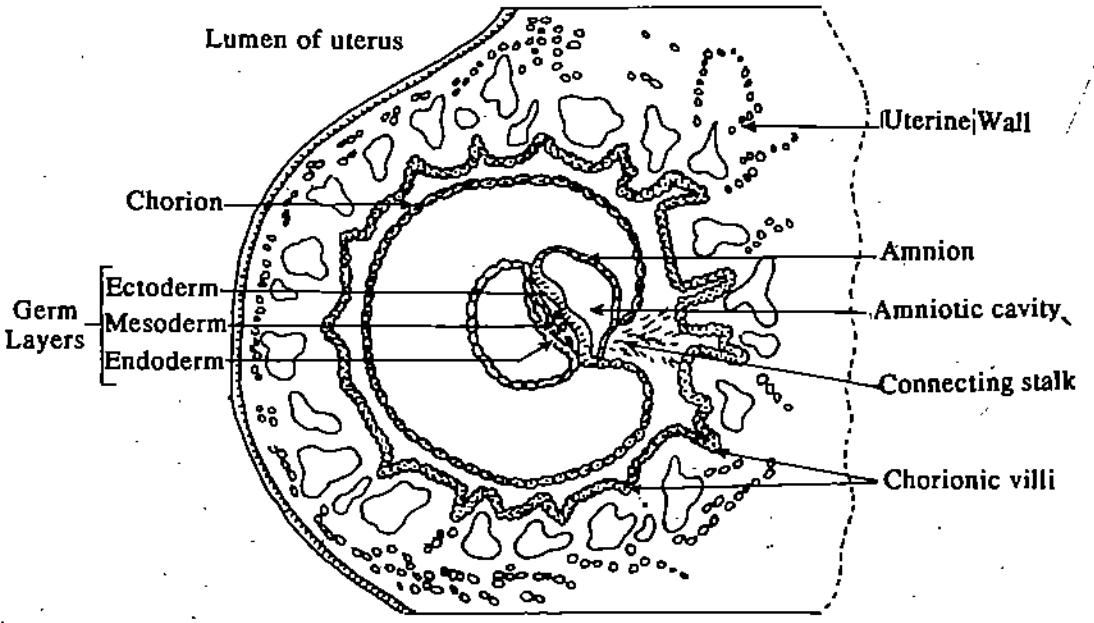


Fig 21.7 : Fully implanted embryo and formation of 3 germ layers

By the 10th day the blastocyst is completely embedded in the uterine wall. This type of implantation in which the embryo gets fully embedded is known as **interstitial implantation**. The trophoblast begins to secrete human chorionic gonadotropin (HCG). HCG causes the corpus luteum to be maintained and to continue to secrete estrogen and progesterone.

HCG secretion which starts shortly after fertilization is excreted in urine. Its presence in urine is used to detect pregnancy. A positive pregnancy test can be detected within about 8 to 10 days of fertilization.

Sometimes implantation may occur outside the uterus at some other location. In that case it is an ectopic pregnancy. The implantation site may be the fallopian tube or even the abdominal cavity. In ectopic pregnancy the embryo has to be surgically removed because if it is not done, it can lead to tubal rupture, internal bleeding, shock and possible death.

At the start of the second week a small cavity appears between the trophoblast and ICM. This is the amniotic cavity which will grow around the embryo and later the foetus. It is a fluid filled cavity which acts as an insulator against shocks, cold and heat. At the same time the ICM also differentiates into two layers, the upper **epiblast** which gives rise to the embryo and the lower **hypoblast** which gives rise to the

extraembryonic membranes. You can see the two primary germ layers and the beginning of third in Fig 21.7.

SAQ 4 Choose the correct term

i) The morula/blastocyst implants in the uterine endometrium

ii) The ICM/trophoblast give rise to the embryo

iii) Ectopic pregnancy is the result of implantation inside/outside the uterus

iv) HCG maintains/degenerates the corpus leuteun

v) Uteroplacental circulation occurs due to development of blood filled space in syncytiotrophoblast/inner cellular layer of trophoblast.

Box 21.2

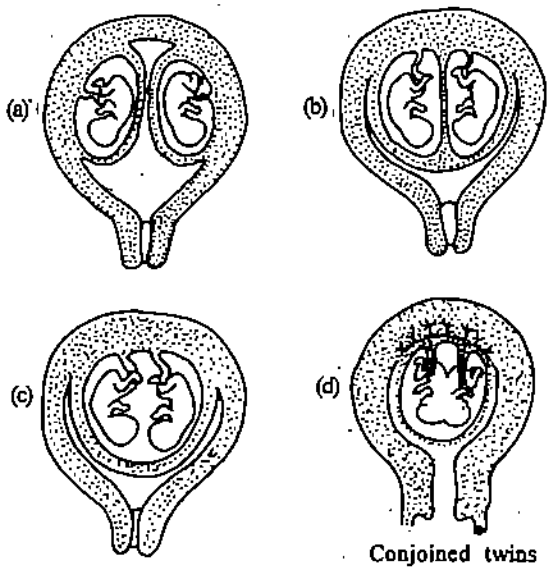
IDENTICAL TWINS

Human twins may be identical, when they are formed from one egg (monozygotic) or fraternal when they are the result of fertilization of two different ova released at the same time and fertilized by different sperms (dizygotic).

From an embryological point of view identical twins are of greater interest. 0.25% of all births may be monozygotic twins. Current evidence indicates that twinning occurs at the blastocyst stage by the splitting of the inner cells mass. The cells of the ICM are considered totipotent *i.e*, they have the potential of forming a single, dual or even a multiple embryonic disc. If by chance, the separation of inner cell mass occurs before day 5, even before the formation of trophoblast, which occurs in 33 percent of identical twins, then they have two separate chorions and amniotic cavities. (Fig. 21.8a). Identical twins that share a common chorion, but two separate amniotic cavities indicate that the split in ICM came between the formation of chorion on day 5 and formation of amnion on day 9 as shown in Fig. 21.8 (b). This is the case in two thirds of identical twins. A small percentage of identical twins are born within a single chorion sharing a single amniotic cavity as shown in Fig 21.8 (c). This indicates that the division occured after day 9 after the implantation of the blastocyst.

As seen in Fig. 21.8 (d), the embryonic disc may divide incompletely *i.e.*, the two new embryonic axes fail to separate completely and the outcome is conjoined twins. The degree of union may be slight or quite extensive and the twins may be joined at any part of their body.

It should be remembered, however, that conjoined twins represent an aberration in development that would normally lead to formation of identical twins. Most conjoined twins do not survive after birth but nowadays it is possible to separate some of them surgically and then one or both may survive to lead fairly normal lives.



Conjoined twins

21.5 EMBRYONIC DEVELOPMENT

The embryonic stage extends from the second week through the 8th week and is characterised by formation of placenta, the development of internal organs and appearance of the major external body structures. During this period the embryo takes on a human shape by morphogenetic processes. The rudiments of various organs get established during the 3rd week but further development will take place from then on to 8th week by organogenesis, a process you are already familiar with. Because of the simultaneous developmental changes taking place during this period the developing embryo is particularly sensitive to teratogens, that is, certain agents like alcohol or drugs etc., can induce malformations in the rapidly forming tissues and organs.

21.5.1 Third Week

During the **third week** of development the ICM separates from the trophoblast and forms the flattened embryonic disc, which at first contains cells of all three germ layers, ectoderm, endoderm and mesoderm and is called the epiblast. From this a lower layer separates to form the endoderm. The second layer, mesoderm, forms by migration of cells through the primitive streak which forms as the embryonic disc elongates. The cells remaining in the upper layer form the ectoderm.

These three germ layers will give rise to all the organs of the body as indicated in Fig. 21.9.

The embryo's heart also begins its development as a pair of microscopic tubes. The cardiovascular system is the first system to become functional in the embryo. At the end of the third week the heart tubes fuse and become linked to the blood vessels in the embryo, body stalk, chorion and yolk sac, to form a primitive blood circulatory system. Chorionic villi (which will form the placenta later) also begin to form during this period.

At 8 to 10 weeks of development doctors can take samples of tissue from chorionic villi to detect genetic defects in the embryo because chorionic cells and foetal cells contain identical genetical information.

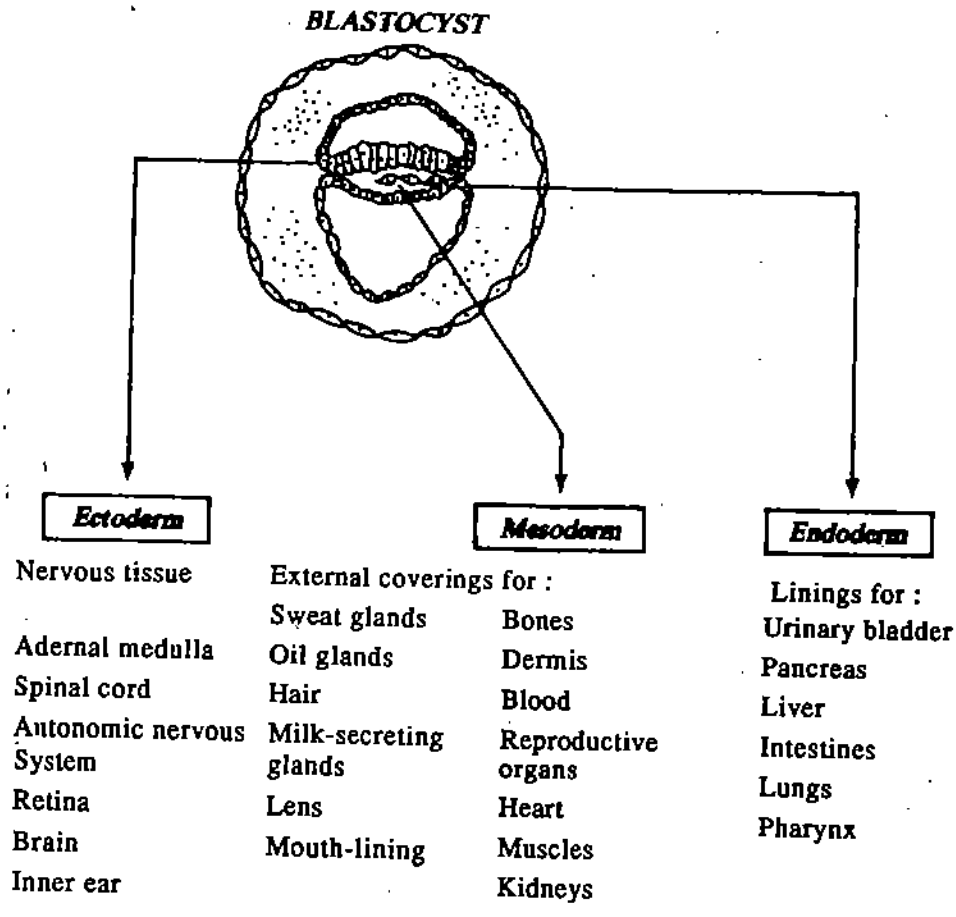


Fig 21.9: The organs of the body form from the three germ layers

21.5.2 Fourth week to Eight Week

The general changes in body shape and plan of the embryo from fourth week to eighth week are shown in Fig 21.10

The **fourth week** embryo is cylindrical and has a blunt head with a very primitive brain (Fig 21.10). Vague rounded elevations on the lateral surfaces of the brain indicate the eye rudiments. The simple tube like heart which arose in the third week is functional, pumping blood through the umblical arteries to the placenta. Heartbeats, however, cannot be recorded yet. Oral and anal openings appear but are nonfunctional. At the end of fourth week the embryo is 1/4 of an inch long. A characteristic feature of the fourth week is the alternating series of elevated ridges and depressions, the **pharyngeal pouches and grooves** respectively. These 4 pharyngeal pouches

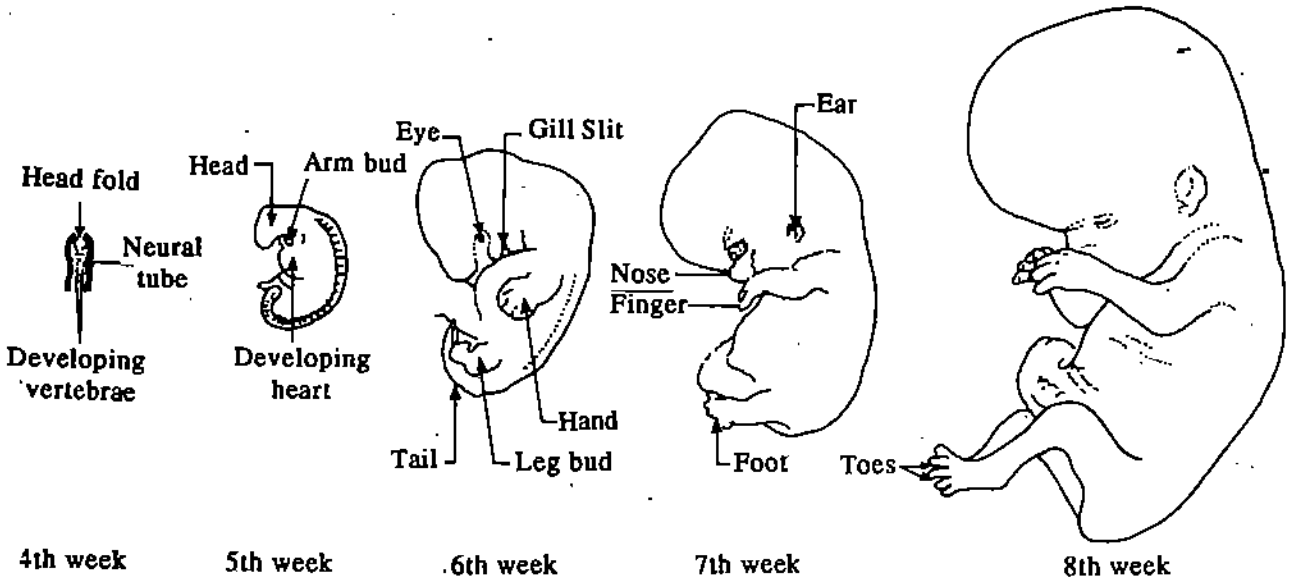


Fig 21.10: Human embryonic development from fourth to eighth week (See text for description)

correspond to the gill arches and grooves to the gill slits of fishes. However, these grooves in humans never become functional or perforated. The pharyngeal pouches in humans form the eustasian tubes (1st pair of pouches), walls of tonsils (2nd pair of pouches), thymus and parathyroids (3rd and 4th pair of pouch).

During the **fifth** week the embryo becomes curled so that the head almost touches the tail (Fig. 21.10). The rudimentary arms and legs (limb buds) appear about the middle of the fifth week. The head is now much larger than the trunk region. The brain is the most prominent feature of the embryo. Nerves spread out in the body, paired gonads form, though not recognizable yet, as testes or ovaries.

In the **sixth week** the head is disproportionately large and the stomach bulges out because of the large liver. Future fingers are seen as indentations on the paddle shaped hands and feet. The hands develop faster than the feet. This is an expression of the general rule that anterior structures grow more rapidly than posterior structures (Fig. 21.10). Rapid growth occurs in the facial region. By the end of the sixth week, the main systems, nervous, muscular, circulatory, excretory, reproductive, digestive and skeletal have been initiated.

After the sixth week the embryo starts looking more human and all the internal organs are formed by the **seventh week** (Fig. 21.10). Along with the development of brain, the head achieves its normal relationship with the body as the neck appears. The nervous system is developed enough to permit reflex actions such as the 'startle response' to touch. By the end of **eight week** of development, the embryo is usually 30 mm in length and weighs less than 5 mg (Fig. 21.10).

21.6 FOETAL DEVELOPMENT

The foetal stage of development begins in the ninth week and lasts till birth. During this period the existing body structures continue to grow and mature and only a few new parts appear. Figure 21.11 shows that the growth rate is rapid during the foetal period and the body proportions also change greatly. You can also see from figure 21.12 that at the beginning of the foetal period the size of the head is disproportionately large and legs short but later the growth of the head slows down.

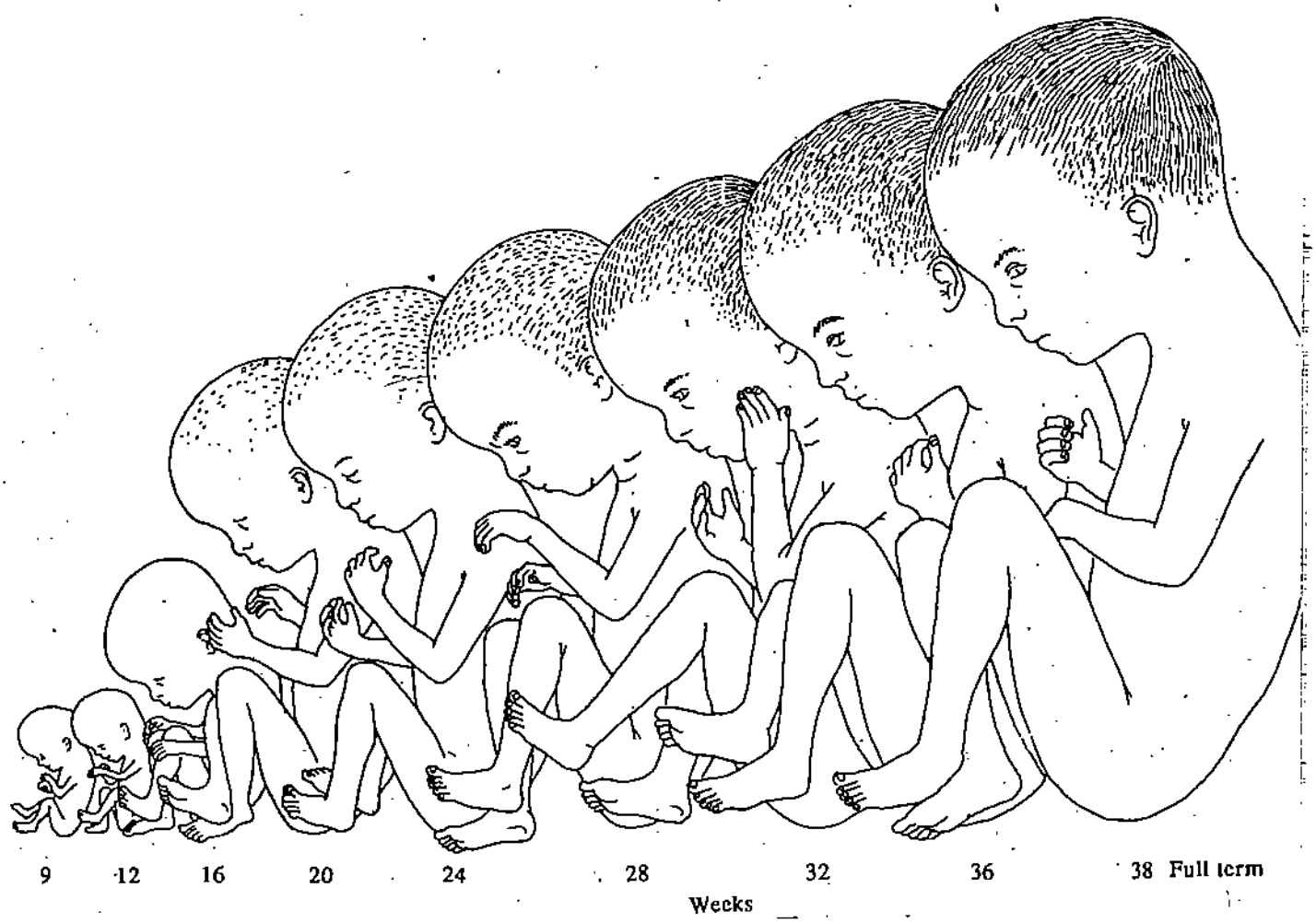


Fig 21.11: Growth during foetal period. The above 9 - 38 week foetuses are less than half actual size.

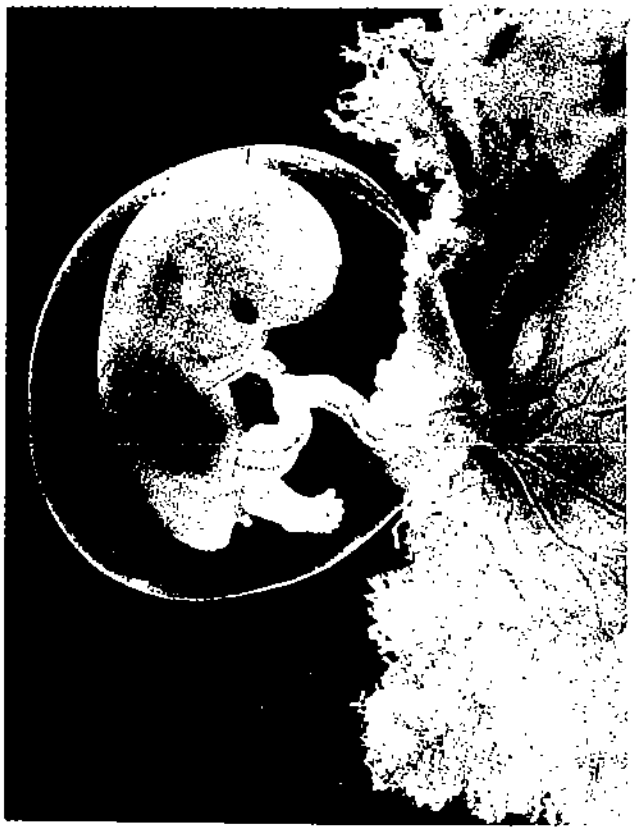


Fig.12.12: 10 week old foetus in the amniotic sac. It can be recognised as a human form and measures about 5 cm. from crown to rump. At the right you can see the placenta which is connected to the foetus by the umbilical cord.

In the **third month,** the arms attain the relative length they will maintain during

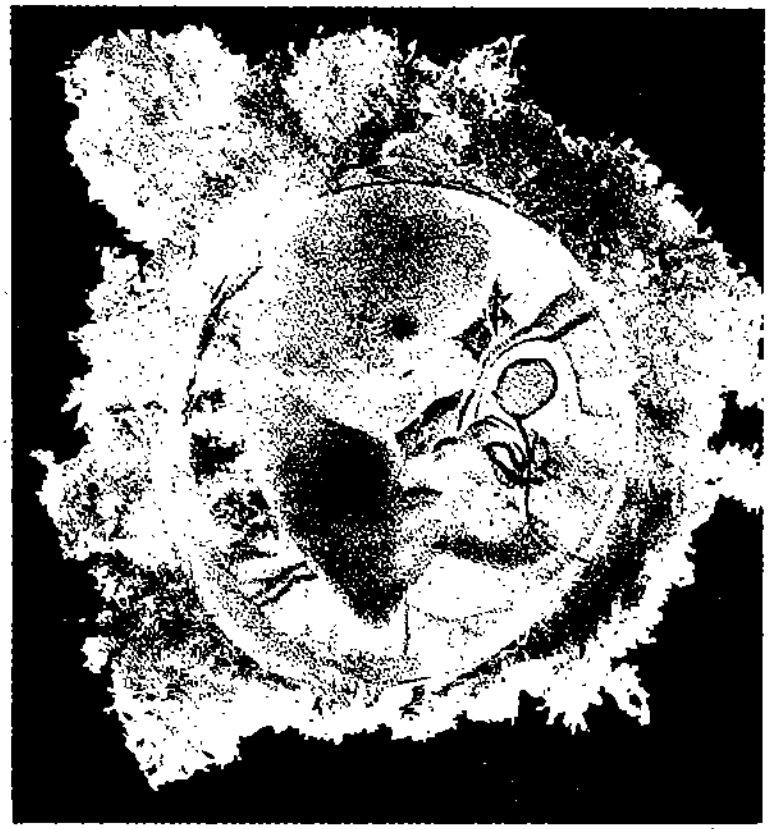


Fig 21.13: 11 week old foetus showing the rib cage. The cartilage cells replacement by bone cells starting by about 9th week. The first movements begin to start around this time.

development. Cartilage is replaced by bone as ossification centres appear in most bones. (Fig. 21.13) By the end of third month, it is possible to distinguish male foetus from the female. The Y chromosome triggers the formation of a protein called the H-Y antigen that causes the differentiation of testes from the indifferent gonads. The testis secretes testosterone that stimulates the growth of external genitalia. In the absence of testosterone, female genitalia form. Estrogen need not be produced by foetal ovaries as there is enough maternal estrogen circulating in the blood.

In the **fourth month,** the body grows rapidly to reach a length of 13-17 cm, the legs lengthen and the heart beat is loud enough to be heard by the physician's stethescope.

In the **fifth month** the rate of growth decreases slightly. The legs achieve their final relative proportion, the skeletal muscles become active and the mother may feel the foetal movements. Some hair appears on the head and the skin is covered by downy hair. The skin is also covered by a cheesy coating made up of dead epidermal cells and secretions of sebaceous glands.

During the **sixth month,** the body gains substantial amount of weight. Eyebrows and eyelashes appear, skin is wrinkled and translucent.

In the **seventh month** fat gets deposited in the subcutaneous tissues. The eyelids that were fused together in the third month reopen. At the end of the seventh month a foetus is about 37 cm in length. If a baby is born in the seventh month it is possible that it may survive.

In the **eighth month,** the testis of the male descends into the scrotal sac from regions near the kidney. During the **ninth month** the foetus reaches a length of about 47 cm. The skin becomes smooth and the body appears chubby due to accumulation of subcutaneous fat. An average full term foetus is about 50 cm long and weighs 2.5 to 3.6 kg. Fingers and toes have well developed nails. Fig. 21.14 shows the full term foetus with its head positioned towards the cervix.

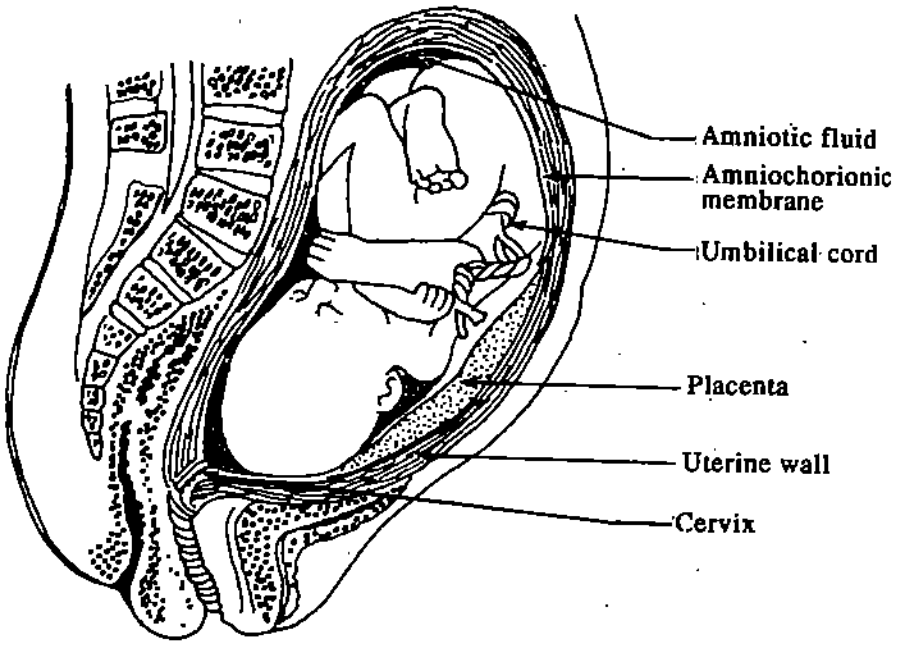


Fig 21.14 : Full Term Foetus

Birth takes place at the end of the third trimester, *38* weeks after conception. The uterus begins a series of powerful contractions. This is due to hormonal as well as mechanical changes in the uterus.

During the last two months of pregnancy estrogen secretion begins to increase while progesterone starts to level off. Since estrogen stimulates and progesterone inhibits contractions, stimulation or contraction dominates. Another hormone oxytocin also stimulates contraction and its levels increase sharply at the beginning of labour. In addition simple stretching of the uterus also increases the contractions. At the same time placental tissue especially the trophoblast exhibits degenerative changes which lead to separation of the foetal and maternal tissues at the time of birth. Now investigations have revealed that at parturition the placenta contains very high concentration of prostaglandins which are powerful stimulators of uterine contractions. These placental prostaglandins are secreted under the influence of foetal hormones. It appears, therefore, that the foetus controls its own birth!Foetal hypothalamus activates the pituitary to release adrenocorticotropin which stimulates the adrenal gland in the foetus to release cortisol. There is evidence that cortisol directs the production of prostaglandins in the placenta and these in turn may influence uterine muscles to contract or cause increased secretion of oxytocin from maternal pituitary gland.

SAQ 5

A. Choose the correct alternative.

i) Growth rate is rapid during the foetal/embryonic period

ii) It is possible to distinguish male foetus from female foetus at the end of second/third month

B. Fill in the blanks

i) foetal heart beat can be heard in the.....month.

ii) foetal movements can be felt by the mother by.....month

iii) eyelids that fused in the third month reopen in the.....month

iv) accumulation of subcutaneous fat to give a chubby appearance takes place in the.....month.

21.7 DEVELOPMENTAL CHANGES AFTER BIRTH

During the birth process, some important developmental changes occur in the infant's respiratory and circulatory systems. These changes occur in response to the change in its environment from aquous to gaseous. The most immediate need at birth is to obtain

oxygen and excrete out carbon dioxide. Therefore, the first breath is critical. Let us examine the changes that take place in the respiratory and circulatory systems.

Respiratory System

The new-born's lungs are collapsed and require powerful breaths to inflate them; airways too are small, offering considerable resistance to flow of air. The surface tension tends to hold the moist membranes together. The lungs of a full term baby secrete surfactant (see Unit 2 LSE-05) which has the unique property of exerting high surface tension when the lungs are expanded but a low surface tension when the lungs are collapsed. Thus after the first powerful breath which expands the lungs, breathing becomes easier, because of the reduced surface tension of collapsed lungs.

The first independent breath may take as much as 10 times the inhalation force required of an adult. As much as 40 per cent of the air inhaled remains inside the lungs so less force is required to expand them in subsequent breaths.

Circulatory System

You have learnt that during foetal life, gas exchange takes place, only through the placenta and not through lungs. Therefore, the foetus has several features in its circulatory systems that are not present in an adult.

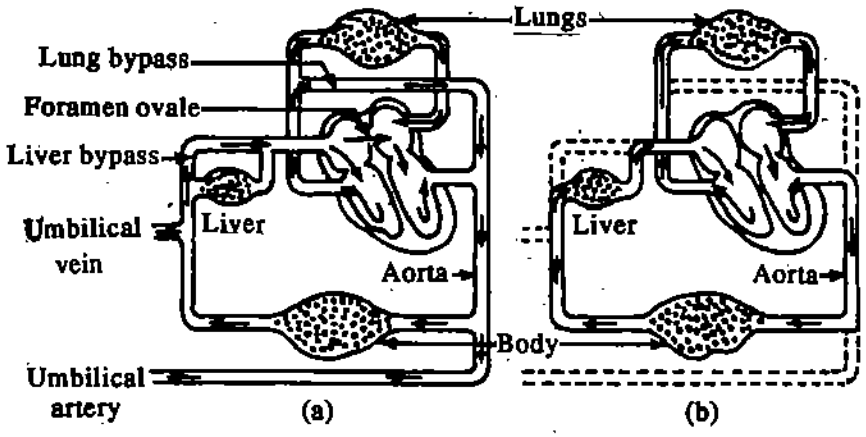


Fig 21.15: Changes that occur in the newborn circulatory system (a) Foetal circulation (b) Infant circulation. In the foetus liver and lungs are bypassed by blood vessels. The foramen ovale permits blood to flow from right to left atria. Infant circulation mirrors adult circulation.

Fig 21.15 is a diagramatic representation of foetal and infant circulation. In the foetus the oxygenated blood flowing back through the umblical vein largely bypasses the liver and goes to the right atrium. The blood then passes to the left atrium directly through an opening called **foramen ovale**. From the left atrium the blood flows to the left ventricle which sends the blood to the head and rest of the body. Blood returning from the head moves through the right atrium to the right ventricle which then does not pump the blood to the lungs but to a shunting vessel the **ductus arteriosis** that connects with the descending aorta. A pair of umblical arteries branch off from the aorta and carry deoxygenated blood to the placenta.

In some new born the foramen ovale fails to close, leading to the blue baby syndrome due to mixing of oxygenated blood with deoxygenated blood. Earlier such babies did not survive more than a few years but now the condition can be corrected by open heart surgery.

At birth the umblical artery and vein collapse when the cord is tied or the placenta separates. As a result there is negative pressure in the right atrium and blood flows back from the left atrium to the right. The flow causes the one way valve to close in the **foramen ovale**. Thus the left and right atria are separated. The shunting vessels that bypassed the liver and the lungs, also collapse and adult pattern of circulation starts. The whole process takes place within a few hours of birth, though the permanent closure of foramen ovale may take upto a year.

Later Changes

We had said in the beginning of the unit that development does not stop once birth has taken place. It continues through the stages of life *i.e.*, infancy, childhood, adolescence and adulthood.

Infancy lasts until about two years of age. The newborn has certain innate reflexes that help it to establish a relationship with its caretakers. During this period sensory and motor development takes place which can be related to his or her's ability to respond to stimuli or perform simple function (Refer to Unit 23 FST-1).

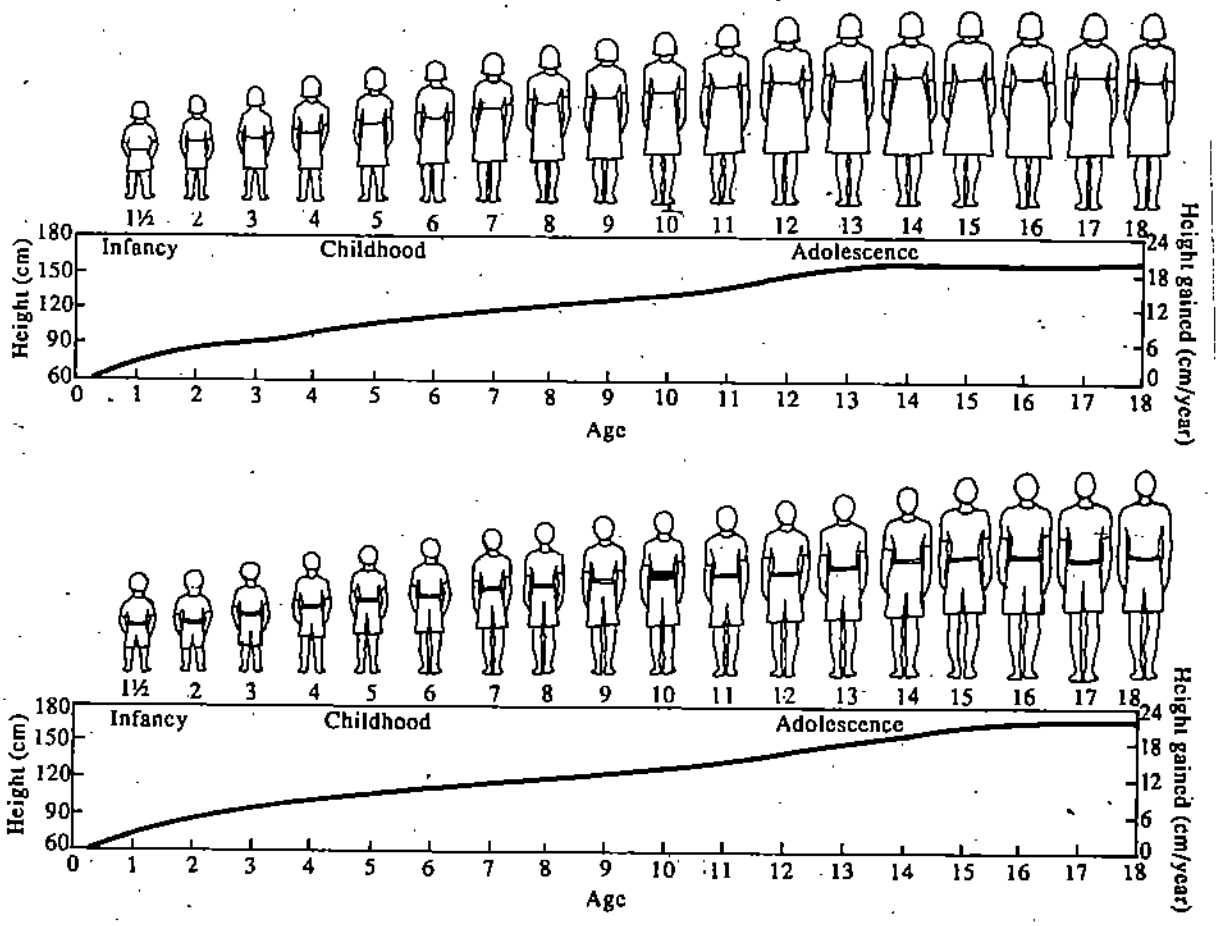


Fig.21.16: Comparative body growth pattern in girls (above) and boys (below) from infancy through adolescence. Note that the male's growth spurt occurs about two years after the females (adapted from 'Growing Up' by J.M. Tanner 1975).

Childhood lasts until puberty which is around 10-12 years in girls and 12-14 years in boys after which adolescence continues till adulthood. The general body growth of boys and girls is given in Fig. 21.16. At the time of puberty the sex organs mature and secondary sexual characters begin to appear. This is related to the level of sex hormones in the body (Refer to Unit-8 and 10 LSE-05).

Actually the hypothalamus pituitary-gonad system functions long before puberty but it is very sensitive to feedback control. When puberty starts, the hypothalamus becomes less sensitive to feedback control and begins to secrete increasing quantities of releasing hormones causing the gonads to develop and start increasing their production of hormones. This sensitivity of the hypothalamus continues to decrease until gonadotropin and sex hormones secretion reaches adult levels.

The sex hormones in combination with other hormones have a profound effect on the body during puberty. There is an acceleration in growth causing changes in weight height, distribution of fat, and body proportions leading to adult appearance. Adulthood is followed by degenerative changes characteristic of ageing.

SAQ 6

i) Why does the newborn need to take a powerful first breath?

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21.8 EXTRA-EMBRYONIC MEMBRANES AND PLACENTA

The extra embryonic membranes as you already know provide nourishment and protection. In Fig. 21.17 you can see how the extra embryonic membranes begin to form. These membranes are essentially similar to the extra-embryonic membranes of reptiles and birds, though the method of origin differs in humans. During the third and fourth week, the **amnion** grows around the embryo enclosing it in a membraneous fluid filled sac within which the growing embryo and later the foetus floats and can move freely. This sac as mentioned in the earlier section is a shock absorber and encloses a fluid, the amniotic fluid, that helps to keep the temperature of foetal environment stable.

The other membrane, the **chorion** develops from the trophoblast cells. The chorion is a highly specialised extraembryonic tissue. It facilitates the transfer of gases, nutrients and wastes between the embryo and the mother. It is the primary part of the placenta which we will study a little later.

Recent studies have indicated yet another function of chorion. It protects the embryo from the immune response of the mother. The human body rejects any kind of foreign tissue implanted in its body, yet it tolerates the embryo which has major histocompatibility antigens from both mother and father. One hypothesis is that the immune system of mother does not reject the embryo or foetus because the chorion produces substances that block the immune response.

Yolk sac develops during the second week of development although there is no yolk in the human egg. It does not provide nourishment to the embryo but it becomes surrounded by mesoderm which forms the blood cells till the liver of the embryo becomes functional in the sixth week. The yolk sac along with the allantois form the umblical cord. Part of the yolk sac also forms the lining of the gut. The **allantois** which forms during the third week of development is a tiny sausage shaped pouch on the yolk sac. It too is responsible for producing blood cells and later develops into the umblical blood vessels.

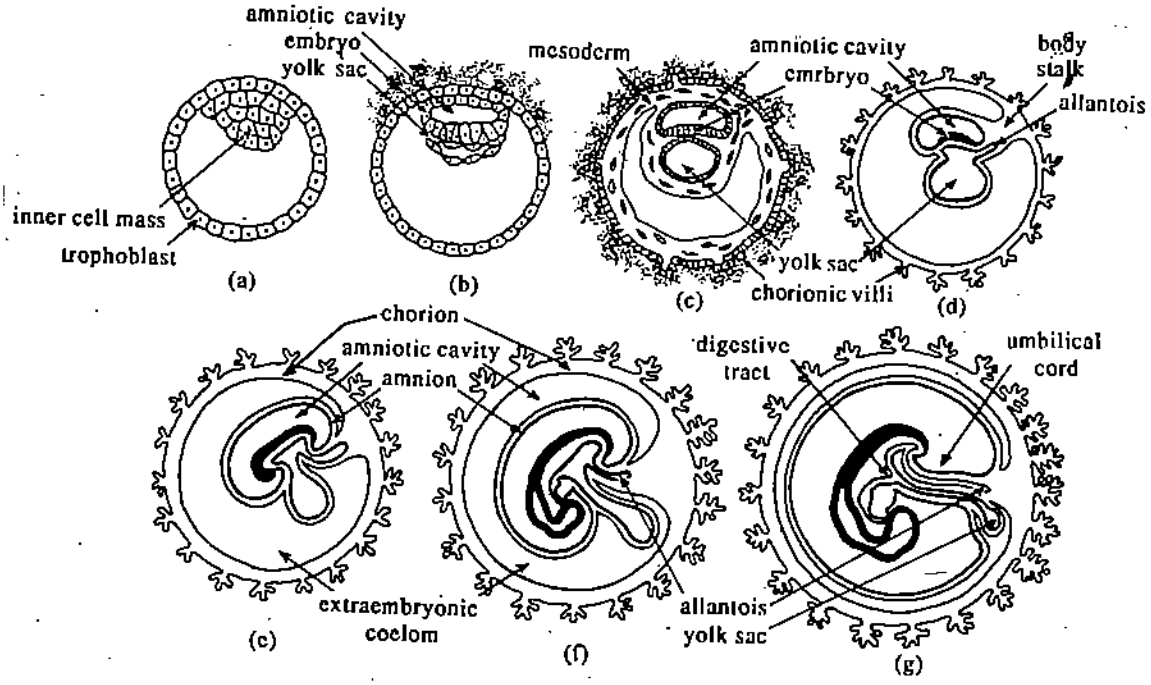

Fig 21.17: Development of extra embryonic membranes and umbilical cord. a) blastocyst and surrounding trophoblast b) amniotic cavity and yolk sac appear c) formation of first chorionic villi d) embryo connected to chorion by body stalke to g) formation of umbilical cord

SAQ 7

Compare the functions of human extra embryonic membranes with those of other vertebrates.

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Placenta

We had said earlier in the unit that in the second week of development a primitive uteroplacental circulation is established. Early in this stage slender projections grow out from the trophoblast into the surrounding endometrium. These are the chorionic villi and they become branched (See Fig. 21.17). By the end of fourth week they are well formed.

As the chorionic villi develop embryonic blood vessels begin to form in them and these vessels are continuous with the vessels in the connecting stalk. Matching the chorionic villi, uterine crypts develop. Until about the end of the eighth week, the chorionic villi cover the entire surface of the former blastocyst. As the embryo and chorion enlarge only those villi that are in contact with the endometrium remain. The others degenerate. The region still in contact with the endometrium forms the disk shaped placenta. The manner in which the trophoblast interacts with the maternal tissue largely determines the morphology of the placenta. Very intimate contact between the mother and embryo is achieved in the placenta found in the humans and rodents. Here the chorionic villi grow deep into uterine tissue and break down the maternal blood vessels until they are bathed in maternal blood. This kind is called the **haemochorial** placenta, in which a thin membrane separates the embryonic blood within the capillary of the chorionic villus from the maternal blood in the crypts of the endometrium. This membrane is the placental membrane and is composed of epithelial membrane of the capillary and the membrane of the villus. It is through this membrane that exchange of gases takes place. Oxygen and nutrients pass from the maternal blood to the embryo and carbon dioxide the other wastes from the foetal blood move across the membrane to the mother's blood.

Though foetal and maternal blood never mix during pregnancy, at the time of birth when the placenta separates (or if a miscarriage occurs) the placental membrane may be broken and some foetal blood may enter the maternal blood stream. This condition can lead to serious consequences in the next pregnancy if the mother is Rh negative and the foetus is Rh positive. The Rh positive cells of the foetus stimulate the maternal tissue to produce anti-Rh agglutins. In case the mother is pregnant with another Rh positive foetus then these anti-Rh agglutins can pass through the placental membrane and react with the foetal red cells causing them to agglutinate. The foetus then develops a condition known as **erythroblastosis foetalis** which is often fatal or if the infant survive it may have severe motor and sensory losses and mental deficiencies.

By the **tenth** week of development, the placenta is fully formed. It begins to secrete estrogen and progesterone. These hormones by their negative feedback on the pituitary and hypothalamus prevent any new follicle from maturing and also maintain the lining of the uterus.

Various substances can move across the membrane by active transport and pinocytosis. The blood of the embryo normally never mixes with that of the mother, however, small molecules and various toxins and viruses do pass through across the placenta and this is the reason that many drugs taken by the mother or certain infections contacted by the mother are passed on to the embryo. We will learn more of this in section 21.9

SAQ 8

1) What is the function of amniotic fluid?

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2) How are substances exchanged between the embryonic and maternal blood?

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21.9 FLAWS IN DEVELOPMENT

The human growth and development despite its complexity works perfectly most of the time. But when development goes awry it creates a giant or dwarf, a child with multiple fingers or toes, an albino or haemophilic or even worse than these. All birth defects are loosely grouped as 'congenital defects'.

Causes for birth defects can be sought in three general areas; defective genes, abnormal arrangements of chromosomes and unfavourable factors occuring in the uterine environment during pregnancy.

The list of hereditary birth defects is long and in some cases the specific chemical defects due to defective genes is known, for example, Haemophilia, sickle cell, thalassemia, anaemia and phenylketonuria. But not all genetic defects are inherited. In some embryos the genetic instructions that govern growth are initially correct but somewhere during cell divisions the instructions become garbled and mishaps of this sort usually involve not a single defective gene but a whole rearrangement, of the cell's genetic material. Down's syndrome is one such example (You learnt in Section 21.2 of this unit about chromosomal aberrations that lead to abnormal development).

You have learnt in section 21.8 that the embryo is very vulnerable to noxious influences. An infection or a toxic substance that can cross the placental barrier can cause serious defect in development.

1) For example, when a pregnant woman drinks alcohol her embryo is exposed to the same concentration of alcohol as is in her blood. The alcohol may produce a set of physical and mental abnormalities known as **foetal alcohol syndrom (FAS).** In this syndrome prenatal and postnatal growth is retarded. Abnormal facial features, reduced head size and mental retardation are seen. As little as 8.5 ml of alcohol per day or engaging in a single drinking binge by a pregnant woman can result in FAS.

2) Infection by German measles (caused by *Rubella* virus) in a pregnant woman can cause congenital malformations in the embryo. If the rubella infection is contacted during the first four or five weeks of pregnancy, the embryo's eyes, ears and heart may be malformed while exposure to this virus during later stages of development may result in defects in the central nervous system.

3) There are many drugs that are apparently harmless to adults but may be injurious to developing embryos. They are not toxins but teratogens as they lead to malformations in the embryo. Perhaps the most unfortunate instance of the effect of a teratogen is the drug thalidomide which was used as a tranquilizer. Within a couple of years of its introduction over 8000 crippled children without limbs were born in Europe and England.

4) The highest sensitivity to such teratogenic agents is during 18-55 days after conception. The peak period being day 30. Pregnant women must, therefore, never use any drugs without the advice of their attending physicians. For further information of teratogens refer to LSE-03 unit-17.

   However, only about 40 percent of birth defects can be definitely attributed to hereditary or environmental causes. Of these too, about 20 percent are the result of external agents that act on the embryo during development. You already know about the effects of teratogens and viral infections during early pregnancy. Exposure to radiations also poses a grave threat to normal development. Large doses of X-rays during the first trimester may cause mental retardation, skeletal malformation, small head size and predispose the person for later development of leukemia.

Physicians can prevent some of the disorders by protecting pregnant women from dangerous drugs or X-rays. In few cases like phenylketonuria they can mitigate or eliminate the impact on development. But an awareness on the part of the mother and rapid research in this field on development may bring forth a cure or some means of prevention in the future.

21.10 SUMMARY

In this Unit you have studied that:

- Human development is a continuous process and can be divided into prenatal and postnatal periods of development.
- Gametogenesis in males starts at puberty and continues throughout life while in females, gametogenesis begins before birth but ova mature only after puberty. Oogenesis continues normally only until the age of 45-50 years beyond which menopause sets in.
- Cyclic changes in the ovary and uterus release the ovum for fertilisation and prepare the uterus for pregnancy.
- Fertilization takes place in the fallopian tube and the period from fertilization to about 2 weeks of development is known as pre-embryonic developmental period in which the implantation of the embryo in the uterine wall takes place.
- Embryonic period of development lasts from two weeks to eight weeks in the first trimester of pregnancy. During this period all the major organs and tissues differentiate from the three germinal layer namely ectoderm, endoderm and mesoderm.
- The foetal period begins at nine weeks. During this period the existing body structures grow and mature rapidly. Body proportions change greatly. Birth takes place at the end of the third trimester, 39 weeks after conception.
- At birth important developmental changes occur in the respiratory and circulatory systems of the newborn that enable it to breath air and separate oxygenated blood from deoxygenated blood so that an adult pattern of circulation starts.
- Development does not stop at birth but continues throughout infancy, childhood, adolescence and adulthood.
- The placenta is haemochorial and forms fully by the tenth week of development. it secretes estrogen and progesterone which maintain the pregnancy. Passage of all nutrients from the maternal blood to foetal blood and exchange of gases takes place across the placental membrane. Various substances and teratogens can cross the placental membrane. Therefore, the embryonic period is especially crucial and sensitive to the effect of drugs and teratogens which can affect the normal development of the embryo.

21.11 TERMINAL QUESTIONS

1) List the sequence of events that occur during fertilization.

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2) How is the placenta formed? What are its functions?

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3) Define the term embryo and foetus

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4) Why is the embryonic period particularly sensitive to teratogens?

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5) Draw a flow chart to show how the three germinal layers are derived from the zygote.

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6) Fill in the blanks with appropriate terms from the text and arrange the developmental events sequentally.

1) The foetus increases its body weight maximum in the..... trimester

2) By the end of ..... trimester, most organ systems are atleast partially furictional

3) After the ..... month the face starts looking more human and the embryo becomes a .....

4) The embryo forms its first organ system in the.....week

5) Cartilage is replaced by bone during the..... month

6) A few days after implantation the ICM becomes a .....ayered embryonic disk separated from the chorion by the .....cavity.

7) The three germ layers form by ..... week of development

8) Following fertilization cleavage, blastulation and implantation occupy the ..... week of development.

21.12 ANSWERS

Self Assessment Questions

1) Greater chance of nondysjunction in chromosomes of ovum because of longer period of suspended meiotic division

2) 1) matches d

2) matches a

3) matches b

4) matches a

3) 1) True ii) False iii) True iv) False

4) i) blastocyst

ii) ICM

iii) Outside the uterus

iv) maintains

v) syncytiotrophoblast

5) A. i) foetal ii) third B. i) fourth ii) fifth iii) seventh iv) eighth

6) to open the collapsed lungs because in the foetus the exchange of gases takes place through the placenta and lungs remain nonfunctional.

7)

In humans	Birds and reptiles
Amnion - fluid filled sac around the embryo. encloses amniotic cavity. Acts as a shock absorber	fluid filled cavity which protects the embryo and prevents dehydration.
Chorion - forms foetal part of placenta; protects embryo against rejection by maternal antibodies. secretes HCG which maintains the corpus luteum till placenta fully formed	controls the overall permeability of the egg. Surrounds amnion, allentois and yolk sac.
Allentois - sac like connected to gut of embryo makes up most of the blood vessels of the embryonic side of placenta	stores wastes until egg hatches; also has respiratory functions.
Yolk sac - No nutrients but bears a clear fluid, manufactures blood cells for early embryo till liver takes up the task; forms part of umblical cord	contains large amount of yolk to nourish the developing embryo. The sac is highly vascularised to carry dissolved nutrients from yolk to embryo.

8) a) To protect the embryo from shocks and provide a stable environment. The embryo is also known to drink the amniotic fluid.

b) Through the selectively permeable membrane of the chorion and capillaries.

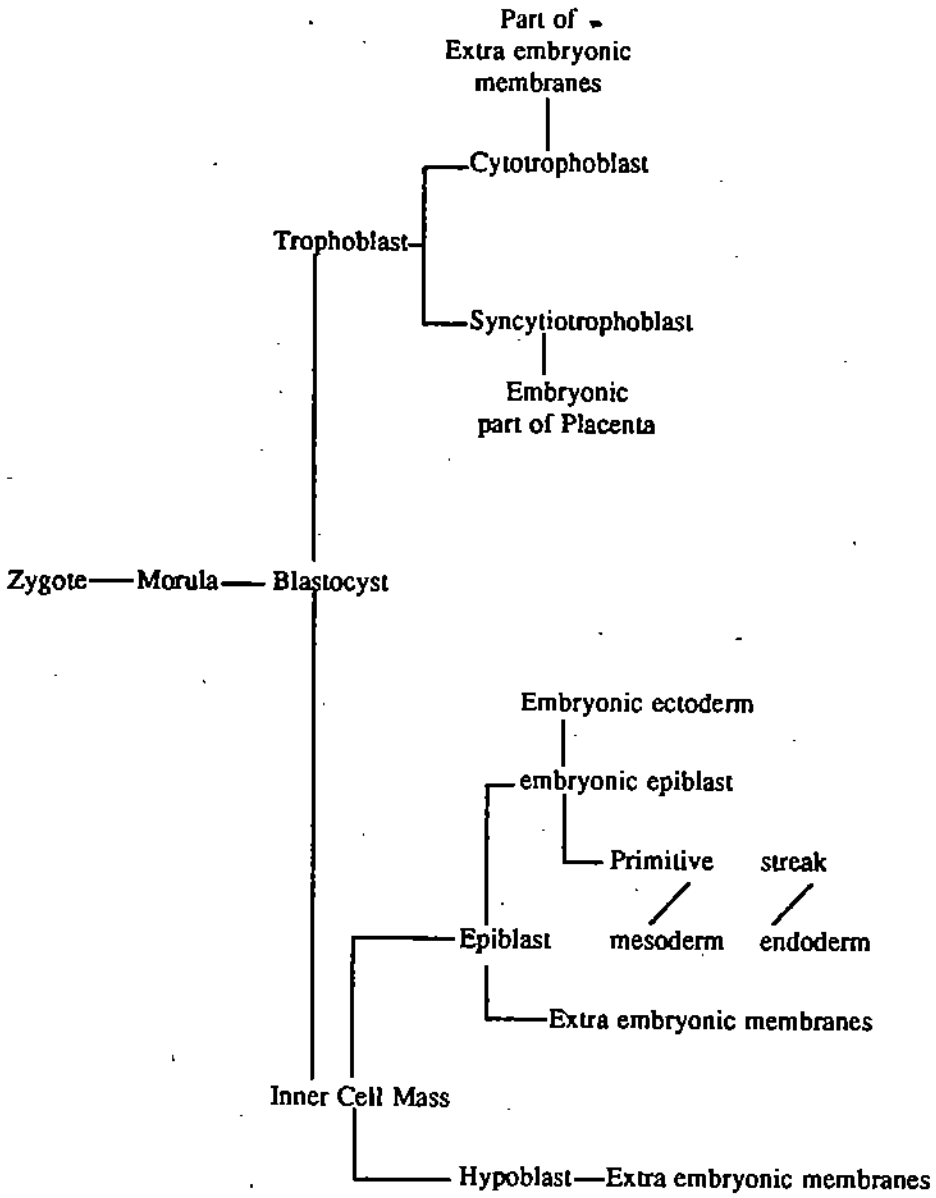
Terminal Questions

1) Refer to section 21.4.2

2) Refer to section 21.5.4

3) The developing individual from two weeks to eight weeks is termed embryo while from eight weeks to birth is referred to as foetus.

4) Because maximum tissue differentiation and organogenesis takes place during the first three months of pregnancy and any agent that will disturb the normal process will result in malformations in the developing individual.



6) Answers for Blanks

1) third

2) first

3) second, foetus

4) third

5) third

6) double; amniotic

7) second

8) first

Sequence for developmental events.

8); 6); 7); 4); 3); 5); 2); 1);

GLOSSARY

Aboral : a region opposite mouth.

Acrosome: covering on the tip of a sperm cell's nucleus containing enzymes necessary for fertilization.

Allantois: an extraembryonic membrane that serves as a source of blood vessels for the umblical cord in mammals. In birds and reptiles it serves as a repository for nitrogenous wastes for the embryo.

***Ambystoma nexicanum*:** species of urodele amphibia also known as aloloti.

Amnion: the innermost extraembryonic membrane: a fluid filled sac around the embryo.

Antenna: a sensory appendage on the head of arthropods: or the second pair of two such pairs of structures in crustaceans.

Apical epidermal cap: multilayered mass of cells that forms a conical bulge at the tip of the amputated urodele limb.

Arterial duct: foetal connection between the pulmonary artery and the aorta, ductus arteriosis.

Atherosclerosis: a pathological condition in which fat deposits are formed on the inner lining of arteries.

Autotomy: the breaking off, of a part of the body at a preformed point by organism itself.

Axolotl:larval stage of any of the several species of genus **Ambystoma.**

Blastocyst: blastula formed by placental mammals. This is the embryonic stage that implants in the wall of the uterus.

Corpus allatum: endocrine gland in insect that produces juvenile hormone.

Corpus luteum: remainder of the follicle after the release of the secondary oocyte. It develops into a gland like structure that produces hormones (progesterone and estogen) that prevent the release of other eggs.

Deurostomía: Animals in which the site of blastopore is far from mouth, which forms a new opening at the anterior end.

Diapause: a period of arrested development in the life cycle of insects and certain other animal in which physiological activity is very low and the animal is highly resistant to unfavourable external conditions.

Differentiation: specialization of embryonic cells.

Ecdysis: shedding of the external cuticular layer; moulting as in insects or crustanceans.

Ecdysone: moulting hormone of arthropods produced by prothoracic gland.

Endopterygote: (endo, within + pteron wing) insects in which wing buds develop internally; has holometabolous metamorphosis

Estrogen: female sex hormones that cause differentiation in the female embryo of internal and external genital anatomy along female lines: responsible for changes in breasts, vagina, uterus, clitoris and pubic bones at puberty.

Exopterygote: (exo-without and pteron feather, wings). Insects in which wing buds develop externally during nymphal instars; has hemimetabolous metamorphosis.

Foetus : unborn mammal after it has completed the development of its organ systems. In humans after 3 months.

Follicle: the sac like structure near the surface of the ovary that encases the soon to be released secondary oocyte.

Gametogenesis: the generation of gametes or sex cells by meiotic cell division.

Genome: a complete haploid set of genes.

Gonadotropic hormone: peptide hormones released from the pituitary gland which regulate gonadial function in vertebrates.

Growth: increase in size of an organism, resulting from an increase in its number of cells.

Histogenesis: formation and development of tissue.

Humerus: bone of the upper arm.

Hypertrophy: abnormal increase in the size of a part of organ.

Imaginal discs: discrete packages of undifferentiated cells in insect endoterygote larva which differentiate during and after metamorphosis to form specific structures.

Imago: the adult and sexually mature insects.

Instars: feeding stages between moults during the larval growth period of insects.

Menopause: the period beginning at about age fifty when the ovaries stop producing viable secondary oocytes.

Mesoglea: the layer of jelly like or cement like material, between the epidermis and gastrodermis in coelentrates such as hydra.

Naiad: an acquatic gill breathing immature insect.

Nematocysts: stinging organelles of hydra and other cnidarians.

Neoteny: the attainment of sexual maturity in larval condition. Also retention of larval characters in adult hood.

Neurosecretory cells: any neuron (cell) of the nervous system that produces a hormone.

Nymph: any immature stage (following hatching) of a hemimetabolous insect that lacks a pupal stage.

Oncogene: gene that contributes to converting normal cells to cancerous state.

Operculum: gill cover in fishes and tadpoles.

Ovulation: cyclic release of secondary oocytes from the surface of ovary every 28 days.

Paedogenesis: reproduction by immature or larval animals.

Pharyngeal arches: columns of mesenchym, between pharyngeal pouches/grooves.

Pharyngeal clefts: region of contact between pharyngeal pouches and grooves that perforate to form the gill.

Pharyngeal grooves: inward projections of the ectodermal pockets in the phyangeal region that approach the phargngeal pouches.

Polar body: the smaller cell formed by unequal meiotic division in the process of oogenesis.

Polyp: sessile stage in the life cycle of cnidarians.

Promoter: region of DNA usually on the 5' side of a gene that is needed for initiation of the transcription of that gene. RNA polymer and other transcription factors bind to the promoter.

Proto-oncogene: a normal cellular gene that when mutated or inappropriately expressed can cause cells to become cancerous. Designated C-onc. They are vital to cell growth and differentiation.

Protostomia: Animals in which blastopore contributes to the formation of mouth.

Puberty: a time in the life of a developing individual characterised by increased production of sex hormones which cause it to become sexually mature.

Puffs: strands of DNA which spread apart at certain location in giant chromosomes of some flies an enlargement of a restricted segment of a polytene chromosome where the DNA is being transcribed.

Pupa: inactive quiescent stage of holometabolous insects. It follows the larval stages and precedes adult stage.

Retrovirus: virus containing RNA as genetic material.

Secondary oocyte: larger of the two cells resulting from lopsided division of primary oocyte in meiosis of oogenesis.

Transformation: conversion to a state of unrestrained growth in culture, resembling or identical with tumerigenic condition. Transformed cells become independent of factors usually needed for cell growth.

Trochophore larva: a free swimming ciliated marine larva, characteristic of most molluses, certain ectoprocts, brachiopods and marine worms.

Ulna: one of the bones of the fore arm of vertebrates.

Urtocle: a protective non cellular, organic layer, secreted by the internal epithelium of many invertebrates.

Xenopus laevis: South African clawed frog.

प्रिय छात्र/छात्रा

इस पाठ्यक्रम के बारे में आपकी राय जानने के लिए हमने यह प्रश्नावली तैयार की है, जो इसी खंड के लिए है। आप के उत्तर हमें पाठ्यक्रम को सुधारने में मदद करेंगे। कृपया इसे भरकर हमें शीघ्र भेज दें।

प्रश्नावली

एल. एस. ई.–06
खंड 4

नामांकन सं. | | | | | | | | | |

1. इकाईयों को पढ़ने में आपको कितने घंटे लगे?

इकाई सं	18	19	20	21
कुल घंटे				

2. इस खंड से संबंधित कार्य को करने के लिए आपको (लगभग) कितने घंटे लगे?

	टी. एम. ए.	सी. एम. ए.
सत्रीय कार्य सं		
कुल घंटे		

3. हमारे विचार से आपके सामने 4 प्रकार की कठिनाईयाँ आई होंगी, उन्हें निम्नलिखित तालिका में दिया गया है। उपयुक्त कालमों में कृपया अपनी कठिनाई पर (√) का निशान लगाइए और सही पृष्ठ संख्या लिखिए।

पृष्ठ सं.	कठिनाइयों के प्रकार			
	प्रस्तुतीकरण स्पष्ट नहीं है	भाषा कठिन है	चित्र स्पष्ट नहीं है	शब्दावली समझाई नहीं गई है

4. हमारा विचार है कि बोध प्रश्नों और अंत में दिये गये प्रश्नों में आपको कुछ कठिनाई हुई होगी। निम्नलिखित तालिका में संभावित कठिनाईयाँ दी हैं। उपयुक्त कालमों से संबंधित इकाइयाँ और प्रश्न संख्या देते हुए अपनी कठिनाईयों पर निशान लगाइए।

इकाई संख्या	कठिनाई का प्रकार					
	बोध प्रश्न संख्या	अंत में दी गई प्रश्न संख्या	प्रश्न स्पष्ट नहीं है	दी गई जानकारी के आधार पर उत्तर नहीं दिया जा सकता	इकाई के अंत में दिया गया उत्तर स्पष्ट नहीं है	दिया गया उत्तर प्रयाप्त नहीं है

5. क्या खंड के अंत में दी गई शब्दावली उपयोगी रही? यदि नहीं, तो निम्न स्थान में कठिन शब्द लिखें।

6. अन्य सुझाव -

Affix
Postage
Stamp

सेवा में,

पाठ्यक्रम संयोजक, एल. एस. ई.-06, परिवर्धन जीवविज्ञान (खंड 2)
विज्ञान विद्यापीठ
इन्दिरा गांधी राष्ट्रीय मुक्त विश्वविद्यालय
मैदान गढ़ी
नई दिल्ली-110068

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